Neuroscience Bulletin

The Official Journal of The Chinese Neuroscience Society
神经科学通报

Volume 33
Number 2
April 2017

Special Issue on Autism Spectrum Disorders

SIBS Springer

www.neurosci.cn

CONTENTS

Neuroscience Bulletin

About the Cover

Head to head
Hand in hand
We lean on each other
In this blue night
Stars twinkle, moonlight hazy

We lean on each other
Me and you, share the same dream
Travel together on an unknown journey
Enjoy together a bowl of cold soft cake
at the shop of childhood

In this blue night
Stars twinkle, moonlight hazy
Me and you, share the same dream

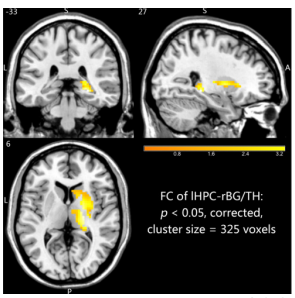
—— *Translation of Ms. Wen Zou's poem*
for the cover image
drawn by Mr. Jingkang Xiao

Children of the Stars; this is what we call children with autism. Parents try every way to reach and understand their world. Scientists try every way to study and understand the disorder. In this Special Issue on Autism Spectrum Disorder, we present a collection of articles on the developmental mechanism, recent technological advancement, and clinical evaluation (especially in China) of autism spectrum disorder. We are honored that Jingkang Xiao, a talented young painter, has contributed in his unique way by providing this cover image for the special issue.

Volume 33 Number 2
April 2017

Editorial

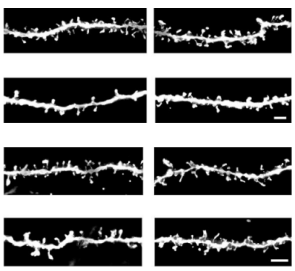
Original Articles



p 130

www.neurosci.cn

CONTENTS



p 143

Reviews

CONTENTS

Neuroscience Bulletin

Letter to the Editor

Editor's Note

—— *Yefei Li*

We the editorial team would like to express our gratitude to Mr. Jingkang Xiao and his loving mother Ms. Wen Zou, for their generous offer of Jingkang's drawing to be the cover of this special issue.

Jingkang Xiao, also known as Kangkang, was born in 1998 and diagnosed as having typical autism with intellectual disability. He could only say the word “mama” when four-and-a-half years old. Like an angel from outer space, he is progressing slowly but steadily every day, every year. He speaks, goes to school, and takes public transportation; he has learned to swim, play basketball, run 5 km, go shopping, play video games on a computer, paint, play piano and cucurbit flute, make toast, and cook. All these little activities that are normal in others’ eyes are, to Kangkang’s parents, magnificent achievements and have brought tremendous joy.

At the age of 15, Kangkang played “You and me” on the cucurbit flute at the opening ceremony of the “Sino-Japanese Autistic Children’s Painting Exhibition” in Tokyo, as a representative of young Chinese autistic painters. Kangkang was called “a healing little painter” when his story was reported by CCTV news. Kangkang enjoys painting and is even more enthusiastic about cuisine. He wants to be a great chef, and enjoy delicious food worldwide.

Through sharing the story of the young man here, we hope to raise more attention to the disease itself and more importantly, the patients and their families' well-being.

Neurosci. Bull. April, 2017, 33(2):125–129
DOI 10.1007/s12264-017-0117-2

www.neurosci.cn
www.springer.com/12264

EDITORIAL

Recent Research Progress in Autism Spectrum Disorder

Xiang Yu[1,2,3] · Zilong Qiu[1,2] · Dai Zhang[4,5,6]

Received: 20 February 2017 / Accepted: 21 February 2017 / Published online: 11 March 2017

On April 2, 2017, the world will celebrate the ninth annual World Autism Awareness Day. In honor of this occasion, Neuroscience Bulletin has put together a Special Issue of reviews and primary research articles focusing on autism spectrum disorder (ASD). ASD is a heterogeneous developmental neurological disorder characterized by deficits in social communication and social interactions, as well as stereotyped, repetitive behavior and/or restricted interests [1]. In addition, affected individuals often have sensory abnormalities and delayed/absent language. The symptoms are present from early childhood, affecting the individual's daily activity and imposing a huge burden on their families and the community at large. While the global burden of ASD is largely unknown, the annual social cost in the United States and the United Kingdom is estimated to be billions of dollars [2, 3].

The term "autism", deriving from the Greek words "*autos*" (self) and "*ismos*" (action), was first used by Leo Kanner in his landmark paper in 1943 [4] to describe children with an "extreme inability to relate to others". In the introductory review "An overview of autism spectrum disorder, heterogeneity and treatment options" [5], Masi *et al*. revisit the history of the diagnosis and characterization of ASD, from Kanner's time to the currently-used Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders [1]. The review progresses to describe the prevalence, etiology, and clinical presentation of ASD, and discusses factors contributing to its heterogeneity, including genetic variability, co-morbidity, and gender. It concludes with evidence for pharmacological and behavioral treatments, highlighting the complexities of conducting clinical trials in ASD populations.

A key factor behind the emerging interest in ASD is its apparently growing prevalence. A recent survey of 8-year old children in the United States estimates ASD occurrence to be as high as 1 in 68 [6]. A review of the global

As we put the final touches on this special issue, we are very excited that the introductory review has already been downloaded (http://link.springer.com/journal/12264) well over 1600 times. We thank Prof. Shumin Duan, Editor in Chief, for the opportunity to organize this issue at such an exciting time for autism research. The issue could not have come together so quickly without concerted efforts from all authors, reviewers and staff members at Neuroscience Bulletin. In particular, we thank Yefei Li for her enthusiastic and professional assistance.

✉ Xiang Yu
yuxiang@ion.ac.cn

✉ Zilong Qiu
zqiu@ion.ac.cn

✉ Dai Zhang
daizhang@bjmu.edu.cn

[1] Institute of Neuroscience, State Key Laboratory of Neuroscience, Center for Excellence in Brain Science and Intelligence Technology, Chinese Academy of Sciences, Shanghai 200031, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

[3] School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China

[4] Institute of Mental Health, Peking University Sixth Hospital, Beijing 100191, China

[5] Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China

[6] PKU-IDG/McGovern Institute for Brain Research, Peking University, Beijing 100871, China

prevalence of autism and other pervasive developmental disorders puts the median estimate of ASD prevalence at the slightly lower rate of 62/10,000 [7], with variations between studies, but no obvious evidence for differences in prevalence between geographic regions and/or ethnicities. This global report [7], commissioned by the World Health Organization, notes that changes in diagnostic concepts, service availability, and awareness of ASD may affect prevalence estimates, and highlights the scarcity of epidemiological studies from low- and middle-income countries.

To better estimate the prevalence of ASD in China, the National Health and Family Planning Commission of the People's Republic of China recently initiated a national population-based study to measure ASD prevalence in children aged 6–12 years in 8 cities (over 120,000 individuals). A suitable screening tool in the Chinese language is essential for conducting this large-scale epidemiological study. From the array of available scales, the Autism Spectrum Rating Scales (ASRS, 6-18 years) [8], translated into Chinese, was selected because of its demonstrated high reliability and validity [9]. In an original article in this Special Issue by Yi Wang and colleagues, exploratory factor analysis was used to assess the psychometric properties of the Chinese version of ASRS [10]. The results showed that the modified Chinese version (MC-ASRS) has a 3-subscale structure comparable to the original US version, although some items have been shifted between subscales. In a companion article by Zhou *et al*., the Chinese norms of the MC-ASRS, including its three sub-scales and the total score, were determined for both parent and teacher ratings [11]. A third study, from Xiu Xu and colleagues, compares the MC-ASRS with the Social Responsiveness Scale, another widely-used tool for screening children with ASD [12]. The results showed that both scales have high reliability and validity, further underscoring the suitability of the MC-ASRS for ASD screening in China. Together, these works set the foundation for large-scale epidemiological studies of ASD in China.

ASD has a strong male bias in its prevalence, on average affecting four times as many males as females [13]. However, sex differences in behavior, the presentation of autistic symptoms, or co-morbid intellectual disability are relatively unknown. An original article from Xiaobing Zou and colleagues explores this question by analyzing the Autism Diagnostic Interview-Revised and Autism Diagnostic Observation Schedule scores for a large cohort of boys and girls [14]. Their results show that girls score significantly higher in socio-emotional reciprocity and lower in restricted and repetitive behaviors than boys. Clarifying sex differences in the diagnosis and clinical phenotypes of ASD could help provide better clinical guidance for early screening, diagnosis, and intervention.

ASD is a heterogeneous disorder with a complex genetic basis. Early twin and family studies have shown that ASD is highly heritable [15], suggesting a strong genetic predisposition. In addition to inherited mutations, recent advances in genetics and genomics have identified a large number of *de novo* copy-number variations and single-base-pair mutations in ASD patients, increasing the estimated proportion of patients with identifiable genetic mutations to 20%–40% [15–20]. As many ASD genes are known to regulate brain development and/or synapse function, theories of ASD relating to synaptic dysfunction, including excitatory/inhibitory imbalance and dysfunctional feedback regulation have been proposed [15, 21–24]. Since deficits in social behavior are hallmarks of ASD, molecules and circuits underlying social behavior have received special attention [25, 26]. Of note are the evolutionarily highly-conserved neuropeptides oxytocin and vasopressin, which have been implicated in ASD through genetic studies and have established roles in regulating social behavior [26–28]. Furthermore, intranasal oxytocin administration is currently being tested as a potential therapy for ASD in clinical trials, with mixed results [29]. Zhang *et al*. [30] review current knowledge regarding associations between ASD and single-nucleotide variants in the human oxytocin and vasopressin signaling pathways, and propose that polymorphisms in these signaling pathways may be important for sub-grouping patients in clinical trials of oxytocin.

In addition to genetic factors, pre-, neo-, and post-natal environmental risk factors have been implicated in the etiology of ASD. Epidemiological studies have identified various pharmaceutical drugs, toxicants, and metabolic and nutritional factors as increasing the ASD risk, especially during the prenatal period [31, 32]. Immunological risk factors, including maternal infection during pregnancy, immune dysregulation, inflammation, and microbial dysbiosis have been consistently reported across multiple studies [31–33]. The interaction between environmental exposures and an individual's genetic susceptibilities, both complex factors by themselves, add yet another layer of complexity to the heterogeneous phenotype of ASD. To delve into this complex problem at some depth, we focused on one aspect of environmental factors contributing to ASD, that of cytokines and the immune system. The review by Guastella and colleagues focuses on the relationship between the immune system, the brain, and behavior, and summarizes previously-identified immune system abnormalities in ASD, focusing on the role of cytokines. They further discuss the use of cytokines as potential biomarkers to define sub-groups of ASD patients [34].

ASD research has not only progressed at the level of genes and molecules, but also at the level of circuits and neural connectivity. Early findings from several structural magnetic resonance imaging studies have shown that toddlers with ASD, aged on average 2–4 years, have a larger brain volume than typically-developing children, an effect that levels off by 6–8 years [35, 36]. These findings contribute to the notion that the trajectory of brain maturation in ASD is atypical and involves an early period of overgrowth, with each brain region having its distinct trajectory [35, 36]. Further structural neuroimaging studies have revealed ASD to be a disorder with general and regional alterations in brain size, while functional neuroimaging studies have highlighted changes in connectivity between brain regions in ASD patients [35, 37–39]. The review by Li *et al*. summarizes recent progress from neuroimaging studies in young ASD children and discusses the applicability of these results in aiding ASD diagnosis [40]. Since ASD is a developmental neurological disorder, neurological changes detected earlier are more likely to represent the causes rather than the effects of ASD pathogenesis. Furthermore, these results could contribute to future diagnosis and treatment strategies.

One approach to further exploration of the application of neuroimaging to ASD research is to interpret its results in combination with molecular markers. An original article from Ji-Sheng Han and colleagues examines correlations between circulating levels of the neuropeptide vasopressin, changes in structural and functional connectivity, and autistic behavior in young children with ASD. They found a significant reduction in the volume of the hypothalamus, where vasopressin neurons reside, as well as enlargement of the left amygdala and left hippocampus, which receive projections from vasopressin neurons [41]. These and other results presented in this article provide evidence for correlated changes in structural and functional connectivity and vasopressin levels in young children with ASD [41]. Neuroimaging can also be used in combination with genome sequencing to characterize rare disorders. The letter by Wen *et al*. [42] identifies an inherited mutation in *SGSH*, encoding N-sulfoglucosamine sulfohydrolase (MIM: 605270), in two brothers with Childhood Disintegrative Disorder, a rare childhood disorder with autistic phenotypes.

A discussion of advances in ASD research would be incomplete without describing the contribution of animal models. Rodent models of ASD, mostly mimicking the genetic abnormalities identified in patients, including loss-of-function mutations, gene duplications, and mis-sense point mutations, have contributed significantly to our understanding of the synaptic, circuit, and behavioral basis of ASD [15, 16, 22, 43, 44]. A number of mouse models of syndromic ASD display social impairment and repetitive behavior, the core features of ASD, although they vary widely in additional co-morbidities, and in alternations in excitatory and inhibitory synaptic transmission in various neuronal circuits. In investigating synaptic alterations in ASD mouse models, Wang *et al*. [45] examined spine density changes in two relatively well-characterized mouse gene-duplication models of ASD, namely the *MECP2* duplication and human chromosome 15q11-13 duplication models. They found that, in the mouse primary somatosensory cortex, 15q11-13 duplication mostly affects spine formation at 1 month of postnatal development, while *MECP2* duplication interferes with spine pruning at 3 months, without significantly affecting spine formation. To study the function of ASD genes in specific circuits and at specific time points during development, it is important to induce the genetic changes with cell-type and spatial–temporal specificity. The review by Hulbert and Jiang discusses the currently-available tools and assays for investigating ASD in rodent models, comprehensively reviewing the genetic tools available through the Cre/LoxP system for inducing genetic alterations in specific cell types and brain regions with temporal control [46]. The authors also summarize the results of published studies using existing mouse models of ASD in combination with these available genetic tools [46]. An exciting recent development in ASD research is the generation of a non-human primate ASD model through *MECP2* overexpression, mimicking the *MECP2* duplication syndrome in humans [47]. The review by Qiu and Li provides an overview of the existing non-human primate models of brain disorders and describes recent advances in gene-editing technology, advances that will likely accelerate the development of other ASD models in non-human primates [48]. Based on the close evolutionary relationships between non-human primates and humans, as well as similarities in their brain structure, non-human primate models of ASD will likely contribute significantly to understanding the circuit basis of ASD, as well as to testing new treatment strategies.

We live in an exciting time for ASD research, with advances at the genetic, molecular, and circuit levels emerging rapidly, and new diagnostic and treatment tools increasing being tested and becoming available. While by no means comprehensive, this Special Issue on ASD, with its reviews and original articles, is intended to provide a flavor of ongoing research progress. We sincerely hope that our efforts will contribute to generating more excitement in ASD research, and ultimately help children and families affected by ASD in a meaningful and fruitful manner.

References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, 5th ed. Arlington, VA: American Psychiatric Publishing 2013.
2. Ganz ML. The lifetime distribution of the incremental societal costs of autism. Arch Pediatr Adolesc Med 2007, 161: 343–349.
3. Knapp M, Romeo Re, Beecham J, Foundation for People with Learning Disabilities. The economic consequences of autism in the UK. Shirley Foundation, King's College London. 2007, 27.
4. Kanner L. Autistic disturbances of affective contact. Nervous Child 1943, 2: 217–250.
5. Masi A, DeMayo MM, Glozier N, Guastella AJ. An overview of autism spectrum disorder, heterogeneity and treatment options. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0100-y.
6. Samuels, Christina A. Prevalence of autism spectrum disorder among children aged 8 years—Autism and developmental disabilities monitoring network, 11 sites, United States, 2010. Education Week 2014, 63: 1–21.
7. Elsabbagh M, Johnson MH. Getting answers from babies about autism. Trends Cogn Sci 2010, 14: 81–87.
8. Goldstein S, Naglieri JA. Autism Spectrum Rating Scales (ASRS). 2009.
9. Zhou H, Zhang L, Wu L, Zou X, Luo X, Xia K, *et al*. Validity and reliability analysis of the Chinese parent version of the Autism Spectrum Rating Scale (6–18 years). Psychiatry Res 2015, 230: 255–261.
10. Zhou H, Zhang L, Luo X, Wu L, Zou X, Xia K, *et al*. Modifying the Autism Spectrum Rating Scale (6–18 years) to a Chinese context: an exploratory factor analysis. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0104-7.
11. Zhou H, Zhang L, Zou X, Luo X, Xia K, Wu L, *et al*. Chinese Norms for the Autism Spectrum Rating Scale. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0105-6.
12. Zhou B, Zhou H, Wu L, Zou X, Luo X, Fombonne E, *et al*. Assessing the accuracy of the modified Chinese Autism Spectrum Rating Scales and social responsiveness scale for screening autism spectrum disorder in Chinese children. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0114-5.
13. Werling DM, Geschwind DH. Sex differences in autism spectrum disorders. Curr Opin Neurol 2013, 26: 146–153.
14. Wang S, Deng H, You C, Chen K, Li J, Tang C, *et al*. Sex differences in diagnosis and clinical phenotypes of Chinese children with autism spectrum disorder. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0102-9.
15. de la Torre-Ubieta L, Won H, Stein JL, Geschwind DH. Advancing the understanding of autism disease mechanisms through genetics. Nat Med 2016, 22: 345–361.
16. Geschwind DH, State MW. Gene hunting in autism spectrum disorder: on the path to precision medicine. Lancet Neurol 2015, 14: 1109–1120.
17. Devlin B, Scherer SW. Genetic architecture in autism spectrum disorder. Curr Opin Genet Dev 2012, 22: 229–237.
18. Krumm N, O'Roak BJ, Shendure J, Eichler EE. A de novo convergence of autism genetics and molecular neuroscience. Trends Neurosci 2014, 37: 95–105.
19. Huguet G, Ey E, Bourgeron T. The genetic landscapes of autism spectrum disorders. Annu Rev Genomics Hum Genet 2013, 14: 191–213.
20. Ronemus M, Iossifov I, Levy D, Wigler M. The role of de novo mutations in the genetics of autism spectrum disorders. Nat Rev Genet 2014, 15: 133–141.
21. Zoghbi HY, Bear MF. Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities. Cold Spring Harb Perspect Biol 2012, 4.
22. Mullins C, Fishell G, Tsien RW. Unifying views of autism spectrum disorders: a consideration of autoregulatory feedback loops. Neuron 2016, 89: 1131–1156.
23. Nelson SB, Valakh V. Excitatory/inhibitory balance and circuit homeostasis in autism spectrum disorders. Neuron 2015, 87: 684–698.
24. Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2003, 2: 255–267.
25. Barak B, Feng G. Neurobiology of social behavior abnormalities in autism and Williams syndrome. Nat Neurosci 2016, 19: 647–655.
26. Stoop R. Neuromodulation by oxytocin and vasopressin. Neuron 2012, 76: 142–159.
27. Insel TR. The challenge of translation in social neuroscience: a review of oxytocin, vasopressin, and affiliative behavior. Neuron 2010, 65: 768–779.
28. Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M. Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. Nat Rev Neurosci 2011, 12: 524–538.
29. Alvares GA, Quintana DS, Whitehouse AJ. Beyond the hype and hope: Critical considerations for intranasal oxytocin research in autism spectrum disorder. Autism Res 2017, 10: 25–41.
30. Zhang R, Zhang HF, Han JS, Han SP. Genes related to oxytocin and arginine-vasopressin pathways: associations with autism spectrum disorders. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0120-7.
31. Matelski L, Van de Water J. Risk factors in autism: Thinking outside the brain. J Autoimmun 2016, 67: 1–7.
32. Rossignol DA, Frye RE. A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. Mol Psychiatry 2012, 17: 389–401.
33. Vuong HE, Hsiao EY. Emerging Roles for the Gut Microbiome in Autism Spectrum Disorder. Biol Psychiatry 2017, 81: 411–423.
34. Masi A, Glozier N, Dale R, Guastella AJ. The immune system, cytokines, and biomarkers in autism spectrum disorder. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0103-8.
35. Ecker C, Bookheimer SY, Murphy DG. Neuroimaging in autism spectrum disorder: brain structure and function across the lifespan. Lancet Neurol 2015, 14: 1121–1134.
36. Courchesne E, Campbell K, Solso S. Brain growth across the life span in autism: age-specific changes in anatomical pathology. Brain Res 2011, 1380: 138–145.
37. Hull JV, Jacokes ZJ, Torgerson CM, Irimia A, Van Horn JD. Resting-State Functional Connectivity in Autism Spectrum Disorders: A Review. Front Psychiatry 2016, 7: 205.
38. Hernandez LM, Rudie JD, Green SA, Bookheimer S, Dapretto M. Neural signatures of autism spectrum disorders: insights into brain network dynamics. Neuropsychopharmacology 2015, 40: 171–189.
39. Mahajan R, Mostofsky SH. Neuroimaging endophenotypes in autism spectrum disorder. CNS Spectr 2015, 20: 412–426.
40. Li D, Karnath HO, Xu X. Candidate biomarkers in children with autism spectrum disorder: a review of MRI studies. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0118-1.
41. Shou XJ, Xu XJ, Zeng XZ, Liu Y, Yuan HS, Xing Y, *et al*. A volumetric and functional connectivity MRI study of brain arginine-vasopressin pathways in autistic children. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0109-2.
42. Wen Z, Cheng TL, Yin DZ, Sun SB, Wang Z, Yu SY, *et al*. Identification of the genetic cause for the childhood disintegrative disorder by whole exome sequencing. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0119-0.

43. Sztainberg Y, Zoghbi HY. Lessons learned from studying syndromic autism spectrum disorders. Nat Neurosci 2016, 19: 1408–1417.
44. Delorme R, Ey E, Toro R, Leboyer M, Gillberg C, Bourgeron T. Progress toward treatments for synaptic defects in autism. Nat Med 2013, 19: 685–694.
45. Wang M, Li H, Takumi T, Qiu Z, Xu X, Yu X, *et al*. Distinct defects in spine formation or pruning in two gene duplication mouse models of autism. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0111-8.
46. Hulbert SW, Jiang YH. Cellular and circuitry bases of autism: lessons learned from the temporospatial manipulation of autism genes in the brain. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0112-7.
47. Liu Z, Li X, Zhang JT, Cai YJ, Cheng TL, Cheng C, *et al*. Autism-like behaviours and germline transmission in transgenic monkeys overexpressing MeCP2. Nature 2016, 530: 98–102.
48. Qiu Z, Li X. Non-human primate models for brain disorders—towards genetic manipulations via innovative technology. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0115-4.

Neurosci. Bull. April, 2017, 33(2):130–142
DOI 10.1007/s12264-017-0109-2

www.neurosci.cn
www.springer.com/12264

ORIGINAL ARTICLE

A Volumetric and Functional Connectivity MRI Study of Brain Arginine-Vasopressin Pathways in Autistic Children

Xiao-Jing Shou[1,2,3] · Xin-Jie Xu[1,2,3] · Xiang-Zhu Zeng[4] · Ying Liu[4] · Hui-Shu Yuan[4] · Yan Xing[5] · Mei-Xiang Jia[6,7] · Qing-Yun Wei[8] · Song-Ping Han[1] · Rong Zhang[1,2,3] · Ji-Sheng Han[1,2,3]

Received: 8 December 2016 / Accepted: 6 February 2017 / Published online: 3 March 2017

Abstract Dysfunction of brain-derived arginine-vasopressin (AVP) systems may be involved in the etiology of autism spectrum disorder (ASD). Certain regions such as the hypothalamus, amygdala, and hippocampus are known to contain either AVP neurons or terminals and may play an important role in regulating complex social behaviors. The present study was designed to investigate the concomitant changes in autistic behaviors, circulating AVP levels, and the structure and functional connectivity (FC) of specific brain regions in autistic children compared with typically developing children (TDC) aged from 3 to 5 years. The results showed: (1) children with ASD had a significantly increased volume in the left amygdala and left hippocampus, and a significantly decreased volume in the bilateral hypothalamus compared to TDC, and these were positively correlated with plasma AVP level. (2) Autistic children had a negative FC between the left amygdala and the bilateral supramarginal gyri compared to TDC. The degree of the negative FC between amygdala and supramarginal gyrus was associated with a higher score on the clinical autism behavior checklist. (3) The degree of negative FC between left amygdala and left supramarginal gyrus was associated with a lowering of the circulating AVP concentration in boys with ASD. (4) Autistic children showed a higher FC between left hippocampus and right subcortical area compared to TDC. (5) The circulating AVP was negatively correlated with the visual and listening response score of the childhood autism rating scale. These results strongly suggest that changes in structure and FC in brain regions containing AVP may be involved in the etiology of autism.

Keywords Autism · Children · Arginine-vasopressin · MRI · Structure · Connectivity · Behavior

Electronic supplementary material The online version of this article (doi:10.1007/s12264-017-0109-2) contains supplementary material, which is available to authorized users.

✉ Rong Zhang
zhangrong@bjmu.edu.cn

✉ Ji-Sheng Han
hanjisheng@bjmu.edu.cn

1 Neuroscience Research Institute, Peking University, Beijing 100191, China

2 Department of Neurobiology, School of Basic Medical Sciences, Peking University, Beijing 100191, China

3 Key Laboratory for Neuroscience, Ministry of Education/ National Health and Family Planning Commission, Peking University, Beijing 100191, China

4 Radiology Department, Peking University Third Hospital, Beijing 100191, China

5 Department of Pediatrics, Peking University Third Hospital, Beijing 100191, China

6 Mental Health Institute, Peking University, Beijing 100191, China

7 Key Laboratory of Ministry of National Health and Family Planning Commission, Beijing 100191, China

8 Sunshine Friendship Rehabilitation Centre, Beijing 100085, China

Introduction

Autism spectrum disorder (ASD) is a developmental disorder with a high incidence and the symptoms may be lifelong. ASD is characterized by impaired social interaction and communication as well as repetitive behaviors. In the United States, ASD affects 14.7 per 1,000 (1/68) children [1], and an estimated prevalence of 4.1 per 1,000 has been

reported in China [2]. Besides, the prevalence is three to five times higher in boys than girls worldwide [1, 3, 4].

Arginine-vasopressin (AVP) and oxytocin (OXT) are nonapeptides with similar primary sequences, mainly synthesized in the hypothalamus. In the last decade, AVP and OXT have attracted more attention as neuropeptides in the central nervous system [5, 6] in addition to their peripheral effects on vascular and uterine smooth muscles. In the brain, AVP and OXT are released through a paracrine process into extra-hypothalamic areas and along the axonal projections to remote nuclei including the amygdala and hippocampus [6, 7], both of which are believed to be involved in certain mental diseases. Several lines of evidence suggest that dysfunction of the AVP and/or OXT system in these areas may be involved in the pathogenesis of ASD. These peptides are known to be heavily involved in the regulation of complex social cognition and behaviors [8–11], and impairment in social interaction is one of the core symptoms in ASD. Both AVP and OXT have been shown to play roles in neural development, and their receptors are highly expressed and constantly remodeled in the immature brain [7, 12–14]. Besides, the distribution of their receptors and the function of AVP/OXT in regulating behaviors are sexually dimorphic, especially during brain development [15–18]. Similar sexual dimorphism has also been noted in children with ASD.

Pharmacological studies have provided strong evidence that interfering with the AVP/OXT system results in changes in social behaviors in normal individuals and affects the symptoms in patients with ASD. Results from brain-imaging studies in healthy people have shown that intranasal AVP enhances emotion processing [19], altruistic interaction [20], and social recognition [21]. Intranasal OXT has been reported to improve trust [22], emotion processing [23–26], altruistic interaction [20], and empathy [27] in healthy participants. Recent studies have demonstrated that intranasal OXT improves the ability to infer the social emotions of others in autistic adults [28] and enhances brain function in children with autism during judgments of social/non-social pictures [29].

However, few studies have explored the relationship between dysfunction of the AVP system and abnormalities of the structure or function of brain regions in autistic patients, especially in very young children. Our previous studies have demonstrated that transcutaneous electrical acupoint stimulation (TEAS) improves autistic behaviors in children, possibly by enhancing the action of the AVP system [30]. In addition, Carson and colleagues recently found that plasma AVP concentrations can be used to predict AVP levels in the cerebrospinal fluid of children, and might serve as a biomarker for social function in children with autism [31, 32]. Therefore, changes in plasma AVP may be used as a less invasive approach to monitoring changes in the central AVP level in children.

Based on these findings, we designed a study using volumetric and functional connectivity measures with magnetic resonance imaging (MRI) techniques in an effort to answer the following questions: (1) whether the nuclei (i.e., hypothalamus, amygdala, and hippocampus) containing AVP perikarya or terminals show morphological abnormalities in children with ASD compared with typically-developing children (TDC); (2) whether these structures have aberrant connections with other areas in children with ASD; and (3) whether brain dysfunction is correlated with changes in the plasma level of AVP. To our knowledge, this is the first study to quantitatively explore a possible association between circulating AVP and the findings from MRI in children with ASD.

Materials and Methods

Participants

This study was approved by Peking University Institutional Review Board (IRB00001052-13079). The purposes and procedures were described orally to the parents of all the children, with question-and-answer sessions. Each participant's parent(s) gave informed consent. Children with ASD were recruited from the Sunshine Friendship Rehabilitation Center for Children with Autism, Beijing, China. Twenty-one children with ASD aged between three and five years were recruited. Nineteen of the 21 completed MRI scanning. Two were excluded before completion of MRI scanning: one with a history of epilepsy, and one who woke up during the scan. Of the 19 scanned children with ASD, 4 were excluded because of abnormal brain structure (one had cavum septum sellucidum, one had a cortical developmental issue, and two had myelin dysplasia). One child's data were discarded in the pre-processing due to head motion (>3 mm or 3°). Thus, high-quality functional and structural MRI data were successfully acquired from a total of 14 children with ASD (12 boys and 2 girls). Fourteen age- and gender-matched TDC (10 boys and 4 girls) were recruited and completed scanning. All participants in the study were Han Chinese.

Diagnoses and Assessments of ASD

All the participants with ASD met the conditions of the International Statistical Classification of Diseases and Related Health Problems, 10th revision [33], and the Diagnostic and Statistical Manual of Mental Disorders IV-Text Revision [34]. The Childhood Autism Rating Scale

(CARS)[35] was used in 13 of the autistic children (one missed the evaluation) by the same experienced psychiatrist (MXJ). Children with ASD were excluded if they also had other neurodevelopmental diseases or psychiatric disorders, or had a history of using antipsychotic drugs. TDC were screened by an experienced pediatric psychiatrist (JSZ) to exclude any mental disorders.

Both children with ASD and TDC were assessed for autistic symptoms by their parent(s) using the following scales, with the exception of one child with ASD whose questionnaire was technically invalid. (1) The Autism Behavior Checklist (ABC), includes a series of atypical behaviors in five subdomains: sensory, relating, body and object use, language, and social and self-help [35, 36]. (2) The Autism Spectrum Quotient Children's Version (AQ-Child), consists of 50 descriptive statements designed to detect autistic traits. And these statements were designed to assess the phenotype in five areas: social skills, attention switching, attention to details, communication, and imagination [37, 38].

Collection of Blood Samples

Blood samples from all autistic children who completed MRI scanning were collected by a pediatric nurse in the morning between 08:00 and 10:30, no more than 7 days after MRI scanning. To minimize the potential impact of eating and drinking, parents were asked to ensure that their child fasted overnight and no more than 100 mL of fluid was taken. Five milliliters of venous blood was drawn into an EDTA tube (Becton, Dickinson and Co., Franklin Lakes, NJ) containing 2500 KIU aprotinin (Sigma-Aldrich, St. Louis, MO) and gently mixed. The samples were kept on ice for 15–30 min and centrifuged at 1600 g for 15 min at 4 °C. The plasma samples were divided into Eppendorf tubes (700 μL/tube) and frozen at −80°C until assay.

Imaging Data Acquisition

To keep the young child's head still during scanning, children were sedated [39, 40] with oral chloral hydrate (50 mg/kg body weight; maximum dose, 1 g). The child was put into the scanner after falling asleep for ~10–15 min.

Images were acquired in a GE Discovery MR750 3.0T scanner at Peking University Third Hospital. T1-weighted anatomical images in the sagittal plane were collected with a 3D fast spoiled gradient echo sequence: repetition time (TR) = 4.9 ms, echo time (TE) = 2 ms, flip angle = 15°, field of view (FOV) = 240 mm, in-plane resolution = 1 × 1 mm^2, slice thickness = 1 mm, 170 slices. Functional images were collected axially using a gradient-echo echo-planar sequence sensitive to BOLD contrast. The acquisition parameters were as follows: TR = 3,000 ms, TE = 20 ms, flip angle = 90°, FOV = 240 mm, in-plane resolution = 2 × 2 mm^2, slice thickness = 3 mm, slice gap = 0.3 mm, 41 slices. Children with abnormal brain structures were excluded by two neuro-radiologists (YL and XZZ).

Structural Imaging Analyses

Structural images were processed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm) and VBM8 toolbox (http://dbm.neuro.unijena.de/vbm) within the MatLab R2014a programming environment (The MathWorks, Natick, MA). Since the participants were pre-school children, a customized template and tissue-probability map (TPM) were created for the subsequent voxel-based morphometric analysis. T1 data from 12 additional age-matched children (2–6 years) acquired with the same sequence were retrieved from the hospital database, giving a total of 40 children, in order to calculate the template and TPM. The customized TPM was calculated in MNI (Montreal Neurological Institute) space with the TOM8 toolbox (http://irc.cchmc.org/software/tom.php) [41]. In addition, an average DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra) template of all the 40 children in MNI space was constructed using the DARTEL suite in SPM8. The customized template was then implemented into VBM8 as the high-dimensional DARTEL approach, while the affine-registered gray matter (GM) segments were warped to the averaged template and modulated. Raw volumes for GM, white matter (WM), cerebrospinal fluid (CSF), and total intracranial volume (TIV) were calculated in native space.

The normalized GM images were smoothed for a kernel with 8-mm full-width-at-half-maximal (FWHM). The two-sample *t*-test was applied to compare the voxel-based gray matter density between groups. The single-voxel threshold was set at $P < 0.01$ and a minimal cluster size of 626 voxels was used to correct for multiple comparisons. The corrected threshold was determined by 5000 Monte Carlo simulations (FWHM = 8 mm, cluster connection radius: rmm = 5, with a mask of whole-brain GM at a resolution of 1.5 × 1.5 × 1.5 mm^3). Parts of the positive results were further analyzed for volume measurement.

Regions of interest (ROIs) including the amygdala, hippocampus, and hypothalamus were extracted from the T1-weighted original space of each participant, manually traced by an experienced rater (XJS) using 3D Slicer (http://www.slicer.org) [42]. The locations of ROIs are described in the supplementary material. There was good test-retest reliability for the amygdala (Cronbach's α = 0.992), hippocampus (Cronbach's α = 0.995), and hypothalamus (Cronbach's α = 0.991).

Functional Imaging Analyses

Pre-processing of the functional images was analyzed with SPM8. The first 10 volumes were discarded and the remaining volumes were then realigned and corrected for slice timing. Each child's T1 and fMRI images were first normalized to the custom template, and the customized template was normalized to the default EPI (echo-planar imaging) template with a voxel size of $3 \times 3 \times 3$ mm^3. After that, the parameter was applied to the normalized T1 and fMRI images, and finally smoothed at FWHM = 8 mm. The inclusion criteria for head motion were <3 mm translation and 3° rotation. One child with ASD was excluded due to head motion.

The functional connectivity (FC) analyses were processed using the REST V1.8 toolkit (http://resting-fmri.sourceforge.net) [43]. To remove the linear trend and reduce the effect of low-frequency drift and high-frequency noise, de-trending and band-pass filtering (0.01–0.08 Hz) were applied. To further control the non-neural noise, the six head motion parameters, the WM signal, the CSF signal and the global mean signal [44] were regressed out as covariates.

Due to the prior significant VBM (voxel-based morphometry) results and knowledge of the structure-function relationships of the central AVP/OXT systems, the left amygdala and left hippocampus were set as seed points based on automated anatomical labeling [45] of template-defined regions. Voxel-wise functional connectivity was calculated for each seed point. Analyses of covariance were then performed in the two groups (TDC and ASD) with covariates of age and gender. Pearson's correlation coefficients between the significant brain regions were computed and normalized to z-values using Fisher's z transformation for further analyses.

The statistical maps were corrected for multiple comparisons using cluster thresholds performed in the AlphaSim module of the REST toolbox. As determined by 5,000 iterations of Monte Carlo simulation (single P value = 0.05, FWHM = 8 mm, cluster connection radius: rmm = 5, with a mask of whole-brain GM with a resolution of $3 \times 3 \times 3$ mm^3), the minimum cluster size was 259 voxels.

Biochemical Analyses

Plasma AVP concentrations were determined by enzyme immunoassay (EIA) (Enzo Life Sciences, Plymouth Meeting, PA). After extraction of AVP with acetone and petroleum ether, the assay was performed according to the manufacturer's instructions. The EIA was highly sensitive with a detection limit of 3.39 pg/mL for AVP. The cross-reactivity with related peptides in human was <0.001%. The r-values of standard curves for the assays were >0.999.

Statistics

Analysis of the non-imaging statistical data was performed in IBM SPSS Statistics 20.0 and Amos 20.0 (IBM, Armonk, NY). The demographics (except for gender) and scale characteristics were all normally distributed (Tables S1 and S2) and were compared between the two groups using the independent-sample t test. Data are expressed as mean ± SD. Gender comparison was made using the χ^2 test. Differences in global tissues (GM, WM, CSF, and TIV) and brain regions were assessed with multivariate analysis of variance. To explore associations between the functional connectivity and behavioral scores or biochemical indices, Pearson correlations were used for normally-distributed data and Spearman correlations were used for non-normally-distributed data.

A structural equation model was used to clarify the relationships among plasma neuropeptides, functional connectivities, and behaviors. A P value >0.05 indicates an acceptance of the assumptive model. The fitness of the model was assessed by the goodness-of-fit index (GFI) and adjusted GFI (AGFI) in which >0.9 was considered to indicate a good fit. A root-mean-square error of approximation (RMSEA) value of ~0.08 or less indicated a reasonable error of approximation.

Results

Demographic Characteristics and Behavioral Measurements

Children with ASD and TDC were matched for age (t = 1.465, P = 0.1548) and gender (χ^2 = 0.849, P = 0.3570). Children with ASD had significantly higher scores in the ABC (F = 6.128, P <0.0001) and AQ-Child (F = 5.617, P <0.0001) than TDC (Table 1), as expected.

Correlations Between Plasma AVP and Symptoms

Spearman correlations were used to explore the correlations between the plasma concentrations of the neuropeptide and behavioral scores. The plasma AVP level was negatively correlated with the visual and listening response score (r = −0.634, P = 0.020) in CARS (Fig. 1). This result implies that an abnormal level of plasma AVP is associated with visual experience and auditory sense impressions.

Structural Differences Between ASD and TDC

In the VBM analyses, no significant differences were found in the raw volumes of GM, WM, CSF, and TIV between the ASD and TDC groups before and after covariates were controlled (Table 2). Voxel-wise whole-brain comparison

Table 1 Demographic characteristics and behavioral measurements of participants.

Variables	TDC	ASD	t or χ^2	P value
Number of participants	14	14	NA	NA
Age range	3.0–5.5	2.9–5.0	NA	NA
Mean age ± SD	4.5 ± 0.74	4.1 ± 0.72	1.465[a]	0.1548
Gender (boy:girl)	10:4	12:2	0.849[b]	0.3570
CARS score (mean ± SD)	NA	35.38 ± 4.00	NA	NA
ABC score (mean ± SD)	10.71 ± 10.63	53.46 ± 22.97	6.128[a]	<0.0001
AQ-Child score (mean ± SD)	17.29 ± 4.92	29.84 ± 6.63	5.617[a]	<0.0001

CARS childhood autism rating scale, *ABC* autism behavior checklist, *AQ-child* autism spectrum quotient children's version, *TDC* typically developing children, *ASD* autism spectrum disorder

[a] Independent-sample t test, t score

[b] χ^2 test, χ^2

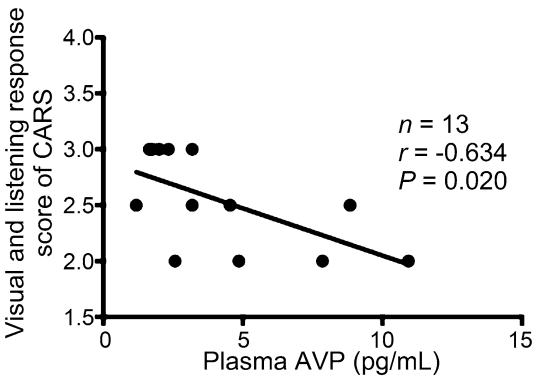


Fig. 1 Correlations between CARS score and plasma AVP level in children with ASD. The visual and listening response score of CARS was negatively correlated with plasma AVP concentration. AVP, arginine-vasopressin; CARS, Childhood Autism Rating Scale.

showed that specific cortical and subcortical areas were significantly enlarged in the ASD group compared to the TDC group (P <0.01, corrected; cluster size >626 voxels): the bilateral insula, putamen, parahippocampus, hippocampus, and amygdala (Fig. 2).

Additional volume measurements of nuclei were then performed according to the voxel-based results, including the bilateral hypothalamus where AVP is synthesized as well as the bilateral amygdala and hippocampus as regions innervated by central AVP neurons. The results showed that the volumes of the left amygdala (1.39 ± 0.18 cm^3 for ASD, 1.27 ± 0.11 cm^3 for TDC; F = 4.520, P = 0.045) and the left hippocampus (3.54 ± 0.44 cm^3 for ASD, 3.24 ± 0.39 cm^3 for TDC; F = 4.548, P = 0.043) were significantly enlarged in children with ASD. When controlling for the effects of age and gender, the volume of the left amygdala remained significantly larger than that in TDC (F = 6.46, P = 0.018), but this was not seen in the left hippocampus (F = 4.019, P = 0.056). Moreover, the volumes of the bilateral hypothalamus were significantly lower (left: F = 13.126, P = 0.001; right: F = 15.253, P = 0.001) in the ASD group (left: 0.50 ± 0.07 cm^3; right: 0.45 ± 0.05 cm^3) than in the TDC group (left: 0.59 ± 0.06 cm^3; right: 0.54 ± 0.07 cm^3), even when the effects of age and gender were carefully controlled (left: F = 12.017, P = 0.002; right: F = 12.006, P = 0.002).

Table 2 Tissue comparison of raw volume using voxel-based morphometric analysis.

Regions	Volume (cm^3)				F score	P value	Corrected for covariates[e]	
	TDC		ASD					
	Mean	SD	Mean	SD			F score	P value
GM[a]	729.43	44.79	708.58	92.34	0.578	0.454	1.220	0.280
WM[b]	378.40	32.92	377.42	69.54	0.002	0.962	0.054	0.818
CSF[c]	165.47	28.69	158.47	31.80	0.374	0.546	0.173	0.681
TIV[d]	1273.30	88.34	1244.47	164.14	0.335	0.568	0.188	0.668

[a] GM, gray matter

[b] WM, white matter

[c] CSF, cerebrospinal fluid

[d] TIV, total intracranial volume

[e] covariates of additional analyses of age and gender; TDC, typically developing children; ASD, autism spectrum disorder.

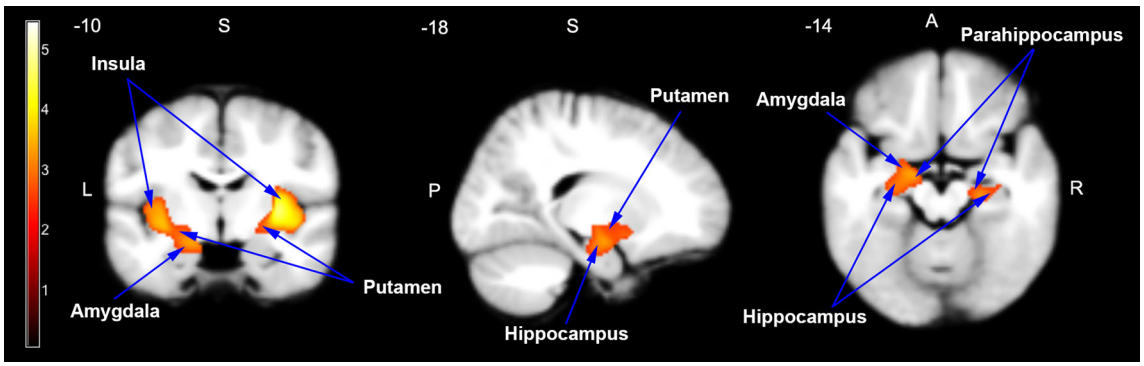


Fig. 2 Enlarged brain areas in children with ASD compared to TDC in the coronal, sagittal, and axial planes. Two clusters were significantly larger in children with ASD than in TDC using voxel-based morphometric analysis. The clusters included the bilateral amygdala, hippocampus, parahippocampus, putamen, and insula. The enlarged brain areas are indicated by arrows on the customized pediatric template. P <0.01, corrected. The cluster in the left hemisphere was 2356 voxels, and the cluster in the right hemisphere was 3790 voxels.

Correlation Between Plasma AVP and Volume of Hypothalamus

Spearman correlation analysis demonstrated that the plasma AVP concentration was positively correlated with the total volume of the hypothalamus (r = 0.574, P = 0.0342). This result suggests that the AVP level is closely related to the morphology of the hypothalamus (Fig. 3).

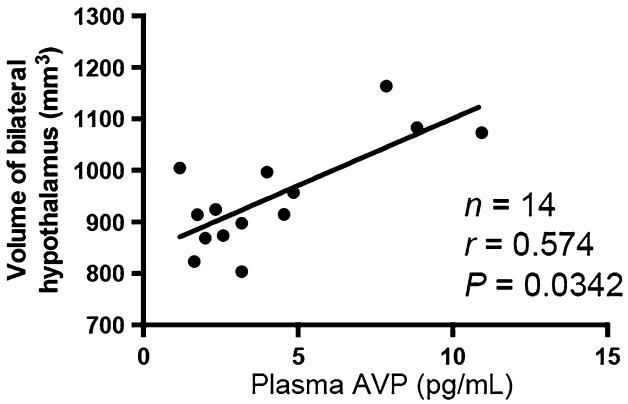


Fig. 3 Correlation between plasma AVP level and the volume of the hypothalamus. The plasma level of AVP was positively correlated with the volume of the bilateral hypothalamus, indicating that a smaller hypothalamus may be predicted by a lower circulating level of AVP.

Analyses of Functional Connectivity

Seed Point Set in the Left Amygdala

The voxel-wise analyses revealed that both the right supramarginal gyrus (rSMG; P <0.05, corrected; cluster size, 358 voxels; peak MNI coordinates, 60, −27, 30) and left supramarginal gyrus (lSMG; P <0.05, corrected; cluster size, 356 voxels; peak MNI coordinates, −54, −21, 24) showed significant negative FC to the left amygdala (lAMG) in children with ASD compared to TDC when controlled for age and gender (Fig. 4A–D). Correlation analyses revealed that in children with ASD, significant correlations existed between the FC of lAMG-rSMG and total ABC score (r = −0.632, P = 0.020), and between the FC of lAMG-rSMG and sensory ABC score (r = −0.677, P = 0.011) (Fig. 4E, F). A strong negative FC was associated with a high sensory score and the total ABC score.

Seed Point Set in the Left Hippocampus

The right basal ganglia and right thalamic area (rBG/TH) were found to have a higher FC to the left hippocampus (lHPC) in children with ASD than TDC (P <0.05, corrected; cluster size, 325 voxels; peak MNI coordinates, 27, −33, −6) with age and gender set as covariates (Fig. 5).

Correlations Between Brain Function and Plasma AVP

We further examined the connection between brain function and plasma levels of AVP in autistic children. Pearson correlation analyses revealed that only in boys with ASD was there a significant positive correlation between the FC of lAMG-lSMG and plasma AVP concentration (r = 0.627, P = 0.029) (Fig. 6A). In all children with ASD, there was a tendency for a negative correlation between the FC of lHPC-rBG/TH and the plasma level of AVP (r = −0.524, P = 0.055) (Fig. 6B). These revealed that in children with ASD, especially boys, a lower plasma AVP level is associated with more aberrant FC.

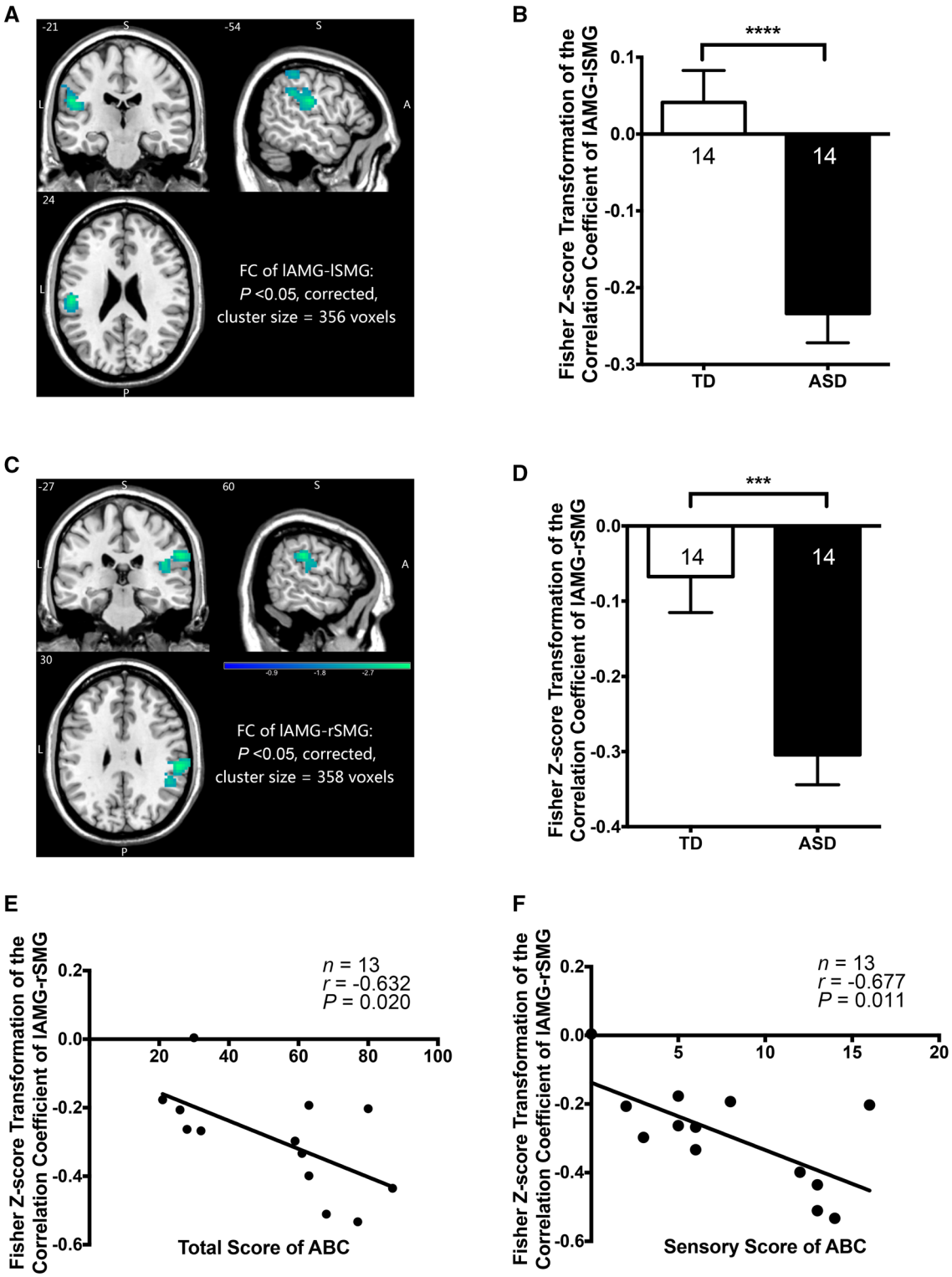


Fig. 4 Functional connectivities (FCs) between left amygdala and bilateral supramarginal gyrus and correlations between the FCs and behavioral scores. **A**, **B** The FC of lAMG-lSMG showed a significantly stronger negative connection in children with ASD than the TDC (P <0.05, controlled for age and gender, corrected; cluster size, 390 voxels, peak MNI coordinates, −54, −18, 24). **C**, **D** The FC of lAMG-rSMG showed a significantly stronger negative connection in children with ASD than the TDC (P <0.05, controlled for age and gender, corrected; cluster size, 358 voxels; peak MNI coordinates, 60, −27, 30). **E**, **F** The FC of lAMG-rSMG was negatively correlated with both the total ABC score and the sensory ABC score. ABC, Autism Behavior Checklist; ASD, autism spectrum disorder; TDC, typically developing children; MNI, Montreal Neurological Institute; lAMG, left amygdala; lSMG, left supramarginal gyrus; rSMG, right supramarginal gyrus.

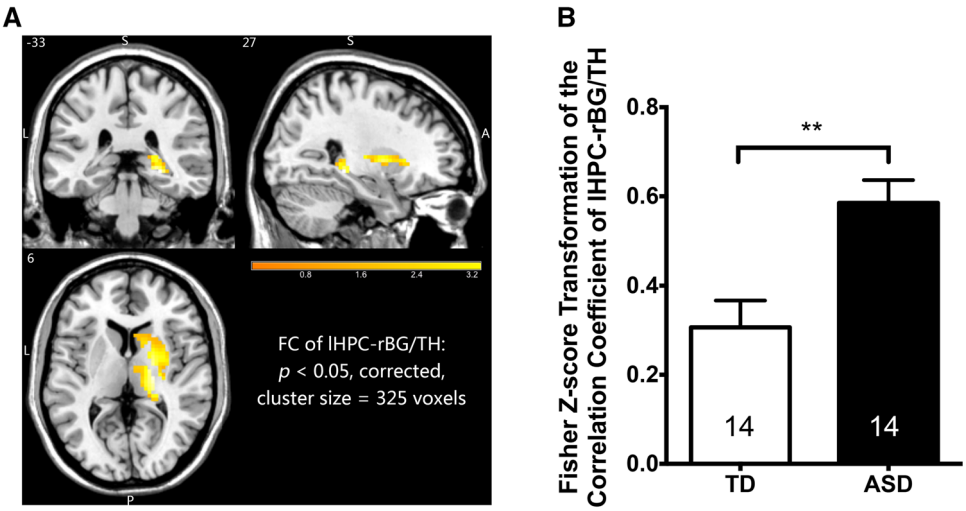


Fig. 5 Functional connectivity between left hippocampus (lHPC) and right basal ganglia/thalamus (rBG/TH) was significantly higher in children with ASD than in TDC. $P < 0.05$, controlled for age and gender, corrected; cluster size, 325 voxels; peak MNI coordinates, 27, −33, −6. ASD, autism spectrum disorder; TDC, typically developing children; MNI,Montreal Neurological Institute.

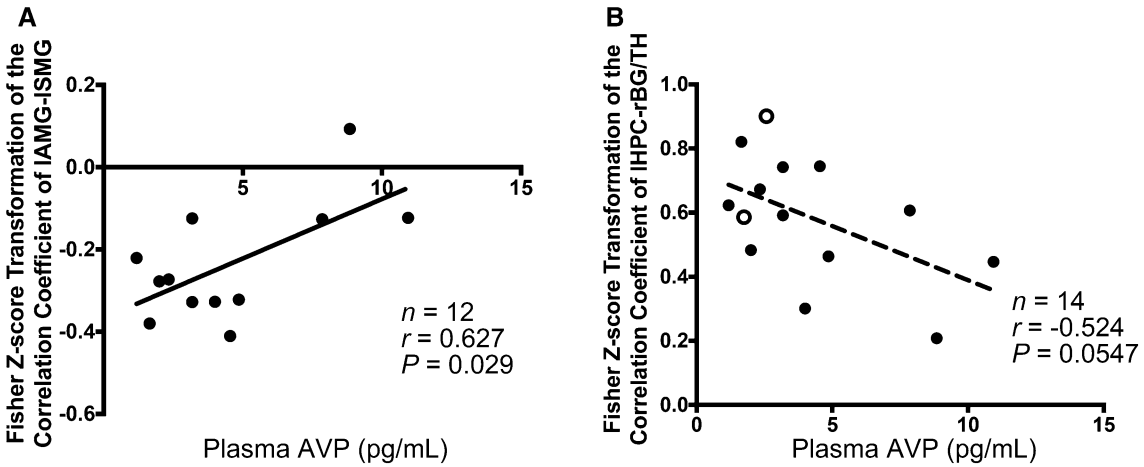


Fig. 6 Correlations between brain functional connectivities (FCs) and plasma AVP concentrations in children with ASD. **A** FC of lAMG-lSMG was positively correlated with plasma AVP concentration in boys with ASD. **B** FC of lHPC-rBG/TH showed a strong trend for negative correlation with plasma AVP concentration in all children with ASD. lSMG, left supramarginal gyrus; lHPC, left hippocampus; rBG/TH, right basal ganglia/thalamus; AVP, arginine-vasopressin. *Filled circles* denote boys; *empty circles* denote girls.

Structural Equation Model in a Hypothetical Pathway

Based on the above results, we constructed a hypothetical pathway in children with ASD (Fig. 7A). The FC value of lAMG-bSMG (bilateral supramarginal gyrus) was the average of the FC values of lAMG-rAMG and lAMG-lSMG. The results seem to fit with a satisfactory model of a pathway with the following data set: $\chi^2 = 0.233$, $P = 0.629$, df = 1, RMSEA <0.001, GFI = 0.990, and AGFI = 0.905.

Discussion

In the present study, we found evidence that the severity of ASD is associated with changes in the morphology and functionality of brain regions where AVP systems are involved. When compared to TDC, the children with ASD showed a decreased GM volume of the hypothalamus where the somata of AVP neurons are located, and an increased volume of the left amygdala and left hippocampus where the terminals of AVP-containing neurons reside. Additional functional analyses revealed a negative FC between the lAMG and bilateral SMG as well as an increased positive FC between the lHPC and rBG/TH in children with ASD. In addition, plasma level of AVP seemed to be useful for predicting the FC between the lAMG and lSMG in boys with ASD.

Aberrant Morphology Suggests Dysfunction of the AVP/OXT Systems

Our data are in line with the results of several previous studies. For example, an enlarged amygdala and

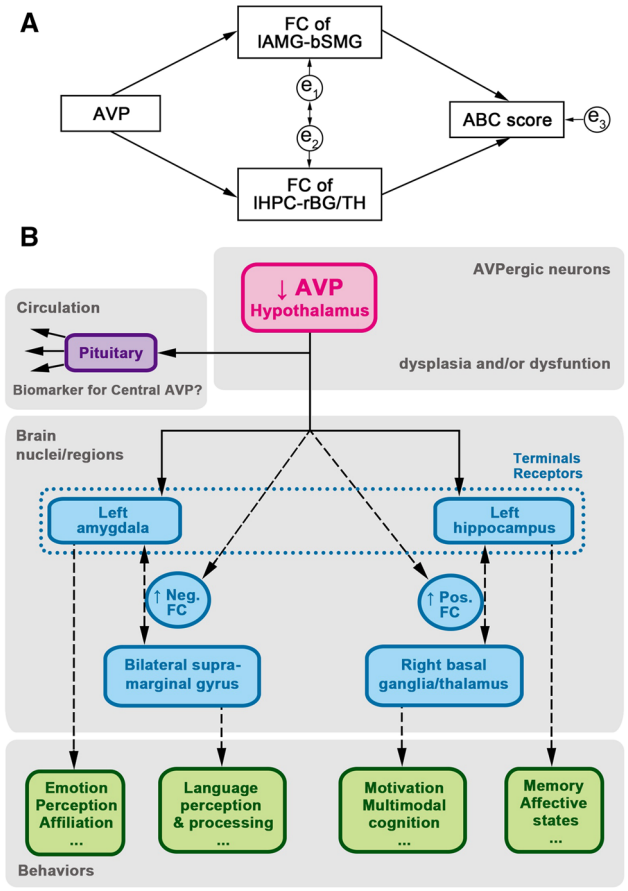


Fig. 7 Diagram of hypothetical central AVP system pathway in children with ASD. (A) Hypothetical statistical model: $\chi^2 = 0.233$, $P = 0.629$, df = 1, RMSEA <0.001, GFI, 0.990; and AGFI, 0.905; e1-e3 indicate that errors could affect the model. (B) Diagram of hypothetical pathway. The blood AVP may serve as a biomarker of central AVP level. A descending AVP level impacts the function of the left amygdala and left hippocampus, which further extend their connectivity to other brain regions, resulting in various behavioral disorders. Solid lines with one-way arrows indicate secretion and projection. Broken lines with one-way arrows indicate a possible impact on function. Broken lines with two-way arrows indicate the possible functional connectivity between brain regions. AGFI, adjusted goodness-of-fit index; AVP, arginine-vasopressin; df, degrees of freedom; FC, functional connectivity; GFI, goodness-of-fit index; RMSEA, root mean square error of approximation.

hippocampus in young children with ASD has been reported where endogenous AVP/OXT [6, 7] and administration of exogenous AVP/OXT seem to have a beneficial effect on children/patients with ASD [10, 46]. In a review, Courchesne and colleagues noted that the amygdala is one of the most markedly overgrown areas in children with autism [47]. Abnormal expansion of the amygdala is likely to occur before 2 years of age [48, 49] and the degree of abnormal expansion is positively correlated with the severity of social and communication impairment [49]. The hippocampus is also enlarged in children with autism, and the abnormal developmental pattern is considered to affect brain function [50–52].

The results of the present study show that volume of the hypothalamus, where the somata of the AVPergic and OXTergic neurons are located, is reduced in children with ASD, even when controlled for covariates. This finding was supported by the previous report that GM in the hypothalamus is diminished in children with autism [53]. Moreover, correlation analysis revealed that a smaller hypothalamic volume was associated with a lower plasma AVP concentration. The reduction in the volume of the hypothalamus might suggest dysplasia of neurons and/or neuropil in the hypothalamus that leads to a low level of AVP/OXT and the manifestation of autistic symptoms. Here, we found no significant difference in whole-brain volume between the ASD and TDC groups, suggesting that the volume changes in the hypothalamus, amygdala, and hippocampus were site-specific.

In addition, using imaging techniques, some genetic studies in humans have demonstrated that the most common phenotype of oxytocin receptor gene mutations (rs2254298 and rs53576) is shrinkage of the hypothalamus [54, 55] and enlargement of the amygdala [55–57]. These two gene mutations have been considered as candidate gene mutations for autism, especially in the Chinese population [58]. Thus, impairments in the AVP/OXT systems might be associated with aberrant brain morphology and function in autism.

Negative Connectivity Between the Amygdala and Supramarginal Gyrus May Affect the Functionality of the "Social Brain"

The amygdala, a hub in the "social brain", seems to play key roles in emotion, social cognition, perception, and affiliation [59]. The supramarginal gyrus, part of the mirror-neuron system in the "social brain", is involved in language perception and processing [60]. It has been reported in a resting-state fMRI study that the amygdala had reduced functional connectivity within and between the default mode network incorporating "social brain" regions under ASD conditions in adults [61]. However, in the present study, the TDC showed extremely week connectivities between the left amygdala and bilateral supramarginal gyrus, which suggested there are different connective patterns in healthy adults and young children. Conversely, children with ASD showed an inhibitory connection between the left amygdala and bilateral supramarginal gyrus. This aberrant connective pattern may perceive information as aversive, causing failure of establishment of the social network. Furthermore, the FC seems to be negatively correlated with the autistic symptoms: a potentiation of the FC was associated with an augmentation

of abnormal behavior and sensory deficit (Fig. 3E, F). It should be pointed out that the changes in connectivity and in GM volume occurred unilaterally in the present study. This is not an unusual phenomenon in connectivity and structural analysis using the fMRI technique since the left and right hemispheres are not identical in structure or function.

Animal experiments have shown that AVP has diverse effects on social behavior across a variety of mammalian species [62], suggesting that endogenous AVP may play a similar role in modulating social communications in humans. The present study revealed a strong association between circulating AVP level and lAMG-lSMG connections: a low plasma AVP concentration was associated with a strong negative lAMG-lSMG connection, a phenomenon that appeared only in boys with ASD. In the rodent, AVP immunoreactivity is more pronounced in the male brain, implying that AVP may contribute more in regulating male social behavior [63, 64]. These data indicate that the function of the amygdala, which is one of the major nuclei innervated by AVP-containing neurons from the hypothalamus, is sexually dimorphic, a well-recognized phenomenon seen in ASD.

Hyper-Connectivity of the Hippocampus and Basal Ganglia/Thalamus May Imply Sustained Immature Brain Function

Apart from the well-known function of hippocampus in learning and memory, this region has also been implicated in the regulation of affective states and emotional behavior. It has also been implied to work through inhibitory connections with many subcortical structures to relieve the stress response [65]. The basal ganglia and thalamus, on the other hand, might play a critical role in the development of emotion, motivation, and multimodal cognition beyond simple sensorimotor function [66]. Similar to our study, some previous studies have found hyper-connectivity in subcortical regions [67–70] in ASD patients, such as the thalamus/basal ganglia and sensorimotor regions. Supekar and colleagues assumed that, in healthy individuals, the functional brain networks in childhood are more strongly connected between subcortical and paralimbic areas, whereas in adolescents and adults a stronger cortico-cortical connectivity may exist among the paralimbic, limbic and association areas [71]. Hence, the hyper-connectivity of the lHPC and rBG/TH may reflect developmental retardation of the autistic brain, which has been regarded as a specific feature in the ASD brain.

Moreover, AVP-containing neurons clearly play an active part in the connectivity between the lHPC and rBG/TH: a higher plasma AVP concentration might predict a lower connectivity between the lHPC and rBG/TH in children with ASD. However, the mechanism is still obscure and deserves further investigation.

A Hypothesized Framework of the Central AVP System in Relation to Social Behavior

In summary, it is hypothesized that in children with ASD, abnormal development and dysfunction of the hypothalamus may impact the brain AVP system that leads to a state of stronger negative-connectivity between the lAMG and bilateral SMG (limbic-association area connectivity) and a state of hyper-connectivity between the lHPC and rBG/TH (limbic-subcortical area connectivity). These aberrant brain connections may eventually result in abnormal emotion, language and social interactions in children with ASD (Fig. 7B).

On the other hand, the peripheral AVP level may be taken as a predictive index to reflect the central AVP level and related symptoms in children with ASD. Carson and colleagues found that AVP concentrations in plasma and CSF are positively correlated in children [31, 32]; lower plasma AVP concentration is correlated with lower social function [32]. Nevertheless, the relationship between central and peripheral AVP levels remains controversial. The results may be influenced by the age of participants and their physiological status during sample collection. In the present study, the age of the participant children had a relatively narrow range and a strictly-controlled dietary status during blood collection. The results showed that the plasma AVP concentration was significantly correlated with the behaviors, hypothalamic volume, and functional connectivities. Besides, our previous interventional study demonstrated that an increased plasma AVP level was positively correlated with behavioral improvement in children with ASD [30]. These data suggest that the peripheral level of AVP may reflect that of the central level.

Limitations and Future Directions

In the present study, efforts were made to explore the differences in brain function between children with ASD and TDC following sedation with oral chloral hydrate. Chloral hydrate is a safe and commonly-used sedative during pediatric examinations, such as MRI and CT, since sometimes it is difficult to complete the procedures under awake or natural sleep conditions, especially in toddlers and preschoolers. There is always a concern whether the abnormalities are artifacts of chloral hydrate. There are only limited studies in literature to show the effects of chloral hydrate on brain activity. Rodent experiments have shown that normal low-frequency fluctuations in the BOLD signal can be detected [72] and visual and auditory cortices

can be activated in children with chloral hydrate-induced sedation [73], suggesting that brain function can be studied under chloral hydrate sedation in certain individuals. Though the inter-hemispheric FC has been shown to be lower in mice treated with alpha-chloralose than in awake mice [74], we believe that the major differences in connectivity detected in the present study were not attributable to chloral hydrate since the TDC group was sedated in the same way. However, caution should be taken when interpreting the data since natural sleep and chloral hydrate-assisted sleep are not the same.

Our sample size was relatively small and future studies with larger sample sizes are warranted to confirm the results with greater confidence. The blood collection and analysis of plasma AVP were done only in the autistic children who had completed the MRI scanning. The interpretation of the data would be more convincing if plasma AVP levels had also been obtained from TDC.

In addition, we focused only on abnormalities of the AVP system in ASD. It would be interesting to investigate both AVP and OXT in future studies, and explore the possible interactions between these two neuropeptides and brain function.

Conclusions

To the best of our knowledge, this is the first study to explore the links among circulating AVP, brain imaging, and behaviors in young children with ASD. The hypothalamus on both sides was found to be shrunken, and this was associated with a lower AVP level. Besides, the left amygdala and left hippocampus were enlarged in children with ASD. All these findings indicate an aberrant morphology of AVPergic neurons. Stronger negative connectivity between the left amygdala and bilateral supramarginal gyrus and stronger positive connectivity between the left hippocampus and right basal ganglia/thalamus were found in children with ASD compared to TDC. These aberrant FCs revealed in autistic children seem to be associated with the degree of severity of symptoms and reduction of plasma AVP. Moreover, AVP might be one of the neurochemical candidates that affect certain brain functions and in turn affect autistic behaviors. These findings may be beneficial for understanding the etiology and neurobiological basis of ASD.

Acknowledgements The co-operation of the families of all the children and the technical support provided by the Department of Radiology, Peking University Third Hospital and the Brainnetome Center, Institute of Automation, Chines Academy of Sciences are highly appreciated. This work was supported by the UULM-PUHSC Joint Center for Neuroscience Fund (BMU20160563).

References

1. Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators; Centers for Disease Control and Prevention (CDC). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill Summ 2014, 63: 1–21.
2. Wang Y. National epidemiological study of autism spectrum disorders in China. Asia Pacific Regional IMPFAR: Shanghai 2015.
3. Huang JP, Cui SS, Han Y, Irva HP, Qi LH, Zhang X. Prevalence and early signs of autism spectrum disorder (ASD) among 18-36 month-old children of Tianjin in China. Biomed Environ Sci 2014, 27: 453–461.
4. Wan Y, Hu Q, Li T, Jiang L, Du Y, Feng L, *et al.* Prevalence of autism spectrum disorders among children in China: a systematic review. Shanghai Arch Psychiatry 2013, 25: 70–80.
5. Landgraf R, Neumann ID. Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. Front Neuroendocrinol 2004, 25: 150–176.
6. Veenema AH, Neumann ID. Central vasopressin and oxytocin release: regulation of complex social behaviours. Prog Brain Res 2008, 170: 261–276.
7. Bales KL, Perkeybile AM. Developmental experiences and the oxytocin receptor system. Horm Behav 2012, 61: 313–319.
8. Ferguson JN, Young LJ, Insel TR. The neuroendocrine basis of social recognition. Front Neuroendocrinol 2002, 23: 200–224.
9. Heinrichs M, von Dawans B, Domes G. Oxytocin, vasopressin, and human social behavior. Front Neuroendocrinol 2009, 30: 548–557.
10. Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M. Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. Nat Rev Neurosci 2011, 12: 524–538.
11. Meyer-Lindenberg A. Impact of prosocial neuropeptides on human brain function. Prog Brain Res 2008, 170: 463–470.
12. Insel TR, O'Brien DJ, Leckman JF. Oxytocin, vasopressin, and autism: is there a connection? Biol Psychiatry 1999, 45: 145–157.
13. Vargas-Martinez F, Uvnas-Moberg K, Petersson M, Olausson HA, Jimenez-Estrada I. Neuropeptides as neuroprotective agents: Oxytocin a forefront developmental player in the mammalian brain. Prog Neurobiol 2014, 123C: 37–78.
14. Hammock EA. Developmental perspectives on oxytocin and vasopressin. Neuropsychopharmacology 2015, 40: 24–42.
15. Dumais KM, Bredewold R, Mayer TE, Veenema AH. Sex differences in oxytocin receptor binding in forebrain regions: correlations with social interest in brain region- and sex- specific ways. Horm Behav 2013, 64: 693–701.
16. Rilling JK, Demarco AC, Hackett PD, Chen X, Gautam P, Stair S, *et al.* Sex differences in the neural and behavioral response to intranasal oxytocin and vasopressin during human social interaction. Psychoneuroendocrinology 2014, 39: 237–248.

17. Aiello TP, Whitaker-Azmitia PM. Sexual differentiation and the neuroendocrine hypothesis of autism. Anat Rec (Hoboken) 2011, 294: 1663–1670.
18. Carter CS. Sex differences in oxytocin and vasopressin: implications for autism spectrum disorders? Behav Brain Res 2007, 176: 170–186.
19. Zink CF, Stein JL, Kempf L, Hakimi S, Meyer-Lindenberg A. Vasopressin modulates medial prefrontal cortex-amygdala circuitry during emotion processing in humans. J Neurosci 2010, 30: 7017–7022.
20. Rilling JK, DeMarco AC, Hackett PD, Thompson R, Ditzen B, Patel R, *et al.* Effects of intranasal oxytocin and vasopressin on cooperative behavior and associated brain activity in men. Psychoneuroendocrinology 2012, 37: 447–461.
21. Zink CF, Kempf L, Hakimi S, Rainey CA, Stein JL, Meyer-Lindenberg A. Vasopressin modulates social recognition-related activity in the left temporoparietal junction in humans. Transl Psychiatry 2011, 1: e3.
22. Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E. Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. Neuron 2008, 58: 639–650.
23. Domes G, Heinrichs M, Glascher J, Buchel C, Braus DF, Herpertz SC. Oxytocin attenuates amygdala responses to emotional faces regardless of valence. Biol Psychiatry 2007, 62: 1187–1190.
24. Domes G, Lischke A, Berger C, Grossmann A, Hauenstein K, Heinrichs M, *et al.* Effects of intranasal oxytocin on emotional face processing in women. Psychoneuroendocrinology 2010, 35: 83–93.
25. Gamer M, Zurowski B, Buchel C. Different amygdala subregions mediate valence-related and attentional effects of oxytocin in humans. Proc Natl Acad Sci U S A 2010, 107: 9400–9405.
26. Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, *et al.* Oxytocin modulates neural circuitry for social cognition and fear in humans. J Neurosci 2005, 25: 11489–11493.
27. Singer T, Snozzi R, Bird G, Petrovic P, Silani G, Heinrichs M, *et al.* Effects of oxytocin and prosocial behavior on brain responses to direct and vicariously experienced pain. Emotion 2008, 8: 781–791.
28. Aoki Y, Yahata N, Watanabe T, Takano Y, Kawakubo Y, Kuwabara H, *et al.* Oxytocin improves behavioural and neural deficits in inferring others' social emotions in autism. Brain 2014, 137: 3073–3086.
29. Gordon I, Vander Wyk BC, Bennett RH, Cordeaux C, Lucas MV, Eilbott JA, *et al.* Oxytocin enhances brain function in children with autism. Proc Natl Acad Sci U S A 2013, 110: 20953–20958.
30. Zhang R, Jia MX, Zhang JS, Xu XJ, Shou XJ, Zhang XT, *et al.* Transcutaneous electrical acupoint stimulation in children with autism and its impact on plasma levels of arginine-vasopressin and oxytocin: a prospective single-blinded controlled study. Res Dev Disabil 2012, 33: 1136–1146.
31. Carson DS, Howerton CL, Garner JP, Hyde SA, Clark CL, Hardan AY, *et al.* Plasma vasopressin concentrations positively predict cerebrospinal fluid vasopressin concentrations in human neonates. Peptides 2014, 61: 12–16.
32. Carson DS, Garner JP, Hyde SA, Libove RA, Berquist SW, Hornbeak KB, *et al.* Arginine vasopressin is a blood-based biomarker of social functioning in children with autism. PLoS One 2015, 10: e0132224.
33. World Health Organization. International Statistical Classification of Diseases and Related Health Problems. 10th revision, 2nd edition. Geneva: World Health Organization, 2004.
34. American Psychiatric Association. Diagnostic criteria from DSM-IV-TR. Washington, DC: American Psychiatric Association, 2000.
35. Rellini E, Tortolani D, Trillo S, Carbone S, Montecchi F. Childhood Autism Rating Scale (CARS) and Autism Behavior Checklist (ABC) correspondence and conflicts with DSM-IV criteria in diagnosis of autism. J Autism Dev Disord 2004, 34: 703–708.
36. Volkmar FR, Cicchetti DV, Dykens E, Sparrow SS, Leckman JF, Cohen DJ. An evaluation of the Autism Behavior Checklist. J Autism Dev Disord 1988, 18: 81–97.
37. Auyeung B, Baron-Cohen S, Wheelwright S, Allison C. The Autism Spectrum Quotient: Children's Version (AQ-Child). J Autism Dev Disord 2008, 38: 1230–1240.
38. Lau WY, Gau SS, Chiu YN, Wu YY, Chou WJ, Liu SK, *et al.* Psychometric properties of the Chinese version of the Autism Spectrum Quotient (AQ). Res Dev Disabil 2013, 34: 294–305.
39. Damaraju E, Phillips JR, Lowe JR, Ohls R, Calhoun VD, Caprihan A. Resting-state functional connectivity differences in premature children. Front Syst Neurosci 2010, 4.
40. Fransson P, Skiold B, Horsch S, Nordell A, Blennow M, Lagercrantz H, *et al.* Resting-state networks in the infant brain. Proc Natl Acad Sci U S A 2007, 104: 15531–15536.
41. Wilke M, Holland SK, Altaye M, Gaser C. Template-O-Matic: a toolbox for creating customized pediatric templates. Neuroimage 2008, 41: 903–913.
42. Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin JC, Pujol S, *et al.* 3D Slicer as an image computing platform for the Quantitative Imaging Network. Magn Reson Imaging 2012, 30: 1323–1341.
43. Song XW, Dong ZY, Long XY, Li SF, Zuo XN, Zhu CZ, *et al.* REST: a toolkit for resting-state functional magnetic resonance imaging data processing. PLoS One 2011, 6: e25031.
44. Macey PM, Macey KE, Kumar R, Harper RM. A method for removal of global effects from fMRI time series. Neuroimage 2004, 22: 360–366.
45. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, *et al.* Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 2002, 15: 273–289.
46. Young LJ, Barrett CE. Neuroscience. Can oxytocin treat autism? Science 2015, 347: 825–826.
47. Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP, *et al.* Mapping early brain development in autism. Neuron 2007, 56: 399–413.
48. Nordahl CW, Scholz R, Yang X, Buonocore MH, Simon T, Rogers S, *et al.* Increased rate of amygdala growth in children aged 2 to 4 years with autism spectrum disorders: a longitudinal study. Arch Gen Psychiatry 2012, 69: 53–61.
49. Schumann CM, Barnes CC, Lord C, Courchesne E. Amygdala enlargement in toddlers with autism related to severity of social and communication impairments. Biol Psychiatry 2009, 66: 942–949.
50. Barnea-Goraly N, Frazier TW, Piacenza L, Minshew NJ, Keshavan MS, Reiss AL, *et al.* A preliminary longitudinal volumetric MRI study of amygdala and hippocampal volumes in autism. Prog Neuropsychopharmacol Biol Psychiatry 2014, 48: 124–128.
51. Groen W, Teluij M, Buitelaar J, Tendolkar I. Amygdala and hippocampus enlargement during adolescence in autism. J Am Acad Child Adolesc Psychiatry 2010, 49: 552–560.
52. Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, Buonocore MH, *et al.* The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. J Neurosci 2004, 24: 6392–6401.
53. Kurth F, Narr KL, Woods RP, O'Neill J, Alger JR, Caplan R, *et al.* Diminished gray matter within the hypothalamus in autism disorder: a potential link to hormonal effects? Biol Psychiatry 2011, 70: 278–282.

54. Tost H, Kolachana B, Verchinski BA, Bilek E, Goldman AL, Mattay VS, *et al.* Neurogenetic effects of OXTR rs2254298 in the extended limbic system of healthy Caucasian adults. Biol Psychiatry 2011, 70: e37–39; author reply e41–32.
55. Tost H, Kolachana B, Hakimi S, Lemaitre H, Verchinski BA, Mattay VS, *et al.* A common allele in the oxytocin receptor gene (OXTR) impacts prosocial temperament and human hypothalamic-limbic structure and function. Proc Natl Acad Sci U S A 2010, 107: 13936–13941.
56. Inoue H, Yamasue H, Tochigi M, Abe O, Liu X, Kawamura Y, *et al.* Association between the oxytocin receptor gene and amygdalar volume in healthy adults. Biol Psychiatry 2010, 68: 1066–1072.
57. Furman DJ, Chen MC, Gotlib IH. Variant in oxytocin receptor gene is associated with amygdala volume. Psychoneuroendocrinology 2011, 36: 891–897.
58. Wu S, Jia M, Ruan Y, Liu J, Guo Y, Shuang M, *et al.* Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. Biol Psychiatry 2005, 58: 74–77.
59. Bickart KC, Dickerson BC, Barrett LF. The amygdala as a hub in brain networks that support social life. Neuropsychologia 2014, 63: 235–248.
60. Gazzaniga MS, Ivry RB, Mangun GR. Cognitive Neuroscience: the Biology of the Mind. Fourth edition. New York, N.Y.: W. W. Norton & Company, Inc., 2013.
61. von dem Hagen EA, Stoyanova RS, Baron-Cohen S, Calder AJ. Reduced functional connectivity within and between 'social' resting state networks in autism spectrum conditions. Soc Cogn Affect Neurosci 2013, 8: 694–701.
62. Bester-Meredith JK, Fancher AP, Mammarella GE. Vasopressin proves es-sense-tial: vasopressin and the modulation of sensory processing in mammals. Front Endocrinol (Lausanne) 2015, 6: 5.
63. Wang Z. Species differences in the vasopressin-immunoreactive pathways in the bed nucleus of the stria terminalis and medial amygdaloid nucleus in prairie voles (Microtus ochrogaster) and meadow voles (Microtus pennsylvanicus). Behav Neurosci 1995, 109: 305–311.
64. Otero-Garcia M, Martin-Sanchez A, Fortes-Marco L, Martinez-Ricos J, Agustin-Pavon C, Lanuza E, *et al.* Extending the socio-sexual brain: arginine-vasopressin immunoreactive circuits in the telencephalon of mice. Brain Struct Funct 2014, 219: 1055–1081.
65. Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception I: The neural basis of normal emotion perception. Biol Psychiatry 2003, 54: 504–514.
66. Nair A, Treiber JM, Shukla DK, Shih P, Muller RA. Impaired thalamocortical connectivity in autism spectrum disorder: a study of functional and anatomical connectivity. Brain 2013, 136: 1942–1955.
67. Iidaka T. Resting state functional magnetic resonance imaging and neural network classified autism and control. Cortex 2015, 63: 55–67.
68. Di Martino A, Kelly C, Grzadzinski R, Zuo XN, Mennes M, Mairena MA, *et al.* Aberrant striatal functional connectivity in children with autism. Biol Psychiatry 2011, 69: 847–856.
69. Lynch CJ, Uddin LQ, Supekar K, Khouzam A, Phillips J, Menon V. Default mode network in childhood autism: posteromedial cortex heterogeneity and relationship with social deficits. Biol Psychiatry 2013, 74: 212–219.
70. Di Martino A, Yan CG, Li Q, Denio E, Castellanos FX, Alaerts K, *et al.* The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. Mol Psychiatry 2014, 19: 659–667.
71. Supekar K, Musen M, Menon V. Development of large-scale functional brain networks in children. PLoS Biol 2009, 7: e1000157.
72. Williams KA, Magnuson M, Majeed W, LaConte SM, Peltier SJ, Hu X, *et al.* Comparison of alpha-chloralose, medetomidine and isoflurane anesthesia for functional connectivity mapping in the rat. Magn Reson Imaging 2010, 28: 995–1003.
73. Altman NR, Bernal B. Brain activation in sedated children: auditory and visual functional MR imaging. Radiology 2001, 221: 56–63.
74. Jonckers E, Delgado y Palacios R, Shah D, Guglielmetti C, Verhoye M, Van der Linden A. Different anesthesia regimes modulate the functional connectivity outcome in mice. Magn Reson Med 2014, 72: 1103–1112.

Neurosci. Bull. April, 2017, 33(2):143–152
DOI 10.1007/s12264-017-0111-8

www.neurosci.cn
www.springer.com/12264

ORIGINAL ARTICLE

Distinct Defects in Spine Formation or Pruning in Two Gene Duplication Mouse Models of Autism

Miao Wang[1,2] · Huiping Li[3] · Toru Takumi[4] · Zilong Qiu[1] · Xiu Xu[3] · Xiang Yu[1,2,5] · Wen-Jie Bian[1,2]

Received: 22 January 2017 / Accepted: 13 February 2017 / Published online: 3 March 2017

Abstract Autism spectrum disorder (ASD) encompasses a complex set of developmental neurological disorders, characterized by deficits in social communication and excessive repetitive behaviors. In recent years, ASD is increasingly being considered as a disease of the synapse. One main type of genetic aberration leading to ASD is gene duplication, and several mouse models have been generated mimicking these mutations. Here, we studied the effects of *MECP2* duplication and human chromosome 15q11-13 duplication on synaptic development and neural circuit wiring in the mouse sensory cortices. We showed that mice carrying *MECP2* duplication had specific defects in spine pruning, while the 15q11-13 duplication mouse model had impaired spine formation. Our results demonstrate that spine pathology varies significantly between autism models and that distinct aspects of neural circuit development may be targeted in different ASD mutations. Our results further underscore the importance of gene dosage in normal development and function of the brain.

Keywords Autism · Autism spectrum disorder · Spine · Spine formation · Spinogenesis · Spine pruning · Gene duplication · MECP2 · 15q11-13 duplication

✉ Xiu Xu
xuxiu@fudan.edu.cn

✉ Xiang Yu
yuxiang@ion.ac.cn

✉ Wen-Jie Bian
bwjflame@gmail.com

[1] Institute of Neuroscience, State Key Laboratory of Neuroscience, CAS Center for Excellence in Brain Science and Intelligence Technology, Chinese Academy of Sciences, Shanghai 200031, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

[3] Department of Child Healthcare, Children's Hospital of Fudan University, Shanghai 201102, China

[4] RIKEN Brain Science Institute, Wako, Saitama 351-0198, Japan

[5] School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China

Introduction

Autism spectrum disorder (ASD) encompasses a wide range of neurological disorders with developmental origins, displaying a variety of symptoms and all including the key characteristics of impaired social interaction and excessive repetitive behaviors and/or restricted interests [1]. These behavioral abnormalities are thought to be caused by alterations in neural circuits. Based on accumulating evidence demonstrating defects in synaptic development and/or function in animal models of ASD and in patients, autism has been considered as the disease of synapse [2–7]. In fact, many genes underlying ASD encode molecules that directly participate in and/or regulate synaptic structure and function, including pre- and post-synaptic scaffolds, subunits of neurotransmitter receptors and synaptic adhesion molecules [4–6, 8–10]. Over 90% of excitatory synapses in the brain are located on dendritic spines [11], which are small and thorn-like protrusions extending from the dendritic shaft. Spines undergo dramatic changes during development, in species ranging from rodents to humans [12–19].

Spines form rapidly during early postnatal life *via* a process called "spinogenesis", which usually lasts 3–4 postnatal weeks in mice [14, 17]. During adolescence, the

brain undergoes a course of spine "pruning" or elimination, to remove excessive synaptic connections and strengthen the physiologically useful/relevant connections [12–16, 18–21]. Both spinogenesis and spine pruning contribute significantly to efficient neural circuit wiring and normal brain function. In addition to their number, the size and shape of spines are also important [22]. It has been shown that the volume of spine head tightly correlates to the area of postsynaptic density, as well as presynaptic vesicle number [23], while the spine neck tunes the postsynaptic response and also shows plasticity upon changes in presynaptic inputs [24, 25]. Thus, measuring the number and morphology of spines is potentially a simple and effective way to assess changes in the synaptic connectivity in ASD.

Up to date, hundreds of genes and genetic alterations have been linked to ASD, including those affecting synaptic function, those regulating gene transcription and post-transcriptional modification, and those involved in other important biological processes [2, 4–6, 8–10, 26–29].

It is of particular interest that copy number variations (CNVs), large nucleotide (one kilobase to a few megabases) duplications or deletions, contribute significantly to ASD. CNVs can occur by inheritance or *de novo* mutation. *De novo* CNV occurs in an offspring whose parents do not have the genetic change and is more common in ASD than inherited cases [9, 26–30]. Of the reported CNVs in ASD, several are highly interesting in that both their deletion and duplication lead to autistic phenotypes. A well-characterized example is the X-linked *Methyl-CpG-binding protein 2* (*MECP2*), loss-of-function of which results in Rett syndrome [31–33] while its duplication leads to many autistic symptoms including lack of eye contact and verbal communication, loss of speech, restricted interests and stereotypic behaviors [33–35]. Another example is the 15q11-13 region of human chromosome 15, which includes a series of imprinting genes, as well as non-imprinting ones. Maternal deletion of this region results in Angelman syndrome, paternal deletion leads to Prader-Willi syndrome, while its duplication represents one of the most frequently reported CNVs in ASD. All the three mutations share ASD features [27, 30, 36]. The observation that deletion and duplication of the same gene or chromosomal region can result in a similar phenotype underscores the importance of gene dosage to neural circuit development and function [5, 26, 27, 29, 30, 33].

In recent years, a number of mouse models of human ASD mutations have been generated. Here we examined two of the better-characterized gene/chromosomal duplication mouse models, the *MECP2*^{Tg1} mouse model of *MeCP2* duplication syndrome [33, 37], and the 15q11-13 duplication mouse model that mimics duplication of human chromosome 15q11-13 region [36, 38]. We quantitated changes in spine density and morphology in these mice in the primary somatosensory and visual cortices at different developmental stages, as indicators of changes in neural circuitry.

Materials and Methods

Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Institute of Neuroscience, Chinese Academy of Sciences (Shanghai, China), under protocol No. NA-003-2016. The hemizygote *MECP2*^{Tg1} mice (full name: FVB-Tg (*MECP2*)1Hzo/J; JAX Stock No: 008679) [37] express full-length human *MECP2* under the endogenous human promoter, with hemizygote males expressing the protein at ~2-fold wildtype levels in the brain [37]. Only male *MECP2*^{Tg1} mice and age-matched wild-type littermates (all on FVB background) of 1 and 3 months were used. The mouse model of human 15q11-13 duplication (on C57/BL6 background) carries an interstitial duplication of 6 Mb on mouse chromosome 7B-C that corresponds to human chromosome 15q11-q13, as previously described [38]. Both male and female mice and age-matched wild-type littermates at postnatal day 14 (P14) and 1 month were used, as the estrous cycle does not affect the spines in female mice at this developmental stage.

Golgi Staining

Golgi staining was performed using the FD Rapid GolgiStain^{TM} Kit (FD NeuroTechnologies, Columbia, MD), according to the manufacturer's instructions. Briefly, mice were deeply anesthetized with 0.7% pentobarbital sodium. The freshly dissected brain was immersed into a mixture containing equal volumes of solution A and B at room temperature for approximately 10 days. The brains were then transferred into solution C for at least 48 h. Coronal sections (150 μm) were prepared with a freezing microtome. Sections were stained using solutions D and E after mounting onto the slides.

Image Acquisition and Analysis

Stained sections were imaged using a Zeiss LSM PASCAL confocal microscope (Carl Zeiss, Jena, Germany), equipped with a 63× oil immersion Plan-Apochromat objective (N.A = 1.4) and at 2× optical zoom. The basal dendrites of layer 2/3 pyramidal neurons in S1BF and V1 were imaged. All images were coded with computer-generated random number sequence (https://www.random.org/sequences/) at the time of acquisition and analyzed blinded to the

experimental condition. Original images were used to measure dendrite branch length and count spine number using Image-Pro Plus (Media-Cybernetics, Silver Spring, MD). Spine density was calculated as the number of spines per micrometer dendrite. Protrusions longer than 3 μm were considered as filopodia and were analyzed separately for P14 mice. The criterion for spine subtype classification was as previously described [15]. The proportion for each spine subtype was calculated as the percentage of total spines on the dendritic segment. Only images with sufficient quality to clearly distinguish and measure the spine shape were used for spine subtype classification. For example images, bright field original images were projected at minimal intensity and inverted, followed by background subtraction and brightness/contrast adjustment, using Fiji/ImageJ (NIH, Bethesda, MD). Paired example images were adjusted with the same parameters.

Statistics

Statistical tests were carried out using GraphPad Prism 5 (GraphPad software, La Jolla, CA). Two-tailed Student's *t*-test was used for comparison between pairs of samples, while one-way ANOVA followed by Tukey's *post hoc* test was used for comparison between multiple samples. For spine subtype classification, two-way ANOVA followed by Bonferroni *post hoc* test was used. Data were collected from 3–6 mice for each condition, up to 10 images per mouse. Results are shown as mean ± SEM, and "*n*" refers to the number of dendrites or neurons. All conditions statistically different from control are indicated: n.s., not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Results

Impaired Spine Pruning and Maturation in the Primary Somatosensory Cortex of *MECP2*^{Tg1} Mice

MECP2 is one of the earliest autism genes identified. Its loss-of-function mutations result in Rett syndrome [32, 33, 39], while its overexpression leads to progressive neurological symptoms with ASD features. Mice with doubled expression of *MECP2* (*MECP2*^{Tg1}) [37] show a series of progressive symptoms including social interaction deficits, aggressiveness, anxiety, behavioral seizures and abnormal electroencephalographic traces [37, 40], similar to those observed in *MECP2* duplication patients. These phenotypes can be rescued by re-normalizing *Mecp2* expression in mice [41]. More recently, transgenic monkeys overexpressing human *MECP2* were shown to recapitulate the key behavioral features of ASD, including impaired social interaction and stereotypic behavior [42]. Importantly, these autism-like behavioral defects were passed onto their offspring through germline transmission [42].

Here we used the *MECP2*^{Tg1} mice [37] to investigate the effect of *MECP2* overexpression on dendritic spine density and morphology in the primary sensory cortices at different developmental stages using Golgi staining. In previous work, we showed that spines in multiple sensory/motor cortices underwent activity-dependent pruning between 1 and 3 months of post-natal development [15]. Since spine pruning is highly development- and activity-dependent in the basal dendrites of layer 2/3 pyramidal neurons in the barrel field of primary somatosensory cortex (S1BF) [15], we first assayed these neurons in *MECP2*^{Tg1} mice. The results showed that spine density was not significantly different between *MECP2*^{Tg1} mice and wildtype littermate controls at 1 month ($P > 0.05$; Fig. 1A, B), indicative of normal spinogenesis. By the age of 3 months, spines in S1BF of wildtype mice have undergone spine pruning, as indicated by the substantial reduction in spine density ($P < 0.001$; Fig. 1A, B). However, in *MECP2*^{Tg1} mice, spine density at 3 months was significantly higher than that of wildtype littermates ($P < 0.01$; Fig. 1A, B), and was only slightly lower than that of 1-month *MECP2*^{Tg1} mice ($P < 0.05$; Fig. 1A, B; percentage reduction in spine density between 1 and 3 months: wildtype, 23.1%; *MECP2*^{Tg1}, 10.4%), suggesting that spine pruning was impaired in *MECP2*^{Tg1} mice.

The pruning of some spines during the transition through adolescence is accompanied by and coordinated with the strengthening and maturation of the surviving ones [15]. To examine whether *MECP2* overexpression also affected spine maturation, we analyzed spine maturity in *MECP2*^{Tg1} mice and wildtype littermates by sorting spines into 4 subtypes based on morphological criteria described previously [15]. Spines with mushroom-like shapes typically contain larger postsynaptic densities and a well-constricted spine neck, and thus are thought to be mature and stable, while thin spines are found to be more motile and immature [18, 21, 22]. We found that at 3 months, the percentage of thin spines significantly increased while that of mushroom spines decreased in *MECP2*^{Tg1} mice, as compared to the wildtype littermates, suggesting that along with the spine pruning defect, more spines failed to mature in *MECP2*^{Tg1} mice. Together, these results showed that both spine pruning and spine maturation were impaired in S1BF of 3-month *MECP2*^{Tg1} mice, while spinogenesis at 1 month was essentially intact.

Impaired Spine Pruning and Maturation in the Primary Visual Cortex of *MECP2*^{Tg1} Mice

To determine whether the defects of spine pruning and maturation observed in S1BF are specific to the

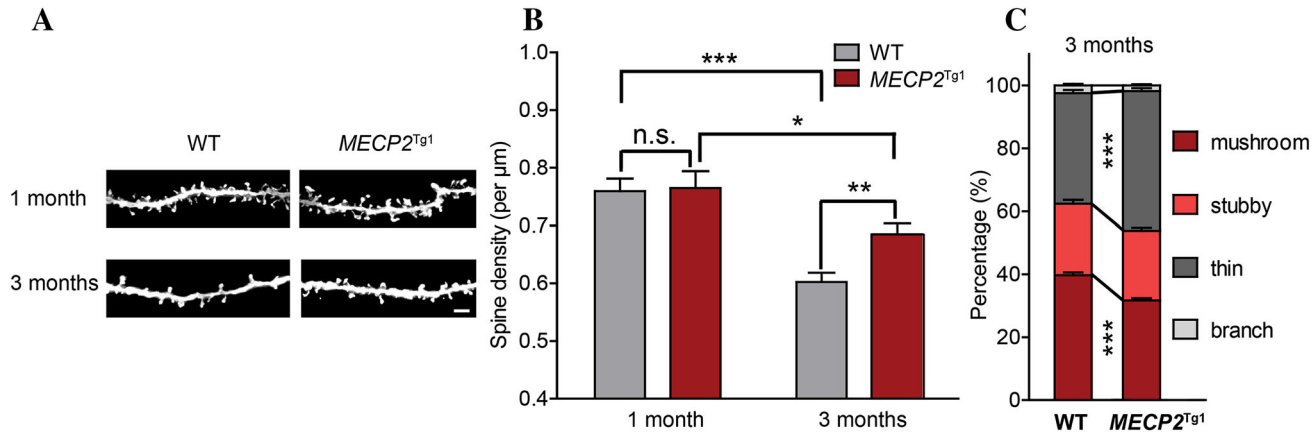


Fig. 1 Spine pruning is impaired in *MECP2*^{Tg1} mice in S1BF. **A** Representative inverted Golgi staining images showing spines in basal dendrites of layer 2/3 pyramidal neurons in S1BF, genotype and age as indicated. *Scale bar* 5 μm. **B** Spine density in wildtype (WT) and *MECP2*^{Tg1} mice at 1 month (WT, $n = 38$; *MECP2*^{Tg1}, $n = 25$) and 3 months (WT, $n = 44$; *MECP2*^{Tg1}, $n = 59$). **C** Spine type classification for WT and *MECP2*^{Tg1} mice at 3 months. Data are presented as mean ± SEM. *n.s.* not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

somatosensory cortex or common to multiple sensory modalities, we further examined spine density and morphology in the primary visual cortex (V1). Similar to our results in S1BF, spine pruning, but not spinogenesis, showed significant defects in V1 of *MECP2*^{Tg1} mice, as demonstrated by the significant differences in spine density between wildtype and *MECP2*^{Tg1} mice at 3 months ($P < 0.001$) but not at 1 month ($P > 0.05$) (Fig. 2A, B). Notably, although spine density in S1BF of *MECP2*^{Tg1} mice was slightly lowered at 3 months as compared with that at 1 month ($P < 0.05$; Fig. 1A, B), in V1 no reductions were observed ($P > 0.05$; Fig. 2A, B; wildtype: 1 month, 0.76 ± 0.02, 3 months, 0.64 ± 0.02; *MECP2*^{Tg1}: 1 month, 0.72 ± 0.04, 3 months, 0.74 ± 0.02). Consistently, an increased portion of thin spines and a decreased portion of mushroom spines were found in V1 of *MECP2*^{Tg1} mice at 3 months (Fig. 2C). The overall pattern and extent of changes in S1BF and V1 were very similar, suggesting that *MECP2* duplication likely results in global defects in spine pruning and maturation in the sensory cortices.

Spinogenesis is Impaired in 15q11-13 Paternal Duplication Mice

Is the spine pruning defect we observed in *MECP2*^{Tg1} mice a common phenotype to multiple ASD models or specific to the *MECP2* duplication? To address this question and further explore spine pathology in ASD, we used another autism mouse model, in which the mouse chromosomal region corresponding to human chromosome 15q11-13 was engineered to be duplicated [38]. Since the duplicated region in these mice contains a series of imprinting genes

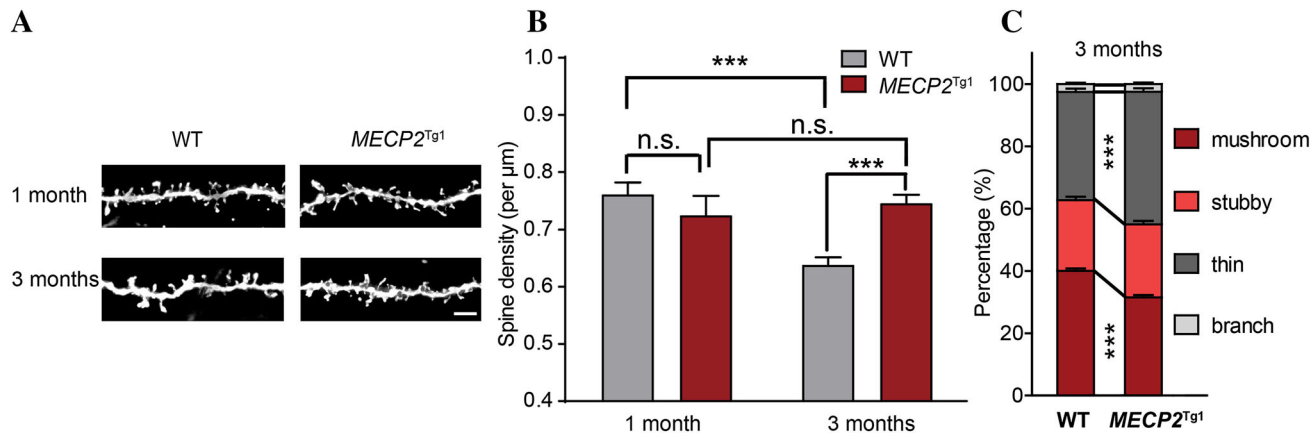


Fig. 2 Spine pruning is impaired in *MECP2*^{Tg1} mice in V1. **A** Representative inverted Golgi staining images showing spines in basal dendrites of layer 2/3 pyramidal neurons in V1, genotype and age as indicated. *Scale bar* 5 μm. **B** Spine density in WT and *MECP2*^{Tg1} mice at 1 month (WT, $n = 32$; *MECP2*^{Tg1}, $n = 26$) and 3 months (WT, $n = 44$; *MECP2*^{Tg1}, $n = 54$). **C** Spine type classification for WT and *MECP2*^{Tg1} mice at 3 months. $^{***}P < 0.001$.

expressed exclusively from the paternal or maternal copy (Fig. 3A) [36, 38], we separately examined spine density in mice carrying paternally (*patDp/+*) or maternally (*matDp/+*) inherited duplication. We found that at 1 month, spine density in *patDp/+* mice was significantly lower than that of wildtype littermates ($P < 0.05$; Fig. 3B, C) in S1BF, indicating impairment of spinogenesis in these *patDp/+* mice. Interestingly, *matDp/+* mice showed no significant differences in spine density as compared to wildtype littermates ($P > 0.05$; Fig. 3D, see also Discussion). To further characterize the defect in spinogenesis in *patDp/+* mice, we assayed spine density at the earlier age of P14 and found no significant differences ($P > 0.05$; Fig. 4A, B). At this age, spine density was also not affected in *matDp/+* mice ($P > 0.05$; Fig. 4A, C).

A considerable portion of spines come from filopodia that extend from the dendritic shaft and probe around for potential presynaptic partners. Once a filopodium "captures" a suitable axonal terminus, it could convert itself into a spine; otherwise, it likely retracts [17]. This transformation from filopodium to spine has been observed in cultured hippocampal neurons, as well as in brain slices, using live imaging [43, 44], and the synapse-like contacts made between axons and filopodia have been observed by electron microscopy [45]. Thus, dendritic filopodia contribute significantly to the formation of spines during early development of the brain, and the number of filopodia in early developmental stage may indicate the potential of a neuron to grow spines during spinogenesis. Since P14 is within the window of rapid filopodial dynamics, we also measured the density of filopodia (protrusions longer than 3 μm) in *patDp/+* mice, and found it to be significantly reduced ($P < 0.001$; Fig. 4D). This reduction likely contributes to the reduction in spine density at 1 month in these mice. Once again, no changes were observed in *matDp/+* mice ($P > 0.05$, Fig. 4E). Together, these results demonstrate a progressive impairment of spinogenesis that selectively occurs in mice with the paternally inherited 15q11-13 duplication.

Spines are Less Mature in 15q11-13 Paternal Duplication Mice

To examine spine maturation in 15q11-13 duplication mice, we analyzed spine morphology in *patDp/+* and *matDp/+* mice at P14 and 1 month. At P14, spines were mostly immature, as suggested by the large portion of thin spines (Fig. 5A, B). We found that in *patDp/+* mice, but not in *matDp/+* mice, the percentage of mushroom spines was reduced and that of thin spines increased

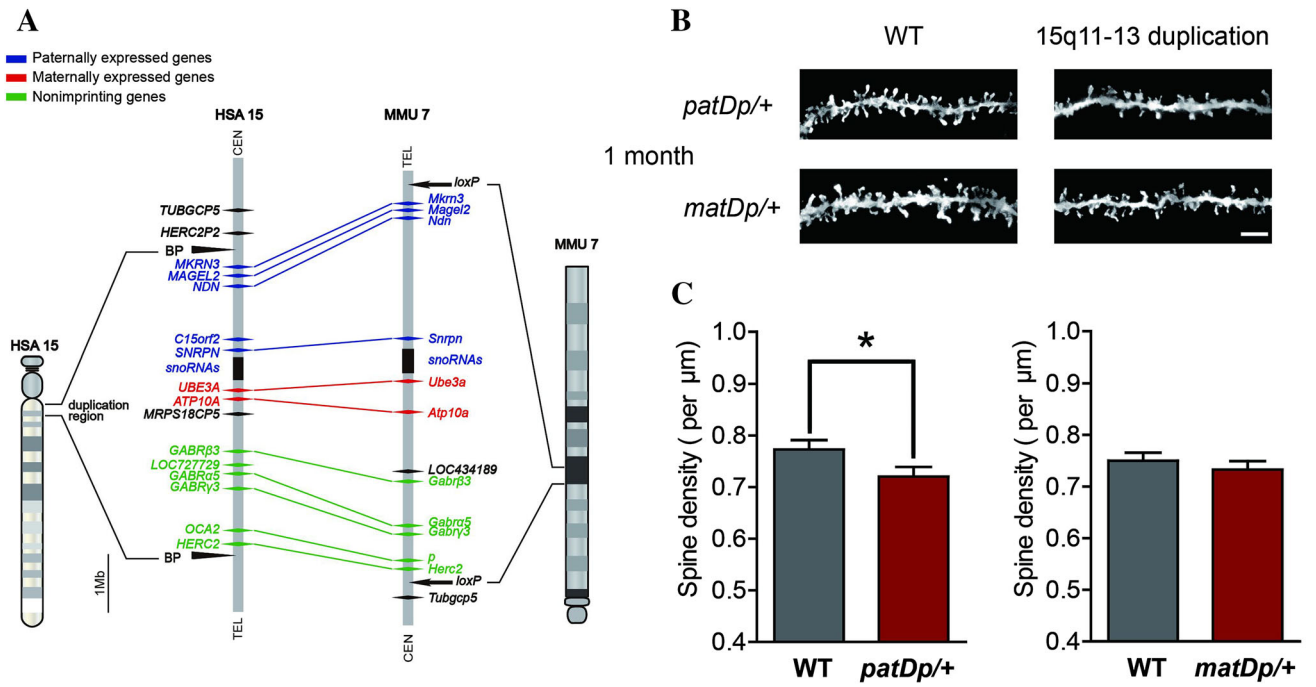


Fig. 3 *patDp/+* mice, but not *matDp/+* mice, show delayed spine maturation at 1 month. **A** Schematic representation of the 15q11-13 duplication region in human chromosome 15 (*left*) and the corresponding region in mouse chromosome 7 (*right*). Genes expressed paternally, maternally, and nonimprinting genes are respectively marked in blue, red and green. Arrowheads indicate the border of duplication segments. Schematic took reference from previous literatures [38, 47]. **B** Representative inverted Golgi staining images showing spines in basal dendrites of layer 2/3 pyramidal neurons in S1BF of 1-month 15q11-13 duplication mice, genotypes as indicated. *Scale bar* 5 μm. **C** Spine density in WT ($n = 34$) and *patDp/+* ($n = 25$) mice at 1 month. **D** Spine density in WT ($n = 38$) and *matDp/+* ($n = 37$) mice at 1 month. *$P < 0.05$.

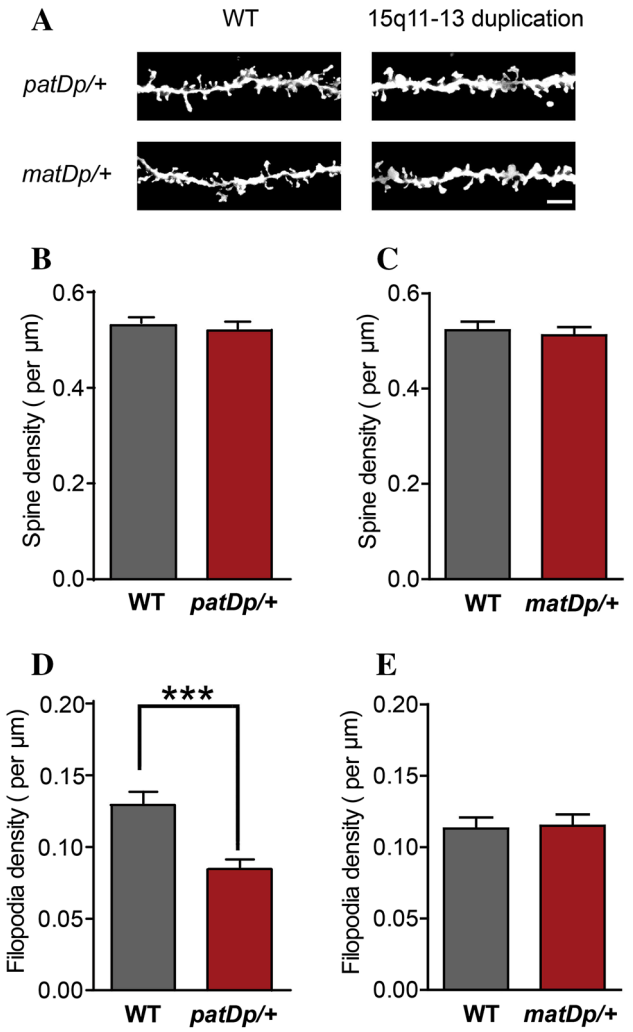


Fig. 4 *patDp*/+ mice, but not *matDp*/+ mice, show impaired filapodia formation at P14. **A** Representative inverted Golgi staining images showing spines in basal dendrites of layer 2/3 pyramidal neurons in S1BF of P14 15q11-13 duplication mice, genotypes as indicated. *Scale bar* 5 µm. **B** Spine density in WT ($n = 29$) and *patDp*/+ ($n = 32$) mice at P14. **C** Spine density in WT ($n = 25$) and *matDp*/+ ($n = 20$) mice at P14. **D** Filopodia density in WT ($n = 29$) and *patDp*/+ ($n = 32$) mice at P14. **E** Filopodia density in WT ($n = 25$) and *matDp*/+ ($n = 20$) mice at P14. $***P < 0.001$.

correspondingly, as compared to wildtype littermates at P14 (Fig. 5A, B), despite similar spine density between the *patDp*/+ mice and wildtype controls. At 1 month, the distribution pattern of spine subtypes was more mature than that at P14 in all genotypes (Fig. 5C, D vs. 5A, B). However, *patDp*/+ mice still possessed more thin spines and less mushroom spines than their wildtype littermates (Fig. 5C). Consistent with the spine density results, spine maturation was not affected in *matDp*/+ mice at 1 month (Fig. 5D). Together, these results demonstrate that in addition to the impairment in generating spines, spines formed in *patDp*/+ mice were also less mature. The absence of these changes in *matDp*/+ mice further

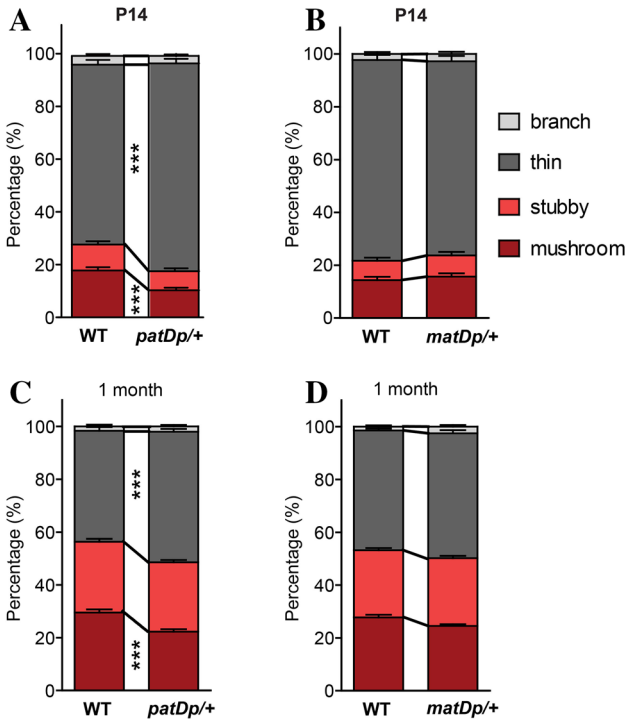


Fig. 5 *patDp*/+ mice, but not matDp/+ mice, show impaired spinogenesis and delayed spine maturation. **A** Spine type classification in P14 WT and *patDp*/+ mice. **B** Spine type classification in P14 WT and *mat*Dp/+ mice. **C** Spine type classification in 1-month WT and *pat*Dp/+ mice. **D** Spine type classification in 1-month WT and *mat*Dp/+ mice. $***P < 0.001$.

underscores the selective impact of this chromosomal duplication depending on its genetic origin.

Discussion

Gene dosage is critical to the normal development and functioning of the brain. This is particularly highlighted in ASD where both loss-of-function and overexpression of gene/chromosomal regions can lead to autistic phenotypes. Here we show that early spinogenesis and later spine pruning are respectively affected in two mouse models of autism with gene duplication, the paternally-inherited 15q11-13 duplication mice and the *MECP2* duplication mice. The distinct spine abnormalities in these two autism models likely reflect the diverse pathologies of the two types of ASDs, with the 15q11-13 duplication primarily impairing the initial establishment of neural connections and the *MECP2* duplication mainly targeting the later refinement of neural circuitry. Our results complement the findings of previous studies and provide further insight into our understanding of diverse pathologies in ASD at the level of synapses and neural circuits. These results raise the importance of time windows for the optimal therapeutic

intervention in the treatment of distinct ASD subtypes. Spinogenesis and spine pruning at the population level in the sensory cortices could further serve as a model for assessing spine pathology in both animal models and postmortem ASD patient tissues, using simple morphological methods such as Golgi staining.

Selective Abnormalities of Spines and Behaviors in 15q11-13 Paternal Duplication Mice

The duplication of chromosomal 15q11-13 region is one of the most frequently identified CNVs in ASD patients. It encompasses a series of imprinting genes including the maternally expressed *Ube3a* and *Atp10a*, the paternally expressed *Snrpn*, *Mkrn3*, *Magel2*, *Necdin* and *snoRNAs*, as well as non-imprinting genes such as a subset of GABA receptor subunits [36, 38] (Fig. 3A). Previous studies have shown that the *patDp/+* mice recapitulate some symptoms of human ASD patients, including impaired social interaction in the three-chamber test, behavioral inflexibility, decreased exploratory activity, impaired cerebellar plasticity and motor learning deficits [27, 36, 38, 46, 47]. Further investigation found that serotonin signaling decreased while dopamine signaling increased in these mice, indicating defects of the neuromodulation systems involved in emotion, motivation and social behaviors [36, 38, 48]. Consistent with the results of these functional analyses, we found that the *patDp/+* mice displayed significant and progressive defects in filopodia formation and spinogenesis (Figs. 3–5), which could lead to inadequate wiring of neural circuitry underlying the above-mentioned functions and behaviors. Consistently, a recent study using two-photon live imaging demonstrated increased spine turnover in *patDp/+* mice [49], suggesting that spines in these mice are more motile and unstable, which may account for the reduced spine density and immature spine morphology observed in these mice (Figs. 3, 5). It is somewhat surprising that all the behavioral, physiological and morphological abnormalities reported thus far [27, 36, 38, 39, 46–49], including ours (Figs. 3–5), were restricted to *patDp/+* mice, while the *matDp/+* mice seemed pretty normal, given that it is the maternal duplication of this chromosomal region that was thought to cause autism in humans [50]. Although a recent detailed human study showed that paternal duplications are also pathogenic and increase risks for ASD, developmental delay and multiple congenital anomalies [51], the "gap" between the mouse model and human patients is still a puzzling. We surmise that it may be due to the differences in imprinting status and epigenetic control of specific genes, in specific brain regions and between species [36]. In fact, some paternally expressed genes were found to be reduced in post-mortem brain tissues of individuals with maternal 15q11-q13 duplication [52], suggesting that gene expression within 15q11-q13 is not based entirely on copy number, and can be influenced by epigenetic mechanisms. Further research is required to elucidate the precise underlying mechanisms.

Distinct Spine Abnormalities in Multiple ASD Mouse Models

ASD shows great diversity in both genetic etiology and clinical manifestation [5, 6, 8, 10, 26, 27]. How to bridge the gap between the genetic causes and the ASD symptoms poses a major challenge to autism research. Previous studies have demonstrated highly distinct spine phenotypes in multiple autism mouse models with gene deletion/mutation. For example, spine pruning defect was found in *Fragile X Mental Retardation 1* (*Fmr1*) knockout mice [53, 54], while decreased spine density was observed in *Mecp2* knockout mice throughout development [55–57]. Additionally, studies using neuronal cultures or transgenic mice have shown that spine density and/or shape were altered after genetic manipulation of proteins implicated in syndromic or non-syndromic autism, including neurexins/neuroligins, Shank2/3, Epac2, Tsc1/2, Ube3A and PTEN [6]. Here we further expand our knowledge of ASD spine pathology to two gene duplication mouse models. We note that our results demonstrating spine pruning defects in the basal dendrites of layer 2/3 neurons in the sensory cortices of $MECP2^{Tg1}$ mice (Figs. 1, 2) are consistent with and complementary to work by Jiang *et al*. in the apical dendrites of layer 5 neurons, where they showed a delayed pruning of spines on this dendritic segment in $MECP2^{Tg1}$ mice [58]. Thus, overproduction of Mecp2 likely results in a global effect across multiple layers of the cerebral cortex, to slow down or inhibit the spine pruning process during neural circuit refinement. We note that at 3 months, the pruning process was completely blocked in V1 (Fig. 2A, B) and only partially impaired in S1BF (Fig. 1A, B). Since mice reared under standard laboratory conditions likely use their tactile sensation more than their visual system, this result is consistent with the activity-dependence of spine pruning [14, 15].

Our existing knowledge of spine pathology in ASD suggests potentially two major classes of abnormalities: one is the insufficient genesis of spines seen in models including *Mecp2* knockout and 15q11-13 paternal duplication, which may result in a less connected and consequently inadequate neural circuitry; the other is defects in spine pruning as seen in *MeCP2* duplication and *Fmr1* knockout models, which may lead to an over-connected and thus less efficient neural circuitry in adulthood. We note that both the defects in spinogenesis and spine pruning, as we identified here, are developmentally regulated.

The spine formation defect in 15q11-13 paternal duplication mice was not significant until 1 month, while the spinogenesis process seemed unaffected before the *MECP2*^{Tg1} mice entered the spine pruning period. The progressive feature of these spine abnormalities is consistent with the gradually emerging and worsening of symptoms often seen in ASD patients. This diversity of spine abnormality in autism mouse models further raises the intriguing question of how distinct alterations of neural circuits during different developmental stages lead to common behavioral manifestations in ASD, including lack of social communications and repetitive behaviors.

Insights from Another Gene Duplication Model

A good analogy of modeling gene-duplication-induced autism in mice is the mouse models of Down syndrome (DS), a disease caused by an extra copy of human chromosome 21 (Hsa21) and characterized by intellectual disability, deficits in learning and memory and early-onset Alzheimer's disease [59–61]. Genes within the duplex region of Hsa21 are synthetic to 3 regions located on mouse chromosome 10 (Mmu10), Mmu16 and Mmu17 [59, 61]. Similar to DS patients, DS mouse models including Ts65Dn [62, 63] and Ts1Cje [64, 65], two most studied mouse models of DS, exhibit deficits in learning and hippocampal-dependent memory, impaired long-term potentiation (LTP) and altered excitatory/inhibitory balance [59, 61]. Interestingly, genes in Hsa21 have been shown to affect spine morphogenesis separately, and DS mouse models Ts65Dn, Ts1Cje and Ts1Rhr (trisomic region: Ts65Dn > Ts1Cje > Ts1Rhr) all show lowered spine density and enlarged spine head with gradually reduced severity (severity: Ts65Dn > Ts1Cje > Ts1Rhr) [59, 66], indicating an additive/synergic effect of these genes on spines. Given that decreased spine density is the shared pathological change in DS and in some ASD mouse models, including *Mecp2* knockout [55–57] and 15q11-13 duplication (Fig. 3), and that intellectual disability also occurs in a significant portion of ASD patients [5, 30, 66], it is interesting to consider the potential crosstalk between ASD genes and DS genes. In fact, it has been recently proposed that the product of one of the DS genes, DS critical region 1 (DSCR1), interacts with the Fragile X mental retardation protein (FMRP) to regulate the local protein synthesis in spines [66]. However, how ASD is linked to DS or other neuropsychiatric disorders mechanistically remains unclear and requires further investigation.

Acknowledgements We thank Sha-Jin Huang and Yuan Lu for excellent technical assistance. This work was supported by grants from the Strategic Priority Research Program of the Chinese Academy of Sciences, China (XDB02010000), the National Natural Science Foundation of China (31530030 and 81371270), and the Science and Technology Commission of Shanghai Municipality, China (16XD1404800).

References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders : DSM-5. Fifth edition. Washington, DC, United States of America, 2013.
2. Ebert DH, Greenberg ME. Activity-dependent neuronal signalling and autism spectrum disorder. Nature 2013, 493: 327–337.
3. Lee E, Lee J, Kim E. Excitation/inhibition imbalance in animal models of autism spectrum disorders. Biol Psychiatry 2016.
4. Mullins C, Fishell G, Tsien RW. Unifying Views of autism spectrum disorders: a consideration of autoregulatory feedback loops. Neuron 2016, 89: 1131–1156.
5. Zoghbi HY, Bear MF. Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities. Cold Spring Harb Perspect Biol 2012, 4.
6. Penzes P, Cahill ME, Jones KA, VanLeeuwen JE, Woolfrey KM. Dendritic spine pathology in neuropsychiatric disorders. Nat Neurosci 2011, 14: 285–293.
7. Nelson SB, Valakh V. Excitatory/inhibitory balance and circuit homeostasis in autism spectrum disorders. Neuron 2015, 87: 684–698.
8. de la Torre-Ubieta L, Won H, Stein JL, Geschwind DH. Advancing the understanding of autism disease mechanisms through genetics. Nat Med 2016, 22: 345–361.
9. Geschwind DH, State MW. Gene hunting in autism spectrum disorder: on the path to precision medicine. Lancet Neurol 2015, 14: 1109–1120.
10. Sztainberg Y, Zoghbi HY. Lessons learned from studying syndromic autism spectrum disorders. Nat Neurosci 2016, 19: 1408–1417.
11. Gray EG. Electron microscopy of synaptic contacts on dendrite spines of the cerebral cortex. Nature 1959, 183: 1592–1593.
12. Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. Science 1986, 232: 232–235.
13. Rakic P, Bourgeois JP, Goldman-Rakic PS. Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. Prog Brain Res 1994, 102: 227–243.
14. Bhatt DH, Zhang S, Gan WB. Dendritic spine dynamics. Annu Rev Physiol 2009, 71: 261–282.
15. Bian WJ, Miao WY, He SJ, Qiu Z, Yu X. Coordinated spine pruning and maturation mediated by inter-spine competition for cadherin/catenin complexes. Cell 2015, 162: 808–822.
16. Elston GN, Oga T, Fujita I. Spinogenesis and pruning scales across functional hierarchies. J Neurosci 2009, 29: 3271–3275.
17. Yuste R, Bonhoeffer T. Genesis of dendritic spines: insights from ultrastructural and imaging studies. Nat Rev Neurosci 2004, 5: 24–34.

18. Zuo Y, Lin A, Chang P, Gan WB. Development of long-term dendritic spine stability in diverse regions of cerebral cortex. Neuron 2005, 46: 181–189.
19. Huttenlocher PR. Neural plasticity: the effects of environment on the development of the cerebral cortex. Cambridge, Massachusetts: Harvard Unversity Press, 2002.
20. Grutzendler J, Kasthuri N, Gan WB. Long-term dendritic spine stability in the adult cortex. Nature 2002, 420: 812–816.
21. Holtmaat AJ, Trachtenberg JT, Wilbrecht L, Shepherd GM, Zhang X, Knott GW, *et al.* Transient and persistent dendritic spines in the neocortex in vivo. Neuron 2005, 45: 279–291.
22. Harris KM, Jensen FE, Tsao B. Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. J Neurosci 1992, 12: 2685–2705.
23. Harris KM, Weinberg RJ. Ultrastructure of synapses in the mammalian brain. Cold Spring Harb Perspect Biol 2012, 4: a005587.
24. Tonnesen J, Katona G, Rozsa B, Nagerl UV. Spine neck plasticity regulates compartmentalization of synapses. Nat Neurosci 2014, 17: 678–685.
25. Yuste R. Electrical compartmentalization in dendritic spines. Annu Rev Neurosci 2013, 36: 429–449.
26. Devlin B, Scherer SW. Genetic architecture in autism spectrum disorder. Curr Opin Genet Dev 2012, 22: 229–237.
27. Huguet G, Ey E, Bourgeron T. The genetic landscapes of autism spectrum disorders. Annu Rev Genomics Hum Genet 2013, 14: 191–213.
28. Krumm N, O'Roak BJ, Shendure J, Eichler EE. A de novo convergence of autism genetics and molecular neuroscience. Trends Neurosci 2014, 37: 95–105.
29. Nakai N, Otsuka S, Myung J, Takumi T. Autism spectrum disorder model mice: Focus on copy number variation and epigenetics. Sci China Life Sci 2015, 58: 976–984.
30. Coe BP, Girirajan S, Eichler EE. The genetic variability and commonality of neurodevelopmental disease. Am J Med Genet C Semin Med Genet 2012, 160C: 118–129.
31. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 1999, 23: 185–188.
32. Chahrour M, Zoghbi HY. The story of Rett syndrome: from clinic to neurobiology. Neuron 2007, 56: 422–437.
33. Lombardi LM, Baker SA, Zoghbi HY. MECP2 disorders: from the clinic to mice and back. J Clin Investig 2015, 125: 2914–2923.
34. Ramocki MB, Peters SU, Tavyev YJ, Zhang F, Carvalho CM, Schaaf CP, *et al.* Autism and other neuropsychiatric symptoms are prevalent in individuals with MeCP2 duplication syndrome. Ann Neurol 2009, 66: 771–782.
35. Ramocki MB, Tavyev YJ, Peters SU. The MECP2 duplication syndrome. Am J Med Genet A 2010, 152A: 1079–1088.
36. Takumi T. The neurobiology of mouse models syntenic to human chromosome 15q. J Neurodev Disord 2011, 3: 270–281.
37. Collins AL, Levenson JM, Vilaythong AP, Richman R, Armstrong DL, Noebels JL, *et al.* Mild overexpression of MeCP2 causes a progressive neurological disorder in mice. Hum Mol Genet 2004, 13: 2679–2689.
38. Nakatani J, Tamada K, Hatanaka F, Ise S, Ohta H, Inoue K, *et al.* Abnormal behavior in a chromosome-engineered mouse model for human 15q11-13 duplication seen in autism. Cell 2009, 137: 1235–1246.
39. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 1999, 23: 185–188.
40. Samaco RC, Mandel-Brehm C, McGraw CM, Shaw CA, McGill BE, Zoghbi HY. Crh and Oprm1 mediate anxiety-related behavior and social approach in a mouse model of MECP2 duplication syndrome. Nat Genet 2012, 44: 206–211.
41. Sztainberg Y, Chen HM, Swann JW, Hao S, Tang B, Wu Z, *et al.* Reversal of phenotypes in MECP2 duplication mice using genetic rescue or antisense oligonucleotides. Nature 2015, 528: 123–126.
42. Liu Z, Li X, Zhang JT, Cai YJ, Cheng TL, Cheng C, *et al.* Autism-like behaviours and germline transmission in transgenic monkeys overexpressing MeCP2. Nature 2016, 530: 98–102.
43. Dailey ME, Smith SJ. The dynamics of dendritic structure in developing hippocampal slices. J Neurosci 1996, 16: 2983-2994.
44. Ziv NE, Smith SJ. Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. Neuron 1996, 17: 91–102.
45. Fiala JC, Feinberg M, Popov V, Harris KM. Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. J Neurosci 1998, 18: 8900–8911.
46. Tamada K, Tomonaga S, Hatanaka F, Nakai N, Takao K, Miyakawa T, *et al.* Decreased exploratory activity in a mouse model of 15q duplication syndrome; implications for disturbance of serotonin signaling. PLoS One 2010, 5: e15126.
47. Piochon C, Kloth AD, Grasselli G, Titley HK, Nakayama H, Hashimoto K, *et al.* Cerebellar plasticity and motor learning deficits in a copy-number variation mouse model of autism. Nat Commun 2014, 5: 5586.
48. Farook MF, DeCuypere M, Hyland K, Takumi T, LeDoux MS, Reiter LT. Altered serotonin, dopamine and norepinepherine levels in 15q duplication and Angelman syndrome mouse models. PLoS One 2012, 7: e43030.
49. Isshiki M, Tanaka S, Kuriu T, Tabuchi K, Takumi T, Okabe S. Enhanced synapse remodelling as a common phenotype in mouse models of autism. Nat Commun 2014, 5: 4742.
50. Cook EH, Jr., Lindgren V, Leventhal BL, Courchesne R, Lincoln A, Shulman C, *et al.* Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. Am J Hum Genet 1997, 60: 928–934.
51. Isles AR, Ingason A, Lowther C, Walters J, Gawlick M, Stober G, *et al.* Parental origin of interstitial duplications at 15q11.2-q13.3 in schizophrenia and neurodevelopmental disorders. PLoS Genet 2016, 12: e1005993.
52. Scoles HA, Urraca N, Chadwick SW, Reiter LT, Lasalle JM. Increased copy number for methylated maternal 15q duplications leads to changes in gene and protein expression in human cortical samples. Mol Autism 2011, 2: 19.
53. Comery TA, Harris JB, Willems PJ, Oostra BA, Irwin SA, Weiler IJ, *et al.* Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. Proc Natl Acad Sci U S A 1997, 94: 5401–5404.
54. Galvez R, Greenough WT. Sequence of abnormal dendritic spine development in primary somatosensory cortex of a mouse model of the fragile X mental retardation syndrome. Am J Med Genet A 2005, 135: 155–160.
55. Xu X, Miller EC, Pozzo-Miller L. Dendritic spine dysgenesis in Rett syndrome. Front Neuroanat 2014, 8: 97.
56. Stuss DP, Boyd JD, Levin DB, Delaney KR. MeCP2 mutation results in compartment-specific reductions in dendritic branching and spine density in layer 5 motor cortical neurons of YFP-H mice. PLoS One 2012, 7: e31896.
57. Chao HT, Zoghbi HY, Rosenmund C. MeCP2 controls excitatory synaptic strength by regulating glutamatergic synapse number. Neuron 2007, 56: 58–65.
58. Jiang M, Ash RT, Baker SA, Suter B, Ferguson A, Park J, *et al.* Dendritic arborization and spine dynamics are abnormal in the

mouse model of MECP2 duplication syndrome. J Neurosci 2013, 33: 19518–19533.
59. Ruparelia A, Pearn ML, Mobley WC. Aging and intellectual disability: insights from mouse models of Down syndrome. Dev Disabil Res Rev 2013, 18: 43–50.
60. Antonarakis SE, Lyle R, Dermitzakis ET, Reymond A, Deutsch S. Chromosome 21 and down syndrome: from genomics to pathophysiology. Nat Rev Genet 2004, 5: 725–738.
61. Liu C, Belichenko PV, Zhang L, Fu D, Kleschevnikov AM, Baldini A, *et al.* Mouse models for Down syndrome-associated developmental cognitive disabilities. Dev Neurosci 2011, 33: 404–413.
62. Davisson MT, Schmidt C, Akeson EC. Segmental trisomy of murine chromosome 16: a new model system for studying Down syndrome. Prog Clin Biol Res 1990, 360: 263–280.
63. Reeves RH, Irving NG, Moran TH, Wohn A, Kitt C, Sisodia SS, *et al.* A mouse model for Down syndrome exhibits learning and behaviour deficits. Nat Genet 1995, 11: 177–184.
64. Sago H, Carlson EJ, Smith DJ, Kilbridge J, Rubin EM, Mobley WC, *et al.* Ts1Cje, a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities. Proc Natl Acad Sci USA 1998, 95: 6256–6261.
65. Sago H, Carlson EJ, Smith DJ, Rubin EM, Crnic LS, Huang TT, *et al.* Genetic dissection of region associated with behavioral abnormalities in mouse models for Down syndrome. Pediatr Res 2000, 48: 606–613.
66. Chang KT, Ro H, Wang W, Min KT. Meeting at the crossroads: common mechanisms in Fragile X and Down syndrome. Trends Neurosci 2013, 36: 685–694.

Neurosci. Bull. April, 2017, 33(2):153–160
DOI 10.1007/s12264-017-0102-9

www.neurosci.cn
www.springer.com/12264

ORIGINAL ARTICLE

Sex Differences in Diagnosis and Clinical Phenotypes of Chinese Children with Autism Spectrum Disorder

Shihuan Wang[1] · Hongzhu Deng[1] · Cong You[1] · Kaiyun Chen[1] · Jianying Li[1] · Chun Tang[1] · Chaoqun Ceng[1] · Yuanyuan Zou[1] · Xiaobing Zou[1]

Received: 9 October 2016 / Accepted: 22 January 2017 / Published online: 25 February 2017

Abstract The aim of this study was to explore the differences between boys and girls in the diagnosis and clinical phenotypes of autism spectrum disorder (ASD) in China's mainland. Children diagnosed with ASD ($n = 1064$, 228 females) were retrospectively included in the analysis. All children were assessed using the Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observation Schedule (ADOS). The results showed that girls scored significantly higher in ADI-R socio-emotional reciprocity than boys, and also scored lower in ADI-R and ADOS restricted and repetitive behaviors (RRBs). Meanwhile, the proportions of girls who satisfied the diagnostic cut-off scores in the ADI-R RRBs domain were lower than in boys ($P < 0.05$). Our results indicated that girls with ASD show greater socio-emotional reciprocity than boys. Girls also tended to show fewer RRBs than boys, and the type of RRBs in girls differ from those in boys. The ADI-R was found to be less sensitive in girls, particularly for assessment in the RRBs domain.

Keywords Autism spectrum disorder · Sex differences · Diagnosis

✉ Hongzhu Deng
denghongzhu@foxmail.com

✉ Xiaobing Zou
zouxb@163.net

[1] Child Developmental and Behavioral Center, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510630, China

Introduction

Autism spectrum disorder (ASD) is a set of heterogeneous neurodevelopmental disorders characterized by developmental delays in social communication and restricted and repetitive behaviors (RRBs) [1]. Based on the most recent epidemiological surveys, the global prevalence of ASD is estimated to be 1%–2% [2, 3]. Males are disproportionately represented at ~4:1 [4, 5]. While epidemiological studies have confirmed the male dominance in ASD, the reason for this is unclear. The original description, diagnostic criteria, and clinical data for ASD were based almost solely on males, with relatively few studies focusing on females. Several studies have reported that females with ASD might exhibit behaviors, cognitive functioning, neuroanatomy, and gene expression patterns different from males [6–8]. However, the characterization of ASD in females is far from complete.

Few studies have explored sex differences within the core clinical phenotypes in children with ASD, and the results are inconsistent. Some studies have reported greater stereotypical play and RRBs in males with ASD. Bölte *et al.* found that males exhibit more RRBs than females in adult high-functioning autism as assessed using the Autism Diagnostic Observation Schedule (ADOS) [9]. Hattier *et al.* also reported a higher frequency of RRBs in adult males regardless of age range as assessed using the Stereotypies subscale of the Diagnostic Assessment for the Severely Handicapped-II [10]. However, some investigators have found no such sex differences in the RRBs domain [11, 12]. In the social communication domain, Frazier *et al.* recently reported that females with ASD (age range, 4–18 years) have greater social communication impairment than males [13]. Hiller *et al.* reported that girls with ASD are more likely to integrate non-verbal and

verbal behaviors, maintain reciprocal conversation, and be able to initiate friendships [14]. In contrast, other studies have found no sex differences in early social-communication skills [15]. Collectively, these studies suggest potential differences in the symptoms of ASD between males and females. However, a clear and consistent picture of the clinical phenotypes of ASD in females has not yet emerged. This may be due to variability in the age of patients, sample sizes, diagnostic criteria, and assessment tools used in previous studies.

Females have been reported to be more likely to experience a lack of diagnosis, delay in diagnosis, and misdiagnosis. Goin-Kochel *et al.* reported that girls were diagnosed later for Asperger's disorder (average 8.9 *vs.* 7.0 years) and pervasive developmental disorder-not otherwise specified (average 5.1 *vs.* 3.9 years), when compared with boys [16]. Koenig and Tsatsanis highlighted that sex differences at the time of presentation have not been sufficiently addressed in validation studies of the key diagnostic instruments, such as the ADI-R and ADOS [17]. There is a paucity of research addressing the validity of diagnostic criteria, particularly in females. In addition, symptom criteria or assessment items may be biased, raising doubts about the criteria and content validity of the ADI-R and ADOS diagnostic algorithms, especially in relation to females.

Few studies have been conducted on sex differences in core clinical phenotypes in children with ASD, specifically in Asian populations. Early abnormal developmental differences between boys and girls with ASD remain unknown. The primary objective of the present study was to explore sex differences in the domains of social communication and RRBs in children with ASD in a large sample from an Asian community. The second objective was to retrospectively analyze the differences in early abnormal development between boys and girls with ASD based on the ADI-R. A third objective was to further explore the differences in diagnostic cut-off scores for ADI-R and ADOS between boys and girls with ASD.

Methods

Participants

The sample retrospectively included 1064 individuals (228 girls and 836 boys). These children were diagnosed with ASD in a single-center clinic—The Child Developmental & Behavioral Center in the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou—between June 2013 and October 2015. The participants selected were 24–83 months old. Inclusion criteria: children who fulfilled the ASD diagnostic criteria based on the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) [18]. Exclusion criteria: children with mental retardation, idiopathic language retardation, or schizophrenia. There were no gender differences in the exclusion samples (11 girls and 47 boys).

Diagnostic Assessment

The Autism Diagnostic Interview-Revised (ADI-R)

The ADI-R [19] is a semi-structured parent/caregiver interview designed to assess and quantify the developmental history of autism-specific behaviors. It contains 93 items, including development of early childhood, language development, communication functioning, social reciprocity, play, and RRBs. The ADI-R diagnostic items constitute 4 domains: social reciprocity (A: cut-off $\geq$10), communication (B: cut-off $\geq$8 for verbal and $\geq$7 for non-verbal individuals), RRBs (C: cut-off $\geq$3), and abnormal development before 36 months (D: cut-off $\geq$1). Verbal children were defined as those who have spontaneous, echoed, or stereotyped language, which on a daily basis, involves phrases of three words or more [19]. The cut-off scores were defined as satisfying the autism diagnostic criteria. The social reciprocity domain (A) consists of non-verbal behaviors to regulate social interaction (A1), developing peer relationships (A2), sharing enjoyment (A3), and socio-emotional reciprocity (A4). The communication domain (B) consists of gesture communication (B1), conversation (B2, only for verbal individuals), repetitive speech (B3, only for verbal individuals), and play (B4). The RRBs domain (C) consists of unusual preoccupation, circumscribed interest, verbal rituals, compulsions/rituals, hand and finger mannerisms, stereotyped body movements, repetitive use of objects/interest in parts of objects, and unusual sensory interest. Abnormal development before 36 months (D) consists of age when parents first noticed developmental abnormalities, age when developmental abnormalities probably first manifested in interviewer's judgment, age of first single words, and age of first phrases. In the ADI-R items, word speech delay is defined as the age at first single words >24 months, and phrase speech delay is defined as the age at first phrase >33 months.

The Autism Diagnostic Observation Schedule (ADOS)

The ADOS is a standardized assessment tool for children with suspected ASD [20]. It involves a semi-structured interview with interspersed activities and tasks intended to elicit behaviors associated with ASD. It covers communication, social reciprocity, play/imagination, and RRBs. Depending on the child's language level, verbal children

received module 2 assessment, while non-verbal children received module 1 assessment. The cut-off scores for satisfying the autism diagnostic criteria were defined in the domains of social reciprocity (A) and communication (B). For module 1, the cut-off for autism was A + B $\geq$ 12, with A $\geq$ 7 and B $\geq$ 4. For module 2, the cut-off for autism was A + B $\geq$ 12, with A $\geq$ 6 and B $\geq$ 5.

Statistical Analyses

Data were analyzed using the Statistical Package for Social Sciences (version 20.0; SPSS Inc., Chicago, IL). The differences in baseline characteristics between boys and girls with ASD were examined using χ^2-test. The scores in the social communication domain were normally distributed, while the scores for different types of RRBs were skewed. Sex differences in the scores for the social communication domain and early abnormal development were tested using Analysis of Covariance, with sex as the fixed factor and age as the covariate. The differences in the scores for different types of RRBs between boys and girls with ASD were determined using Mann–Whitney U tests. The differences in cut-off scores with respect to social reciprocity, communication, and RRBs between boys and girls with ASD were examined using χ^2-test. $P < 0.05$ was considered statistically significant. Effect Size (ES) was used to estimate the sex effect.

Results

Demographic Characteristics

The baseline demographic characteristics are listed in Table 1. There were no statistically significant age differences between boys and girls for both verbal and non-verbal children. Word and phrase speech delay was more frequently reported in girls than in boys ($\chi^2 = 21.82$, 7.67; $P < 0.05$; ES = 0.14, 0.09). While most children were diagnosed with autism, only 7.46% of girls and 7.66% of boys were diagnosed with Asperger's disorder. There were no sex differences in the distribution of diagnoses.

Sex Differences in Social Reciprocity and Communication Domains

No significant between-group differences were found in total social reciprocity scores based on ADI-R and ADOS in verbal and non-verbal children (Table 2). However, detailed analysis of social reciprocity revealed that verbal and non-verbal girls with ASD scored higher in terms of ADI-R socio-emotional reciprocity than boys ($P = 0.049$, 0.001; ES = 0.22, 0.38).

No sex-based differences were found in total verbal communication scores based on ADI-R and ADOS in verbal children (Table 3). However, verbal girls with ASD scored higher in ADI-R gesture communication than boys ($P < 0.001$; ES = 0.40), and non-verbal girls scored higher in the ADOS communication domain than boys ($P = 0.006$; ES = 0.32). In addition, verbal girls scored lower in ADI-R repetitive speech than boys ($P = 0.003$; ES = 0.29).

Sex Differences in RRBs Domain

Girls with ASD (3.59 $\pm$ 1.87) scored lower than boys (4.55 $\pm$ 2.06) in total RRBs based on the ADI-R ($F = 39.03$, $P < 0.001$; ES = 0.32), and girls with ASD (2.02 $\pm$ 1.47) also scored lower in RRBs than boys (2.30 $\pm$ 1.41) based on the ADOS ($F = 7.73$, $P = 0.006$; ES = 0.13). Based on the ADI-R, non-verbal girls with

Table 1 Baseline demographic characteristics of children with ASD.

	Girls with ASD Mean (SD)	Boys with ASD Mean (SD)	Effect size	t/χ^2	*P*
n	228	836	0.03	0.84	0.361
Verbal (%)	134 (58.77%)	463 (55.38%)			
Non-verbal (%)	94 (41.23%)	373 (44.62%)			
Age in months					
Verbal	52.04 (17.86)	55.07 (16.32)	0.18	1.85	0.065
Non-verbal	35.49 (10.79)	36.37 (10.84)	0.06	0.71	0.48
Word speech delay (*n*, %)	155 (67.98)	423 (50.60)	0.14	21.82	<0.001
Phrase speech delay (*n*, %)	103 (45.18)	294 (35.17)	0.09	7.67	0.006
Diagnosis (*n*, %)					
Autism	204 (89.47)	761 (91.03)	0.06	0.51	0.474
Asperger's disorder	17 (7.46)	64 (7.66)	0.01	0.01	0.92
PDD-NOS	7 (3.07)	11 (1.31)	0.18	3.32	0.069

ASD, autism spectrum disorder; PDD-NOS, pervasive developmental disorder-not otherwise specified; SD, standard deviation.

Table 2 Descriptive statistics for social reciprocity domain in girls and boys with ASD.

Scores	Girls with ASD Mean (SD)	Boys with ASD Mean (SD)	Effect size	*F*	*P*
Non-verbal behaviors to regulate social interaction (A1)					
Verbal	3.20 (1.22)	3.04 (1.18)	0.13	1.69	0.194
Non-verbal	3.69 (1.25)	3.56 (1.30)	0.10	0.86	0.353
Develop peer relationships (A2)					
Verbal	4.13 (1.85)	4.13 (1.90)	0.00	1.17	0.279
Non-verbal	3.20 (1.60)	3.17 (1.55)	0.02	0.33	0.568
Share enjoyment (A3)					
Verbal	3.77 (1.48)	3.62 (1.52)	0.10	0.46	0.499
Non-verbal	4.59 (1.27)	4.60 (1.35)	0.01	0.01	0.907
Socio-emotional reciprocity (A4)					
Verbal	4.99 (1.98)	4.62 (1.60)	0.22	3.89	0.049
Non-verbal	6.35 (1.84)	5.70 (1.65)	0.38	11.08	0.001
ADI-R social reciprocity domain (A)					
Verbal	16.10 (4.83)	15.41 (4.02)	0.16	3.30	0.070
Non-verbal	17.83 (4.57)	17.03 (4.21)	0.19	3.12	0.078
ADOS social reciprocity domain (A)					
Verbal	8.93 (2.74)	8.80 (2.80)	0.04	0.13	0.715
Non-verbal	9.86 (2.33)	9.42 (2.68)	0.17	1.97	0.161

ADI-R Social reciprocity domain A = A1 + A2 + A3 + A4.

ASD, autism spectrum disorder; SD, standard deviation.

Table 3 Descriptive statistics for the communication domain in girls and boys with ASD.

Scores	Girls with ASD Mean (SD)	Boys with ASD Mean (SD)	Effect size	*F*	*P*
Gesture communication (B1)					
Verbal	4.12 (2.06)	3.27 (2.07)	0.40	14.54	<0.001
Non-verbal	5.78 (1.71)	5.62 (1.92)	0.08	0.52	0.473
Conversation (B2)					
Verbal	2.69 (1.38)	2.90 (1.44)	0.14	1.70	0.192
Repetitive speech (B3)					
Verbal	2.78 (1.01)	3.10 (1.16)	0.29	9.04	0.003
Play (B4)					
Verbal	3.87 (1.31)	4.03 (1.33)	0.12	1.42	0.233
Non-verbal	4.99 (1.07)	4.97 (1.01)	0.02	0.04	0.85
ADI-R communication domain (B)					
Verbal	13.46 (3.44)	13.30 (3.63)	0.05	0.05	0.824
Non-verbal	10.77 (2.36)	10.59 (2.50)	0.07	0.39	0.533
ADOS communication domain (B)					
Verbal	5.87 (1.88)	5.50 (2.07)	0.18	2.32	0.128
Non-verbal	6.56 (1.58)	5.99 (1.85)	0.32	7.65	0.006

ADI-R communication domain: B (verbal) = B1 + B2 + B3 + B4; B (non-verbal) = B1 +B4.

ASD, autism spectrum disorder; SD, standard deviation.

ASD scored higher than boys in hand and finger mannerisms and stereotyped body movements ($Z = 2.13$, 2.22; $P = 0.033$, 0.026). Conversely, non-verbal boys with ASD scored higher than girls in unusual preoccupation, repetitive use of objects, and interest in parts of objects ($Z = 2.15$, 7.95; all $P < 0.05$). In addition, verbal boys with ASD scored higher than girls in unusual preoccupation, circumscribed interest, verbal rituals, repetitive use of objects, and interest in parts of objects ($Z = 2.83$, 2.54, 2.98, 9.22; all $P < 0.05$) (Table 4).

Sex Differences in Early Abnormal Development

Based on the ADI-R, the age when parents first noticed developmental abnormalities in both verbal and non-verbal girls was later than in boys ($F = 34.06$, 51.09; all $P < 0.001$; ES = 0.45, 0.54). Meanwhile, the age when developmental abnormalities probably first manifested in the interviewer's judgment in both verbal and non-verbal girls was also later than in boys ($F = 114.27$, 115.56; $P < 0.001$; ES = 0.44, 0.56). Furthermore, the age at which single words and phrases were first spoken by verbal girls was higher than that of boys ($F = 6.94$, 8.16; $P = 0.009$, 0.004; ES = 0.25, 0.26) (Table 5).

Sex Differences in Diagnostic Cut-off Scores

The differences in diagnostic cut-off scores in boys and girls with ASD are summarized in Table 6. A lower proportion of verbal girls with ASD satisfied the cut-off scores for ASD relative to boys (89.85%) in the ADI-R repetitive stereotyped behaviors domain ($\chi^2 = 20.53$, $P < 0.001$, ES = 0.19). A lower proportion of non-verbal girls (73.40%) satisfied the cut-off scores for ASD relative to boys (84.72%) in the same domain ($\chi^2 = 6.64$, $P = 0.010$, ES = 0.12).

Discussion

Sex Differences in Core Clinical Phenotypes in Children with ASD

An important finding emerging from our study is the strong suggestion that both verbal and non-verbal girls with ASD have greater socio-emotional reciprocity impairment than boys, while non-verbal girls show more serious communication impairment than boys. Socio-emotional reciprocity includes use of the body to communicate, offering comfort, quality of expression of social interest, appropriate facial expressions, and appropriateness of social response. Holtmann *et al.* examined sex differences using the ADI-R and ADOS for participants with high-

Table 4 Descriptive statistics for repetitive stereotyped behaviors domain in girls and boys with ASD.

Scores	Girls ($n = 228$)			Boys ($n = 836$)			Z	P
	0	1	2	0	1	2		
Unusual preoccupation								
Verbal	90 (39.47%)	33 (14.47%)	11 (4.82%)	252 (30.14%)	137 (16.39%)	74 (8.85%)	2.83	0.005
Non-verbal	60 (26.32%)	28 (12.28%)	6 (2.63%)	200 (23.92%)	116 (13.88%)	57 (6.82%)	2.15	0.032
Circumscribed interest								
Verbal	68 (29.82%)	54 (23.68%)	12 (5.26%)	201 (24.04%)	158 (18.90%)	104 (12.44%)	2.54	0.011
Non-verbal	64 (28.07%)	25 (10.96%)	5 (2.19%)	285 (34.09%)	62 (7.42%)	26 (3.11%)	1.45	0.141
*Verbal rituals**								
Verbal	91 (39.91%)	37 (16.23%)	6 (2.63%)	261 (31.22%)	126 (15.07%)	76 (9.09%)	2.98	0.003
Compulsions/rituals								
Verbal	58 (25.44%)	55 (24.12%)	21 (9.21%)	257 (30.74%)	110 (13.16%)	96 (11.48%)	1.45	0.148
Non-verbal	54 (23.68%)	31 (13.60%)	9 (3.95%)	247 (29.55%)	69 (8.25%)	57 (6.82%)	0.97	0.331
Hand and finger mannerisms								
Verbal	87 (38.16%)	36 (15.79%)	11 (4.82%)	325 (38.88%)	83 (9.93%)	55 (6.58%)	0.77	0.442
Non-verbal	51 (22.37%)	28 (12.28%)	15 (6.58%)	248 (29.67%)	80 (9.57%)	45 (5.38%)	2.13	0.033
Stereotyped body movements								
Verbal	81 (35.53%)	39 (17.11%)	14 (6.14%)	256 (30.62%)	114 (13.64%)	93 (11.12%)	1.67	0.096
Non-verbal	34 (14.91%)	40 (17.54%)	20 (8.77%)	201 (24.04%)	92 (11.00%)	81 (9.69%)	2.22	0.026
Repetitive use of objects/interest in parts of objects								
Verbal	72 (31.58%)	50 (21.93%)	12 (5.26%)	73 (8.73%)	206 (24.64%)	184 (22.01%)	9.22	<0.001
Non-verbal	37 (16.23%)	43 (18.86%)	14 (6.14%)	33 (3.95%)	145 (17.34%)	195 (23.33%)	7.95	<0.001
Unusual sensory interest								
Verbal	58 (25.44%)	67 (29.39%)	9 (3.95%)	213 (25.48%)	254 (30.38%)	26 (3.11%)	0.45	0.881
Non-verbal	26 (14.40%)	48 (21.05%)	20 (8.77%)	114 (13.64%)	219 (26.20%)	40 (4.78%)	1.68	0.093

*$P < 0.05$; all comparisons between boys and girls with ASD (autism spectrum disorder).

Scores for different types of RRBs are ranked data; differences in skewed scores between boys and girls with ASD compared using Mann–Whitney U tests.

Table 5 Comparison of early abnormal development in girls and boys with ASD.

Age (months)	Girls with ASD Mean (SD)	Boys with ASD Mean (SD)	Effect size	*F*	*P*
Age when parents first noticed developmental abnormalities					
Verbal	34.71 (12.80)	29.73 (10.61)	0.45	34.06	<0.001
Non-verbal	27.27 (11.03)	21.94 (6.69)	0.54	51.09	<0.001
Age when developmental abnormalities probably first manifest in interviewer's judgment					
Verbal	32.21 (9.72)	24.50 (7.32)	0.44	114.27	<0.001
Non-verbal	27.45 (8.88)	20.26 (5.85)	0.56	115.56	<0.001
Age of first single words					
Verbal	23.81 (8.11)	21.79 (7.85)	0.25	6.94	0.009
Age of first phrases					
Verbal	31.25 (9.51)	28.98 (8.29)	0.26	8.16	0.004

ASD, autism spectrum disorder; SD, standard deviation.

functioning autism matched for age (range, 5–20 years), and found that females have greater impairment in playing with the peer group and social problems as per the reports of parents based on ADI-R [21]. A recent study by Howe *et al.* revealed that verbal girls with ASD show greater impairment of social communication than males, based on the ADOS [22]. A possible explanation for this could be related to lower cognitive function in girls with ASD. Previous studies have suggested that girls with ASD have lower cognitive ability than boys [23]; Frazier also pointed out that females with a lower IQ have greater communication impairment [13]. The results of the present study suggest that girls with ASD exhibit a clinical phenotype different from that in boys.

To date, very few studies have documented differences in RRBs between girls and boys. In the present study, we found that girls with ASD showed fewer RRBs than boys, using both the ADI-R and ADOS. We also found that girls with ASD exhibited more stereotyped body movements (e.g. repetitive circling and jumping up and down) and hand and finger mannerisms (mechanical play with the hand) than boys, while boys exhibited more unusual preoccupations (e.g. with metal objects, lights, and traffic signs), verbal rituals (e.g. questioning knowingly and forcing others to speak), repetitive use of objects, and interest in parts of objects (e.g. playing with wheels and turning the lights on and off). In addition, boys with ASD exhibited more repetitive speech than girls. These results suggest that girls with ASD show different types of RRBs than boys, and that girls more commonly develop special repetitive stereotyped behaviors.

Girls with ASD are more likely to mask atypical interest, and this would not be considered an RRB in girls. For example, parents may report that their daughter likes to play with dolls. However, when probed about exactly how she 'played', it could become apparent that every session involved repeated brushing of hair, with little flexibility or imagination. This condition can be misinterpreted as an imaginative game for girls, rather than as an RRB [24]. Moreover, some special characteristics of RRBs in girls were absent from the diagnostic algorithms. For example, ASD girls often carry the same books when going outside, which may also be considered an RRB, but this is not included among the diagnostic criteria in the ADI-R [25]. In addition, some activities in boys are more likely to be considered RRBs. For example, parents may report that their son likes to play with trains or dinosaurs. While this may be considered a "special interest", on further inquiry it may be a little stronger without affecting other interests [26]. Consequently, clinicians should carefully look for RRBs in ASD children to identify those common to both boys and girls. The notion that girls show fewer RRBs may be a "protective" factor for girls that in turn makes a formal diagnosis of ASD more difficult. Szatmari *et al.* suggested that this "protective" mechanism may have an underlying genetic component, consistent with the gene-threshold model for girls with ASD [27]. This model assumes that the threshold for ASD in females is higher than in males [28]. In other words, females require a greater genetic load to manifest autistic behaviors. As a result, once females are formally diagnosed, their cognitive function and behavioral characteristics tend to be more severe than in males.

Sex Differences in Identification and Diagnosis in Children with ASD

Our results revealed that the age when parents first noticed developmental abnormalities and the age when developmental abnormalities probably first manifested in the interviewer's judgment in girls were later than in boys. Furthermore, the age at which single words/phrases were first spoken was also later in girls than in boys. Collectively, the results suggest that early abnormal development

Table 6 Descriptive statistics for cut-off scores in girls and boys with ASD.

	Girls with ASD Satisfied cut-off scores (*n*)	Boys with ASD Satisfied cut-off scores (*n*)	Effect size	χ^2	*P*
ADI-R social reciprocity (A)					
Verbal	123 (91.79%)	447 (96.54%)	0.10	5.44	0.020
Non-verbal	88 (93.62%)	361 (96.78%)	0.07	2.03	0.154
ADI-R communication (B)					
Verbal	127 (94.78%)	450 (97.19%)	0.06	1.87	0.171
Non-verbal	91 (96.81%)	351 (94.10%)	0.05	1.09	0.297
ADI-R RRBs (C)					
Verbal	100 (74.63%)	416 (89.85%)	0.19	20.53	<0.001
Non-verbal	69 (73.40%)	316 (84.72%)	0.12	6.64	0.010
ADI-R abnormal development before 36 months (D)					
Verbal	130 (97.01%)	458 (98.92%)	0.06	2.54	0.111
Non-verbal	93 (98.93%)	370 (99.20%)	0.01	0.06	0.807
ADOS communication (B)					
Verbal	128 (95.52%)	426 (92.01%)	0.06	1.92	0.166
Non-verbal	93 (98.94%)	370 (99.20%)	0.01	0.06	0.807
ADOS social reciprocity (A)					
Verbal	131 (97.76%)	452 (97.62%)	0.00	0.01	0.926
Non-verbal	94 (100.00%)	366 (98.12%)	0.06	1.79	0.181
ADOS communication + social reciprocity (A + B)					
Verbal	130 (97.01%)	432 (93.30%)	0.07	2.59	0.107
Non-verbal	94 (100.00%)	364 (97.59%)	0.07	2.31	0.128

and behavioral characteristics for girls are not as easy to identify and are liable to be missed by both parents and evaluators. This may lead to delayed diagnosis of ASD in girls. Shattuck *et al.* reported that the age at which the diagnosis of ASD is made in girls is significantly later than in boys (average 6.1 *vs.* 5.6 years) [29]. Previous studies have reported no obvious sex differences in core symptoms after controlling for age and IQ. However, girls with ASD tend to show more emotional problems, attention deficit, and thought problems [14]. This suggests that girls are diagnosed only when they exhibit more behavioral problems. One possible explanation for this difference is that boys are comparatively more likely to exhibit hyperactivity and repetitive use of objects, and exhibit interest in parts of objects to trigger detection and identification by parents or clinicians. In contrast, the characteristic behaviors in ASD girls are not always as overt and thus are liable to be missed. Clinical symptoms in high-functioning autistic girls (e.g. those exhibiting fewer RRBs) are particularly prone to be missed or misdiagnosed.

We also revealed that the proportion of both verbal and non-verbal girls who satisfied the cut-off scores in the RRBs domain was lower than in boys when assessed using the ADI-R. The ADI-R may be less sensitive for diagnosing ASD in girls, particularly in the RRBs domain. Girls with ASD may be under-identified due to RRBs not satisfying the cut-off scores for diagnosis. Wilson *et al.* noted that sex affects the diagnosis and evaluation of ASD, suggesting that females and males demonstrate distinct clinical phenotypes [26]. As such, sex differences need to be incorporated into the current diagnostic tools. This viewpoint has been articulated by several clinicians. There is therefore a call for tailoring the current diagnostic and assessment tools to address sex differences, in order to improve the diagnostic rate of ASD in girls.

Conclusions

Our findings suggest that girls with ASD show greater socio-emotional reciprocity, and non-verbal girls suffer more communication impairment than boys. Girls tend to show fewer RRBs than boys, and the types of RRBs for girls may be different from those for boys. Early abnormal development and behavioral characteristics in girls are not easy to recognize. In addition, the ADI-R is less sensitive for girls, particularly assessment in the RRBs domain. Clarifying sex differences in diagnosis and clinical phenotype will assist in answering the question of why fewer girls are diagnosed with ASD than boys, and may provide clinical guidance for early screening, diagnosis, and intervention.

Acknowledgements This work was supported by the National Natural Science Foundation of China (81471017) and a Scientific Project of the Ministry of Health of China (201302002).

References

1. Lai MC, Lombardo MV, Baron-Cohen S. Autism. Lancet 2014, 383: 896–910.
2. Elsabbagh M, Divan G, Koh YJ, Kim YS, Kauchali S, Marcín C, *et al.* Global prevalence of autism and other pervasive developmental disorders. Autism Res 2012, 5: 160–179.
3. Kim YS, Leventhal BL, Koh YJ, Fombonne E, Laska E, Lim EC, *et al.* Prevalence of autism spectrum disorders in a total population sample. Am J Psychiatry 2011, 168: 904–912.
4. Blumberg SJ, Bramlett MD, Kogan MD, Schieve LA, Jones JR, Lu MC. Changes in prevalence of parent-reported autism spectrum disorder in school-aged U.S. children: 2007 to 2011–2012. Natl Health Stat Report 2013, 20: 1–11.
5. Fombonne E. Epidemiology of autistic disorder and other pervasive developmental disorders. J Clin Psychiatry 2005, 66 Suppl 10: 3–8.
6. Beacher FD, Minati L, Baron-Cohen S, Lombardo MV, Lai MC, Gray MA, *et al.* Autism attenuates sex differences in brain structure: a combined voxel-based morphometry and diffusion tensor imaging study. AJNR Am J Neuroradiol 2012, 33: 83–89.
7. Lai MC, Lombardo MV, Suckling J, Ruigrok AN, Chakrabarti B, Ecker C, *et al.* Biological sex affects the neurobiology of autism. Brain 2013, 136: 2799–2815.
8. Lai MC, Lombardo MV, Ruigrok AN, Chakrabarti B, Wheelwright SJ, Auyeung B, *et al.* Cognition in males and females with autism: similarities and differences. PLoS One 2012, 7: e47198.
9. Bölte S, Duketis E, Poustka F, Holtmann M. Sex differences in cognitive domains and their clinical correlates in higher-functioning autism spectrum disorders. Autism 2011, 15: 497–511.
10. Hattier MA, Matson JL, Tureck K, Horovitz M. The effects of gender and age on repetitive and/or restricted behaviors and interests in adults with autism spectrum disorders and intellectual disability. Res Dev Disabil 2011, 32: 2346–2351.
11. Banach R, Thompson A, Szatmari P, Goldberg J, Tuff L, Zwaigenbaum L, *et al.* Brief report: relationship between nonverbal IQ and gender in autism. J Autism Dev Disord 2009, 39: 188–193.
12. Carter AS, Black DO, Tewani S, Connolly CE, Kadlec MB, Tager-Flusberg H. Sex differences in toddlers with autism spectrum disorders. J Autism Dev Disord 2007, 37: 86–97.
13. Frazier TW, Georgiades S, Bishop SL, Hardan AY. Behavioral and cognitive characteristics of females and males with autism in the simons simplex collection. J Am Acad Child Adolesc Psychiatry 2014, 53: 329–340.
14. Hiller RM, Young RL, Weber N. Sex differences in autism spectrum disorder based on DSM-5 criteria: evidence from clinician and teacher reporting. J Abnorm Child Psychol 2014, 42: 1381–1393.
15. Reinhardt VP, Wetherby AM, Schatschneider C, Lord C. Examination of sex differences in a large sample of young children with autism spectrum disorder and typical development. J Autism Dev Disord 2015, 45:697–706.
16. Goin-Kochel RP, Abbacchi A, Constantino JN. Lack of evidence for increase genetic loading for autism among families of affected females: a replication from family history data in two large samples. Autism, 2007; 11: 279–286.
17. Koenig K, Tsatsanis KD. Pervasive developmental disorders in girls. In Bell DJ, Foster SL, Mash EJ (Eds.), Handbook of behavioral and emotional problems in girls. New York, NY, US: Kluwer Academic/Plenum Publishers, 2005, 211–237.
18. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, revised (4th ed.). Washington, DC: American Psychiatric Association, 2000.
19. Rutter M, Le Couteur A, Lord C. Autism Diagnostic Interview-Revised Manual. Los Angeles: Western Psychological Services, 2003.
20. Lord C, Rutter M, DiLavore PC, Risi S. Autism Diagnostic Observation Schedule: ADOS manual. Los Angeles, CA: Western Psychological Services, 2002.
21. Holtmann M, Boelte S, Poustka F. Autism spectrum disorders: sex differences in autistic behaviour domains and coexisting psychopathology. Dev Med Child Neurol 2007, 49: 361–366.
22. Howe YJ, O'Rourke JA, Yatchmink Y, Viscidi EW, Jones RN, Morrow EM. Female autism phenotypes investigated at different levels of language and developmental abilities. J Autism Dev Disord 2015, 45: 3537–3549.
23. Bhasin TK, Schendel D. Sociodemographic risk factors for autism in a US metropolitan area. J Autism Dev Disord 2007, 37: 667–677.
24. Lai MC, Lombardo MV, Auyeung B, Chakrabarti B, Baron-Cohen S. Sex/gender differences and autism: setting the scene for future research. J Am Acad Child Adolesc Psychiatry 2015, 54: 11–24.
25. Halladay AK, Bishop S, Constantino JN, Daniels AM, Koenig K, Palmer K, *et al*. Sex and gender differences in autism spectrum disorder: summarizing evidence gaps and identifying emerging areas of priority. Mol Autism 2015, 13: 6–36.
26. Wilson CE, Murphy CM, McAlonan G, Robertson DM, Spain D, Hayward H, *et al*. Does sex influence the diagnostic evaluation of autism spectrum disorder in adults? Autism 2016, 20: 808–819.
27. Szatmari P, Liu XQ, Goldberg J, Zwaigenbaum L, Paterson AD, Woodbury-Smith M, *et al*. Sex differences in repetitive stereotyped behaviors in autism: implications for genetic liability. Am J Med Genet B Neuropsychiatr Genet 2012, 159B: 5–12.
28. Tsai L, Stewart MA, August G. Implication of sex differences in the familial transmission of infantile autism. J Autism Dev Disord 1981, 11: 165–173.
29. Shattuck PT, Durkin M, Maenner M, Newschaffer C, Mandell DS, Wiggins L, *et al*. Timing of identification among children with an autism spectrum disorder: findings from a population-based surveillance study. J Am Acad Child Adolesc psychiatry 2009, 48: 474–483.

Neurosci. Bull. April, 2017, 33(2):161–167
DOI 10.1007/s12264-017-0105-6

www.neurosci.cn
www.springer.com/12264

ORIGINAL ARTICLE

Chinese Norms for the Autism Spectrum Rating Scale

Hao Zhou[1,11] · Lili Zhang[1] · Xiaobing Zou[2] · Xuerong Luo[3] · Kun Xia[4] · Lijie Wu[5] · Yimin Wang[1] · Xiu Xu[6] · Xiaoling Ge[7] · Yong-Hui Jiang[8] · Eric Fombonne[9] · Weili Yan[10] · Yi Wang[1]

Received: 26 October 2016 / Accepted: 22 January 2017 / Published online: 24 February 2017

Abstract This study aimed to establish norms for the modified Chinese version of the Autism Spectrum Rating Scale (ASRS). Participants were recruited from Shanghai, Harbin, Guangzhou, and Changsha, China, and their parents and teachers were invited to complete the Chinese Parent version and the Teacher version of the ASRS. In both versions, boys had significantly higher sub-scale scores and total score (T-score) by 1–3 and 4–5 points respectively, than girls (both $P < 0.001$). Age had weak correlations with some sub-scores and the T-score (r ranged from -0.1859 to 0.0738), and some reached significance ($P < 0.03$). The correlations appeared stronger and were more common in females. The T-score based on Chinese norms ideally correlated with the score based on the United States norms in boys and girls for both versions. Norms for the Chinese version of the ASRS for children aged 6–12 years are proposed and may be helpful for screening individuals with autism spectrum disorders from the general population of children.

Keywords Autism spectrum disorders · Autism spectrum rating scale · Norm · Children

Introduction

Autism spectrum disorders (ASDs) are a set of heterogeneous neurodevelopmental conditions characterized by early-onset developmental impairments in social communication and unusually restricted, repetitive behaviors and interests [1]. Epidemiological studies have identified various risk factors, but none has been shown to be necessary or sufficient for the development of autism [2]. Understanding of the gene-environment interplay in autism is still at an early stage and needs further research. Meta-analysis [3] has shown that individuals with autism have a mortality

Hao Zhou and Lili Zhang have contributed equally to this work.

✉ Weili Yan
yanwl@fudan.edu.cn

✉ Yi Wang
yiwang@shmu.edu.cn

1 Division of Neurology, Children's Hospital of Fudan University, Shanghai 201102, China

2 Child Development Center, The Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510000, China

3 Department of Psychiatry, The Second Xiangya Hospital of Central South University, Changsha 410008, China

4 State Key Laboratory of Medical Genetics, Changsha 400078, China

5 School of Public Health, Harbin Medical University, Harbin 150081, China

6 Department of Child Healthcare, Children's Hospital of Fudan University, Shanghai 201102, China

7 Children's Hospital of Fudan University, Shanghai 201102, China

8 Division of Medical Genetics, Department of Pediatrics and Neurobiology, Duke University School of Medicine, Durham, NC 27710, USA

9 Oregon Health and Science University, Portland, OR 97239, USA

10 Department of Clinical Epidemiology, Children's Hospital of Fudan University, Shanghai 201102, China

11 Pediatric Department, Guizhou Provincial People's Hospital, Guiyang 550002, China

risk 2.8-times higher than that of unaffected people of the same age and gender. Higher childhood intelligence, communicative phrase speech before age 6, and fewer childhood social impairments predict a better outcome. Yet, even for individuals without intellectual disabilities, the outcome of social communication in adulthood is often unsatisfactory in terms of quality of life and achievement of occupational potential [4].

The global prevalence of autism has increased 20- to 30-fold since the earliest epidemiologic studies were conducted in the late 1960s and early 1970s, and now the reported worldwide population prevalence is ~1% [5], which is a major concern for those who care for affected children and their families. The Centers for Disease Control and Prevention (USA) set up the Autism and Developmental Disabilities Monitoring network to periodically monitor ASD prevalence [6]. In China, the official prevalence of ASD is not yet known. Estimates can be obtained from large-scale surveys, but care should be taken when selecting screening instruments based on various characteristics of the target sample and on the purpose of a study.

Several scales exist to screen for autistic traits in the general population, including the Social Communication Questionnaire (developed in 1999) [7], the Autism Spectrum Screening Questionnaire (developed in 1999) [8], the Autism Spectrum Quotient (developed in 2001) [9], the Childhood Autism Screening Test (developed in 2002) [10], and the Social Responsiveness Scale (developed in 2003) [11]. The Autism Spectrum Rating Scale (ASRS) is a newer scale developed in 2009 to identify youths who are most likely to need additional evaluation or services for ASD and related issues [12]. In this study, we constructed ASRS norms based on several advantages of the scale as a screening tool for ASD. First, the ASRS is not just a screening tool; it is also helpful in guiding diagnostic decisions and can be used for treatment planning, ongoing monitoring of the response to intervention, and program evaluation. In addition, the ASRS was designed for both young children aged 2–5 years and youths aged 6–18 years, from a diverse group of individuals.

Finally, comparisons with other instruments are easy due to the availability of standard scores. As the prevalence of ASD and the risk of over- and under-diagnosis are increasing in China, a valid, reliable, and carefully-crafted tool for screening and treatment assessment is needed. The norms of a Chinese version of the ASRS are expected to meet this need [12]. Previous studies have also shown that the cultural setting can impinge on the performance of scales [13, 14]. Therefore, the aim of this study was to propose Chinese norms, before its application for national screening in the general Chinese population.

Methods

Study Population

This study was conducted from January to July, 2014. Community-based participants (aged 6–12 years) were selected as the general sample to ensure its representativeness by using convenient cluster sampling. Four community-based samples were selected from Shanghai, Harbin, Guangzhou, and Changsha. From each site, one administrative street containing >400 children aged 6–12 years was chosen; all children with residency were recruited and comprised the reference sample.

The protocol was approved by the Research Ethics Committee of the Children's Hospital of Fudan University. Both the parental and teachers' consent was in written form.

Measurements

Description of the Instrument

The ASRS contains screening, DSM-IV-TR, and treatment scales, with a total of 71 items. The screening scale comprises 60 items of the total 71 including Social/Communication (SC), Unusual Behaviors (UB), and Self-Regulation (SR). The DSM-IV-TR scale contains 34 items of the total 71 and a higher score indicates a higher chance of a diagnosis of autism by a psychiatrist. The treatment scale has 69 items of the total 71 and includes 8 scales.

Each scale yields a raw score by summing the relevant items. This raw score is subsequently transformed into a standardized score with a mean of 50 and a standard deviation (SD) of 10. The T-score incorporates the information from the 3 screening scales; the 3 standardized scale scores are first summed, and then transformed into a single score with a mean of 50 and a SD of 10. In that way, each of the screening scales contributes an equal weight to the overall summary T-score. Further details of the scoring procedures and interpretation of scores can be found in the ASRS manual [12].

Development of the Chinese Version of the ASRS

A pilot study was first conducted to establish the reliability and validity of the Chinese ASRS, and they were found to be excellent. As in the original research in the USA that developed the ASRS, we conducted an exploratory factor analysis to confirm the factor structure of the ASRS in a Chinese sample; this can be found in a companion paper entitled "Modifying the Autism Spectrum Rating Scale (6–18 years) to a Chinese Context: An Exploratory

Analysis" in this issue [15]. Based on the same selection criteria of factor loading, >0.30, our analysis retained 59 items (as compared to 60 in the US study) loading on a comparable 3-factor structure. The content of the 3 factors was similar to that of the original US study, and therefore the factor names were retained. The only difference was that the numbers of items for each factor were different in the China validation sample, with SC, UB, and SR now having 21, 14 and 24 items. The DSM-IV-TR scale was based on expert judgment as to which items in the ASRS closely map each of the diagnostic criteria for PDD. Therefore, the DSM-IV-TR scale was used as recommended in the original US manual.

Procedure

With the approval of Multi-Health Systems, we prepared a Chinese version of the ASRS by the usual translation-back-translation approach, and the pilot study allowed us to confirm the linguistic appropriateness [16]. Researchers were trained before the scales were distributed. Most parents were asked to complete the questionnaire at home, and they were subsequently collected by researchers. Other questionnaires completed by parents were collected by the teachers in a sealed envelope. Teacher ratings were collected directly from the school. Parents and teachers completed the scales at the same time.

Basic personal information about the child's date of birth, gender, and school was requested. The child's age was calculated as the difference between the date of questionnaire completion or return and the birth date. Rating scores were excluded if the child was older or younger than the target age-range. All scores were entered online using a database created from the original scoring method. Quality control of the data was performed before further analysis.

Quality Control

A detailed schedule for data collection was developed and implemented in the four sites. All research staff was trained in the administration and scoring of the questionnaires. To facilitate data entry and checks, we established an online multi-center database that was accessible to the teams at each center to promptly upload and check data. All rating scores were scrutinized for errors or missing information. Before data analysis, a few parental ASRS questionnaires were excluded for reasons including errors on the birth date and an older or younger than the target age range. Analyses were subsequently performed with or without the excluded questionnaires.

Statistical Analysis

Data analyses were performed using Stata 11 software (version 11.0, College Station, TX). Conventional descriptive analyses were used to present the site and gender distribution of the study sample, and the differences in raw score distributions of the three factors SC, SR, and UB. Student's *t* test was used to test for gender differences. Analyses of variance (ANOVA) were used to examine differences among sites. Multiple linear regression analyses were used to assess the effects of gender, age and site on ASRS scores. Participants aged 6–12 years were treated as one age group. All ASRS subscale scores and T-scores were normalized to a normal distribution with a mean of 50 and standard deviation of 10. The agreement of the T-score normal distribution for the Chinese population with that for US norms was tested by Pearson correlation analysis. All *P* values were two-sided and *P* values <0.05 were deemed statistically significant.

Results

In this study, 2053 children were eligible for inclusion in the general sample. After exclusion of questionnaires due to various errors, 1625 parental questionnaires were available for the normative sample (830 boys and 795 girls; mean age, 8.85 ± 1.78 years). In addition, after exclusion of questionnaires with various errors, 1514 teacher questionnaires were finally available (772 boys and 742 girls; mean age, 8.96 ± 1.75 years). All teachers or caregivers had known the students for at least 1 month.

Demographic characteristics of the sample are shown in Tables 1 and 2. The participants' age and gender did not differ significantly between the parent and teacher groups.

In the parent and teacher versions of the ASRS, boys had significantly higher raw scores in SC, UB, SR, T-score, and DSM-TR by 1–3 for parents rating, and 4–5 points for teachers than girls ($P < 0.001$). Age showed a weak correlation with some sub-scores and T-score (r ranged from −0.1859 to 0.0738), and some were significant ($P < 0.03$; Table 3). The correlations were stronger and more common in females. ANOVA revealed slight site differences in the raw scores of subscales (Table 4).

The T-score of the reference sample showed a significant correlation with T-scores that were computed based on American ASRS norms for the parent version ($r = 0.9674$ for boys and 0.9664 for girls, $P < 0.001$; Fig. 1A, B). For the teacher version, the correlation coefficient values were 0.9715 and 0.9683, respectively (Fig. 2A, B).

Table 1 Age and gender distribution of the reference sample.

Age	Parent rating			Teacher rating		
	Male *n* (%)	Female *n* (%)	Total	Male *n* (%)	Female *n* (%)	Total
6	84 (53.16)	74 (46.84)	158	56 (52.34)	51 (47.66)	107
7	164 (52.23)	150 (47.77)	314	160 (55.94)	126 (44.06)	286
8	128 (51.61)	120 (48.39)	248	117 (49.16)	121 (50.84)	238
9	155 (52.36)	141 (47.64)	296	146 (50.34)	144 (49.66)	290
10	109 (45.80)	129 (54.20)	238	111 (48.47)	118 (51.53)	229
11	125 (50.20)	124 (49.80)	249	111 (47.03)	125 (52.97)	236
12	65 (53.28)	57 (46.72)	122	71 (55.47)	57 (44.53)	128
Total	830 (51.08)	795 (48.92)	1625	772 (50.99)	742 (49.01)	1514
χ^2	4.0905			6.3404		
P	0.664			0.386		

Table 2 Gender distribution of the reference sample by study site.

City	Parent rating		Teacher rating	
	Male *n* (%)	Female *n* (%)	Male *n* (%)	Female *n* (%)
Shanghai	216 (49.32)	222 (50.68)	203 (46.77)	231 (53.23)
Guangzhou	228 (52.29)	208 (47.71)	227 (52.06)	209 (47.94)
Changsha	182 (51.12)	174 (48.88)	187 (52.53)	169 (47.47)
Harbin	204 (51.65)	191 (48.35)	155 (53.82)	133 (46.18)
Total	830 (51.08)	795 (48.92)	772 (50.99)	742 (49.01)
χ^2	0.5963		4.5476	
P	0.897		0.208	

Table 3 Pearson correlation analyses of ASRS scale scores with age by gender.

Sub-scales	Male		Female	
	r	*P*	r	*P*
Parent rating				
SC_R	0.0738	0.0342	0.0305	0.3926
UB_R	−0.0250	0.4722	−0.1075	0.0025
SR_R	−0.0967	0.0054	−0.1859	<0.0001
T-score	−0.0113	0.7468	−0.0987	0.0057
DSM_R	0.0114	0.7429	−0.0543	0.1269
Teacher rating				
SC_R	−0.0638	0.0779	−0.1162	0.0016
UB_R	−0.0093	0.7960	−0.0524	0.1536
SR_R	−0.0882	0.0149	−0.1341	0.0002
T-score	−0.0607	0.0954	−0.1303	0.0004
DSM_R	−0.0724	0.0449	−0.1545	<0.0001

DSM diagnostic and statistical manual of mental disorders, *r* correlation coefficient, *R* raw score, *SR* self-regulation, *SC* social/communication, *T-score* standardized total score, *UB* unusual behaviours.

Discussion

In this study, we obtained norms for the ASRS sub-scales and T-score from a culturally and linguistically representative community-based sample of Chinese 6–12 year-old children, which could be used to determine which young people are most likely to require additional evaluation or services for ASD and related issues.

We found that gender had a significant effect on SC, UB, SR, T-score, and DSM-IV-TR as both parents and teachers rated males higher than females. This supports previous evidence that boys and girls have social and communication developmental trajectories with different profiles, boys typically displaying higher levels of difficulty in social and communicational skills. These gender differences are consistent with research findings that ASDs occur far more frequently in males than in females with a prevalence ratio of 4.5:1 [5, 6, 17, 18]. Many researchers have focused on mechanisms that explain the contribution of gender differences to the risk of ASDs [19, 20] such as the Extreme Male Brain theory [21–24]. However, further research is needed to fully understand the origins of this robust difference.

The results of the current study show minor age effects in the ASRS scores for both parental and teacher ratings, which is consistent with the findings in the ASRS norm study [12], indicating that developmental trends in the scores are very small. Despite the fact that initial signs and symptoms typically emerge in the early developmental period, consistently before age 3, some social deficits and behavioral patterns might not be recognized as symptoms

Table 4 Site and gender differences in raw sub-scale scores and T-score.

Sub-scales	City				Gender	
	Shanghai $n = 438$	Guangzhou $n = 436$	Changsha $n = 356$	Harbin $n = 395$	Male $n = 830$	Female $n = 791$
Parent rating						
SC_R	27.53 ± 12.71	36.98 ± 13.28	32.18 ± 13.07	26.04 ± 14.07	31.80 ± 14.09	29.61 ± 13.76
UB_R	29.32 ± 10.39	31.78 ± 11.02	32.70 ± 9.44	26.68 ± 10.49	30.74 ± 10.58	29.38 ± 10.65
SR_R	20.43 ± 8.84	24.21 ± 9.19	22.69 ± 7.93	18.34 ± 8.44	22.92 ± 9.02	19.86 ± 8.56
T-score	48.26 ± 9.98	53.75 ± 9.61	52.16 ± 8.49	45.88 ± 9.73	51.29 ± 10.00	48.65 ± 9.83
DSM_R	39.78 ± 12.98	46.26 ± 12.30	44.21 ± 11.68	37.28 ± 13.10	43.29 ± 12.99	40.40 ± 12.94
Teacher rating						
SC_R	31.61 ± 17.15	40.01 ± 16.05	33.55 ± 17.56	27.04 ± 16.76	36.37 ± 17.51	30.82 ± 16.98
UB_R	27.00 ± 11.73	27.22 ± 12.19	31.18 ± 11.38	27.26 ± 12.04	29.54 ± 12.12	26.59 ± 11.60
SR_R	20.42 ± 11.21	23.23 ± 10.74	21.81 ± 10.22	19.13 ± 10.71	24.02 ± 10.69	18.54 ± 10.31
T-score	48.91 ± 10.23	52.05 ± 9.90	51.15 ± 9.44	47.03 ± 9.58	52.06 ± 9.93	47.89 ± 9.63
DSM_R	40.15 ± 15.84	45.44 ± 15.88	43.39 ± 15.27	37.20 ± 15.74	44.80 ± 16.13	38.88 ± 15.25

R raw score, *SR* self-regulation, *SC* social/communication, *T-score* standardized total score, *UB* unusual behaviours.

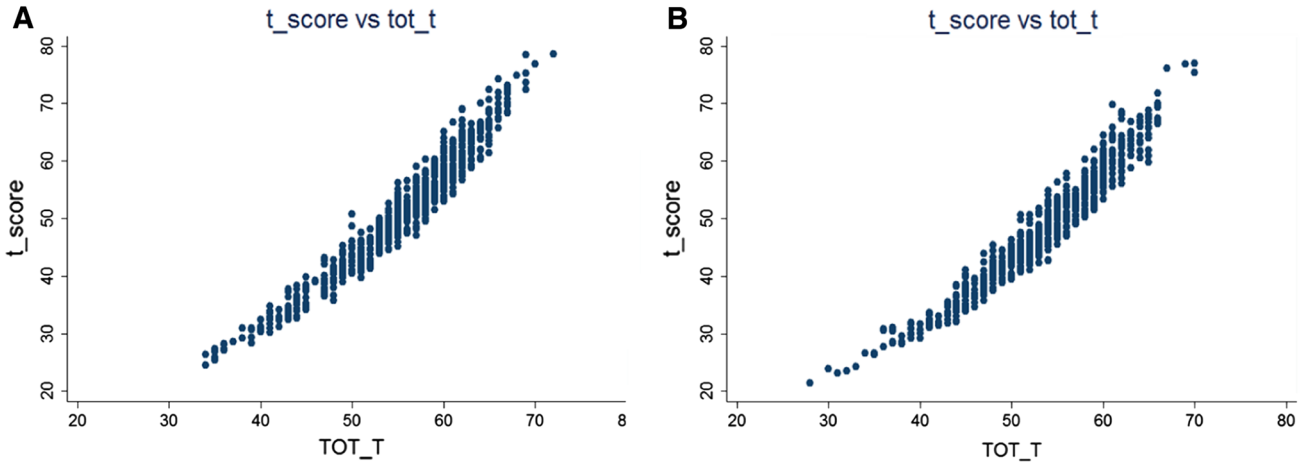


Fig. 1 Correlations between the T-score based on the Chinese norm and that based on the US norm (parent ratings) for boys (**A**), and girls (**B**) for the parent version. t_score, T-score calculated by Chinese norm; tot_t, T-score calculated based on the US norm. $r = 0.9674$, $P < 0.001$ for boys and $r = 0.9664$, $P < 0.001$ for girls.

of ASD until a child is unable to meet social, educational, occupational, or other important life-stage demands [25]. This finding supports the proposal that the norm is to cover Chinese children aged 6–12 years.

Although the representativeness of the reference samples for developing norms was ensured by including 4 cities in China, Shanghai, Guangzhou, Changsha, and Harbin, cultural and economic differences may exist. Uniform protocols were applied for data collection. The results showed balanced age and gender distributions of the 4 sub-samples; however, mean raw subscale scores and standard deviations showed slight differences among the 4 cities. This may reflect sample differences across the 4 sites. As we did not have individual data on respondents with regard to profession, education, or other variables that may influence scores, we were unable to further investigate the source of these differences. However, the 4 sites were selected in regions that differ slightly with respect to cultural background and level of economic development. It is likely that these differences reflect true variability in the population that was appropriately reflected in our normative sample. After statistical normalization, combination of the 4 sub-samples helped to enhance the representativeness of the reference study sample.

Based on exploratory factor analysis, we made slight changes to items and structure of the scales (refer to the companion paper entitled "Modifying the Autism Spectrum Rating Scale (6–18 years) to a Chinese Context: An Exploratory Analysis" [15]). The present data provide encouraging evidence in support of use of the ASRS, given

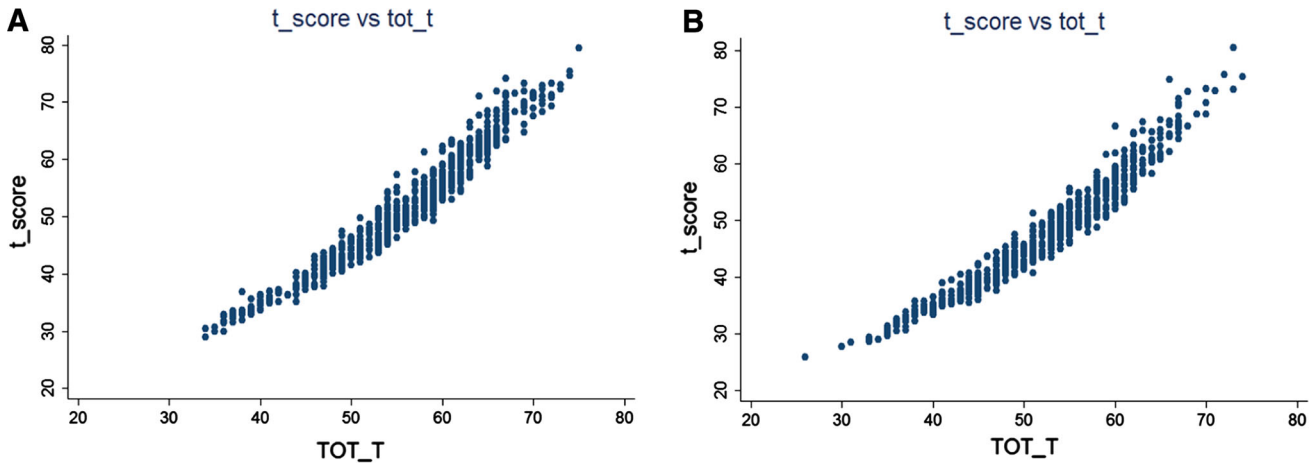


Fig. 2 Correlations between the T-score based on the Chinese norm and that based on US norm (teacher ratings) for boys (**A**) and girls (**B**) for the teacher version. t_score, T-score calculated by Chinese norm; tot_t, T-score calculated based on the US norm. $r = 0.9715$, $P < 0.001$ for boys and $r = 0.9683$, $P < 0.001$ for girls.

an excellent positive correlation with the US norm data. This shows that the slightly-modified ASRS is suitable for screening ASD in the Chinese cultural environment.

One limitation of this study is that we only selected urban populations as the reference sample in this study, and it was relatively limited. Therefore, it is necessary to include rural populations in further studies.

In conclusion, we have established the Chinese norm referenced criteria for ASRS, adopting the theoretical approach used for other languages and settings. The excellent correlation between our normative data and those in the USA demonstrated the high quality of this scale. The normative data will be useful in the screening and clinical evaluation of school-aged children in China.

Acknowledgements This work was supported by the National Health and Family Planning Commission of China (201302002; ClinicalTrials.gov Number NCT 02200679), the Shanghai International Cooperation Ministry of Science Projects, China (14430712200), and the Development Project of Shanghai Peak Discipline-Integrated Chinese and Western Medicine. We thank all the parents who participated in this study.

References

1. Grzadzinski R, Huerta M, Lord C. DSM-5 and autism spectrum disorders (ASDs): an opportunity for identifying ASD subtypes. Mol Autism 2013, 4: 12.
2. Diaz-Anzaldua A, Diaz-Martinez A. Genetic, environmental, and epigenetic contribution to the susceptibility to autism spectrum disorders. Rev Neurol 2013, 57: 556–568.
3. Woolfenden S, Sarkozy V, Ridley G, Coory M, Williams K. A systematic review of two outcomes in autism spectrum disorder—epilepsy and mortality. Dev Med Child Neurol 2012, 54: 306–312.
4. Billstedt E, Gillberg IC, Gillberg C. Autism after adolescence: population-based 13- to 22-year follow-up study of 120 individuals with autism diagnosed in childhood. J Autism Dev Disord 2005, 35: 351–360.
5. Kim YS, Leventhal BL, Koh YJ, Fombonne E, Laska E, Lim EC, *et al.* Prevalence of autism spectrum disorders in a total population sample. Am J Psychiatry 2011, 168: 904–912.
6. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill Summ 2014, 63: 1–21.
7. Berument SK, Rutter M, Lord C, Pickles A, Bailey A. Autism screening questionnaire: diagnostic validity. Br J Psychiatry 1999, 175: 444–451.
8. Ehlers S, Gillberg C, Wing L. A screening questionnaire for Asperger syndrome and other high-functioning autism spectrum disorders in school age children. J Autism Dev Disord 1999, 29: 129–141.
9. Baron-Cohen S, Wheelwright S, Skinner R, Martin J, Clubley E. The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. J Autism Dev Disord 2001, 31: 5–17.
10. Scott FJ, Baron-Cohen S, Bolton P, Brayne C. The CAST (Childhood Asperger Syndrome Test): preliminary development of a UK screen for mainstream primary-school-age children. Autism 2002, 6: 9–31.
11. Constantino JN, Davis SA, Todd RD, Schindler MK, Gross MM, Brophy SL, *et al.* Validation of a brief quantitative measure of autistic traits: comparison of the social responsiveness scale with the autism diagnostic interview-revised. J Autism Dev Disord 2003, 33: 427–433.
12. Goldstein S, Naglieri JA. Autism Spectrum Rating Scales (ASRS?). Tonawanda, NY: Multi-Health Systems, 2009.
13. Vandborg PK, Hansen BM, Greisen G, Mathiasen R, Kasper F, Ebbesen F. Follow-up of extreme neonatal hyperbilirubinaemia in 5- to 10-year-old children: a Danish population-based study. Dev Med Child Neurol 2015, 57: 378–384.
14. Fombonne E, Marcin C, Bruno R, Tinoco CM, Marquez CD. Screening for autism in Mexico. Autism Res 2012, 5: 180–189.

15. Zhou H, Zhang L, Luo X, Wu L, Zou X, Xia K, *et al.* Modifying the Autism Spectrum Rating Scale (6–18 years) to a Chinese context: an exploratory factory analysis. Neurosci Bull 2017, 33. doi:10.1007/s12264-017-0104-7.
16. Zhou H, Zhang L, Wu L, Zou X, Luo X, Xia K, *et al.* Validity and reliability analysis of the Chinese parent version of the Autism Spectrum Rating Scale (6–18 years). Psychiatry Res 2015, 230: 255–261.
17. Blenner S, Augustyn M. Is the prevalence of autism increasing in the United States? BMJ 2014, 348: g3088.
18. Taylor B, Jick H, Maclaughlin D. Prevalence and incidence rates of autism in the UK: time trend from 2004–2010 in children aged 8 years. BMJ Open 2013, 3: e003219.
19. Begeer S, Mandell D, Wijnker-Holmes B, Venderbosch S, Rem D, Stekelenburg F, *et al.* Sex differences in the timing of identification among children and adults with autism spectrum disorders. J Autism Dev Disord 2013, 43: 1151–1156.
20. Hall J, Philip RC, Marwick K, Whalley HC, Romaniuk L, McIntosh AM, *et al.* Social cognition, the male brain and the autism spectrum. PLoS One 2012, 7: e49033.
21. Baron-Cohen S. Empathizing, systemizing, and the extreme male brain theory of autism. Prog Brain Res 2010, 186: 167–175.
22. Wen W, Wen SW. Expanding upon the 'extreme male brain' theory of autism as a common link between other major risk factors: a hypothesis. Med Hypotheses 2014, 82: 615–618.
23. Bejerot S, Eriksson JM, Bonde S, Carlstrom K, Humble MB, Eriksson E. The extreme male brain revisited: gender coherence in adults with autism spectrum disorder. Br J Psychiatry 2012, 201: 116–123.
24. Krahn TM, Fenton A. The extreme male brain theory of autism and the potential adverse effects for boys and girls with autism. J Bioeth Inq 2012, 9: 93–103.
25. Zwaigenbaum L, Bryson S, Lord C, Rogers S, Carter A, Carver L, *et al.* Clinical assessment and management of toddlers with suspected autism spectrum disorder: insights from studies of high-risk infants. Pediatrics 2009, 123: 1383–1391.

Neurosci. Bull. April, 2017, 33(2):168–174
DOI 10.1007/s12264-017-0114-5

www.neurosci.cn
www.springer.com/12264

ORIGINAL ARTICLE

Assessing the Accuracy of the Modified Chinese Autism Spectrum Rating Scale and Social Responsiveness Scale for Screening Autism Spectrum Disorder in Chinese Children

Bingrui Zhou[1] · Hao Zhou[2] · Lijie Wu[3] · Xiaobing Zou[4] · Xuerong Luo[5] · Eric Fombonne[6] · Yi Wang[2] · Weili Yan[7] · Xiu Xu[1]

Received: 13 December 2016 / Accepted: 9 February 2017 / Published online: 3 March 2017

Abstract The reported prevalence of autism spectrum disorder (ASD) has been increasing rapidly in many parts of the world. However, data on its prevalence in China are largely missing. Here, we assessed the suitability of the modified Chinese version of a newly-developed ASD screening tool, the Modified Chinese Autism Spectrum Rating Scales (MC-ASRS) in screening for ASD in Chinese children aged 6–12 years, through comparison with the Social Responsiveness Scale (SRS) that has been widely used for ASD screening. We recruited the parents/caregivers of 1588 typically-developing children and 190 children with ASD aged 6–12 years to complete the MC-ASRS and SRS, and evaluated the validity of both scales in discriminating children with ASD from those developing typically. The results showed that MC-ASRS performed as well as SRS in sensitivity, specificity, and area-under-the-curve (both >0.95) in receiver operating characteristic analysis, with a fair false-negative rate. These results suggest that MC-ASRS is a promising tool for screening for children with ASD in the general Chinese population.

Keywords Autism spectrum disorder · Screening accuracy · ROC analysis · Modified Chinese Autism Spectrum Rating Scale · Social Responsiveness Scale

✉ Weili Yan
yanwl@fudan.edu.cn

✉ Xiu Xu
xuxiu1006@126.com; xuxiu@shmu.edu.cn

1 Department of Child Healthcare, Children's Hospital of Fudan University, Shanghai 201102, China

2 Department of Neurology, Children's Hospital of Fudan University, Shanghai 201102, China

3 School of Public Health, Harbin Medical University, Harbin 150081, China

4 Child Development Center, The Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510630, China

5 Department of Psychiatry, The Second Xiangya Hospital of Central South University, Changsha 410011, China

6 Oregon Health and Science University, Portland, OR 97239, USA

7 Department of Clinical Epidemiology, Children's Hospital of Fudan University, Shanghai 201102, China

Introduction

In the past several decades, autism spectrum disorder (ASD) has become an increasingly important issue of concern worldwide. ASD consists of an array of disorders characterized by impairment in reciprocal social interaction and communication skills, and the presence of repetitive stereotypic behaviors/restricted interests [1, 2], with variability in symptom pattern, severity, associated cognitive and language ability, and prognosis [3]. Many studies have suggested that early identification, diagnosis, and intervention can ameliorate the prognosis of ASD [4–7].

The prevalence of ASD reported in various countries and regions has increased dramatically since 2000. Studies have suggested an estimated prevalence of ASD of ~1% in the general population [8]. In China, most reported epidemiological studies of ASD have been regional, with relatively small samples. Furthermore, the targeted clinical cases were variable, with most studies screening for children with classical autism, and some for those with ASD [9]. In addition, a lack of standard diagnostic instruments to assess the positively-screened individuals made the results less valid. The Autism Diagnostic Observation Schedule

(ADOS) and Autism Diagnostic Interview-Revised (ADI-R) have generally been recommended for case confirmation in ASD epidemiology studies [10]. The shortcomings noted above make it difficult to directly compare the prevalence estimates from existing Chinese studies with those from recent studies in other parts of the world. With the support of a national program, we will conduct a multi-site epidemiological investigation of ASD in Chinese school-aged children (6–12 years old) using standard procedures of screening and diagnosis, making the prevalence comparable to existing results from developed countries. Above all, we needed to identify a screening instrument appropriate for our targeted population.

Currently in China, screening instruments available for 6–12-year-old children are mainly the Autism Behavior Checklist (ABC), Autism Spectrum Screening Questionnaire (ASSQ), and Social Communication Questionnaire (SCQ). There has been almost no research using the ABC in developed countries, making it difficult to make comparisons. Although a previous study showed good sensitivity and specificity of the ASSQ in differentiating children with ASD from healthy controls, as well as children with attention deficit/hyperactivity disorder (ADHD) and childhood-onset schizophrenia [11], the ASSQ was designed to identify children with high-functioning ASD, particularly Asperger syndrome. The positive rate using the ASSQ for level-1 screening may underestimate the prevalence of ASD. The SCQ is more commonly used in level-2 screening to discriminate ASD from other developmental disorders [12]. In recent years, the Social Responsiveness Scale has been introduced in the Taiwan region and shows good reliability and validity in Taiwan children [13].

The Autism Spectrum Rating Scale (ASRS) is a screening tool developed by Goldstein and Naglieri in 2009 [14], designed to measure autism-related behaviors in children and adolescents aged 2–18 years. The ASRS has full-length and short versions, both of which can be completed by parents or teachers. The full-length ASRS (6–18 years) consists of 3 scales: the ASRS scale for screening, the DSM-IV-TR scale for guiding diagnostic decisions, and the treatment scale for monitoring the effectiveness of intervention. The available age-range of the ASRS is large and appropriate for follow-up studies. In previous work, Zhou *et al.* [15, 16] translated the ASRS into Chinese and assayed its suitability for screening children with ASD from the general population. The results showed that the modified Chinese version of the ASRS, the MC-ASRS, shows good reliability and validity [16]. In the current study, we compared the screening accuracy of the MC-ASRS with that of the widely-used SRS in discriminating ASD cases in school-aged children, to further investigate the applicability of the MC-ASRS in first-level screening for ASD in China.

Methods

Study Design and Participants

The study was conducted from January to July, 2014, and enrolled children diagnosed with ASD according to DSM-V from both clinics and local autism rehabilitation centers, and typically-developing healthy children from communities, all aged 6–12 years. The children were recruited from Shanghai, Guangzhou, Harbin, and Changsha, representing four main areas of China, to ensure data quality and representativeness.

The ASD children were recruited from both clinics and local autism rehabilitation centers in the four cities. A clinical diagnosis of ASD was made according to the DSM-V criteria and confirmed by senior developmental pediatricians using the ADOS and ADI-R. Individuals were excluded if they were diagnosed with symptomatic autism (such as Rett syndrome and fragile X syndrome), inherited metabolic diseases, mental retardation caused by secondary brain injury, or psychiatric diseases such as schizophrenia and schizoaffective disorder.

Healthy, typically-developing age-matched children were recruited from communities in the 4 cities using convenient cluster sampling, to represent a healthy group without ASD. Parents of children with visual and/or auditory impairment and nervous system diseases were excluded.

Instruments

MC-ASRS (Full-Length Form, 6–18 Years, Parent Rating)

The original full-length ASRS (6–18 years) uses a five-point Likert scale ranging from 'Never' (0) to 'Very Frequently' (4) according to the frequency of the corresponding behavior, and has good psychometric properties [14]. It includes 71 items consisting of 3 scales, the ASRS scale for ASD screening, the DSM-IV-TR scale, and the treatment scale. In the ASRS scale, three subscales consisting of 60 items are used: Unusual Behaviors (UB, 24 items), Social/Communication (SC, 19 items), and Self-Regulation (SR, 17 items), the scores of which are raw scores. All raw scores are combined into a single composite score, the T-score [17]. A higher T-score indicates more obvious ASD features. The T-scores of ASRS follow a normal distribution with a normative mean of 50 and standard deviation of 10 [15], and the cut-off point is set to 60 (mean + 1 SD) [14].

With the permission of Goldstein and Naglieri and with approval by the Multi-Health System, our colleagues Zhou

et al., in the team of the Research Special Fund for Public Welfare Industry of Health of China, translated the original ASRS into Chinese using a two-way procedure. They then found it to be a reliable and valid tool for screening ASD traits in general Chinese children, but its construct validity was not entirely satisfactory [15]. Therefore, they conducted exploratory factor analyses, after which they retained the original three-factor solution but excluded 12 items because of low factor loading (<0.3) or cross-loading, resulting in the Modified Chinese ASRS (MC-ASRS) that includes 59 items in the ASRS scale. The DSM-IV-TR and treatment scales of the MC-ASRS were retained from the original version. Then, confirmatory factor analyses for the MC-ASRS and the unmodified Chinese ASRS were performed in the same new Chinese sample. The results show that the model-fitting indices of the MC-ASRS are better than those of the unmodified version with the same cut-off T-score of 60, indicating that the MC-ASRS has better construct validity [16, 18].

The Chinese Version of the Social Responsiveness Scale (Chinese SRS) - Parent

The original SRS was developed by Constantino and colleagues in 2002[19]. It consists of 65 items divided into 5 subscales: Social Awareness, Social Cognition, Social Communication, Social Motivation, and Autistic Mannerisms. It was designed to assess the social behavior of children aged 4–18 years. The SRS uses a four-point Likert-type questionnaire reported by the individual himself/herself or the caregiver according to the frequency of each behavior ("0" never to "3" always). A higher score indicates more severe social deficits and autistic behaviors. The raw SRS score can be converted to a T-score, but it is recommended to use the total raw score in research, in order to increase the comparability between studies [20]. Therefore, we used the raw SRS score in the current study. SRS scores are highly correlated with ADI-R scores (r = 0.65–0.77) [19]. The SRS performs well in psychometric properties across different cultures [13, 21, 22], including the Chinese version in the Taiwan region. The recommended cut-off for the raw in the Chinese version of SRS when used for screening for ASD in low-risk populations in China is 60 [23].

Procedures

The research protocol was approved by the Research Ethics Committee of the Children's Hospital of Fudan University ([2012] No. 185) before data collection. Parents of eligible children were invited to participate in the study and received a folder containing an informed consent letter, a general information sheet, the MC-ASRS and SRS scales, and guidance notes. Parents who signed the informed consent completed the two scales on two separate days with an intervening period of no more than two weeks. The order in which the MC-ASRS and SRS were assigned to the parents was done by simple randomization. For comparison with the SRS, only the parent version of the MC-ASRS was included in the analysis.

Data were entered after the questionnaires were retrieved from the sites by two separate groups of trained staff. Clinicians who administered the ADOS and ADI-R assessments were trained and certified.

Statistical Analysis

We retrieved 1596 questionnaires from the general sample and 190 from the ASD sample. In the general sample, 3 individuals were excluded from analysis because both the MC-ASRS and SRS were ≥70, indicating high likelihood of ASD [24]. Indeed, further assessment using the ADOS and ADI-R confirmed that these children have ASD. In addition, 5 individuals in the clinical sample were excluded because they failed to complete both scales. Finally, the data from 1778 participants (1593 from the general population and 185 clinical ASD cases) were analyzed.

Descriptive statistics were computed for the scores on the selected instruments. Unpaired t-tests were used to compare means, and the χ^2 test was used to assess differences in proportions. First, differences between the scores in children with ASD and typically-developing children were investigated using independent sample t-tests when the distribution was robustly normal, or using the Mann-Whitney test when it was skewed. Then, using clinical diagnosis by DSM-V as the reference standard and the general sample as a typical control, we performed receiver-operator-characteristic (ROC) area-under-the-curve (AUC) analyses using the same cut-off score of 60, to assess and compare the screening accuracy of the MC-ASRS and the SRS. Based on these results, we further calculated and compared the sensitivities, specificities, positive and negative predictive values, and positive and negative likelihood ratios for the MC-ASRS and SRS. Sensitivity was calculated as the percentage of children with ASD who tested positive, while specificity was calculated as the percentage of children without ASD who tested negative. The positive predictive value (PPV) was determined as the percentage of all children testing positive who were later diagnosed with ASD, while the negative predictive value (NPV) was the percentage of all children testing negative who did not have ASD. The 95% confidence interval (95% CI) was computed by the Wilson method. Stata SE 11.0 was used to conduct the statistical analyses.

Results

The demographic characteristics of the two samples are shown in Table 1. The mean age of all participants was 8.8 years (SD = 1.8), and those of the clinical ASD cases and general sample showed no significant differences (8.9 ± 1.8 *vs* 8.4 ± 1.9, $P = 0.178$). The sex ratio of the clinical group was 7.26:1 (male:female), while that of the general sample was 1.05:1. The percentages of participants from each site showed no significant differences.

As expected, the clinical sample scored significantly higher than the community sample on both the MC-ASRS and the SRS (both $P < 0.001$, Table 2).

In general, with the same cut-off point of 60, the MC-ASRS and SRS performed similarly in screening for ASD cases in the general sample. The sensitivity of MC-ASRS was a little lower than SRS (MC-ASRS vs SRS, 93% *vs* 96.8%), while the specificity was in the opposite direction (83.2% *vs* 82.2%). The NPVs were both very high (≥99%), suggesting that it was very unlikely that a child scoring <60 would be diagnosed as having ASD, while the PPVs were relatively low, indicating high false-positive rates of both instruments. The positive likelihood ratios were similar, but the negative likelihood ratio of SRS was lower than that of MC-ASRS (Table 3).

The performance of the MC-ASRS and SRS were compared mainly through the AUCs under ROC curves. Both performed well in distinguishing ASD cases from typically-developing children (both AUCs >0.95), SRS being slightly better than MC-ASRS (MC-ASRS 0.9522 *vs* SRS 0.9719, $P = 0.0011$) (Fig. 1).

At all four sites, parents were the main administrators, but the proportion of mothers administering the scales was higher than that of fathers. In considering the possible discrepancy resulting from the father or the mother administering the scales, we separately calculated and compared the AUCs of the ROC curves for the two subsamples. The results showed no significant differences in the AUC of the MC-ASRS between the fathers or the mothers administering the questionnaire, with a higher AUC for the mothers completing the SRS than the fathers (Table 4).

Discussion

Screening Accuracy of the MC-ASRS and SRS

In a previous study, the original ASRS was translated into Chinese [15] and modified based on the results of exploratory and confirmatory factor analysis to achieve a better construct validity for the Chinese population aged 6–12 years [16]. Also, the norm of the MC-ASRS in 6–12-year-old Chinese children was established [25]. Here, we investigated the screening accuracy of the MC-ASRS in a multicenter study, by comparing it with the SRS, a widely-

Table 1 Demographic characteristics of the general sample and ASD cases.

	General sample			ASD cases		
	n	χ^2	*P*	*n*	χ^2	*P*
City (%)	1596			190		
1	415			57		
2	412			41		
3	345			42		
4	424			50		
Sex (% male)	816 (51.13%)			167 (87.89%)		
6 years	82 (53.95%)	4.3697	0.627	35 (89.74%)	2.7481	0.840
7 years	158 (51.97%)			31 (86.11%)		
8 years	128 (51.82%)			24 (88.89%)		
9 years	153 (52.40%)			27 (90.00%)		
10 years	107 (45.34%)			18 (85.71%)		
11 years	125 (50.40%)			23 (92.00%)		
12 years	63 (53.85%)			9 (75.00%)		
Administrator (%)	1561			179		
Father	510 (32.67%)			33 (18.44%)		
Mother	1010 (64.70%)			125 (69.83%)		
Grandfather	17 (1.09%)			5 (2.79%)		
Grandmother	14 (0.90%)			14 (7.82%)		
Other	10 (0.64%)			2 (1.12%)		

Table 2 MC-ASRS and SRS scores in the general sample and ASD cases.

Scales	General sample (n = 1593)	ASD cases (n = 185)	Effect size*	t value	P
ASRS scale					
T-Score	47.89 ± 7.85	67.06 ± 8.73	−19.17 ± 0.62	−31.07	<0.001
SC	24.30 ± 11.95	50.01 ± 13.54	−25.72±0.94	−27.29	<0.001
UB	27.74 ± 10.79	46.84 ± 13.52	−19.10±0.86	−22.15	<0.001
SR	16.91 ± 7.52	29.46 ± 9.10	−12.54±0.60	−20.99	<0.001
DSM-IV-TR scale	41.86 ± 13.07	74.80 ± 14.91	−32.94 ± 1.03	−27.72	<0.001
SRS score	43.15 ± 18.22	103.33 ± 25.70	−60.17 ± 1.49	−40.49	<0.001

MC-ASRS Modified Chinese Autism Spectrum Rating Scales, *SC* Social Communication, *SR* Self Regulation, *SRS* Social Responsiveness Scale, *UB* Unusual Behavior.

* Difference ± SE. The SC, UB, SR, DSM-IV-TR, and SRS scores are raw, and the T-score of MC-ASRS is composite.

Table 3 Comparison of diagnostic accuracy between the MC-ASRS and SRS scales in screening for autism spectrum disorder in children aged 6–12 years.

	MC-ASRS	SRS
True positives	172	179
False negatives	13	6
False positives	268	284
True negatives	1325	1309
Sensitivity (95% CI)	93.0% (88.3–96.2)	96.8% (93.1–98.8)
Specificity (95% CI)	83.2% (81.2–85.1)	82.2% (80.2–84.0)
Positive likelihood ratio (95% CI)	553% (492–621)	543% (487–605)
Negative likelihood ratio (95% CI)	8.45%(5.0–14.3)	3.95% (1.8–8.7)
Odds ratio (95% CI)	65.4(36.9–116.0)	138.0 (61.6–307.0)
Positive predictive value (95% CI)	39.1% (34.5–43.8)	38.7% (34.2–43.3)
Negative predictive value (95% CI)	99.0% (98.3–99.5)	99.5% (99.0–99.8)
False-positive rate, %	16.8%	17.8%

MC-ASRS Modified Chinese Autism Spectrum Rating Scales, *SRS* Social Responsiveness Scale, *95% CI* 95% confidence interval.

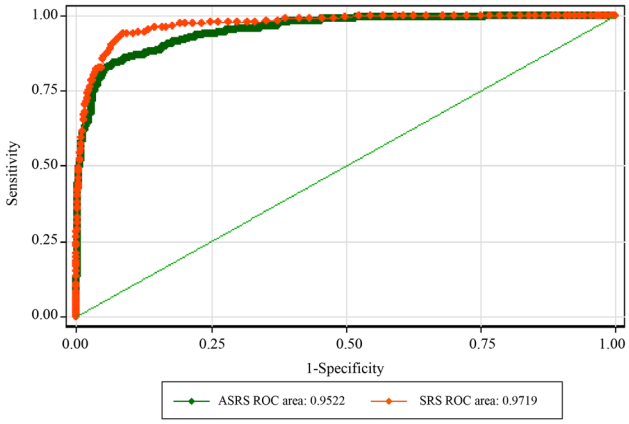


Fig. 1 ROC curves and AUCs of MC-ASRS T-scores and total raw SRS scores. *AUC* area-under-the-curve, *ROC* receiver operating characteristic, *MC-ASRS* Modified Chinese Autism Spectrum Rating Scales, *SRS* Social Responsiveness Scale.

used tool for ASD screening. Our results showed that the MC-ASRS effectively identified children diagnosed with ASD using the DSM-V criteria, with screening accuracy similar to that of the SRS. When separated into two subsamples of administrators (father and mother), the results of both instruments were still both excellent.

As a newly-developed scale, the screening accuracy of the ASRS has not been systematically examined. We found good to excellent sensitivity and specificity for the cut-off T-score of 60 on the parent report. As to the parent-reported SRS, the estimate of sensitivity with regard to ASD classification according the DSM-V criteria was similar to that of a previous study [23], using samples with only typically-developing children and children with ASD.

Predictive values depend upon the prevalence of the targeted disease [26], while the likelihood ratios are relatively independent and also more steady when used in evaluating screening accuracy. In the current study, the NPVs of the two instruments were similarly high, while the PPVs were almost equally low, partly because of the low prevalence of ASD classification in our sample (185/1778, 10.4%). It was noted that the NLR of the MC-ASRS was higher than that of the SRS, indicating that the MC-ASRS has slightly greater but still acceptable potential [27] to misjudge an ASD case for a typical child than the SRS.

Characteristics of the Two Scales

The parent version of the SRS is a widely-used scale designed to evaluate the social ability of children in the

Table 4 AUCs of ROCs of the MC-ASRS and SRS for main administrators.

Scales	Administrator	*n*	AUC	SE	95% CI	χ^2	*P*
MC-ASRS	Father	543	0.9502	0.0199	0.9111–0.9892	0.11	0.8021
	Mother	1135	0.9506	0.0099	0.9312–0.9698		
SRS	Father	543	0.9435	0.0217	0.9010–0.9859	15.24	0.0001
	Mother	1135	0.9777	0.0054	0.9671–0.9883		

AUC area-under-the-curve, *MC-ASRS* Modified Chinese Autism Spectrum Rating Scales, *ROC* receiver operating characteristic, *SRS* Social Responsiveness Scale.

general population for screening purposes. There have been many diagnostic validity studies of the SRS in different countries [12, 13, 21, 28–31]. In the USA, German, and Chinese studies, the total SRS score performs well in differentiating children with ASD from typically-developing children. Our study concurred with these results. However, studies, including the original validation study [19], have also suggested that the SRS has lower screening accuracy in a complicated group of other mental disorders (such as intellectual disability, language disorder, ADHD, and ODD/CD), especially in children with a lower IQ and with greater behavioral problems [12]. The reason could be great overlap of communication and social interaction symptoms in children with ASD and other mental disorders and insufficient items focused on repetitive and restricted behaviors (RRBs) – another pivotal and characteristic domain of ASD – in the SRS. The majority of items (53/65) in the SRS describe normal or abnormal responses in social situations, focusing on the severity of the social communication deficit, while 12 items describe autistic mannerisms. The score generated by the SRS is an index of impairments in reciprocal social behaviors; some items are even geared toward other domains focusing on social aspects [32]. Furthermore, several items are descriptive of common symptoms of ASD, as well as of other neuropsychiatric disorders. Therefore, some disorders with social impairment showed overlapping SRS scores and could not be efficaciously differentiated from ASD [33].

The ASRS is a relatively new screening tool specifically for autistic traits. The scales include items related to the comprehensive symptoms and associated behaviors of ASD, including Asperger's Syndrome and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS). The structure of the scales is consistent with the 3 symptomatic domains of the criteria, and all domains are covered in significant proportions. Even in the UB subscale, there are three key areas about RRBs: language stereotypes, behavioral rigidity, and sensory sensitivity, which could improve the ability to discriminate between children with ASD and children with other psychiatric disorders. The original ASRS study showed that the scores on the ASRS can effectively distinguish individuals with ASD from typically-developing individuals and those with other diagnoses [17]. However, further research is necessary in different countries and cultures to assess how the ASRS performs when differentiating children with ASD from those with other developmental neurological disorders.

Strengths, Limitations, and Prospects

To our knowledge, the current study is the first to explore the screening accuracy of the MC-ASRS, and compare it with the SRS, another well-established ASD screening scale. The strengths of our study include a relatively large sample size and wide age-range, which could enhance the validity of the scales in subsequent studies. One limitation of this study is that the sample did not include children with other diagnoses, especially other developmental neurological disorders.

The screening accuracy of the two instruments may have been overestimated since children in the case group had been previously diagnosed and received special education, resulting in their parents' or caregivers' having a better understanding of the disorder and responding well to the items on the questionnaires, as compared to parents without previous knowledge of ASD. Therefore, when using the ASRS and SRS to screen for autistic traits in the general population, caution should be exercised. In future studies, it would be better to recruit the clinical subjects and complete the questionnaires during the first visit to reduce bias. In summary, the MC-ASRS shows good performance in screening for children with ASD in the general Chinese population aged 6–12 years, with effectiveness similar to the SRS. Further larger-scale and more sophisticated studies are needed to determine its suitability in screening children with ASD from those with other developmental neurological disorders.

Acknowledgements This work was supported by the National Health and Family Planning Commission of China (201302002) and the National Natural Science Foundation of China (81371270; ClinicalTrials.gov number NCT 02200679). We would like to thank all of the children, parents, and teachers who participated in this study.

References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th ed revised. Washington, DC: American Psychiatric Association, 2000.
2. American Psychiatric Association. Neurodevelopmental disorders. Autism spectrum disorder. Diagnostic and statistical manual of mental disorders (5th ed.) 2013.
3. Cholemkery H, Kitzerow J, Rohrmann S, Freitag CM. Validity of the social responsiveness scale to differentiate between autism spectrum disorders and disruptive behaviour disorders. Eur Child Adolesc Psychiatry 2014, 23: 81–93.
4. Rogers SJ, Vismara LA. Evidence-based comprehensive treatments for early autism. J Clin Child Adolesc Psychol 2008, 37: 8–38.
5. Bradshaw J, Steiner AM, Gengoux G, Koegel LK. Feasibility and effectiveness of very early intervention for infants at-risk for autism spectrum disorder: a systematic review. J Autism Dev Disord 2015, 45: 778–794.
6. Williams LW, Matson JL, Beighley JS, Konst M. Ethnic disparities in early autism assessment: a large scale screening study of infants and toddlers. J Dev Phys Disabil 2015, 27: 141–148.
7. Granpeesheh D, Dixon DR, Tarbox J, Kaplan AM, Wilke AE. The effects of age and treatment intensity on behavioral intervention outcomes for children with autism spectrum disorders. Res Autism Spectr Disord 2009, 3: 1014–1022.
8. Baron-Cohen S, Scott FJ, Allison C, Williams J, Bolton P, Matthews FE, *et al.* Prevalence of autism-spectrum conditions: UK school-based population study. Br J Psychiatry 2009, 194: 500–509.
9. Sun X, Allison C, Matthews FE, Sharp SJ, Auyeung B, Baron-Cohen S, *et al.* Prevalence of autism in mainland China, Hong Kong and Taiwan: a systematic review and meta-analysis. Mol Autism 2013, 4.
10. Sun X, Allison C, Auyeung B, Matthews FE, Baron-Cohen S, Brayne C. What is available for case identification in autism research in mainland China? Res Autism Spectr Disord 2013, 7: 579–590.
11. Guo YQ, Tang Y, Rice C, Lee LC, Wang YF, Cubells JF. Validation of the Autism Spectrum Screening Questionnaire, Mandarin Chinese Version (CH-ASSQ) in Beijing, China. Autism 2011, 15: 713–727.
12. Charman T, Baird G, Simonoff E, Loucas T, Chandler S, Meldrum D, *et al.* Efficacy of three screening instruments in the identification of autistic-spectrum disorders. Br J Psychiatry 2007, 191: 554–559.
13. Gau SSF, Liu LT, Wu YY, Chiu YN, Tsai WC. Psychometric properties of the Chinese version of the Social Responsiveness Scale. Res Autism Spectr Disord 2013, 7: 349–360.
14. Goldstein S, Naglieri JA. Autism Spectrum Rating Scales (ASRS^{TM}). Tonawanda, NY: Multi-Health Systems, 2009.
15. Zhou H, Zhang LL, Wu LJ, Zou XB, Luo XR, Xia K, *et al.* Validity and reliability analysis of the Chinese parent version of the Autism Spectrum Rating Scale (6-18 years). Psychiatry Res 2015, 230: 255–261.
16. Zhou H, Zhang LL, Yan WL, Xu X, Wang Y. Construct validity of the Autism Spectrum Rating Scale of the Revised Chinese Version. Chin J Contemp Pediatr 2015, 17: 1282–1285.
17. Simek AN, Wahlberg AC. Test Review: Autism Spectrum Rating Scales. J Psychoeduc Assess 2010, 29: 191–195.
18. Zhou H, Zhang L, Luo X, Wu L, Zou X, Xia K, *et al.* Modifying the Autism Spectrum Rating Scale (6-18 years) to a Chinese Context: An Exploratory Analysis. Neurosci Bull 2017.
19. Constantino JN, Davis SA, Todd RD, Schindler MK, Gross MM, Brophy SL, *et al.* Validation of a brief quantitative measure of autistic traits: Comparison of the social responsiveness scale with the autism diagnostic interview-revised. J Autism Dev Disord 2003, 33: 427–433.
20. Constantino JN, Gruber CP. Social Responsiveness Scale (SRS). Los Angeles: Western Psychological Services, 2005.
21. Boelte S, Poustka F, Constantino JN. Assessing autistic traits: cross-cultural validation of the social responsiveness scale (SRS). Autism Res 2008, 1: 354–363.
22. Fombonne E, Marcin C, Bruno R, Manero Tinoco C, Diaz Marquez C. Screening for autism in Mexico. Autism Res 2012, 5: 180–189.
23. Chen Q, Zou X. The revision and application of social responsiveness scale—a preliminary study. Pediatrics 2009, Master.
24. Simek AN, Wahlberg AC. Test review: autism spectrum rating scales. J Psychoeduc Assess 2011, 29: 191–195.
25. Zhou H, Zhang L, Zou X, Luo X, Xia K, Wu L, *et al.* Chinese norms for the autism spectrum rating scale. Neurosci Bull 2017.
26. Altman DG, Bland JM. Diagnostic tests 2: Predictive values. BMJ 1994, 309: 102.
27. Jaeschke R, Guyatt GH, Sackett DL, Guyatt G, Bass E, Brilledwards P, *et al.* Users' guides to the medical literature. III. How to use an article about a diagnostic test. B. What are the results and will they help me in caring for my patients? The Evidence-Based Medicine Working Group. JAMA 1994, 271: 703–707.
28. Fombonne E. Estimated prevalence of autism spectrum conditions in Cambridgeshire is over 1%. Evid Based Ment Health 2010, 13: 32.
29. Constantino JN, Lavesser PD, Zhang Y, Abbacchi AM, Gray T, Todd RD. Rapid quantitative assessment of autistic social impairment by classroom teachers. J Am Acad Child Adolesc Psychiatry 2007, 46: 1668–1676.
30. Wang J, Lee LC, Chen YS, Hsu JW. Assessing autistic traits in a Taiwan preschool population: cross-cultural validation of the Social Responsiveness Scale (SRS). J Autism Dev Disord 2012, 42: 2450–2459.
31. Duvekot J, van der Ende J, Verhulst FC, Greaves-Lord K. The screening accuracy of the parent and teacher-reported social responsiveness scale (SRS): comparison with the 3Di and ADOS. J Autism Dev Disord 2015, 45: 1658–1672.
32. Norris M, Lecavalier L. Screening accuracy of Level 2 autism spectrum disorder rating scales. A review of selected instruments. Autism 2010, 14: 263–284.
33. Cholemkery H, Mojica L, Rohrmann S, Gensthaler A, Freitag CM. Can autism spectrum disorders and social anxiety disorders be differentiated by the social responsiveness scale in children and adolescents? J Autism Dev Disord 2014, 44: 1168–1182.

Neurosci. Bull. April, 2017, 33(2):175–182
DOI 10.1007/s12264-017-0104-7

www.neurosci.cn
www.springer.com/12264

ORIGINAL ARTICLE

Modifying the Autism Spectrum Rating Scale (6–18 years) to a Chinese Context: An Exploratory Factor Analysis

Hao Zhou[1,11] · Lili Zhang[1] · Xuerong Luo[2] · Lijie Wu[3] · Xiaobing Zou[4] · Kun Xia[5] · Yimin Wang[1] · Xiu Xu[6] · Xiaoling Ge[7] · Yong-Hui Jiang[8] · Eric Fombonne[9] · Weili Yan[10] · Yi Wang[1]

Received: 26 October 2016 / Accepted: 22 January 2017 / Published online: 25 February 2017

Abstract The purpose of this study was to explore the psychometric properties of the Chinese version of the autism spectrum rating scale (ASRS). We recruited 1,625 community-based children and 211 autism spectrum disorder (ASD) cases from 4 sites, and the parents of all participants completed the Chinese version of the ASRS. A robust weighted least squares means and variance adjusted estimator was used for exploratory factor analysis. The 3-factor structure included 59 items suitable for the current sample. The item reliability for the modified Chinese version of the ASRS (MC-ASRS) was excellent. Moreover, with 60 as the cut-off point, receiver operating characteristic analysis showed that the MC-ASRS had excellent discriminate validity, comparable to that of the unmodified Chinese version (UC-ASRS), with area under the curve values of 0.952 (95% CI: 0.936–0.967) and 0.948 (95% CI: 0.930–0.965), respectively. Meanwhile, the confirm factor analysis revealed that MC-ASRS had a better construct validity than UC-ASRS based on the above factor solution in another children sample. In conclusion, the MC-ASRS shows better efficacy in epidemiological screening for ASD in Chinese children.

Keywords Autism spectrum disorder · Screening · Epidemiology · Exploratory factor analysis · Children

Introduction

Autism spectrum disorder (ASD) is a group of heterogeneous neurodevelopmental disorders characterized by deficits in social interaction and reciprocal communication, as well as restricted and repetitive interests and behaviors [1]. ASD has become a major worldwide issue in public health because its prevalence has significantly increased in many

Electronic supplementary material The online version of this article (doi:10.1007/s12264-017-0104-7) contains supplementary material, which is available to authorized users.

✉ Yi Wang
yiwang@shmu.edu.cn

1 Department of Neurology, Children's Hospital of Fudan University, Shanghai 201102, China

2 Department of Psychiatry, The Second Xiangya Hospital of Central South University, Changsha 410008, China

3 School of Public Health, Harbin Medical University, Harbin 150081, China

4 Child Development Center, The Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510000, China

5 State Key Laboratory of Medical Genetics, Changsha 400078, China

6 Department of Child Healthcare, Children's Hospital of Fudan University, Shanghai 201102, China

7 Children's Hospital of Fudan University, Shanghai 201102, China

8 Division of Medical Genetics, Department of Pediatrics and Neurobiology, Duke University School of Medicine, Durham, NC 27710, USA

9 Institute on Development and Disability, Oregon Health and Science University, Portland, OR 97239, USA

10 Department of Clinical Epidemiology, Children's Hospital of Fudan University, Shanghai 201102, China

11 Pediatric Department of Guizhou Provincial People's Hospital, Guiyang 550002, China

countries over the last few decades [2–4]. However, the causes of the progressive increase in the prevalence of ASD are not entirely clear. Potential contributing factors are changes in diagnostic criteria, increased attention within the medical community, and greater awareness among parents [5]. The etiology of autistic conditions nevertheless remains poorly understood, and the prevalence rate has varied substantially between studies and over time [6, 7].

ASD is an important cause of childhood disability worldwide. The prevalence of disability caused by autism is 2.38 per 10,000 individuals between 0 and 17 years old and 6.39 per 10,000 individuals between 4 and 6 years in the Chinese population [8]. However, at the national level, the prevalence of ASD in children in mainland China remains unknown. The usual approach to conducting a nation-wide epidemiological investigation of ASD in the Chinese general pediatric population (6–12 years) is to screen a representative population in order to identify children suspected of having ASD and to conduct next-step clinical assessments with systematic methods in order to obtain an accurate estimate of prevalence. A questionnaire-based epidemiologic study is an easy and efficient method of screening for ASD in the general population because it is easy to carry out and relatively inexpensive. However, the use of questionnaires relies on each participant's understanding of the instructions for each individual item, which may vary according to the cultural context in different samples [9, 10]. Factor analysis has been widely used to investigate the latent structure of ASD questionnaires in different populations in cross-cultural environments [11, 12].

Most studies of the factor structure of ASD questionnaires have used Western populations. To date, Chinese versions of several screening tools for autism have been developed [13]; however, only a few studies have conducted factor analysis of these assessment tools. One study used samples of school-aged students recruited from primary school and participants from clinical settings to explore the Social Responsiveness Scale in a Chinese population [14]. The results supported a 4-factor structure for the Chinese version of this scale. Gau *et al.* conducted factor analysis and revealed a 3-factor structure for a social communication questionnaire in Chinese children [15]. Another study examined the Autism Spectrum Quotient, which involved 5 factors in the general Chinese population [16]. However, these studies were all based on populations in the Taiwan region. So far, only one study has conducted a factor analysis of a screening tool for ASD in the Chinese population in China's mainland. Specifically, Sun *et al.* conducted a factor analysis of the Mandarin Chinese version of the Childhood Autism Spectrum Test in normal children and cases of autism; the results revealed a two-factor solution [17].

The Autism Spectrum Rating Scale (ASRS) is an ASD screening instrument developed by Goldstein and Naglieri [18]. It is available for two age ranges: 2–5 and 6–18 years. It is a newly-developed screening tool, and the only factor analysis of the ASRS has been conducted in a US population [18]. In a previous study, we demonstrated that the Chinese version of the ASRS is a useful instrument for screening autism in Chinese children. However, the construct validity of this version did not achieve the optimal value, with all values of the model fit <0.9 [19]. Therefore, to explore whether factor analysis of the ASRS in a sample of Chinese children is necessary, we measured the latent structure of the Chinese version (6–18 years old) and assessed the modified version in a different cultural environment, before its application in a national ASD screening program for children aged 6–12 years.

Materials and Methods

Participants

The samples were from a pilot national epidemiological study of ASD in Chinese school-aged children, conducted from January to July, 2014. To ensure data quality and that the sample was representative, participants were recruited from four cities geographically representative of China with a well-established base for epidemiological research: Shanghai, Harbin, Guangzhou, and Changsha.

The participants comprised two subsamples: (1) a community-based sample drawn from the parents of 2,053 children aged 6–12 years in Shanghai, Harbin, Guangzhou, and Changsha; and (2) a clinical sample of the parents of 211 individuals with autism. The children with ASD were recruited from the outpatients of participating institutions (The Children's Hospital of Fudan University, Shanghai; The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou; The Second Xiangya Hospital of Central South University, Changsha; and Harbin Medical University, Harbin). All children with ASD had a clinical diagnosis made by a pediatrician according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.

Screening Instrument

The ASRS was introduced to China using standard questionnaire translation procedures with the approval of Multi-Health Systems [20], and a previous study confirmed that the method is reliable [19]. The ASRS includes screening,

Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR), and treatment scales, with a total of 71 items. In factor analyses, a 3-factor solution was most commonly found with the ASRS in western population. Three-factors comprising 60 items of the total 71 were generated for screening: Social/Communication (SC, 19 items), Unusual Behaviors (UB, 24 items), and Self-Regulation (SR, 17 items). These 3 scales were combined into a single composite score, the T-score, which was developed for screening purposes. The DSM-IV-TR scale contained 34 items based on expert experience from the total of 71 items, and a high score indicates that the child has a higher chance of being diagnosed as autistic by a psychiatrist. Finally, the treatment scale had a total of 69 out of the 71 items and included 8 subscales based on expert experience, which are: Peers Socialization (PS, 9 items), Adult Socialization (AS, 6 items), Social/Emotional Reciprocity (SER, 13items), Atypical Language (AL, 6 items), Stereotypy (ST, 5 items), Behavioral Rigidity (BR, 8 items), Sensory Sensitivity (SS, 6 items), and Attention (AT, 11 items). This can be used for ongoing monitoring of the clinical status of children with ASD.

Study Procedure

The parents who gave written consent were invited to complete the Chinese version of the ASRS. Each parent was given a booklet that contained an information sheet, questionnaire, consent form, and guidance notes. Contact information for the research team was provided along with the scale in case parents had questions about the forms. The program was approved by the Ethics Review Board of the Children's Hospital of Fudan University ([2012] No. 185).

Statistics

The Chinese version of the ASRS was distributed to the parents of all eligible children in a pilot study. In all, 369 questionnaires were not returned, and 59 lacked basic information (e.g., name and date of birth). Another 160 questionnaires from the community-based sample had missing items: 126 (7.5%) had <5 missing items, and 34 (2%) had ≥ 5. In addition, 24 questionnaires from the clinical sample had missing items: 18 (8.5%) had <5, and 6 (2.8%) had ≥ 5. In total, 1,465 questionnaires from the community-based sample and 187 from the clinical sample were available for analysis.

The raw scores were used for factor analysis. We used the statistical package MPlus version 7.0 (Muthén & Muthén, Los Angeles, CA) to test the factor structure with exploratory factor analysis (EFA) [21]. Items in the ASRS were measured with 5-point Likert scales, and the variables were categorical. EFA was conducted with Geomin (oblique) rotation, which is a proper method for extracting categorical variables in factor analysis. The factor structure of the Chinese version of the ASRS was estimated using a robust weighted least squares means and variance-adjusted estimator [22]. This approach is considered to be more accurate for exploring the latent structure of questionnaires by identifying the factor structure of categorical variables than other methods based on continuous variables [23]. The χ^2 goodness-of-fit test, the root mean square error of approximation (RMSEA), the comparative fit index (CFI), the Tucker–Lewis index (TLI), and the standardized root mean square residual (SMSR) were used to estimate the factor structure [24].

We selected the number of factors to retain via the Kaiser criterion, where components with eigenvalues >1, and the scree test, where components with eigenvalues before the 'elbow' of a scree plot, were retained [25]. The literature indicates that using both approaches is more accurate at identifying the correct number of factors than using only one method. In particular, the number of factors to retain can be overestimated if one method is used.

We considered factor loadings ≥ 0.3 to be outstanding, and an item was removed from further analysis if it had a factor loading <0.3 or cross-loading <0.1 [18]. In addition, to ensure that each factor was well measured, factors with <3 items were removed.

We used the standard ASRS T-score to conduct further analyses. Cronbach's alpha was used to test item reliability [26], and receiver operating characteristic (ROC) curves were used to assess the performance of the questionnaire, as ROC analysis is a helpful method for determining the validity of questionnaires [27]. Specifically, we used ROC analysis to measure the discriminate validity of the Chinese version of the ASRS and computed the area under the curve (AUC) and 95% confidence intervals (CI). Ultimately, the sensitivity and specificity of the Chinese version of the ASRS for screening for ASD were analyzed.

Results

Demographic Characteristics of the Samples

A total of 1,465 questionnaires from the community-based sample and 187 questionnaires from the clinical sample were included in the analysis. The community-based sample included 752 boys (51.3%) with a mean age of 8.8 ± 1.8 years, and the clinical sample included 161 boys (86.1%) with a mean age of 8.9 ± 1.9 years. Those excluded were missing basic information and data necessary for statistical analysis. Statistics regarding age, sex, and site distribution are shown in Tables 1 and 2.

The mean scores for the ASRS by type of sample and by gender are summarized in Table 3. The total score and SC,

Table 1 Age and sex distribution of the reference sample.

Age	Community-based sample ($n = 1465$)			Clinical sample ($n = 187$)		
	Male *n* (%)	Female *n* (%)	Total	Male *n* (%)	Female *n* (%)	Total
6	125 (54.6)	104 (45.4)	229	44 (89.8)	5 (10.2)	49
7	123 (50.8)	119 (49.1)	242	28 (87.5)	4 (12.5)	32
8	134 (55.6)	107 (44.4)	241	18 (81.8)	4 (18.2)	22
9	118 (47.0)	133 (53.0)	251	25 (92.6)	2 (7.4)	27
10	99 (48.3)	106 (52.7)	205	17 (85.0)	3 (15.0)	20
11	106 (52.7)	95 (48.3)	201	17 (77.3)	5 (22.7)	22
12	47 (49.0)	49 (51.0)	96	12 (80.0)	3 (20.0)	15
Total	752 (51.3)	713 (48.7)	1465	161 (86.1)	26 (13.9)	187
χ^2	5.76			3.82		
P	0.45			0.70		

Table 2 Site distribution of the reference sample.

City	Community-based sample ($n = 1465$)			Clinical sample ($n = 187$)		
	Male *n* (%)	Female *n* (%)	Total	Male *n* (%)	Female *n* (%)	Total
Shanghai	183 (49.7)	185 (50.3)	368	47 (85.5)	8 (14.5)	55
Guangzhou	221 (52.1)	203 (47.9)	424	40 (87.0)	6 (13.0)	46
Changsha	166 (50.0)	166 (50.0)	332	40 (85.1)	7 (14.9)	47
Harbin	182 (53.4)	159 (46.6)	341	34 (87.2)	5 (12.8)	39
Total	752 (51.3)	713 (48.7)	1465	161 (86.1)	26 (13.9)	187
χ^2	1.29			0.12		
P	0.73			0.99		

Table 3 Mean ASRS scores by sample type and gender.

	Community-based sample ($n = 1465$)			Clinical sample ($n = 187$)			$P^{\#}$
	All	Boys ($n = 747$)	Girls	All	Boys ($n = 166$)	Girls	
Total score	54.82 ± 7.0	55.78 ± 6.82	53.82 ± 7.04	69.23 ± 6.30	69.0 ± 6.47	71.48 ± 4.2	<0.011
SC	56.50 ± 9.44	57.36 ± 9.42	55.61 ± 9.40	74.49 ± 8.14	74.27 ± 8.45	76.20 ± 4.88	<0.011
SR	47.37 ± 8.36	48.71 ± 8.24	57.75 ± 6.40	59.90 ± 7.00	59.54 ± 8.23	65.62 ± 4.68	<0.011
UB	58.22 ± 6.31	58.69 ± 6.18	45.97 ± 8.26	64.84 ± 6.0	64.75 ± 6.11	62.76 ± 4.05	<0.011

[#] *t* test results for comparisons of means between community-based and clinical samples. All $P < 0.001$ for all community children *versus* all children with ASD; all community boys *vs* all boys with ASD; all community girls vs all girls with ASD. *SC* Social Communication, *UB* Unusual Behavior, *SR* Self Regulation.

UB, and SR sub-scores were higher in the clinical sample than in the community-based sample. These significant differences still existed between the two samples for males and females (all $P < 0.001$).

Exploratory Factor Analysis

The results of the EFA revealed thirteen factors with eigenvalues >1. While a break was apparent in the slope of plotted eigenvalues, the shape of the curve suggested that three factors were appropriate for the present sample (Fig. 1). Therefore, our model fit statistics were based on a three-factor structure, and each factor was extracted (Table 4).

Among the 71 items, 12 were excluded: items 2 (becomes bothered by some fabrics or tags in clothes), 3 (seeks the company of other children), 4 (shows little emotion), 7 (has problems waiting his/her turn), 11 (avoids looking at people who speak to him/her), 14 (has trouble talking with other children), 26 (repeats or echoes what others have said), 34 (avoids looking at adults when a problem occurs), 46 (flaps his/her hands when excited), 52

Fig. 1 Screen plot.

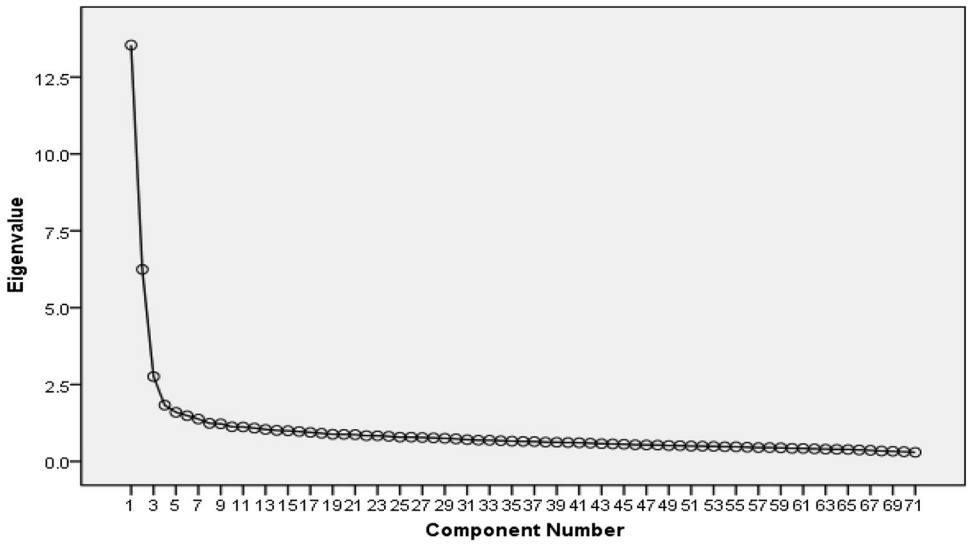


Table 4 Model fit statistics by factor solutions from the exploratory factor analysis (71 items).

Factors	χ^2			RMSEA	CFI	TLI	SMSR	Eigenvalues
	χ^2	*df*	*P*					
1	23546.570	2414	0.000	0.077	0.669	0.659	0.101	17.102
2	10707.564	2344	0.000	0.049	0.869	0.861	0.053	6.827
3	7348.148	2275	0.000	0.039	0.921	0.913	0.040	3.037

Index criteria for a model of good fit: RMSEA < 0.05, CFI > 0.90, TLI > 0.90, SMSR < 0.08. *CFI* comparative fit index, *RMSEA* root mean square error of approximation, *SMSR* standardized root mean square residual, *TLI* Tucker–Lewis index.

(has problems paying attention to fun tasks), 59 (has trouble talking with adults), and 68 (reverses pronouns [e.g., you for me]). Items 2, 3, 4, 11, 46, and 52 were excluded because their factor loading was <0.30. Item 7 had a cross-loading on factors 2 (0.300) and 3 (0.232); item 14 had a cross-loading on factors 1 (0.376) and 3 (0.463); item 26 had a cross-loading on factors 2 (0.281) and 3 (0.304); item 34 had a cross-loading on factors 2 (0.327) and 3 (0.271); item 59 had a cross-loading on factors 1 (0.378) and 3 (0.463); and item 68 had a cross-loading on factors 1 (0.289) and 3 (0.342). Thus, these items were excluded as well.

Factor 1, "SC", included 21 items (5, 8, 9, 10, 12, 15, 23, 28, 31, 32, 33, 39, 42, 43, 45, 47, 55, 56, 61, 69, and 70); factor 2, "SR", included 14 items (1, 6, 16, 17, 27, 30, 35, 36, 37, 44, 57, 58, 60, and 71), and factor 3, "UB", included 24 items (13, 18, 19, 20, 21, 22, 24, 25, 29, 38, 40, 41, 48, 49, 50, 51, 53, 54, 62, 63, 64, 65, 66, and 67). Thus, 59 items were retained for further analysis and the EFA was performed again on them. The model remained stable and met the criteria for the goodness-of-fit indices (RMSEA = 0.041, CFI = 0.926, TLI = 0.950, SRMR = 0.045). The item loadings for each factor of the Chinese version of the ASRS are shown in Table S1.

We conducted a confirm factor analysis based on the above factor solution in another population of normal children. The sample came from a primary school in the Minhang District of Shanghai: 671 children aged 6–12 years. The results revealed that this modified Chinese version (MC-ASRS) had a better construct validity than the unmodified version (UC-ASRS) [28].

Item Reliability of the Chinese Version of the ASRS

We used the Cronbach's alpha to test the item reliability [29]. The item reliability for the 59 items was 0.926 for the MC-ASRS and 0.915 for the UC-ASRS. Moreover, for the SC, SR, and UB subscales, Cronbach's alpha was 0.908, 0.873, and 0.857 for the MC-ASRS and 0.87, 0.863, and 0.846, for the UC-ASRS, respectively. These results indicated that, regarding the item structure, the MC-ASRS had relatively better reliability (for the three subscales and total scores) than the UC-ASRS for the Chinese population (Table 5).

Table 5 Comparison of Cronbach's alpha for each factor and the total score between the UC-ASRS and the MC-ASRS.

Factors	UC-ASRS	Cronbach's alpha	MC-ASRS	Cronbach's alpha
SC	19	0.87	21	0.908
SR	17	0.863	14	0.873
UB	24	0.846	24	0.857
Total	60	0.915	59	0.929

MC-ASRS modified Chinese version of the Autism Spectrum Rating Scale, *UC-ASRS* unmodified Chinese version of the scale, *SC* Social Communication, *SR* Self Regulation, *UB* Unusual Behavior.

Optimal Cut-Offs of the Chinese Version of the ASRS

A previous study suggested that the ROC curve is a reliable method to determine the ideal cut-offs for questionnaires in psychiatric research on children [30]. Using an approach to determine the optimal sensitivity and specificity, we found that the conventionally-used cut-off of 60 (mean + 1 SD) for the MC-ASRS achieved a sensitivity of 94.2% and a specificity of 82.0%, in the current sample. The original study developing the ASRS suggested using a cut-off of 60 for the USA version [18]. Thus, we used a cut-off of 60 to compute the sensitivity and specificity of the UC-ASRS. The results (sensitivity 94.7%, specificity 77%) showed that, compared with the MC-ASRS, the UC-ASRS had a relatively equal sensitivity and a slightly lower specificity.

Discriminate Validity of the Chinese Version of the ASRS

We performed ROC analysis to test the overall discriminate validity of both the MC-ASRS and the UC-ASRS (Fig. 2). Using the same cut-off of 60, we found that both versions yielded AUCs > 0.9, with an AUC of the total score of 0.952 (95% CI: 0.936–0.967) for the MC-ASRS and 0.948 (95% CI: 0.930–0.965) for the UC-ASRS, indicating equally excellent discriminate validity for screening children with ASD. We performed further analysis separately on each gender and found that the scales performed even better among girls: AUC = 0.991; 95% CI: 0.980–1.000 for the MC-ASRS and 0.996; 95% CI: 0.991–1.000 for the UC-ASRS (Figs. S1 and S2).

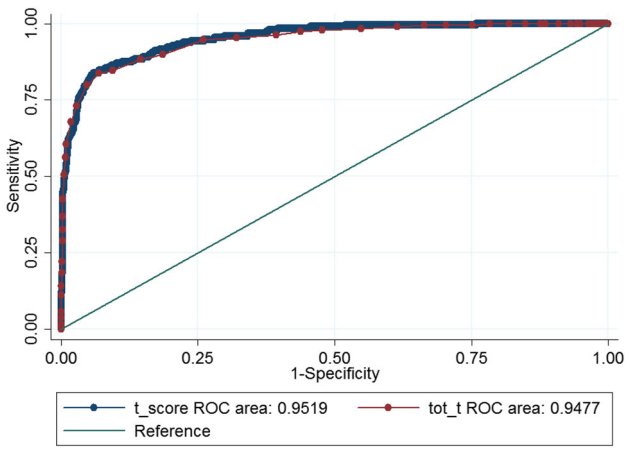


Fig. 2 Receiver Operating Characteristic (ROC) curves for the total score for the MC-ASRS and UC-ASRS. MC-ASRS, modified Chinese version of the Autism Spectrum Rating Scale; UC-ASRS, unmodified Chinese version of the ASRS; t_score, total score of the MC-ASRS, tot_t, total score of the UC-ASRS.

Discussion

A standard approach to determining the efficacy of an assessment tool is to determine whether scores on the scale are significantly higher for a clinical sample than for the general population. This indicates that the tool is able to easily identify cases in the general population. The ASRS is a newly-developed screening tool, and prior research has demonstrated that scores on its subscales are significantly higher in children with ASD than in normal children in the US population [18]. The current study demonstrates the efficacy of ASRS based on a Chinese sample.

EFA revealed the underlying structure of the MC-ASRS, which consisted of three domains related to the quality of ASD screening in the present sample. An EFA of the ASRS suggested that a 3-factor solution, comprising 60 of the total 71 items, was suitable for screening in western population. However, the MC-ASRS retained 59 items loaded on a comparable 3-factor structure. Moreover, the content of the 3 factors was similar to that of those in the original US version [18]. The only difference was that a change in the numbers of items contained in each factor was justified for the Chinese sample. The content of each factor may have differed between the MC-ASRS and the UC-ASRS for two reasons.

First, some items shifted from one factor to another in the MC-ASRS compared with the UC-ASRS. Second, in the MC-ASRS, some items in the UC-ASRS were removed, and other items from the 71-item total were added. These adjustments may have been justified because of cultural differences that may have affected the understanding of each concept. For instance, items 3 "will seek the company of other children" and 4 "shows little

emotion" were removed from the MC-ASRS. In Western culture, a child exhibiting such behaviors may be considered to lack social skills, and his or her parents might think that the child is introverted and shy; in Chinese culture, however, such behavior is considered normal. The differences between the two versions were very similar for the SR and UB subscales, which may be attributed to different understanding of the same concepts between cultures, especially since the concepts of SR and UB are easy to confuse in Chinese culture. Thus, the shifting of many items between the UB and SR subscales is understandable. Expert judgments were required in the factor analysis when items shifted from one factor to another, which may have influenced the results. Our expert team thought that the MC-ASRS would be more suitable for a Chinese cultural environment. Previous studies have also demonstrated that cross-cultural influences may affect the factor structure of a questionnaire and that modifying questionnaires for different cultural backgrounds may be important [31, 32].

The EFA identified 12 items as potential candidates for deletion because of poor factor loadings in the MC-ASRS. Experts have suggested that as many items in the questionnaire as possible should be retained in a factor analysis. In this study, we deleted 12 items. The need to delete so many may be associated with the design of the ASRS questionnaire. Many well-informed autism scales have been designed mainly for screening. Initially, Dr. Sam Goldstein developed the ASRS not only for screening but also for diagnosis and monitoring the treatment of children with ASD. Therefore, the ASRS contains more items than other screening instruments for ASD. The UC-ASRS retained 60 items in the ASRS screening scale via EFA [18]. However, item assignment to the DSM-IV-TR and treatment scales was based on the content of the items, clinical experience, and the judgment of experts.

The analysis of item reliability demonstrated that Cronbach's alpha for each factor and the total score was slightly better for the MC-ASRS than for the UC-ASRS. The cross-cultural environment is known to affect the performance of a questionnaire [33]. The high AUC values in the ROC analysis indicated that the discriminate validity of the MC-ASRS was strong and as high as that of the UC-ASRS in the Chinese reference sample. The results revealed that the MC-ASRS had excellent item reliability and discriminate validity and that the MC-ASRS had equal sensitivity and better specificity than the UC-ASRS. The confirm factor analysis based on the factor solution in another population of normal children [28] also demonstrated that the MC-ASRS had a better construct validity than the UC-ASRS, supporting its use as a reliable screening tool for ASD in children and adolescent populations in China.

Limitations

The samples in our study were drawn from 4 cities. Differences in culture, language, and diversity are the most probable causes of the disparities in factor structure between the MC-ASRS and the UC-ASRS. Using EFA, we were unable to explore the specific contributions of each of these types of difference. As currently the EFA of the ASRS is conducted only in the US population, a comparison between the present results and those of other studies with respect to these issues cannot be made.

It is important to note that caution should be exercised in interpreting our results. Owing to missing data, the final analysis did not include all of the collected questionnaires, but the vast majority were included; thus, the exclusion of these ASRS questionnaires is unlikely to have affected the results of the EFA. The criteria used to determine salient loadings, the factor extraction and rotation methods, the methods of analysis, and the criteria used for indices of model fit may have affected the factor structure. However, we conducted EFA with reference to previous research methods [34].

Conclusion

This is the first multisite study to use both community-based and clinical samples to test the MC-ASRS with EFA. The 3-factor solution of the MC-ASRS was stable and reliable, and it showed excellent discriminate validity, as well as good sensitivity and specificity. Our results thus demonstrated that the MC-ASRS is a useful and reliable tool for screening for the symptoms of autism in Chinese children.

Acknowledgements This study was supported by the National Health and Family Planning Commission of the People's Republic of China (201302002; ClinicalTrials.gov number NCT 02200679), the Shanghai International Cooperation Ministry of Science Projects (14430712200), and the Development Project of Shanghai Peak Discipline-Integrated Chinese and Western Medicine. We thank all of the parents who participated in this study.

References

1. Mittal VA, Walker EF. Diagnostic and statistical manual of mental disorders. Psychiatry Res 2011, 189: 158–159.
2. Blumberg SJ, Bramlett MD, Kogan MD, Schieve LA, Jones JR, Lu MC. Changes in prevalence of parent-reported autism

spectrum disorder in school-aged U.S. children: 2007 to 2011–2012. Natl Health Stat Report 2013, (65): 1–11.
3. Williams JG, Higgins JP, Brayne CE. Systematic review of prevalence studies of autism spectrum disorders. Arch Dis Child 2006, 91: 8–15.
4. Elsabbagh M, Divan G, Koh YJ, Kim YS, Kauchali S, Marcin C, *et al*. Global prevalence of autism and other pervasive developmental disorders. Autism Res 2012, 5: 160–179.
5. Levy SE, Mandell DS, Schultz RT. Autism. Lancet 2009, 374: 1627–1638.
6. Neggers YH. Increasing prevalence, changes in diagnostic criteria, and nutritional risk factors for autism spectrum disorders. ISRN Nutr 2014, 514026. doi: 10.1155/2014/514026.
7. Fombonne E. Epidemiology of pervasive developmental disorders. Pediatr Res 2009, 65: 591–598.
8. Li N, Chen G, Song X, Du W, Zheng X. Prevalence of autism-caused disability among Chinese children: a national population-based survey. Epilepsy Behav 2011, 22: 786–789.
9. Soto S, Linas K, Jacobstein D, Biel M, Migdal T, Anthony BJ. A review of cultural adaptations of screening tools for autism spectrum disorders. Autism 2015,19: 646–661.
10. Varma A, Iskandar JW. Challenges in diagnosis of autism and the struggle of using western screening tools in different cultures. Psychiatrist's perspective. Indian Pediatr 2014, 51: 356–357.
11. Posserud B, Lundervold AJ, Steijnen MC, Verhoeven S, Stormark KM, Gillberg C. Factor analysis of the Autism Spectrum Screening Questionnaire. Autism 2008, 12: 99–112.
12. Frazier TW, Youngstrom EA, Kubu CS, Sinclair L, Rezai A. Exploratory and confirmatory factor analysis of the autism diagnostic interview-revised. J Autism Dev Disord 2008, 38: 474–480.
13. Sun X, Allison C, Auyeung B, Matthews FE, Baron-Cohen S, Brayne C. What is available for case identification in autism research in mainland China? Res Autism Spectr Disord 2013, 7: 579–590.
14. Gau SS, Liu L, Wu Y, Chiu Y, Tsai W. Psychometric properties of the Chinese version of the social responsiveness scale. Res Autism Spectr Disord 2013, 7: 349–360.
15. Gau SS, Lee C, Lai M, Chiu Y, Huang Y, Kao J, *et al*. Psychometric properties of the Chinese version of the Social Communication Questionnaire. Res Autism Spectr Disord 2011, 5: 809–818.
16. Lau WY, Gau SS, Chiu YN, Wu YY, Chou WJ, Liu SK, *et al*. Psychometric properties of the Chinese version of the Autism Spectrum Quotient (AQ). Res Dev Disabil 2013, 34: 294–305.
17. Sun X, Allison C, Auyeung B, Matthews FE, Norton S, Baron-Cohen S, *et al*. Psychometric Properties of the Mandarin Version of the Childhood Autism Spectrum Test (CAST): An Exploratory Study. J Autism Dev Disord 2014, 44: 1565–1576.
18. Goldstein S, Naglieri JA. Autism Spectrum Rating Scales (ASRS^{TM}). Tonawanda, NY: Multi-Health Systems, 2009.
19. Zhou H, Zhang L, Wu L, Zou X, Luo X, Xia K, *et al*. Validity and reliability analysis of the Chinese parent version of the Autism Spectrum Rating Scale (6-18 years). Psychiatry Res 2015, 230: 255–261.
20. Kvamme OJ, Mainz J, Helin A, Ribacke M, Olesen F, Hjortdahl P. Interpretation of questionnaires. An translation method problem. Nord Med 1998, 113: 363–366.
21. Muthén LK, Muthén BO. Mplus User's Guide Version 6. Los Angeles, CA: Muthén & Muthén, 2010.
22. Muthén B, Du Toit SH, Spisic D. Robust inference using weighted least squares and quadratic estimating equations in latent variable modeling with categorical and continuous outcomes. Psychometrika 1997, 75: 1–45.
23. Nussbeck FW, Eid M, Lischetzke T. Analysing multitrait-multimethod data with structural equation models for ordinal variables applying the WLSMV estimator: what sample size is needed for valid results? Br J Math Stat Psychol 2006, 59:195–213.
24. Wichmann FA, Hill NJ. The psychometric function: I. Fitting, sampling, and goodness of fit. Percept Psychophys 2001, 63: 1293–1313.
25. Parsian N. Developing and validating a questionnaire to measure spirituality: A psychometric process. Glob J Health Sci 2009, 1: p2.
26. Tavakol M, Dennick R. Making sense of Cronbach's alpha. Int J Med Edu 2011, 2:53.
27. Hajian-Tilaki K. Receiver operating characteristic (roc) curve analysis for medical diagnostic test evaluation. Caspian J Intern Med 2013, 4: 627–635.
28. Zhou H, Zhang LL, Yan WL, Xu X, Wang Y. Construct validity of the Autism Spectrum Rating Scale of the Revised Chinese Version. Zhongguo Dang Dai Er Ke Za Zhi 2015, 17: 1282–1285.
29. Santos JRA. Cronbach's alpha: A tool for assessing the reliability of scales. J Ext 1999, 37:1–5.
30. Fombonne E. The use of questionnaires in child psychiatry research: measuring their performance and choosing an optimal cut-off. J Child Psychol Psychiatry 1991, 32: 677–693.
31. LaFromboise TD, Coleman HL, Hernandez A. Development and factor structure of the Cross-Cultural Counseling Inventory—Revised. Prof Psychol Res Pr 1991, 22: 380–388.
32. Hale WW, Crocetti E, Raaijmakers QA, Meeus WH. A meta-analysis of the cross-cultural psychometric properties of the Screen for Child Anxiety Related Emotional Disorders (SCARED). J Child Psychol Psyc 2011, 52: 80–90.
33. McCrae RR, Costa PT, Del Pilar GH, Rolland J, Parker WD. Cross-cultural assessment of the Five-Factor Model The Revised NEO Personality Inventory. J Cross-Cultural Psychol 1998, 29:171–188.
34. Bölte S, Poustka F, Constantino JN. Assessing autistic traits: cross-cultural validation of the social responsiveness scale (SRS). Autism Res 2008, 1:354–363.

Neurosci. Bull. April, 2017, 33(2):183–193
DOI 10.1007/s12264-017-0100-y

www.neurosci.cn
www.springer.com/12264

REVIEW

An Overview of Autism Spectrum Disorder, Heterogeneity and Treatment Options

Anne Masi[1] · Marilena M. DeMayo[1] · Nicholas Glozier[1] · Adam J. Guastella[1]

Received: 18 November 2016 / Accepted: 10 January 2017 / Published online: 17 February 2017

Abstract Since the documented observations of Kanner in 1943, there has been great debate about the diagnoses, the sub-types, and the diagnostic threshold that relates to what is now known as autism spectrum disorder (ASD). Reflecting this complicated history, there has been continual refinement from DSM-III with 'Infantile Autism' to the current DSM-V diagnosis. The disorder is now widely accepted as a complex, pervasive, heterogeneous condition with multiple etiologies, sub-types, and developmental trajectories. Diagnosis remains based on observation of atypical behaviors, with criteria of persistent deficits in social communication and restricted and repetitive patterns of behavior. This review provides a broad overview of the history, prevalence, etiology, clinical presentation, and heterogeneity of ASD. Factors contributing to heterogeneity, including genetic variability, comorbidity, and gender are reviewed. We then explore current evidence-based pharmacological and behavioral treatments for ASD and highlight the complexities of conducting clinical trials that evaluate therapeutic efficacy in ASD populations. Finally, we discuss the potential of a new wave of research examining objective biomarkers to facilitate the evaluation of sub-typing, diagnosis, and treatment response in ASD.

Keywords Autism Spectrum Disorder · Diagnosis · Heterogeneity · Treatment

Introduction

Autism spectrum disorders (ASDs) are complex, pervasive, and multifactorial neurodevelopmental conditions. Observation of aberrant behavior forms the basis of diagnosis, with criteria focused on impairments in social communication and interaction, and restricted, repetitive patterns of behavior, interests, or activities [1]. Heterogeneity of presentation is a hallmark [2–4] with comorbid psychiatric and medical morbidities frequently reported. Commonly identified psychiatric and cognitive comorbidities with ASD include social anxiety disorder, oppositional defiant disorder, attention-deficit/hyperactivity disorder, and intellectual disability [5–7]. Medical conditions frequently reported include immune system abnormalities, gastrointestinal disorder, mitochondrial dysfunction, sleep disorders, and epilepsy [8–10].

The substantial direct and indirect effects of ASDs extend across many different sectors including health, education, social care, housing, employment, welfare benefits, and labor markets, with a high economic burden extending to adulthood and often carried by families [11, 12]. With forecasts of annual direct medical, non-medical, and productivity costs projected to reach close to $US 500 billion by 2025 in the United States alone [13], the importance of adequate care, support structures for affected individuals and their families, and efficacious treatments to improve functioning and outcomes cannot be underestimated.

Diagnosis, Prevalence, and Etiology

In 1943, Leo Kanner published a report entitled "Autistic disturbances of affective contact", detailing eleven case studies of children (eight males and three females) aged

✉ Adam J. Guastella
adam.guastella@sydney.edu.au

[1] Autism Clinic for Translational Research, Brain and Mind Centre, Central Clinical School, Sydney Medical School, University of Sydney, Sydney, NSW, Australia

from 2 years and 4 months to 11 years who had presented to his clinics [14]. Kanner described observations of these children as having an extreme inability to relate to others that appeared to be present throughout infancy. Kanner drew a distinction between this syndrome and that of "childhood schizophrenia" based on the time of onset, as childhood schizophrenia was explained as withdrawal following typical development. Along with this desire for aloneness, Kanner also observed unusual language development, with an aptitude for nouns and learning nursery rhymes, a failure to develop the communicative aspects of speech, a tendency to show echolalia, and a tendency to interpret things literally, along with sensory sensitivities and repetitive behaviors.

In 1944, Hans Asperger published a paper describing what he termed "autistic psychopathy". This paper described children who primarily had difficulties with nonverbal communication and related social skills. This paper would eventually be considered as important as Kanner's work in the development of the concept of autism, since the core symptoms were the same as those identified by Kanner but in higher-functioning individuals [15, 16]. As it was published in Germany, in German, during the Second World War, it was not widely read and did not enter the English-speaking medical community until the 1970s [17]. In 1981, Lorna Wing provided a history of the syndrome proposed by Asperger, though she renamed it "Asperger syndrome" to remove the connotations of "psychopathy" [17]. She acknowledges in her introduction the similarities between the criteria proposed by Kanner and Asperger, noting "the argument continues as to whether they are varieties of the same underlying abnormality". Wing described and refined Asperger's initial diagnostic criteria, and highlighted the continuum in criteria ranging from the lower functioning "Kanner's autism" to "Asperger's syndrome" to typically-developing individuals, who display some of the criteria of Asperger's syndrome [17].

Infantile autism as an endorsed medical diagnosis first appeared in the Diagnostic and Statistical Manual of Mental Disorders, third edition (DSM-III) [18], and described a subgroup of pervasive developmental disorder (PDD) [19]. The criteria outlined for Infantile Autism required an onset prior to the age of 30 months, a failure of responsiveness to others, gross deficits in language development, and bizarre responses to environmental stimuli, with an absence of schizophrenic symptoms. The criteria were broadened in the DSM-III-R to recognize the pervasive nature of the disorder and that it was not limited to infants, as the criteria for Infantile Autism excluded a subgroup of higher-functioning individuals who displayed the deficits described but did not evidence the symptoms early enough in life to receive the diagnosis. The revision of Infantile Autism to Autistic Disorder in the DSM-III-R recognized the broader spectrum of functioning with 8 out of 16 possible criteria required for diagnosis. The age of onset was specified as either during infancy or early childhood, with a childhood onset specifier (after 36 months). These criteria allowed for the identification of potentially less-impaired individuals to receive a diagnosis [20].

The DSM-IV was released in 1994 with criteria similar to the DSM-III-R for the diagnosis of Autistic Disorder, though the childhood onset specifier was removed as onset before 36 months of age was required. The DSM-IV introduced a formal set of criteria for Asperger's Syndrome using some of the criteria outlined by Wing [17]. The criteria for Asperger's Syndrome described a condition with impairments in social interaction, communication, and imagination, similar to that described by the Autistic Disorder criteria, but without the impairments in language or cognition [21].

The move to the DSM-5 was marked by broadening of the definition and reduction in the specificity of autism-related symptoms [1], heralding substantial changes to the diagnostic criteria. Diagnoses of Autistic Disorder, Asperger's Syndrome and Pervasive Developmental Disorder—Not Otherwise Specified (PDD-NOS), were removed as diagnostic classifications and collapsed into two diagnoses, Autism Spectrum Disorder and Social Communication Disorder. This latest modification reflected growing concerns about the validity of the Asperger's diagnosis, given evidence that it was frequently interchanged across time with Autistic Disorder [22]. Individuals who would have previously received a diagnosis of Asperger's Syndrome were generally thought to receive a diagnosis of "Autism Spectrum Disorder without language or cognitive impairment" (DSM-5). The current DSM-5 criteria for Autism Spectrum Disorder are listed in Table 1, with specifiers for current severity summarized in Table 2 [1]. For DSM-5, Social (Pragmatic) Communication Disorder (SCD) was introduced and required persistent difficulties in the social use of verbal and nonverbal communication, without impairments relating to restricted, repetitive behavior. It is expected that those who were previously diagnosed as PDD-NOS, and do not meet the DSM-5 Autism Spectrum Disorder criteria, would be more frequently diagnosed with SCD [23]. The new criteria were expected to enable greater standardization of diagnosis. Previously, a multisite observational study revealed significant variation in clinical diagnoses of specific ASDs, despite similar distributions of scores on standardized measures across sites, supporting the transition from the subgroupings used in DSM-IV to the current dimensional descriptors of the core features of social communication and interaction, and restricted, repetitive behaviors [24]. With the release of DSM-5, it is clear that many of the debates initiated by Kanner's, Asperger's, and Lorna Wing's work remain. There

Table 1 Diagnostic criteria for Autism Spectrum Disorder

	Social communication	Restricted repetitive behavior
Criteria	Persistent deficits in social communication and social interaction across multiple contexts, currently or by history	Restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least two of the following:
Illustrative examples of symptoms	(1) Deficits in social-emotional reciprocity, ranging from abnormal social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions, or affect, to failure to initiate or respond to social interactions (2) Deficits in nonverbal communicative behaviors used for social interaction, ranging from poorly integrated verbal and nonverbal communication, to abnormalities in eye contact and body language or deficits in understanding and use of gestures, to a total lack of facial expressions and nonverbal communication (3) Deficits in developing, maintaining, and understanding relationships, ranging from difficulties adjusting behavior to suit various social contexts, to difficulties in sharing imaginative play or in making friends, to absence of interest in peers	(1) Stereotyped or repetitive motor movements, use of objects, or speech. (2) Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behavior (3) Highly restricted, fixated interests that are abnormal in intensity or focus (4) Hyper- or hyporeactivity to sensory input or unusual interest in sensory aspects of the environment
	Symptoms must be present in the early developmental period. Symptoms may not become fully manifest until social demands exceed limited capacities, or may be masked by learned strategies in later life	
	Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning	
	These disturbances are not better explained by intellectual disability or global developmental delay	
Specifiers	With or without accompanying intellectual impairment	
	With or without accompanying language impairment	
	Associated with a known medical or genetic condition or environmental factor	
	Associated with another neurodevelopmental, mental, or behavioral disorder	
	With catatonia	

continues to be great debate about the number of different diagnoses with the term Autism Spectrum Disorder, the lack of clarity over the relationship between functioning levels of autism and impaired cognitive function, and the diagnostic relevance and need for treatment for those individuals who appear to show higher levels of occupational and intellectual functions.

The diagnostic criteria discussed above have been developed primarily with Western participants. Even though it is regarded as heavily influenced by biological factors and a developmental condition, research has recently highlighted that social and cultural factors influence diagnostic rates and the cultural acceptability of the tools used to make the diagnoses [25]. For instance, in the United States, general developmental delays or impaired language skills are common symptoms that result in a diagnosis [25]. Given that the diagnosis is based on social and contextual observations, it is not surprising that phenotypes and tools do not transfer as easily to other cultures. For example, in India, language may not be typically incorporated in the diagnostic criteria as boys acquire language skills later than girls [26]. In many Asian cultures, direct eye contact with elders is viewed as a sign of disrespect, thus the reduced eye-contact as a diagnostic feature may be seen a less atypical in these cultures [25]. There is an important need for a growing body of research addressing these cross-cultural factors in the diagnosis of ASD [25, 27]. Perhaps this research will highlight the universal features of autism that reduce the influence of contextual factors in the diagnostic criteria.

The prevalence of ASDs has, however, been increasing. In Asia, the average prevalence before 1980 was $\sim$1.9 cases per 10,000, rising to 14.8 between 1980 and 2010 [28]. A review of epidemiological studies published between 1996 and 2001, and conducted in the United Kingdom, United States and in Scandinavia and Japan, indicated that the prevalence was likely to be within the range of 30–60 cases per 10,000 [29]. More recent estimates are as high as 1 in 68, based on 8-year old children in the United States [30]. However, a combination of the broadening of diagnostic criteria previously discussed, and the methodology employed in epidemiologic surveys, including changes in the assessment process, response rates, and differences in sample size, publication year, and geographic location, suggests that it may not be informative to estimate trends over time [31]. Increases in prevalence estimates may represent changes in the concepts, definitions, service availability, and awareness of ASDs in both the lay and professional public [32]. While a recent review of epidemiologic surveys does not support

differences in prevalence across geographic regions or variability based on ethnicity or socioeconomic factors, the paucity of comprehensive datasets from low-income countries impacts the ability to detect these effects [33]. Consequently, investigations of any disproportionate impact of environmental factors on prevalence relating to specific regions are difficult to characterize and the global burden of ASD is difficult to quantify.

The etiology of ASD is commonly described as a genetic predisposition combined with an environmental impact [34]. The body of research identifying genetic deletions and duplications, inherited and *de novo*, and rare and common variants in ASD is expansive. Evidence for genetic variants in the etiology of ASD includes genes involved in intellectual disability and neuropsychiatric disorder, common pathway genes and ASD-risk genes, multigenic contributions from rare or common variations, DNA mutations, and environmental effects on gene expression and/or protein function [35]. Rare genetic risk factors, including those resulting in ASD-related syndromes (e.g. Fragile X), chromosomal abnormalities, and penetrant genes are estimated to contribute to ~20% of ASDs [35]. At least 5% of non-syndromic, idiopathic, and primarily simplex ASD are caused by *de novo* copy-number variants [36]. It is estimated that 400–1000 genes are likely to lead to a susceptibility to autism [37, 38]. Genetic influences are thought to converge on a smaller number of key pathways and developmental stages of the brain [39]. Despite the extensive research in this field, the genetic etiology for at least 70% of cases of ASD remains unknown [36]. Pre-, neo-, and post-natal environmental risk factors have also been implicated [40, 41]. For example, deficits in social interaction and language and the presence of restricted and stereotyped patterns of behavior have all been demonstrated in a mouse model of maternal infection, considered a prenatal environmental risk factor for autism [42]. Decreased levels of neurotrophic factors, which support the growth, survival, and differentiation of developing and mature neurons, have been identified as an environment risk in the neonatal period [43]. In addition, during the postnatal period, it has been proposed that a vulnerable physiology may be particularly susceptible to environmental influences [44], such as the burden of organic pollutants which has been found to be associated with the severity of autism-related symptoms [45]. It is also thought that gene-environment interactions may be involved in the etiology of ASD, although the evidence to date is derived predominantly from animal models [46].

Symptomatology, Clinical Presentation, and Severity

The symptomatology of ASD is extensive and pervasive with a variable onset that could be considered a dimensional process [47]. While ASD is considered a lifelong condition [48], there are a range of prognoses with the recent identification of an optimal outcome whereby children previously diagnosed with an ASD were no longer considered to meet the diagnostic criteria [49]. The identification of this outcome challenges the concept that ASD phenotypes are stable and insensitive to treatment and suggests that developmental trajectories can diverge significantly [50]. The classification of ASD severity is based on the required levels of support to assist with impairments in social communication and social interaction, and restricted, repetitive patterns of behavior, interests, or activities (APA 2013) (Tables 1 and 2). However, there are concerns that conceptualizations of severity based on required levels of support could result in inconsistencies

Table 2 Current severity specifiers for Autism Spectrum Disorder

Severity level	Social communication	Restricted, repetitive behaviors
Level 3 Requiring very substantial support	Severe deficits in verbal and nonverbal social communication skills cause severe impairments in functioning, very limited initiation of social interactions, and minimal response to social overtures from others	Inflexibility of behavior, extreme difficulty coping with change, or other restricted/repetitive behaviors markedly interfere with functioning in all spheres. Great distress/difficulty changing focus or action
Level 2 Requiring substantial support	Marked deficits in verbal and nonverbal social communication skills; social impairments apparent even with supports in place; limited initiation of social interactions; and reduced or abnormal responses to social overtures from others	Inflexibility of behavior, difficulty coping with change, or other restricted/repetitive behaviors appear frequently enough to be obvious to the casual observer and interfere with functioning in a variety of contexts Distress and/or difficulty changing focus or action.
Level 1 Requiring support	Without supports in place, deficits in social communication cause noticeable impairments. Difficulty initiating social interactions, and clear examples of atypical or unsuccessful responses to social overtures of others. May appear to have decreased interest in social interactions	Inflexibility of behavior causes significant interference with functioning in one or more contexts Difficulty switching between activities. Problems of organization and planning hamper independence

when there are mixed levels of impairment across cognitive, adaptive, and autism-related symptoms and result in site-specific applications of ASD categories [51]. Symptoms associated with ASD range from slight to profound impairment where deficits can impair all daily living functions. The severity of symptoms increases when demands in certain environments exceed the individual's capacity to function at a required level. The spectrum of need in terms of supports and services can be vast, with the ability to function across skill areas required for daily living and across the lifespan often independent of the severity of autistic symptoms. The difficulties associated with the accurate assessment of functioning, an important factor in understanding the impact of severity on outcomes, is currently being addressed with the development of the International Classification of Functioning, Disability and Health core sets for ASD [52]. The core set is a shortlist of categories selected to encompass aspects of functioning most relevant when describing a person with ASD.

Interestingly, the onset of ASD symptoms has been a focus of research that has identified an early onset pattern and a regressive onset pattern in which children appear to develop typically before losing skills and developing autism-like symptoms [53]. However, in-depth review of these conceptualizations concludes that the onset of ASD, or symptom emergence, is better considered a dimensional process and a continuum in which the early onset and regression patterns describe two extremes [47].

Heterogeneity

Heterogeneity in etiology, phenotype, and outcome are hallmarks of ASD. These factors contribute to a clinical heterogeneity which manifest as diverse deficits or impairments in behavioral features and communicative functioning. The marked heterogeneity of ASDs has led to suggestions that rather than a single disorder, it could be constructive to reframe ASDs as 'the autisms', thereby giving consideration to multiple etiologies and distinct clinical entities [54]. The heterogeneity of clinical entities is in part a function of the multiple genes involved, the myriad of environmental factors impacting the developmental course of symptom expression, and the co-occurrence of medical and mental health dysfunctions in ASDs. Heterogeneity complicates the quest for personalized medicine in ASD. Three factors contributing to the heterogeneity of ASD, genetic variability, comorbidity, and gender, are now considered.

Genetic Variation in ASD

Genetic variability is considered a major contributor to the heterogeneity of ASD. High-throughput genomic methods are rapidly increasing the pool of ASD genes and in doing so expanding the genetic variability associated with ASD heterogeneity [55]. Large datasets have not identified significant genome-wide associations with specific common variants, and associated analyses suggest that common variants exert weak effects on the risk for ASD [56]. The genetic architecture in ASD varies substantially, from a single penetrant mutation being enough to cause ASD, to an accumulation of over one thousand low-risk alleles [57]. Rare variants affecting ASD risk collectively encompass hundreds of genes [58], while copy-number variant data and *de novo* protein-altering mutations suggest extreme locus heterogeneity [59]. Furthermore, the combined effect of common low-impact genetic variants has also been associated with ASD [60]. Large numbers of genes implicated in ASD are thought to converge on common pathways affecting neuronal and synaptic homeostasis [61], and play critical roles in fundamental developmental pathways [39, 59]. For example, mutation of a single copy of SHANK3, a synaptic scaffolding protein, has been associated with language and social communication impairment in individuals with ASD [62]. In contrast, pleiotropic effects have been identified whereby the same deleterious genetic variant increases the risk for ASD and other neuropsychiatric syndromes [63, 64]. Finally, findings from pathway network analyses of gene ontologies suggest that, in addition to contributing to the core features of ASD, associated genes may contribute to vulnerabilities in important molecular mechanisms leading to multiple systemic comorbidities that also overlap with other conditions [65].

Comorbidity in ASD

Characterizing the heterogeneity of ASD is further complicated by the occurrence of comorbidities. A recent study described comorbidities of $> 14{,}000$ participants with an ASD and highlighted the burden of comorbidity across multiple health care systems [66]. Comorbid psychopathologies significantly over-represented in ASD include anxiety [67], depression [68], ADHD [69], and intellectual disability [5, 7]; and medical comorbidities include seizures [70], sleep difficulties [71], gastrointestinal disorders [72], mitochondrial dysfunction [73], and immune system abnormalities [74].

The presence of one or more of these comorbidities is likely to be associated with more severe autism-related symptoms. For example, 11%–39% of individuals with ASD also have epilepsy and these individuals are more likely to have severe social impairments than those diagnosed with ASD only [75]. Comorbid sleep disturbance is indicated in 50%–80% of children with ASD and is correlated with daytime problem behaviors [76, 77].

Furthermore, sleep problems exacerbate the severity of core ASD symptoms [78, 79] and sleep disturbance is associated with behavioral dysregulation in children with ASD [80]. Aberrant behaviors are correlated with gastrointestinal problems in young children with ASD [81], and markers of mitochondrial dysfunction are significantly correlated with autism severity [73]. The role of immune system abnormalities in ASD is a significant focus of ongoing research. Altered immunity involving cytokines, immunoglobulins, inflammation, cellular activation, and autoimmunity have all been implicated in ASD [82]. Furthermore, altered levels of cytokines have been associated with the severity of behavioral impairments [83–85]. There is limited characterization of these associations between comorbidities in general and the severity of autism-related symptoms due to the complex nature of these relationships. For example, it has been proposed that precise characterization of the immune system's role in the biology of autism requires an understanding of whether these relationships underlie the pathophysiology of ASD in a causative way, whether they create vulnerabilities to other causative factors such as pathogens, or whether a third factor underlies the pathology of ASD and the aberrant immune response in ASD [86]. Improved characterization of comorbidities is imperative for the development of a comprehensive understanding of ASD heterogeneity and may lead to the identification of distinct subgroups of ASD and subgroup-specific treatments [87].

Gender

The male bias in ASD prevalence is most frequently reported as 4 males diagnosed to every 1 female [32, 88]. Intellectual functioning and sex-differential genetic and hormonal factors may modify this ratio [88]. Many theories have been proposed to explain the gender distribution, including the "extreme male brain" theory [89, 90]. The basis of this theory is that a normal male cognitive profile encompasses individuals who are better at systemizing (the drive to analyze or construct systems) than empathizing, and that autism can be considered an extreme of the normal male profile. A potential mechanism for this theory is an elevation of fetal sex steroids, which is supported by a recent study reporting that amniotic fluid steroid hormones were elevated in males who later were diagnosed with ASD [91]. However, in recent times a 'female protective model' has been proposed based on genetic studies. For example, a recent DNA study showed that girls display resilience to genetic insults in that they are more likely to have more extreme neurodevelopmentally related genetic mutations, including both copy-number variants and single-nucleotide variants, than males presenting with the same symptoms [92]. An alternative perspective is that females are underidentified and there may be a gender bias in the diagnostic criteria [93]. A large-scale study has found that females had greater impairments than males, presenting with more social communication and interaction symptoms, lower cognitive and language abilities, poorer adaptive function, and increased externalizing behavior and irritability, suggesting that females require more severe symptoms to be diagnosed as ASD [94]. However, females with ASD have been identified as having fewer repetitive behaviors than males [95], but equivalent impairments in social and communication skills [96].

There is an increased risk of ASD for a child with an older sibling who has been diagnosed with the condition. Previous investigations have estimated the recurrence risk to be between 3% and 18.7% [97]. Predictors of an ASD diagnosis in a younger sibling include male gender of the infant and the number of affected older siblings. In a large sample using a prospective design, the recurrence rate for multiplex families has been reported at 32.2% [97]. In a more recent and larger retrospective study, a 14-fold increase in ASD risk in younger siblings was found to be comparable across gestational age at birth and the child's ethnicity, with the risk higher for younger boys regardless of the gender of the older sibling with ASD [98]. A higher recurrence risk has been identified in families with at least one affected female proband compared to families with only male probands, suggesting female protective mechanisms may be operating in families with high genetic recurrence risk rates [99].

Treatment Options in ASD

Despite significant economic and societal costs, there are limited treatment options to ameliorate the symptoms associated with ASDs, including both symptoms related to diagnostic criteria and those that are considered to be a function of comorbid mental and medical conditions known to exacerbate the severity of presentation. While there are promising indications for new medical treatments for autism [100], a recent systematic review found that while many children with ASDs are treated with medical interventions, there is minimal evidence to support the benefit of most treatments [101]. There are numerous challenges for the identification of effective treatments for ASD. Systematic reviews highlight the possibility that genetic, environmental, cognitive, and social heterogeneity in the ASD phenotype produce highly variable study samples which reduce the potential effect size of an intervention [102]. Other factors contributing to the difficulties in identifying efficacious treatments include small sample sizes, the lack of significantly impaired study participants and the use of outcome measures that are not

uniformly adopted or used as intended [102]. Cross-cultural differences, including what may be considered deviations from typical behavior in a particular culture but not in another culture, further complicate the quest for treatment options across the ASD population [103]. In addition, up to 30% of child ASD participants may respond to placebo treatments [104], which could contribute to reduced active intervention effect sizes.

Behavioral interventions undertaken early in life, using an intensive delivery format, are considered the current gold-standard treatment for behavioral symptoms associated with ASDs [105]. However, methodologically weak studies with few participants and short-term follow-ups are common in this field [106, 107]. Furthermore, early intensive behavioral interventions are expensive to implement and require extensive resources to execute effectively, making them inaccessible for many children with ASD and their families. Alternatively, only two pharmaceuticals are approved by the US Food and Drug Administration (FDA), risperidone and aripiprazole, for the treatment of symptoms associated with ASD. Risperidone, an adult antipsychotic, was approved in 2006 for the symptomatic treatment of irritability, including aggression, deliberate self-injury, and tantrums, in autistic children and adolescents. Risperidone, which acts by blocking the brain's receptors for dopamine and serotonin, was found to be safe and effective for short-term treatment, with improvements observed in stereotypic behavior and hyperactivity [108]. However, significant side-effects are associated with risperidone use, including weight gain from increased appetite, drowsiness, and increased levels of the hormone prolactin, which is produced by the pituitary gland and which can have a feminizing effect on both females and males [109]. The frequency of side-effects appears to be dose-related [110], and while weight gain is common, somnolence more significantly influences the discontinuation of treatment [111]. In 2009, following evaluation of short-term efficacy and safety, the FDA also approved aripiprazole, a third-generation atypical antipsychotic, for the treatment of irritability associated with ASD in children and adolescents [112, 113]. Adverse events include sedation, fatigue, vomiting, increased appetite, somnolence, and tremor [114], with discontinuation commonly due to aggression and weight gain [112]. Aripiprazole is known as a dopamine system stabilizer and is less likely to elevate serum prolactin levels and induce extrapyramidal symptoms than risperidone [115].

The heterogeneity of ASD has implications for the assessment of treatment efficacy [116]. The design of treatment trials would benefit from the selection of treatment subgroups that maximize homogeneity in ways that improve the detection of efficacious interventions [87]. An improved understanding of the biological basis of the inherent heterogeneity in ASD is crucial in order to facilitate the identification of well-characterized subgroups. Investigation of underlying medical and psychopathological comorbidities associated with ASD such as immune system aberrations [82], mitochondrial dysfunction [73], gastrointestinal dysfunction [72], sleep disorders [117, 118], epilepsy [119], depression, and anxiety [120] may provide a means of characterizing the heterogeneity of ASD.

The treatment response in randomized controlled trials for ASDs continues to be primarily based on the observation of clinically relevant behaviors. Focusing entirely on behaviorally-defined diagnostic criteria and response to treatment risks a two-dimensional phenomenological approach to ASDs. The multi-dimensional aspects of presentation are increasingly recognized as aberrations in the biological pathways at the molecular and cellular levels, with alterations in circuitry linked to behavior [121, 122]. Furthermore, the limitations of the clinician paradigm as a standard for the diagnosis of ASD [24] support a move beyond this historical model to one increasingly guided by biological measurement [123]. Concurrently, there has been a greater focus on the importance of objective rather than subjective indicators of response, such as biomarkers, and the possibility of biological signatures contributing to the definition of ASD subgroups [123, 124]. Genomics, neuroimaging, and pathophysiological markers relating to mitochondrial function, oxidative stress, and immune function all offer potential as biomarkers to reduce the diagnostic heterogeneity and improve the prediction of treatment response [125].

The initiation of the Research Domain Criteria project by the National Institute of Mental Health also supports this paradigm-shift towards a diagnostic system founded on a deeper understanding of the biological and psychosocial bases of psychiatric disorders, and the requirement for research across multiple units of analysis including genes, neural circuits, and behavior [126]. Whilst this objective is still in the development phase it represents a shift towards precision or personalized medicine based on etiology and pathophysiology, which will hopefully ultimately parse out the issues contributing to heterogeneity. A precision or personalized medicine approach recognizes the importance of aligning treatment and support, care, and services to individual needs and outcomes. Individual outcomes drive community outcomes which drive societal outcomes.

Conclusions

A concerning issue in ASD research, particularly for people with ASD and their carers requiring support, is the paucity of approved evidence-based treatment options

available to ameliorate the core and associated symptoms of ASD. While the hallmark heterogeneity of ASDs may be a major contributing factor, it should not impede an understanding of ASD subgroups, associated markers of pathological states, and cross-cultural factors that are imperative to advancing this field of research. The diagnosis of ASDs continues to be entirely based on the observation of behaviors, or what is externally visible. However, there is now a greater recognition of complex symptomatology including medical and mental health comorbidities, due to the recent identification of relationships between comorbidities and the severity of autism-related symptoms. The identification of objective rather than subjective measures of response, such as biomarkers, and the possibility of biological signatures contributing to the definition of subgroups of ASDs will advance the quest for personalized medicine and treatment models in this highly heterogeneous population.

Acknowledgements We acknowledge a NHMRC career development fellowship (APP1061922) and a Project Grant (1043664) to Adam J. Guastella.

References

1. APA. Diagnostic and Statistical Manual of Mental Disorders. Fifth Edition. Washington, DC: American Psychiatric Association, 2013.
2. Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet 2008, 9: 341–355.
3. Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. Trends Neurosci 2008, 31: 137–145.
4. Geschwind DH. Advances in autism. Annu Rev Med 2009, 60: 367–380.
5. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. J Am Acad Child Adolesc Psychiatry 2008, 47: 921–929.
6. Mannion A, Leader G. An investigation of comorbid psychological disorders, sleep problems, gastrointestinal symptoms and epilepsy in children and adolescents with autism spectrum disorder: A two year follow-up. Res Autism Spectr Disord 2016, 22: 20–33.
7. Matson JL, Shoemaker M. Intellectual disability and its relationship to autism spectrum disorders. Res Dev Disabil 2009, 30: 1107–1114.
8. Bauman ML. Medical comorbidities in autism: Challenges to diagnosis and treatment. Neurotherapeutics 2013, 7: 320–327.
9. Mannion A, Leader G. Comorbidity in autism spectrum disorder: A literature review. Res Autism Spectr Disord 2013b, 7: 1595–1616.
10. Zerbo O, Leong A, Barcellos L, Bernal P, Fireman B, Croen LA. Immune mediated conditions in autism spectrum disorders. Brain Behav Immun 2015, 46: 232–236.
11. Buescher AV, Cidav Z, Knapp M, Mandell DS. Costs of autism spectrum disorders in the United Kingdom and the United States. JAMA Pediatrics 2014, 168: 721–728.
12. Ganz ML. The lifetime distribution of the incremental societal costs of autism. Arch Pediatr Adolesc Med 2007, 161: 343–349.
13. Leigh JP, Du J. Brief report: Forecasting the economic burden of autism in 2015 and 2025 in the United States. J Autism Dev Disord 2015, 45: 4135–4139.
14. Kanner L. Autistic disturbances of affective contact. Nervous Child 1943, 2: 217–250.
15. Asperger H. Die Autistischen Psychopathen im Kindesalter. Arch Psychiatr Nervenkr 1944, 117: 76–136. Trans. U. FRITH in U. FRITH (ed.). Autism and Asperger Syndrome. Cambridge: Cambridge University Press, 1991: 37–92.
16. Silberman S. NeuroTribes: The Legacy of Autism and How to Think Smarter About People Who Think Differently. Allen & Unwin, 2015.
17. Wing L. Asperger's syndrome: a clinical account. Psychol Med 1981, 11: 115–129.
18. APA. Diagnostic and Statistical Manual of Mental Disorders. Third Edition. Washington, DC: American Psychiatric Association, 1980.
19. Volkmar FR, Cohen DJ, Paul R. An evaluation of DSM-III criteria for infantile autism. J Am Acad Child Psychiatry 1986, 25: 190–197.
20. Volkmar F, Bregman J, Cohen D, Cicchetti D. DSM-III and DSM-III-R diagnoses of autism. Am J Psychiatry 1988, 145: 1404–1408.
21. APA. Diagnostic and Statistical Manual of Mental Disorders. Fourth Edition. Washington, DC: American Psychiatric Association, 1994.
22. Volkmar FR, Reichow B. Autism in DSM-5: progress and challenges. Mol Autism 2013, 4: 13.
23. Kim YS, Fombonne E, Koh YJ, Kim SJ, Cheon KA, Leventhal BL. A comparison of DSM-IV pervasive developmental disorder and DSM-5 autism spectrum disorder prevalence in an epidemiologic sample. J Am Acad Child Adolesc Psychiatry 2014, 53: 500–508.
24. Lord C, Petkova E, Hus V, Gan W, Lu F, Martin D, *et al.* A multisite study of the clinical diagnosis of different autism spectrum disorders. Arch Gen Psychiatry 2012, 69: 306–313.
25. Bernier R, Mao A, Yen J. Psychopathology, families, and culture: autism. Child Adolesc Psychiatr Clin N Am 2010, 19: 855–867.
26. Daley TC, Sigman MD. Diagnostic conceptualization of autism among Indian psychiatrists, psychologists, and pediatricians. J Autism Dev Disord 2002, 32: 13–23.
27. Daley TC. The need for cross-cultural research on the pervasive developmental disorders. Transcult Psychiatry 2002, 39: 531–550.
28. Sun X, Allison C. A review of the prevalence of autism spectrum disorder in Asia. Res Autism Spectr Disord 2010, 4: 156–167.
29. Rutter M. Incidence of autism spectrum disorders: changes over time and their meaning. Acta Paediatr 2005, 94: 2–15.
30. CDC. Prevalence of autism spectrum disorder among children aged 8 years–autism and developmental disabilities monitoring network, 11 sites, United States, 2010. Morbidity and Mortality Weekly Report: Surveillance Summaries 2014, 63: 1–14.
31. Fombonne E. Epidemiology of autistic disorder and other pervasive developmental disorders. J Clin Psychiatry 2005, 66 Suppl 10: 3–8.
32. Fombonne E. Epidemiology of pervasive developmental disorders. Pediatr Res 2009, 65: 591–598.

33. Elsabbagh M, Divan G, Koh YJ, Kim YS, Kauchali S, Marcín C, *et al.* Global prevalence of autism and other pervasive developmental disorders. Autism Res 2012, 5: 160–179.
34. Newschaffer CJ, Croen LA, Daniels J, Giarelli E, Grether JK, Levy SE, *et al.* The epidemiology of autism spectrum disorders. Annu Rev Public Health 2007, 28: 235–258.
35. Devlin B, Scherer SW. Genetic architecture in autism spectrum disorder. Curr Opin Genet Devel 2012, 22: 229–237.
36. Schaaf Christian P, Zoghbi Huda Y. Solving the autism puzzle a few pieces at a time. Neuron 2011, 70: 806–808.
37. Ronemus M, Iossifov I, Levy D, Wigler M. The role of de novo mutations in the genetics of autism spectrum disorders. Nat Rev Genet 2014, 15: 133–141.
38. He X, Sanders SJ, Liu L, De Rubeis S, Lim ET, Sutcliffe JS, *et al.* Integrated model of de novo and inherited genetic variants yields greater power to identify risk genes. PLoS Genetics 2013, 9: e1003671.
39. Krishnan A, Zhang R, Yao V, Theesfeld CL, Wong AK, Tadych A, *et al.* Genome-wide prediction and functional characterization of the genetic basis of autism spectrum disorder. Nat Neurosci 2016, 19: 1454–1462.
40. Patterson PH. Maternal infection and immune involvement in autism. Trends Mol Med 2011, 17: 389–394.
41. Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. Pediatrics 2011, 128: 344–355.
42. Malkova NV, Yu CZ, Hsiao EY, Moore MJ, Patterson PH. Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. Brain Behav Immun 2012, 26: 607–616.
43. Abdallah MW, Mortensen EL, Greaves-Lord K, Larsen N, Bonefeld-Jorgensen EC, Norgaard-Pedersen B, *et al.* Neonatal levels of neurotrophic factors and risk of autism spectrum disorders. Acta Psychiatr Scand 2013, 128: 61–69.
44. Herbert MR. Contributions of the environment and environmentally vulnerable physiology to autism spectrum disorders. Curr Opin Neurol 2010, 23: 103–110.
45. Boggess A, Faber S, Kern J, Kingston HM. Mean serum-level of common organic pollutants is predictive of behavioral severity in children with autism spectrum disorders. Sci Rep 2016, 6: 26185.
46. Chaste P, Leboyer M. Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin Neurosci 2012, 14: 281–292.
47. Ozonoff S, Heung K, Byrd R, Hansen R, Hertz-Picciotto I. The onset of autism: patterns of symptom emergence in the first years of life. Autism Res 2008, 1: 320–328.
48. Howlin P, Goode S, Hutton J, Rutter M. Adult outcome for children with autism. J Child Psychol Psychiatry 2004, 45: 212–229.
49. Fein D, Barton M, Eigsti IM, Kelley E, Naigles L, Schultz RT, *et al.* Optimal outcome in individuals with a history of autism. J Child Psychol Psychiatry 2013, 54: 195–205.
50. Szatmari P, Georgiades S, Duku E, Bennett TA, Bryson S, Fombonne E *et al.* Developmental trajectories of symptom severity and adaptive functioning in an inception cohort of preschool children with autism spectrum disorder. JAMA Psychiatry 2015, 72: 276–283.
51. Weitlauf AS, Gotham KO, Vehorn AC, Warren ZE. Brief Report: DSM-5 "Levels of Support:" a comment on discrepant conceptualizations of severity in ASD. J Autism Dev Disord 2014, 44: 471–476.
52. Bolte S, de Schipper E, Robison JE, Wong VC, Selb M, Singhal N, *et al.* Classification of functioning and impairment: the development of ICF core sets for autism spectrum disorder. Autism Res 2014, 7: 167–172.
53. Volkmar FR, Cohen DJ. Disintegrative disorder or "late onset" autism. J Child Psychol Psychiatry 1989, 30: 717–724.
54. Geschwind DH, Levitt P. Autism spectrum disorders: developmental disconnection syndromes. Curr Opin Neurobiol 2007, 17: 103–111.
55. Willsey AJ, State MW. Autism spectrum disorders: from genes to neurobiology. Curr Opin Neurobiol 2015, 30: 92–99.
56. Anney R, Klei L, Pinto D, Almeida J, Bacchelli E, Baird G, *et al.* Individual common variants exert weak effects on the risk for autism spectrum disorders. Hum Mol Genet 2012, 21: 4781–4792.
57. Bourgeron T. From the genetic architecture to synaptic plasticity in autism spectrum disorder. Nat Rev Neurosci 2015, 16: 551–563.
58. Pinto D, Delaby E, Merico D, Barbosa M, Merikangas A, Klei L, *et al.* Convergence of genes and cellular pathways dysregulated in autism spectrum disorders. Am J Hum Genet 2014, 94: 677–694.
59. O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, *et al.* Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 2012, 485: 246–250.
60. Klei L, Sanders SJ, Murtha MT, Hus V, Lowe JK, Willsey AJ, *et al.* Common genetic variants, acting additively, are a major source of risk for autism. Mol Autism 2012, 3: 9.
61. Huguet G, Ey E, Bourgeron T. The genetic landscapes of autism spectrum disorders. Ann Rev Genomics Hum Genet 2013, 14: 191–213.
62. Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, *et al.* Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet 2007, 39: 25–27.
63. Kim YS, State MW. Recent challenges to the psychiatric diagnostic nosology: a focus on the genetics and genomics of neurodevelopmental disorders. Int J Epidemiol 2014, 43: 465–475.
64. Geschwind DH. Genetics of autism spectrum disorders. Trends Cogn Sci 2011, 15: 409–416.
65. Wen Y, Alshikho MJ, Herbert MR. Pathway network analyses for autism reveal multisystem involvement, major overlaps with other diseases and convergence upon MAPK and calcium signaling. PLoS One 2016, 11: e0153329.
66. Kohane IS, McMurry A, Weber G, MacFadden D, Rappaport L, Kunkel L, *et al.* The co-morbidity burden of children and young adults with autism spectrum disorders. PLoS One 2012, 7: e33224.
67. White SW, Oswald D, Ollendick T, Scahill L. Anxiety in children and adolescents with autism spectrum disorders. Clin Psychol Rev 2009, 29: 216–229.
68. Magnuson KM, Constantino JN. Characterization of depression in children with autism spectrum disorders. J Dev Behav Pediatr 2011, 32: 332–340.
69. Ghanizadeh A. Co-morbidity and factor analysis on attention deficit hyperactivity disorder and autism spectrum disorder DSM-IV-derived items. J Res Med Sci 2012, 17: 368–372.
70. Tuchman R, Cuccaro M, Alessandri M. Autism and epilepsy: historical perspective. Brain Dev 2010, 32: 709–718.
71. Goldman SE, Surdyka K, Cuevas R, Adkins K, Wang L, Malow BA. Defining the sleep phenotype in children with autism. Dev Neuropsychol 2009, 34: 560–573.
72. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. Pediatrics 2014, 133: 872–883.
73. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. Mol Psychiatry 2012, 17: 290–314.
74. Ashwood P, Wills S, Van de Water J. The immune response in autism: a new frontier for autism research. J Leukoc Biol 2006, 80: 1–15.

75. Ko C, Kim N, Kim E, Song DH, Cheon KA. The effect of epilepsy on autistic symptom severity assessed by the social responsiveness scale in children with autism spectrum disorder. Behav Brain Funct 2016, 12: 20.
76. Goldman SE, McGrew S, Johnson KP, Richdale AL, Clemons T, Malow BA. Sleep is associated with problem behaviors in children and adolescents with autism spectrum disorders. Res Autism Spectr Disord 2011, 5: 1223–1229.
77. Sikora DM, Johnson K, Clemons T, Katz T. The relationship between sleep problems and daytime behavior in children of different ages with autism spectrum disorders. Pediatrics 2012, 130: S83–90.
78. Cohen S, Conduit R, Lockley SW, Rajaratnam SMW, Cornish KM. The relationship between sleep and behavior in autism spectrum disorder (ASD): a review. J Neurodev Disord 2014, 6: 44.
79. Schreck KA, Mulick JA, Smith AF. Sleep problems as possible predictors of intensified symptoms of autism. Res Dev Disabil 2004, 25: 57–66.
80. Mazurek MO, Sohl K. Sleep and behavioral problems in children with autism spectrum disorder. J Autism Dev Disord 2016, 46: 1906–1915.
81. Chaidez V, Hansen RL, Hertz-Picciotto I. Gastrointestinal problems in children with autism, developmental delays or typical development. J Autism Dev Disord 2014, 44: 1117–1127.
82. Goines P, Van de Water J. The immune system's role in the biology of autism. Curr Opin Neurol 2010, 23: 111–117.
83. Ashwood P, Enstrom A, Krakowiak P, Hertz-Picciotto I, Hansen RL, Croen LA, *et al.* Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes. J Neuroimmunol 2008, 204: 149–153.
84. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 2011a, 25: 40–45.
85. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Wate J. Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. J Neuroimmunol 2011b, 232: 196–199.
86. Gesundheit B, Rosenzweig JP, Naor D, Lerer B, Zachor DA, Procházka V, *et al.* Immunological and autoimmune considerations of autism spectrum disorders. J Autoimmun 2013, 44: 1–7.
87. Ousley O, Cermak T. Autism spectrum disorder: defining dimensions and subgroups. Curr Dev Disord Rep 2014, 1: 20–28.
88. Werling DM, Geschwind DH. Sex differences in autism spectrum disorders. Curr Opin Neurol 2013, 26: 146–153.
89. Baron-Cohen S. The extreme male brain theory of autism. Trends Cogn Sci 2002, 6: 248–254.
90. Baron-Cohen S, Knickmeyer RC, Belmonte MK. Sex differences in the brain: implications for explaining autism. Science 2005, 310: 819–823.
91. Baron-Cohen S, Auyeung B, Norgaard-Pedersen B, Hougaard DM, Abdallah MW, Melgaard L, *et al.* Elevated fetal steroidogenic activity in autism. Mol Psychiatry 2015, 20: 369–376.
92. Jacquemont S, Coe BP, Hersch M, Duyzend MH, Krumm N, Bergmann S, *et al.* A higher mutational burden in females supports a "female protective model" in neurodevelopmental disorders. Am J Hum Genet 2014, 94: 415–425.
93. Dworzynski K, Ronald A, Bolton P, Happe F. How different are girls and boys above and below the diagnostic threshold for autism spectrum disorders? J Am Acad Child Adolesc Psychiatry 2012, 51: 788–797.
94. Frazier TW, Georgiades S, Bishop SL, Hardan AY. Behavioral and cognitive characteristics of females and males with autism in the Simons Simplex Collection. J Am Acad Child Adolesc Psychiatry 2014, 53: 329–340.e321–323.
95. Szatmari P, Liu XQ, Goldberg J, Zwaigenbaum L, Paterson AD, Woodbury-Smith M, *et al.* Sex differences in repetitive stereotyped behaviors in autism: implications for genetic liability. Am J Med Genet B Neuropsychiatr Genet 2012, 159b: 5–12.
96. Mandy W, Chilvers R, Chowdhury U, Salter G, Seigal A, Skuse D. Sex differences in autism spectrum disorder: evidence from a large sample of children and adolescents. J. Autism Dev Disord 2012, 42: 1304–1313.
97. Ozonoff S, Young GS, Carter A, Messinger D, Yirmiya N, Zwaigenbaum L, *et al.* Recurrence risk for autism spectrum disorders: a Baby Siblings Research Consortium study. Pediatrics 2011, 128: e488–495.
98. Xie F, Peltier M, Getahun D. Is the risk of autism in younger siblings of affected children moderated by sex, race/ethnicity, or gestational age? J Dev Behav Pediatr 2016, 37: 603–609.
99. Werling DM, Geschwind DH. Recurrence rates provide evidence for sex-differential, familial genetic liability for autism spectrum disorders in multiplex families and twins. Mol Autism 2015, 6: 27.
100. Yatawara CJ, Einfeld SL, Hickie IB, Davenport TA, Guastella AJ. The effect of oxytocin nasal spray on social interaction deficits observed in young children with autism: a randomized clinical crossover trial. Mol Psychiatry 2016, 21: 1225–1231.
101. McPheeters ML, Warren Z, Sathe N, Bruzek JL, Krishnaswami S, Jerome RN, *et al.* A systematic review of medical treatments for children with autism spectrum disorders. Pediatrics 2011, 127: e1312–e1321.
102. Siegel M, Beaulieu AA. Psychotropic medications in children with autism spectrum disorders: a systematic review and synthesis for evidence-based practice. J Autism Dev Disord 2012, 42: 1592–1605.
103. Freeth M, Sheppard E, Ramachandran R, Milne E. A cross-cultural comparison of autistic traits in the UK, India and Malaysia. J Autism Dev Disord 2013, 43: 2569–2583.
104. King BH, Wright D, Handen BL, Sikich L, Zimmerman AW, McMahon W, *et al.* Double-blind, placebo-controlled study of amantadine hydrochloride in the treatment of children with autistic disorder. J Am Acad Child Adolesc Psychiatry 2001, 40: 658–665.
105. Eldevik S, Hastings RP, Hughes JC, Jahr E, Eikeseth S, Cross S. Meta-analysis of Early Intensive Behavioral Intervention for children with autism. J Clin Child Adolesc Psychol 2009, 38: 439–450.
106. Ospina MB, Krebs Seida J, Clark B, Karkhaneh M, Hartling L, Tjosvold L, *et al.* Behavioural and developmental interventions for autism spectrum disorder: a clinical systematic review. PLoS One 2008, 3: e3755.
107. Spreckley M, Boyd R. Efficacy of applied behavioral intervention in preschool children with autism for improving cognitive, language, and adaptive behavior: a systematic review and meta-analysis. J Pediatr 2009, 154: 338–344.
108. McCracken JT, McGough J, Shah B, Cronin P, Hong D, Aman MG, *et al.* Risperidone in children with autism and serious behavioral problems. N Engl J Med 2002, 347: 314–321.
109. Anderson GM, Scahill L, McCracken JT, McDougle CJ, Aman MG, Tierney E, *et al.* Effects of short-and long-term risperidone treatment on prolactin levels in children with autism. Biol Psychiatry 2007, 61: 545–550.
110. Kent J, Kushner S, Ning X, Karcher K, Ness S, Aman M, *et al.* Risperidone dosing in children and adolescents with autistic

disorder: a double-blind, placebo-controlled study. J Autism Dev Disord 2013, 43: 1773–1783.
111. Lemmon ME, Gregas M, Jeste SS. Risperidone use in autism spectrum disorders: a retrospective review of a clinic-referred patient population. J Child Neurol 2011, 26: 428–432.
112. Marcus RN, Owen R, Kamen L, Manos G, McQuade RD, Carson WH, *et al.* A placebo-controlled, fixed-dose study of aripiprazole in children and adolescents with irritability associated with autistic disorder. J Am Acad Child Adolesc Psychiatry 2009, 48: 1110–1119.
113. Owen R, Sikich L, Marcus RN, Corey-Lisle P, Manos G, McQuade RD, *et al.* Aripiprazole in the treatment of irritability in children and adolescents with autistic disorder. Pediatrics 2009, 124: 1533–1540.
114. Robb AS, Andersson C, Bellocchio EE, Manos G, Rojas-Fernandez C, Mathew S, *et al.* Safety and tolerability of aripiprazole in the treatment of irritability associated with autistic disorder in pediatric subjects (6–17 years Old): Results from a pooled analysis of 2 studies. Prim Care Companion CNS Disord 2011, 13: e1–e9.
115. Kirino E. Efficacy and safety of aripiprazole in child and adolescent patients. Eur Child Adolesc Psychiatry 2012, 21: 361–368.
116. Rizzo R, Pavone P. Aripiprazole for the treatment of irritability and aggression in children and adolescents affected by autism spectrum disorders. Expert Rev Neurother 2016, 16: 867–874.
117. Krakowiak P, Goodlin-Jones B, Hertz-Picciotto I, Croen LA, Hansen RL. Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study. J Sleep Res 2008, 17: 197–206.
118. Richdale AL, Schreck KA. Sleep problems in autism spectrum disorders: prevalence, nature, & possible biopsychosocial aetiologies. Sleep Med Rev 2009, 13: 403–411.
119. Woolfenden S, Sarkozy V, Ridley G, Coory M, Williams K. A systematic review of two outcomes in autism spectrum disorder–epilepsy and mortality. Dev Med Child Neurol 2012, 54: 306–312.
120. Strang JF, Kenworthy L, Daniolos P, Case L, Wills MC, Martin A, *et al.* Depression and anxiety symptoms in children and adolescents with autism spectrum disorders without intellectual disability. Res Autism Spectr Disord 2012, 6: 406–412.
121. Kim H, Lim CS, Kaang BK. Neuronal mechanisms and circuits underlying repetitive behaviors in mouse models of autism spectrum disorder. Behav Brain Funct 2016, 12: 1–13.
122. Fuccillo MV. Striatal circuits as a common node for autism pathophysiology. Front Neurosci 2016, 10: 27.
123. Constantino JN, Charman T. Diagnosis of autism spectrum disorder: reconciling the syndrome, its diverse origins, and variation in expression. Lancet Neurol 2016, 15: 279–291.
124. Goldani AAS, Downs SR, Widjaja F, Lawton B, Hendren RL. Biomarkers in autism. Front Psychiatry 2014, 5: 100.
125. Bent S, Hendren RL. Improving the prediction of response to therapy in autism. Neurotherapeutics 2010, 7: 232–240.
126. Insel TR. The NIMH Research Domain Criteria (RDoC) Project: precision medicine for psychiatry. Am J Psychiatry 2014, 171: 395–397.

Neurosci. Bull. April, 2017, 33(2):194–204
DOI 10.1007/s12264-017-0103-8

www.neurosci.cn
www.springer.com/12264

REVIEW

The Immune System, Cytokines, and Biomarkers in Autism Spectrum Disorder

Anne Masi[1] · Nicholas Glozier[1] · Russell Dale[2] · Adam J. Guastella[1]

Received: 27 November 2016 / Accepted: 22 January 2017 / Published online: 25 February 2017

Abstract Autism Spectrum Disorder (ASD) is a pervasive neurodevelopmental condition characterized by variable impairments in communication and social interaction as well as restricted interests and repetitive behaviors. Heterogeneity of presentation is a hallmark. Investigations of immune system problems in ASD, including aberrations in cytokine profiles and signaling, have been increasing in recent times and are the subject of ongoing interest. With the aim of establishing whether cytokines have utility as potential biomarkers that may define a subgroup of ASD, or function as an objective measure of response to treatment, this review summarizes the role of the immune system, discusses the relationship between the immune system, the brain, and behavior, and presents previously-identified immune system abnormalities in ASD, specifically addressing the role of cytokines in these aberrations. The roles and identification of biomarkers are also addressed, particularly with respect to cytokine profiles in ASD.

Keywords Autism Spectrum Disorder · Cytokine · Immune system

✉ Adam J. Guastella
adam.guastella@sydney.edu.au

[1] Autism Clinic for Translational Research, Brain and Mind Centre, Central Clinical School, Sydney Medical School, University of Sydney, Sydney, New South Wales 2050, Australia

[2] Childrens Hospital at Westmead Clinical School, University of Sydney, Sydney, New South Wales 2145, Australia

Introduction

Autism Spectrum Disorders (ASDs) are complex, pervasive neurodevelopmental conditions with a largely unknown etiology and a significant male bias. ASDs are behaviorally defined and characterized by deficits in social communication and interaction, and the presence of restricted, repetitive patterns of behavior, interests, or activities [1]. Developmental trajectories and patterns of severity vary substantially, with all facets of daily functioning potentially impacted. The vast clinical heterogeneity is a hallmark. Comorbid psychiatric and medical conditions are frequently reported, including social anxiety disorder, attention deficit disorder, immune system abnormalities, gastrointestinal disorders, mitochondrial dysfunction, sleep disorders, and epilepsy [2–6]. The multifaceted nature of the condition has resulted in investigations aiming to characterize biological subtypes of ASD. However, an understanding of the biological mechanisms driving pathophysiology is evolving. Immune system aberrations, including altered cytokine profiles, are believed to have a role in ASD [7, 8]. Accumulating evidence of alterations in central and peripheral immune system functioning supports the proposal that there is a subgroup of individuals with ASD who have some form of immune system dysregulation [9]. Altered cytokine levels may facilitate the identification of ASD subtypes as well as provide biological markers of the response to effective treatments.

The Immune System

The immune system is a complicated group of defense mechanisms that are triggered in order to protect an organism from disease- or illness-causing pathogens,

which include bacteria, viruses, fungi, and parasites. An antigen, considered foreign by the host's body, is a molecule that stimulates the immune system to produce antibodies which function to identify and neutralize or remove the antigen. Many interconnected organs, molecules, cells, and pathways play roles in a fully functional immune system, which is comprised of two interconnected systems of immunity: innate and adaptive [10]. These two systems work together to protect the body from pathogens.

Innate immunity refers to first-line defense mechanisms that respond to an infection immediately or within hours of the host being attacked by a pathogen. The innate immune response relies on physical barriers, such as the epithelial layers of the skin and mucosal and glandular tissue surfaces connected to the body's openings, as well as chemical barriers, which include soluble antimicrobial proteins and peptides, and an acidic pH. When pathogens breach these barriers, cellular innate immune responses are triggered through a pathogen-recognition process involving an array of cells with cell surface and intracellular receptors. Groups of pathogens present with characteristic pathogen-associated molecular patterns, which are recognized by pattern recognition receptors expressed by many different immune cells [11]. Some cells are activated to phagocytose and degrade the pathogen, a process through which macrophages and neutrophils engulf and destroy extracellular microbes. Cell receptors can also be activated, causing the cells to produce antimicrobial substances that eliminate the pathogens. Other cellular activation processes lead to the production of cytokines and chemokines, which are proteins that recruit cells, molecules, and fluid to sites of infection. This procedure results in the physiological changes known as inflammation [10]. Other effectors of innate immunity are natural killer (NK) cells that, through cytokine production or cytotoxicity, contain infections until an adaptive immune response is initiated.

When innate immunity is insufficient, for example when features of certain pathogens allow them to evade the defense mechanisms of the innate immune system, the adaptive immune response is activated. The adaptive response is initiated a few days after initial exposure to the pathogen and breach of the physical or chemical barriers. This response is more comprehensive and antigen-specific than the innate response. The adaptive immune system is able to recognize, eliminate, and remember pathogens. The innate immune system is not considered to have this memory component. However, demonstrations of NK cell memory in viral infections indicate that these cells have attributes of both innate and adaptive immunity [12]. There are two types of adaptive immune response, both carried out by lymphocytes. The first type of response is an antibody response carried out by B lymphocytes, or B cells, which, when activated by an antigen, secrete antibodies, also known as immunoglobulins. B cells are formed in the bone marrow. The second type of adaptive immune response is a cell-mediated immune response where activated T cells specifically recognize and neutralize or eliminate antigens. T cells mature in the thymus, which is a lymphoid organ of the immune system. Residual B and T cells remaining after antigen exposure function as memory cells that are activated by subsequent pathogen challenges.

The innate and adaptive immune responses discussed above are characteristic of a regulated immune system contributing to the maintenance of homeostasis and preventing disruptions of normal functions of the body. A regulated immune system requires an optimal balance of pro- and anti-inflammatory signaling. Inflammation may not be a problem in isolation, but if an unregulated or dysregulated immune system responds to physiological changes initiated by pathogens, then the inflammation may be problematic. An aberrant immune system may manifest as upregulation of the inflammatory/immune response or as immune deficiency compromising host defense. Allergies, asthma, and autoimmune disorders are all conditions associated with immune dysfunction.

Cytokines and the Immune System

Cytokines are cell-signaling molecules that facilitate communication among cells of both the innate and adaptive immune systems. They are primary regulators of inflammation, coordinating the response to infection and associated immune challenges and are involved in a multitude of biological processes. As part of an integrated network, cytokines stimulate and modulate immune system activity and induce their own synthesis and the synthesis of other cytokines. They are typically soluble molecules although some remain cell-bound. Cytokines can be broadly classified into three groups based on the type of immune response: adaptive immunity, pro-inflammatory signaling, and anti-inflammatory signaling [13]. Chemokines are a subpopulation of cytokines that initiate the recruitment of well-defined leukocyte subsets through chemical stimuli [14]. Cells attracted to the chemokine follow a signal of increasing concentration towards the source of the chemokine, usually an infected or damaged cell. As cell signaling molecules, cytokines bind to receptors on the plasma membrane and elicit effects through the activation of an intracellular signaling cascade. Cytokines can be further classified based on the distance between the cell secreting the signaling ligand and the cell receiving the chemical signal [10]. Endocrine action is when cytokines pass through the bloodstream before reaching the target. Cytokines that act near the secreting cell are paracrine. Autocrine action is when cells can secrete a signal that is

received through its own receptors. Cytokines have been characterized as belonging to one of six groups based on cytokine and cytokine receptor structure, but members may exhibit diverse functionality. The cytokine families and some of the members of each group are detailed in Table 1 [10].

T and B lymphocytes mediate adaptive immunity. However, it is the T helper cells that are required for almost all adaptive immune responses [15]. They help activate B cells to secrete antibodies and macrophages to eliminate pathogens. Naïve T helper cells are differentiated into functional types defined by their pattern of cytokine production and function: Type 1 T helper (Th1), Type 2 T helper (Th2), T-regulator, and Th17 cells [16]. Proliferation and differentiation are functions of the particular cytokine milieu and signaling requirements during T cell receptor activation. Differentiation into either Th1 or Th2 effector cells then determines the nature of the subsequent adaptive immune responses activated by effector cells [17]. Th1 cells produce interferon (IFN)-γ, their signature cytokine and interleukin (IL)-2, while many also produce tumor necrosis factor (TNF)-α. Th2 signature cytokines are IL-4, IL-5, and IL-13, but these cells also make TNF-α, and some produce IL-9 and modest amounts of IL-2 [16]. Immune regulation is thought to require homeostasis between Th1 and Th2 activity [15]. If either Th1 or Th2 dominates, then the other response may be suppressed. Lower proportions of Th1 cells and higher proportions of Th2 cells have been found in children with ASD compared to healthy controls, providing evidence of an imbalance of Th1- and Th2-like cytokines in ASD [18]. In addition, analysis of peripheral blood mononuclear cells from children with ASD showed increased activation of both the Th1 and Th2 arms of the adaptive immune response, with a Th2 dominance and no compensatory increase in expression of the regulatory cytokine IL-10 [19].

Each cytokine can be produced by a single cell type or multiple cell types. For example, Th1 cells produce IFN-γ, IL-2, and TNF-β, while Th2 cells produce IL-4, IL-5, IL-6, IL-9, and IL-10 [20]. However, granulocyte macrophage colony-stimulating factor (GM-CSF) can be produced by multiple cell types, including macrophages, endothelial cells, and fibroblasts [21]. Similarly, IFN-β can be produced by multiple cell types including fibroblasts and epithelial cells. Cytokines can act on a single or multiple cell types. For example, IL-12 acts on Th1 cells, while IL-1 acts on T cells, B cells, macrophages, endothelial cells, fibroblasts, and epithelial cells, and all interferons act on multiple cell types. Cytokines also exhibit redundancy, meaning multiple cytokines exert the same biological

Table 1 Details of six cytokine families and examples of family members.

Cytokine Family	Examples	Characteristics
Interleukin 1 family	IL-1α, IL-β, IL-RA	Induce responses against infection that are primarily pro-inflammatory
		IL-RA antagonizes effects of IL-1α and IL-β
Hematopoietin (Class I cytokine) family	IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-12p40, IL-12p70, IL-13, IL-15, IL-23, GM-CSF, G-CSF	Largest family of cytokines, all members sharing a 4-helix bundle structure
		Sequence and functional diversity
		Diverse effects include proliferation, differentiation, and antibody secretion
Interferon (Class II cytokine) family	IFN-γ, IL-10	Mediate early antiviral responses
		Activate macrophages, interact with cells of the adaptive immune system, and support the generation of Th1 cells
Tumor necrosis factor family	TNF-α, TNF-β1	Expressed in either soluble or membrane-bound form
		Cause apoptosis
Interleukin 17 family	IL-17	Primarily pro-inflammatory
Chemokine family	IL-8, Eotaxin, IP-10 (CXCL10), MCP-1(CCL2), MIP-1α (CCL3), MIP-1β (CCL4), RANTES (CCL5)	Secondary pro-inflammatory mediators
		Stimulate recruitment of well-defined leukocyte subsets
		Promote chemoattraction, movement of immune system cells into, within, and out of lymphoid organs

CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C) ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN-γ, interferon-γ; IL, interleukin; IL.1RA, IL-1 receptor antagonist; IP-10, IFNγ-inducible protein 10; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; RANTES, regulated on activation, normal T cell expressed and secreted; Th, T helper; TNF-α, tumor necrosis factor-α.

action. Furthermore, an individual cytokine can have multiple effects on a target cell. IFN-γ induces antiviral proteins, upregulates major histocompatibility complex Class I antigens, stimulates NK and IL-12 production, and induces antiproliferative effects [22]. Increased levels of IFN-γ and IL-12 can induce inflammation whereas increased levels of transforming growth factor (TGF)-β, IL-4, and IL-10 can downregulate inflammation [23]. The classification of the biological action of cytokines as either pro-inflammatory or anti-inflammatory may be dependent on the amount of cytokine, the nature of the target cell, the nature of the activating signal, the nature of produced cytokines, and the timing and sequence of cytokine action [24].

The Immune System, the Nervous System, Behavior, and the Role of Cytokines

The immune system and the nervous system are intricately interconnected. The functional status of the immune system affects a multitude of biological processes, including brain function and development, which can be affected when the innate and adaptive immune responses are dysregulated [25]. Sickness behavior, a term used to describe changes in the subjective experience and behavior occurring in a physically ill person [26], provides an example of how, through multiple mechanisms, the immune system can influence brain function and subsequent behavior [25]. Nonspecific symptoms of sickness behavior include fever, nausea, reduced appetite, fatigue, irritability, and withdrawal from physical and social environments [27]. Sickness behavior is considered an organized and evolved strategy to facilitate the role of fever in fighting infection. It is initiated by pro-inflammatory cytokines that are produced at the site of infection by activated accessory immune cells and is characterized by endocrine, autonomic, and behavioral changes [27]. The brain recognizes cytokines such as the pro-inflammatory cytokines IL-1α, IL-1β, TNF-α, and IL-6 as molecular signals of sickness [28]. Furthermore, TNF-α, IL-6, and IL-1β can cross the blood-brain barrier and act on the hypothalamus where they promote fever and sickness behavior [29]. The similarities of symptom expression in sickness behavior and depression have led to the hypothesis that cytokines and inflammatory factors are involved in the pathophysiology of neuropsychiatric disorders, and this has been a catalyst for extensive research into the pathways and mechanisms through which the immune system influences the brain and behavior [30]. Interestingly, another example of a relationship between symptom and cytokine expression involves immunotherapy in cancer patients, in whom prolonged exposure to the proinflammatory cytokine IL-2 results in dose- and time-related cognitive dysfunction and altered behavior [31].

The literature identifying abnormal cytokine profiles in depression, bipolar disorder, and schizophrenia [32–34] collectively suggests that cytokines induce both sickness behavior and neuropsychiatric symptoms, and that inflammation is a key pivotal factor in psychopathology [35]. In addition, a systematic review evaluating pro-inflammatory markers in almost 4,000 children and adolescents with neuropsychiatric and neurodevelopmental disorders, including ASD, identified preliminary evidence of the role of inflammation in these conditions and an association with a pro-inflammatory state [36]. Furthermore, growth in research into the role of inflammation has led to the redefinition of many diseases, such as heart disease, Alzheimer's disease, type 1 diabetes, type 2 diabetes, and obesity, as inflammatory disorders [13]. A possible mechanism for the role of inflammation in these disorders is the alteration of the structural and functional integrity of the central nervous system (CNS) by cytokines, thereby contributing to the pathology of neuro-inflammation and neuropsychological disorders.

Peripheral cytokine signals are thought to access the brain through three pathways: humoral (with antibody involvement), neural, and cellular [30]. These communication pathways involve at least five mechanisms: (1) passage of cytokines through leaky regions of the blood-brain barrier; (2) active transport *via* saturable cytokine-specific transport molecules on brain endothelium; (3) activation of endothelial cells, which release second messengers within the brain parenchyma; (4) transmission of cytokine signals *via* afferent nerve fibers, including the vagus; and (5) entry into the brain parenchyma of peripherally-activated monocytes which release cytokines.

Cytokines may influence behavior through effects on neurotransmitter function, neuroendocrine activity, neurogenesis, and alterations to brain circuitry [30]. For example, cytokines have been shown to increase the release and decrease reuptake of the excitatory neurotransmitter glutamate, which can result in the pathological process of excitotoxicity [37]. This type of mechanism could support a model for some types of ASD; the model postulates an increased excitation/inhibition ratio in key neural systems, such as sensory, mnemonic, social, and emotional systems [38].

An alternate communication pathway has recently been proposed based on the groundbreaking work by Louveau and colleagues who identified functional lymphatic vessels in the CNS that carry fluid and immune cells from the cerebrospinal fluid, and in doing so discovered a pathway for immune cells to exit the CNS [39]. While the anatomy and functional importance of these pathways and systems have yet to be characterized in humans, this work provides

a new perspective on the possible etiology of neuroinflammatory and neurodegenerative conditions. These findings also provide an impetus for further consideration of the relationship between immune responses and behaviors in other conditions that are characterized by immune system dysfunction, such as ASD.

An understanding of the pathways and mechanisms through which the immune system affects behavior is primarily based on findings in animal models. For example, mice deficient in T cells have cognitive deficits [25]. Interestingly, an altered activation profile for T cells has been identified in ASD, with these perturbations of T cell function possibly modulating behavior and core features of ASD [40]. Recently, social behavior as operationalized within an animal model, has been shown to be influenced by meningeal immunity [41]. Mice deficient in adaptive immunity, specifically an absence of interferon IFN-γ, display both hyper-connectivity in the prefrontal cortex (PFC) and significant social deficits. This is particularly interesting, given that hyperactivity in the PFC in the context of social stimuli is known to be a feature of social impairment in ASD [42]. Filiano and colleagues demonstrated that CNS neurons respond to IFN-γ derived from meningeal T cells, elevating tonic GABAergic inhibition [41]. This process prevents aberrant hyper-excitability in the PFC and restores social behaviors through IFN-γ. Previously, IFN-γ released from T cells was thought to predominantly stimulate and modulate immune responses to infection. Interestingly, NK cells, recognized as major producers of cytokines including IFN-γ [12] in physiological and pathological conditions, are dysfunctional in ASD [43]. While a novel finding, the regulation of neural activity and social behavior through IFN-γ provides further evidence for the interconnectedness of the immune system, the nervous system, and behavior.

Immune System Deregulation in ASD

Evidence suggesting a pathophysiological relationship between the immune system and ASD was first presented over 40 years ago [44]. Subsequent research investigating the complex relationship between the immune system and ASD symptomatology has identified numerous potential interactions and proposed associated mechanisms at both the systemic and cellular levels [7, 45]. One of these areas of research focuses on the prenatal period. Maternal immune activation refers to the defensive response of the mother's immune system to an invading pathogen. A large population-based study found that acute immune activation caused by maternal viral infection during the first trimester increases the risk of ASD in children [46]. Furthermore, a recent meta-analysis of >40,000 ASD cases showed that maternal infection during pregnancy is associated with an increased risk of ASD in the offspring, with hospitalization during infection heightening the risk [47]. Moderators of this risk include the type of infectious agent, the timing of infectious exposure, and the site of infection. A recent review has proposed that maternal infection leads to the release of pro-inflammatory cytokines and activation of Th17 cells in the mother's bloodstream and that the immune status and genetic predisposition of the fetus determine its vulnerability to maternal immune activation, a process considered a disease primer [48]. Peripheral cytokine profiles at birth, including elevated IL-1β and IL-4, are associated with an ASD diagnosis later in childhood and vary with ASD symptom severity [49]. Elevation of IL-1β and IL-4 may reflect a prenatal immune challenge, and an association with both ASD risk and cognitive developmental outcomes suggests the possibility of a global impact of early cytokine dysregulation [49]. Familial autoimmunity has also been implicated in the pathogenesis of ASD, with an increased risk of ASD in children with a maternal history of rheumatoid arthritis and celiac disease, and an increased risk of infantile autism has been identified in children with a family history of type 1 diabetes [50].

Another area of focus on immune involvement in the pathogenesis and maintenance of ASD is the postnatal period. Altered cytokine profiles have been consistently linked to ASD in children during this period [7]. In high-functioning male children with ASD, the plasma levels of IL-1β, IL-1 receptor antagonist (IL-1RA), IL-5, IL-8, IL-12(p70), IL-13, and IL-17 are elevated relative to matched controls [51]. IL-1β, a pro-inflammatory cytokine, activates neutrophils and macrophages to phagocytose invading pathogens [52]. IL-1RA inhibits the activities of IL-1β, suggesting that the levels of IL-1RA might be a function of a negative feedback regulator role in response to the elevation of IL-1β [51]. IL-1β is involved in the production of IL-17 [53] and IL-17 is a potent mediator of the production of IL-8, a chemokine with important roles in the innate immune response. IL-5 and IL-13 stimulate B cells to secrete immunoglobulins including IgE, which is a mediator of allergic inflammation. IL-12(p70) is a pro-inflammatory cytokine that enhances Th1 and NK cell responses [54]. In addition to elevated expression of IL-1β, as identified by Suzuki and colleagues, IL-6, IL-12, TNF-α, and IL-23 are also elevated in ASD compared to healthy controls, suggesting a dysregulated immune response [55]. TNF-α is a central regulator of inflammation and is elevated in the cerebrospinal fluid of children with ASD [56]. IL-6 is typically regarded as a pro-inflammatory cytokine and has been identified as a cytokine the brain recognizes as a molecular signal of sickness [28]. However, it also has regenerative or anti-inflammatory activity, and is involved in the regulation of metabolic and neural processes [57].

Finally, a further example of immune abnormalities in the postnatal period involves activation of the monocytic and Th1 arm of the immune response, *via* increased IL-1RA and increased IFN-γ, respectively, and this has been found in children with ASD [58].

Immune-mediated mechanisms have also been hypothesized as reflecting a chronic state of specific cytokine activation [59]. Immunocytochemical studies have identified marked activation of microglia and astroglia associated with the increased production of two cytokines by neuroglia, macrophage chemoattractant protein (MCP)-1, and TGF-β1 [59]. In addition, a unique profile of pro-inflammatory cytokines has been identified in cerebrospinal fluid [59]. Another post-mortem study also demonstrated significant increases in pro-inflammatory and Th1 cytokines relative to matched controls [60]. Elevation of IL-6 in ASD, both centrally and peripherally, has been frequently reported [59–62]. In a mouse model with elevated IL-6 in the brain, Wei and colleagues have shown that IL-6 can modulate autism-like behaviors through impairments of synapse formation, dendritic spine development, and neuronal circuit balance [63]. Acute and chronic psychological stress and alterations in sleep duration and quality, a commonly reported comorbidity in ASD [64], increase the concentrations of IL-6 [65]. This evidence for abnormal cytokine profiles in ASD suggests that immune system disturbances may be active and continuous contributors to the presentation of ASD. It is this accumulation of evidence that has acted as the catalyst for efforts to characterize possible subgroups of ASD patients who present with immune system abnormalities or dysfunction and altered patterns of symptom presentation [9, 66].

Associations between changes in peripheral cytokine expression and the severity of behavioral impairments and associated symptoms have been identified in children with ASD (Table 2). Reduced levels of the regulatory cytokine TGF-β1 are associated with reduced adaptive behavior and worsening behavioral symptoms [67, 68]. While TGF-β1 is involved in cell growth and differentiation, organ development, migration, and apoptosis, its major role is to control inflammation. This negative correlation with behavioral impairment suggests that there is an ongoing inflammatory process in children with ASD who present with worsening behavioral profiles [67]. Increased levels of the chemokines MCP-1, RANTES (regulated on activation, normal T cell expressed and secreted), and eotaxin are associated with more impaired behaviors and adaptive functioning [69]. Chemokines are expressed in the developing brain and regulate neuronal cell migration, proliferation, and differentiation. They are also involved in communication between neurons and microglia [70]. Elevated IL-1β and IL-6 have been associated with increased stereotypical behaviors [62]. Dysregulation of IL-1β, a pro-inflammatory cytokine expressed early in an immune response, is implicated in impairments in memory and learning [71]. IL-1β induces and inhibits neural progenitor cell proliferation in the CNS, which can contribute to region-specific deviant brain growth in ASD [72]. As previously highlighted, IL-6 is elevated in most inflammatory states and has been implicated in a wide range of conditions. Elevation of IL-8 and IL-12p40 is also associated with greater impairment of aberrant behaviors including lethargy and stereotypy as measured by the Aberrant Behavior Checklist (ABC) [62]. In addition, as the expression of IL-8 decreases, cognitive and adaptive ability improves [62]. IL-8 is a chemoattractant cytokine, attracting and activating neutrophils in regions of inflammation [73] and hence may contribute to the pathogenesis of inflammatory diseases [74].

Other cellular markers of immune dysfunction identified in children with ASD include significantly higher absolute numbers of B cells and NK cells, and increased markers of cellular activation compared to healthy controls [75]. These findings suggest an immune response activation that leads to an increased frequency of NK cells and activated B cells and T cells. Increased levels of the IgG4 subclass have been identified in children with ASD [76]. The IgG4 subclass has features and biological function different from other subclasses of IgG, acting as a blocking antibody that binds strongly to antibody receptors rather than a protective antibody. A correlation between the severity of behavioral measures and reduced levels of immunoglobulin has also been found, suggesting suboptimal humoral function in children with ASD [77]. Furthermore, an elevated prevalence of other immune-related comorbidities, including autoimmune diseases, allergies, and psoriasis, has been found in children with ASD compared to healthy controls [6]. Overall, these relationships between immune dysfunction and behavioral symptoms associated with an ASD presentation suggest an ongoing relationship impacting the severity of the condition in children with an ASD diagnosis.

Altered Cytokine Profiles as Potential Biomarkers in ASD

To facilitate improved communication about measurements of disease and treatment effects, an expert working group convened by the National Institutes of Health Director's Initiative on Biomarkers and Surrogate Endpoints (USA) proposed the following definition of a biological marker or biomarker: a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [78]. Additional applications of biomarkers have been proposed,

Table 2 Cytokines and associations with severity of autism-related symptoms.

Cytokine	*n* (ASD)	Medium	Measures	Finding	Study
TGF-β1	75	Plasma	ABC, VABS	Reduced levels associated with reduced adaptive behavior and worsened behavioral symptoms including stereotypy, irritability, and hyperactivity	Ashwood *et al.* [67]
Eotaxin	80	Plasma	ABC, MSEL, VABS	Increased levels associated with increased severity of lethargy, stereotypy, and hyperactivity (ABC)	Ashwood *et al.* [69]
				Increased levels associated with greater impairments in visual reception, fine motor skills, and receptive and expressive language (MSEL)	
				Increased levels associated with greater impairments in communication, daily living skills, socialization, and motor skills (VABS)	
MCP-1	80	Plasma	ABC, MSEL, VABS	Increased levels associated with greater impairments in visual reception, fine motor skills and expressive language (MSEL)	Ashwood *et al.* [69]
				Increased levels associated with greater impairments in daily living skills (VABS)	
RANTES	80	Plasma	ABC, MSEL, VABS	Increased levels associated with increased severity of lethargy, stereotypy, and hyperactivity (ABC)	Ashwood *et al.* [69]
				Increased levels associated with greater impairments in visual reception, fine motor skills, and expressive language (MSEL)	
				Increased levels associated with greater impairments in communication and socialization (VABS)	
IL-1β	97	Plasma	ADI-R, ABC, MSEL, VABS	Increased levels associated with increased stereotypy (ABC)	Ashwood *et al.* [62]
IL-4	97	Plasma	ADI-R, ABC, MSEL, VABS	Increased levels associated with greater impairments in non-verbal communication (ADI-R)	Ashwood *et al.* [62]
IL-6	97	Plasma	ADI-R, ABC, MSEL, VABS	Increased levels associated with increased stereotypy (ABC)	Ashwood *et al.* [62]
IL-8	97	Plasma	ADI-R, ABC, MSEL, VABS	Increased levels associated with increased hyperactivity, stereotypy, and lethargy (ABC)	Ashwood *et al.* [62]
				Decreased levels associated with improved visual reception, receptive language, and expressive language (MSEL)	
				Decreased levels associated with improved daily living skills (VABS)	
IL-12p40	97	Plasma	ADI-R, ABC, MSEL, VABS	Increased levels associated with increased stereotypy and lethargy (ABC)	Ashwood *et al.* [62]
TGF-β1	30	Plasma	CARS	Reduced levels associated with increasing severity	El Gohary *et al.* [68]

ABC, Aberrant Behavior Checklist; ADI-R, Autism Diagnostic Interview—Revised; IL, interleukin; MCP-1, monocyte chemotactic protein-1; MSEL, Mullen Scales of Early Learning; RANTES, regulated on activation, normal T cell expressed and secreted; TGF-β1, transforming growth factor-β1; VABS, Vineland Adaptive Behavior Scales; WAIS-R, Wescheler Adult Intelligence Scale-Revised.

such as use as a diagnostic tool for the identification of those patients with a disease or abnormal condition, and for the prediction and monitoring of the clinical response to an intervention. Specifically, with respect to using biomarkers to guide better treatment of schizophrenia and other psychotic disorders, Banati and Hickie have proposed clinically useful properties of biomarkers, including diagnostically non-specific, quantitative, longitudinal, plausibly linked to underlying pathophysiology, and predictive of risk of impairment [79]. They also highlight the clinical importance of the role of biomarkers in guiding treatment selection, and demonstrating a correlation between active interventions and the short-term clinical response.

Numerous biomarkers have been proposed for ASD, including biochemical, morphological, immunological, hormonal, neurophysiological, neuroanatomical, and neuropsychological markers [80]. A recent clinical trial in ASD involving an immunomodulatory pharmacological intervention demonstrates a correlation between an active intervention and a relevant short-term clinical response. Greater improvement in symptoms of irritability, hyperactivity, stereotypic behavior, social withdrawal, and inappropriate speech was achieved when risperidone was used adjunctively with pentoxifylline, an immune-modulating drug and pro-inflammatory cytokine inhibitor [81]. Furthermore, another adjunct treatment trial in children with ASD using risperidone and pioglitazone showed reductions in the severity of symptoms of irritability, social withdrawal, and hyperactivity in the adjunct treatment group compared to the risperidone-only group, indicating positive effects of pioglitazone [82]. Pioglitazone is a

peroxisome proliferator-activated receptor, which inhibits the production of pro-inflammatory cytokines and chemokines by microglia [83, 84]. Furthermore, risperidone was found to be more effective when given adjunctively with celecoxib, a nonsteroidal anti-inflammatory drug, with significant improvements in the irritability, social withdrawal, and stereotypy subscales of the ABC [85]. However, in a recent open-label study of risperidone treatment for children and adolescents with ASD, the plasma levels of eotaxin and MCP-1 showed statistically significant decreases after treatment, although these changes were not significantly associated with changes in severity measures [86]. Eotaxin and MCP-1 are pro-inflammatory and are elevated in brain specimens from patients with ASD [59]. Overall, the results of these trials suggest that treating immune-related symptoms may contribute to behavioral changes in ASD.

The clinical trials of immune-modulating agents, taken together with previous examples of immune system perturbations in children with ASD, suggest that cytokines are worthy of consideration as potential biomarkers of a subgroup of individuals with an ASD diagnosis and more severe behavioral outcomes. However, the validity of peripheral sampling of blood cells as a relevant biomarker and as a surrogate for a CNS sample is still debated. Given that peripheral blood cells comprise the major cellular components of the immune system, they could be considered suitable for the assessment of immune-related markers [87]. In addition, accessibility and speed of sampling for regular assessments and analysis of peripheral blood sampling are significant advantages over, for example, cerebrospinal fluid sampling and brain imaging.

A National Research Council (USA) report on precision medicine, which is the tailoring of medical treatment to the individual characteristics of each patient, highlights a critical need for the deconstruction of current diagnostic groups using biomarkers to help identify the subgroups for which treatment is highly effective [88, 89]. In addition to physical signs and symptoms, it is recommended that conditions should also be defined by their underlying molecular causes and other factors, which would represent an emergence of a new taxonomy based on biomedical research and an extensive patient data network. A data network would be needed to integrate current research on the molecular composition of conditions with clinical data on individual patients in an effort to drive precision medicine. Precision medicine also refers to the classification of individuals into subpopulations that differ in their susceptibility to a particular condition in the biology and/or prognosis of those conditions they may develop, or in their response to a specific treatment [89]. Similarly, the long-term aim of the Research Domain Criteria (RDoC) project of the National Institute of Mental Health (USA) is precision medicine for psychiatry through the adoption of a diagnostic system based on a comprehensive understanding of the biological and psychosocial bases of conditions, unhindered by the limitations of diagnostic categories [88].

A robust biological system is one that maintains its state and functions against external and internal perturbations [90]. It is possible that a robust immune system is protective and that while the mechanisms leading to abnormal function have yet to be established, it is tempting to consider that a regulated and functional immune system, both pre- and postnatally, is a prerequisite for a normal functioning brain [91]. Consistent with the RDoC agenda, the non-specific association found between inflammation and neuropsychiatric disorders, including ASD, and the identification of common reliable inflammatory markers across those different disorders warrant further investigations to determine if these processes play a role in the etiology of symptom dimensions or symptom domains that overlap or are shared by different conditions [35]. A diagnosis of ASD continues to be behaviorally defined. However, the body of research and accumulating evidence with respect to immune system perturbations in ASD suggest that a broader approach should be taken in order to understand biological systems as they pertain to ASD and associated behaviors.

Conclusion

The hallmark heterogeneity of ASD is a key reason for the focus of researchers on the identification of potential biological measures as a means of describing subsets within ASD, and thereby facilitating the targeting of more individualized therapies. Previously discussed cytokine aberrations in ASD have highlighted a possible relationship between cytokine aberration and ASD. Altered cytokine levels may facilitate the identification of ASD subtypes that share similar traits and profiles, as well as provide biological markers that facilitate monitoring of the benefits of active treatments over the time-course of clinical trials. Biological markers, as objective measures of the response to treatment in clinical trials, will assist with the identification of efficacious interventions. Furthermore, the identification of objective markers of a pathological state related to a subgroup in ASDs may assist in reducing the heterogeneity of participants in clinical trials, and this may lead to the identification of more targeted treatments for autism-related symptoms.

Acknowledgements We acknowledge a National Health and Medical Research Council Career Development Fellowship (APP1061922) and a Project Grant (1043664) to AJG.

References

1. APA. Diagnostic and Statistical Manual of Mental Disorders. Fifth Edition. Washington, DC: American Psychiatric Association, 2013.
2. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. J Am Acad Child Adolesc Psychiatry 2008, 47: 921–929.
3. Mannion A, Leader G. An investigation of comorbid psychological disorders, sleep problems, gastrointestinal symptoms and epilepsy in children and adolescents with autism spectrum disorder: A two year follow-up. Res Autism Spectr Disord 2016, 22: 20–33.
4. Mannion A, Leader G. Comorbidity in autism spectrum disorder: A literature review. Res Autism Spectr Disord 2013b, 7: 1595–1616.
5. Bauman ML. Medical comorbidities in autism: Challenges to diagnosis and treatment. Neurotherapeutics 2010, 7: 320–327.
6. Zerbo O, Leong A, Barcellos L, Bernal P, Fireman B, Croen LA. Immune mediated conditions in autism spectrum disorders. Brain Behav Immun 2015, 46: 232–236.
7. Goines P, Van de Water J. The immune system's role in the biology of autism. Curr Opin Neurol 2010, 23: 111–117.
8. Masi A, Quintana DS, Glozier N, Lloyd AR, Hickie IB, Guastella AJ. Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. Mol Psychiatry 2015, 20: 440–446.
9. Mead J, Ashwood P. Evidence supporting an altered immune response in ASD. Immunol Lett 2015, 163: 49–55.
10. Owen JA, Punt J, Stranford SA, Jones PP, Kuby J. Kuby Immunology. New York: W.H. Freeman, 2013.
11. D'Elia RV, Harrison K, Oyston PC, Lukaszewski RA, Clark GC. Targeting the "cytokine storm" for therapeutic benefit. Clin Vaccine Immunol 2013, 20: 319–327.
12. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, *et al.* Innate or adaptive immunity? the example of natural killer cells. Science 2011, 331: 44–49.
13. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 2014, 1843: 2563–2582.
14. Graves DT, Jiang Y. Chemokines, a family of chemotactic cytokines. Crit Rev Oral Biol Med 1995, 6: 109–118.
15. Depino MA, de los Angeles Robinson-Agramonte M. Understanding on neuroimmunology in autism spectrum disorder. Translational approaches to autism spectrum disorder. Springer International Publishing, 2015: 155–180.
16. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations. Annu Rev Immunol 2010, 28: 445–489.
17. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular biology of the cell. 4th edition. New York: Garland Science; 2002. Helper T Cells and Lymphocyte Activation.
18. Gupta S, Aggarwal S, Rashanravan B, Lee T. Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. J Neuroimmunol 1998, 85: 106–109.
19. Molloy CA, Morrow AL, Meinzen-Derr J, Schleifer K, Dienger K, Manning-Courtney P, *et al.* Elevated cytokine levels in children with autism spectrum disorder. J Neuroimmunol 2006, 172: 198–205.
20. Romagnani S. T-cell subsets (Th1 versus Th2). Ann Allergy Asthma Immunol 2000, 85: 9–21.
21. Shi Y, Liu CH, Roberts AI, Das J, Xu G, Ren G, *et al.* Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: what we do and don't know. Cell Res 2006, 16: 126–133.
22. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol 2004, 75: 163–189.
23. Xu N, Li X, Zhong Y. Inflammatory cytokines: potential biomarkers of immunologic dysfunction in autism spectrum disorders. Mediators Inflamm 2015, 2015: 10.
24. Cavaillon JM. Pro- versus anti-inflammatory cytokines: Myth or reality. Cell Mol Biol (Noisy-le-grand) 2001, 47: 695–702.
25. Filiano AJ, Gadani SP, Kipnis J. Interactions of innate and adaptive immunity in brain development and function. Brain Res 2015, 1617: 18–27.
26. Hart BL. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 1988, 12: 123–137.
27. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008, 9: 46–56.
28. Dantzer R. Cytokine, sickness behavior, and depression. Immunol. Allergy Clin North Am 2009, 29: 247–264.
29. Dantzer R. Cytokine-Induced Sickness Behavior: Where Do We Stand? Brain Behav Immun 2001, 15: 7–24.
30. Capuron L, Miller AH. Immune system to brain signaling: neuropsychopharmacological implications. Pharmacology & therapeutics 2011, 130: 226–238.
31. Denicoff KD, Rubinow DR, Papa MZ, Simpson C, Seipp CA, Lotze MT, *et al.* The neuropsychiatric effects of treatment with interleukin-2 and lymphokine-activated killer cells. Ann Intern Med 1987, 107: 293–300.
32. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, *et al.* A meta-analysis of cytokines in major depression. Biol Psychiatry 2010, 67: 446–457.
33. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol Psychiatry 2011, 70: 663–671.
34. Modabbernia A, Taslimi S, Brietzke E, Ashrafi M. Cytokine alterations in bipolar disorder: a meta-analysis of 30 studies. Biol Psychiatry 2013, 74: 15–25.
35. Capuron L, Castanon N. Role of inflammation in the development of neuropsychiatric symptom domains: evidence and mechanisms. Curr Top Behav Neurosci 2017, 31: 31–44.
36. Mitchell RH, Goldstein BI. Inflammation in children and adolescents with neuropsychiatric disorders: a systematic review. J Am Acad Child Adolesc Psychiatry 2014, 53: 274–296.
37. Tilleux S, Hermans E. Neuroinflammation and regulation of glial glutamate uptake in neurological disorders. J Neurosci Res 2007, 85: 2059–2070.
38. Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2003, 2: 255–267.
39. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, *et al.* Structural and functional features of central nervous system lymphatic vessels. Nature 2015, 523: 337–341.
40. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Altered T cell responses in children with autism. Brain Behav Immun 2011, 25: 840–849.
41. Filiano AJ, Xu Y, Tustison NJ, Marsh RL, Baker W, Smirnov I, *et al.* Unexpected role of interferon-γ in regulating neuronal connectivity and social behaviour. Nature 2016, 535: 425–429.

42. Forbes CE, Grafman J. The role of the human prefrontal cortex in social cognition and moral judgment. Annu Rev Neurosci 2010, 33: 299–324.
43. Enstrom AM, Lit L, Onore CE, Gregg JP, Hansen R, Pessah IN, *et al.* Altered gene expression and function of peripheral blood natural killer cells in children with autism. Brain Behav Immun 2009, 23: 124–133.
44. Stubbs EG, Crawford ML. Depressed lymphocyte responsiveness in autistic children. J Autism Child Schizophr 1977, 7: 49–55.
45. Meltzer A, Van de Water J. The role of the immune system in autism spectrum disorder. Neuropsychopharmacology 2017, 42: 284–298.
46. Atladottir HO, Thorsen P, Ostergaard L, Schendel DE, Lemcke S, Abdallah M, *et al.* Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. J Autism Dev Disord 2010, 40: 1423–1430.
47. Jiang HY, Xu LL, Shao L, Xia RM, Yu ZH, Ling ZX, *et al.* Maternal infection during pregnancy and risk of autism spectrum disorders: A systematic review and meta-analysis. Brain Behav Immun 2016, 58: 165–172..
48. Estes ML, McAllister AK. Maternal immune activation: Implications for neuropsychiatric disorders. Science 2016, 353: 772–777.
49. Krakowiak P, Goines PE, Tancredi DJ, Ashwood P, Hansen RL, Hertz-Picciotto I, *et al.* Neonatal cytokine profiles associated with autism spectrum disorder. Biol Psychiatry 2017, 81: 442–451..
50. Atladottir HO, Pedersen MG, Thorsen P, Mortensen PB, Deleuran B, Eaton WW, *et al.* Association of family history of autoimmune diseases and autism spectrum disorders. Pediatrics 2009, 124: 687–694.
51. Suzuki K, Matsuzaki H, Iwata K, Kameno Y, Shimmura C, Kawai S. Plasma cytokine profiles in subjects with high-functioning autism spectrum disorders. PLoS One 2011, 6: e20470.
52. Netea MG, Simon A, van de Veerdonk F, Kullberg B-J, Van der Meer JWM, Joosten LAB. IL-1β Processing in Host Defense: Beyond the Inflammasomes. PLoS Pathog 2010, 6: e1000661.
53. Li L, Kim J, Boussiotis VA. IL-1beta-mediated signals preferentially drive conversion of regulatory T cells but not conventional T cells into IL-17-producing cells. J Immunol 2010, 185: 4148–4153.
54. Verma ND, Hall BM, Plain KM, Robinson CM, Boyd R, Tran GT, *et al.* Interleukin-12 (IL-12p70) Promotes Induction of Highly Potent Th1-Like CD4(+)CD25(+) T Regulatory Cells That Inhibit Allograft Rejection in Unmodified Recipients. Front Immunol 2014, 5: 190.
55. Ricci S, Businaro R, Ippoliti F, Lo Vasco VR, Massoni F, Onofri E, *et al.* Altered cytokine and BDNF levels in autism spectrum disorder. Neurotox Res 2013, 24: 491–501.
56. Chez MG, Dowling T, Patel PB, Khanna P, Kominsky M. Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children. Pediatr Neurol 2007, 36: 361–365.
57. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 2011, 1813: 878–888.
58. Croonenberghs J, Bosmans E, Deboutte D, Kenis G, Maes M. Activation of the inflammatory response system in autism. Neuropsychobiology 2002, 45: 1–6.
59. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 2005, 57: 67–81.
60. Li X, Chauhn A, Shiekh AM, Patil S, Chauhn V, Li X-M, *et al.* Elevated immune response in the brain of autistic patients. J Neuroimmunol 2009, 207: 111–116.
61. Wei H, Zou H, Sheikh AM, Malik M, Dobkin C, Brown WT, *et al.* IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. J Neuroinflammation 2011, 8: 52.
62. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 2011a, 25: 40–45.
63. Wei H, Chadman KK, McCloskey DP, Sheikh AM, Malik M, Brown WT, *et al.* Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. Biochim Biophys Acta 2012, 1822: 831–842.
64. Miano S, Giannotti F, Cortesi F. Sleep disorders and autism spectrum disorder. In: Mazzone L, Vitiello B (Eds.). Psychiatric symptoms and comorbidities in autism spectrum disorder. Springer International Publishing, 2016: 111–128.
65. Rohleder N, Aringer M, Boentert M. Role of interleukin-6 in stress, sleep, and fatigue. Ann N Y Acad Sci 2012, 1261: 88–96.
66. McDougle CJ, Landino SM, Vahabzadeh A, O'Rourke J, Zurcher NR, Finger BC, *et al.* Toward an immune-mediated subtype of autism spectrum disorder. Brain Res 2015, 1617: 72–92.
67. Ashwood P, Enstrom A, Krakowiak P, Hertz-Picciotto I, Hansen RL, Croen LA, *et al.* Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes. J Neuroimmunol 2008, 204: 149–153.
68. El Gohary TM, El Aziz NA, Darweesh M, Sadaa ES. Plasma level of transforming growth factor β 1 in children with autism spectrum disorder. Egyptian Journal of Ear, Nose, Throat and Allied Sciences 2015, 16: 69–73.
69. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. J Neuroimmunol 2011b, 232: 196–199.
70. Biber K, Vinet J, Boddeke HWGM. Neuron-microglia signaling: Chemokines as versatile messengers. J Neuroimmunol 2008, 198: 69–74.
71. Goines PE, Ashwood P. Cytokine dysregulation in autism spectrum disorders (ASD): possible role of the environment. Neurotoxicol Teratol 2013, 36: 67–81.
72. Courchesne E. Brain development in autism: Early overgrowth followed by premature arrest of growth. Ment Retard Dev Disabil Res Rev 2004, 10: 106–111.
73. Bickel M. The role of interleukin-8 in inflammation and mechanisms of regulation. J Periodontol 1993, 64: 456–460.
74. Qazi BS, Tang K, Qazi A. Recent advances in underlying pathologies provide insight into interleukin-8 expression-mediated inflammation and angiogenesis. Int J Inflam 2011, 2011: 908468.
75. Ashwood P, Corbett BA, Kantor A, Schulman H, Van de Water J, Amaral DG. In search of cellular immunophenotypes in the blood of children with autism. PLoS One 2011c, 6: e19299.
76. Enstrom A, Krakowiak P, Onore C, Pessah IN, Hertz-Picciotto I, Hansen RL, *et al.* Increased IgG4 levels in children with autism disorder. Brain Behav Immun 2009, 23: 389–395.
77. Heuer L, Ashwood P, Schauer J, Goines P, Krakowiak P, Hertz-Picciotto I, *et al.* Reduced levels of immunoglobulin in children with autism correlates with behavioral symptoms. Autism Res 2008, 1: 275–283.
78. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clin Pharmacol Ther 2001, 69: 89–95.
79. Banati R, Hickie IB. Therapeutic signposts: using biomarkers to guide better treatment of schizophrenia and other psychotic disorders. Med J Aust 2009, 190: S26.

80. Ruggeri B, Sarkans U, Schumann G, Persico A. Biomarkers in autism spectrum disorder: the old and the new. Psychopharmacology (Berl.) 2014, 231: 1201–1216.
81. Akhondzadeh S, Fallah J, Mohammadi MR, Imani R, Mohammadi M, Salehi B*, et al.* Double-blind placebo-controlled trial of pentoxifylline added to risperidone: effects on aberrant behavior in children with autism. Prog. Neuropsychopharmacol. Biol Psychiatry 2010, 34: 32–36.
82. Ghaleiha A, Rasa SM, Nikoo M, Farokhnia M, Mohammadi M-R, Akhondzadeh S. A pilot double-blind placebo-controlled trial of pioglitazone as adjunctive treatment to risperidone: Effects on aberrant behavior in children with autism. Psychiatry Res 2015, 229: 181–187.
83. Kobayashi T, Notoya K, Naito T, Unno S, Nakamura A, Martel-Pelletier J*, et al.* Pioglitazone, a peroxisome proliferator-activated receptor gamma agonist, reduces the progression of experimental osteoarthritis in guinea pigs. Arthritis Rheum 2005, 52: 479–487.
84. Mandrekar-Colucci S, Karlo JC, Landreth GE. Mechanisms underlying the rapid peroxisome proliferator-activated receptor-γ-mediated amyloid clearance and reversal of cognitive deficits in a murine model of Alzheimer's disease. J Neurosci 2012, 32: 10117–10128.
85. Asadabadi M, Mohammadi MR, Ghanizadeh A, Modabbernia A, Ashrafi M, Hassanzadeh E*, et al.* Celecoxib as adjunctive treatment to risperidone in children with autistic disorder: a randomized, double-blind, placebo-controlled trial. Psychopharmacology (Berl.) 2013, 225: 51–59.
86. Choi JE, Widjaja F, Careaga M, Bent S, Ashwood P, Hendren RL. Change in plasma cytokine levels during risperidone treatment in children with autism. J Child Adolesc Psychopharmacol 2014, 24: 586–589.
87. Hayashi-Takagi A, Vawter MP, Iwamoto K. Peripheral biomarkers revisited: integrative profiling of peripheral samples for psychiatric research. Biol Psychiatry 2014, 75: 920–928.
88. Insel TR. The NIMH research domain criteria (RDoC) project: precision medicine for psychiatry. Am J Psychiatry 2014, 171: 395–397.
89. National Academy of Sciences. The national academies collection: reports funded by national institutes of health. Washington (DC): National Academies Press (US), 2011.
90. Kitano H. Biological robustness. Nat Rev Genet 2004, 5: 826–837.
91. Derecki N, Privman E, Kipnis J. Rett syndrome and other autism spectrum disorders—brain diseases of immune malfunction? Mol Psychiatry 2010, 15: 355–363.

Neurosci. Bull. April, 2017, 33(2):205–218
DOI 10.1007/s12264-017-0112-7

www.neurosci.cn
www.springer.com/12264

REVIEW

Cellular and Circuitry Bases of Autism: Lessons Learned from the Temporospatial Manipulation of Autism Genes in the Brain

Samuel W. Hulbert[1] · Yong-hui Jiang[1,2,3]

Received: 10 January 2017 / Accepted: 12 February 2017 / Published online: 7 March 2017

Abstract Transgenic mice carrying mutations that cause Autism Spectrum Disorders (ASDs) continue to be valuable for determining the molecular underpinnings of the disorders. Recently, researchers have taken advantage of such models combined with Cre-*loxP* and similar systems to manipulate gene expression over space and time. Thus, a clearer picture is starting to emerge of the cell types, circuits, brain regions, and developmental time periods underlying ASDs. ASD-causing mutations have been restricted to or rescued specifically in excitatory or inhibitory neurons, different neurotransmitter systems, and cells specific to the forebrain or cerebellum. In addition, mutations have been induced or corrected in adult mice, providing some evidence for the plasticity and reversibility of core ASD symptoms. The limited availability of Cre lines that are highly specific to certain cell types or time periods provides a challenge to determining the cellular and circuitry bases of autism, but other technological advances may eventually overcome this obstacle.

Keywords Autism · Mouse models · Behavior · Cre-*loxP* · Cerebellum · Critical period

✉ Yong-hui Jiang
yong-hui.jiang@duke.edu

[1] Department of Neurobiology, School of Medicine, Duke University, Durham, NC 27710, USA

[2] Department of Pediatrics, School of Medicine, Duke University, Durham, NC 27710, USA

[3] Program in Genetics and Genomics, School of Medicine, Duke University, Durham, NC 27710, USA

Introduction

The neurobiological basis of Autism Spectrum Disorders (ASDs) has been a growing area of research during the past few decades. While substantial progress has been made to uncover the molecular underpinnings of the disorders, the cell types and circuits underlying autistic behaviors remain largely unknown [1]. This knowledge, however, is critical to developing targeted therapy. Neuroimaging in human patients has provided correlates between the function of certain brain regions and behavior [2, 3]. Such studies are limited by the phenotypic heterogeneity that is characteristic of ASDs, the individuals available for study, the low resolution of imaging techniques, and the inability to manipulate molecules and circuits in the human brain. For these reasons, it is technically challenging to establish causality between circuits and behavior in studies involving humans. However, animal models allow researchers to determine causation because the genetic background and environmental factors can be controlled. Furthermore, the use of laboratory rodents allows for more invasive studies that would not be feasible or ethical in humans.

Transgenic mice with ASD-causing mutations that are present in the germline have provided clues to the molecular underpinnings of the disorders, and some degree of overlap is seen across multiple mouse models [4]. However, germline, or conventional, mutant mice do not provide enough evidence to causally link a particular brain region or circuit to ASD-like behaviors. More recently, several groups have taken advantage of tools that allow for the manipulation of genes across space and time in animal models to begin dissecting the cell types, brain regions, and associated circuits contributing to ASD phenotypes. In this review, we have focused on studies that utilize conditional gene-expression technology combined with mouse models

of ASD that have high construct validity (i.e. the molecular consequences of the mutations mimic those observed in humans) and report phenotypes that have strong face validity (i.e. appear to mimic the behavioral features of the disorders) for the core symptoms of ASDs, impaired social communication and repetitive behaviors.

Assays for ASD-Related Behaviors

Currently, ASDs are diagnosed purely on the assessment of behavioral features [5]. Therefore, behavioral analysis is an important part of the strategy to model human mutations and understand the cellular and circuitry bases of the disorders. To help achieve this goal, a number of assays that test behaviors resembling the core symptoms of ASDs have been developed for laboratory mice [6, 7]. The behaviors mentioned in this review are briefly described here and are summarized in Table 1.

Tests to Assess Social Communication

The three chamber test consists of two phases. The first phase has a caged stranger mouse in one of the chambers, a novel object in the opposing chamber, and a neutral central chamber [8]. The second phase has the caged mouse from the first phase in one chamber, a neutral central chamber, and a novel caged mouse in the opposing chamber. The first phase is considered a test for "sociability" where wild-type mice tend to spend more time in the chamber with the caged stranger mouse than in either other chamber. The second phase is a test for "preference for social novelty" or "social preference," where most wild-type mice spend more time with the novel stranger mouse than with the familiar stranger mouse or in the neutral chamber.

The habituation/dishabituation test involves introducing the test mouse to the same contained stimulus mouse over several days and then introducing a novel contained stimulus mouse [9]. Wild-type mice tend to spend less time engaging with the familiar mouse over time, but have increased interactions with the novel mouse. This is a measure of social recognition.

In the partition test, the test mouse and a stimulus mouse are in an arena on opposite sides of a clear acrylic barrier with holes [10, 11]. The amount of time the test mouse spends near the partition is a measure of social behavior.

In direct social interaction tests, the test mouse and stimulus mouse are allowed to freely interact. The type, duration, and amount of contacts are scored [12]. One variant of this test is resident-intruder, where the stranger mouse is introduced into the test mouse's home cage [13].

Ultrasonic vocalizations (USVs) can be recorded in pups (~postnatal days 3–12) and in adults [14]. For pups, this simply involves briefly separating the pups from their mother. For adults, the most common test involves exposing male mice to females in estrous or their urine.

Table 1 Summary of behavioral assays.

Assay	Resemblance to core feature of ASDs	Quantitative measurements
Three chamber test	Social communication deficits	Phase 1: "Sociability" - preference for social stimulus (stranger mouse) over an inanimate object
		Phase 2: "Social novelty" - preference for an unfamiliar mouse over the stranger mouse used in the sociability phase
Habituation/dishabituation	Social communication deficits	Time spent interacting with the same mouse during the trial period over several days and time spent with a novel mouse on the last trial
Partition test	Social communication deficits	Time spent near a barrier that separates the test mouse from a stranger mouse
Direct social interaction tests	Social communication deficits	Duration, type (e.g. aggressive), and amount of social interactions
Ultrasonic vocalizations	Social communication deficits	Number, duration, and complexity of vocalizations
Nest-building	Social communication deficits	Amount of nesting material used, size of nest, or numerical scoring system
Self-grooming	Repetitive behavior	Time spent grooming or number of grooming bouts
Jumping	Repetitive behavior	Time spent jumping or number of jumping bouts
Digging	Repetitive behavior	Time spent digging or number of digging bouts
Hole board test	Repetitive behavior	Number of total hole-pokes or number of consecutive (2+) pokes in the same hole
Marble-burying test	Repetitive behavior	Number of marbles buried

The number, duration, and complexity of USVs can be compared.

Nest-building is considered to be another measure of social behavior. The test mouse is given a small amount of cotton or other material to build a nest in its home cage. The amount of material used, the size of the nest, or a scoring system can be used to compare experimental groups [15].

Tests to Assess Repetitive Behavior

Spontaneous repetitive behaviors include excessive self-grooming, jumping, or digging [16]. The time spent engaging in each behavior or number of bouts can be compared.

The hole board test also measures repetitive behavior. Mice are placed on an apparatus with circular holes arranged in a grid [17]. Mice that display repetitive behavior have more nose-pokes into the holes, or explore the same hole repeatedly.

Finally, in the marble burying test, the test mouse is placed in a clean cage with marbles positioned on top of bedding [18]. Mice with repetitive behavior bury more marbles in the bedding than their wild-type littermates.

Tools to Manipulate Gene Expression

The Cre-*loxP* system is the approach taken most often to manipulate gene expression in a cell-type-specific or time-dependent manner [19]. For cell-type-specific manipulations, this method typically involves breeding mice that contain the *Cre recombinase* transgene under the control of a specific promoter with mice that have the target gene flanked by *loxP* sequences (floxed) [20]. Deletions, and rarely duplications, of the target DNA occur when the *loxP* sequences are oriented in the same direction in either the *cis* or *trans* configuration, whereas inversions occur if the *loxP* sequences are oriented in opposite directions. For more precisely-timed manipulations, the Cre recombinase protein is fused to a mutated estrogen receptor, so that tamoxifen treatment causes translocation into the nucleus and subsequent site-specific recombination [21]. Other, but less common, methods for inducible gene expression include the Tet-Off (tTA) and Tet-On (rtTA) systems, where doxycycline treatment represses and activates transcription, respectively [22].

While extraordinarily useful for temporospatial manipulation of genes in mice, the Cre-*loxP* system is not without its limitations. The major limiting factor is the availability of Cre lines that meet the needs of an experiment, in terms of timing and spatial distribution of Cre expression. The specificity and efficiency of recombination relies on the promoter, which may be expressed at various levels in off-target tissues or cell-types. One way to circumvent this problem is to deliver a lentivirus or adeno-associated virus construct containing Cre to mice with the target gene floxed [23]. While this method may limit the expression of Cre to a particular brain region, it is more difficult to control the expression of Cre in different cell types within a particular region. Another potential problem is that Cre expression itself can induce a phenotype by causing recombination at cryptic *loxP* sites in the mouse genome [24], or by affecting the expression of surrounding genes, depending on the insertion site of the transgene. Therefore it is important that proper controls are implemented to compare the phenotypes.

New advances in gene-editing technologies may eventually overcome the limitations of the Cre-*loxP* and similar systems. For example, it has been demonstrated that CRISPR/Cas9 can be used to edit the genome of adult mice and rescue disease phenotypes [25]. Moreover, methods are being developed to increase the cell-type specificity of CRISPR/Cas9-mediated mutations [26]. However, off-target mutations using this system remain a concern and must be assessed individually [27]. Thus, some refinement of this newer technology is necessary before using it to answer questions about the neuroanatomical bases of ASDs.

The Roles of Excitatory and Inhibitory Neurons in ASDs

The two major classes of neurons in the brain are excitatory neurons, those which depolarize the neurons they project onto and thus make them more likely to produce action potentials, and inhibitory neurons, those which hyperpolarize their outputs and make them less likely to produce action potentials. It has been proposed that a common mechanism underlying ASDs is an imbalance between inhibitory and excitatory synaptic transmission in particular circuits. This may reflect a relative increase of function in excitatory neurons compared to that of inhibitory neurons [28], or *vice versa*. Although support for this hypothesis is currently somewhat limited, studies have begun to address it.

One important question is whether disrupting the function of either major class of neurons is sufficient to produce phenotypes related to ASDs and, if so, which types of neurons within these major classes contribute to the phenotypes. Another important question is whether different ASD-causing mutations affect the same neuronal types and circuits, or have shared circuit-level mechanisms. This may translate into whether patients with different mutations require individualized therapy in the clinic. Fortunately, the advent of conditional gene expression technologies and the

production of multiple mouse models of ASDs with high construct validity have made it possible to start exploring these questions.

Manipulations in Large Groups of Excitatory (Glutamatergic) Neurons

Most of the published work on conditional gene expression for ASDs involves manipulation of the mouse version of the genes that are mutated in syndromic autism, such as Rett syndrome (*MECP2*), tuberous sclerosis complex (*TSC1* or *TSC2*), *PTEN*-related disorders, and Angelman syndrome (*UBE3A*). One group found that restricting the deletion of *Mecp2* (*loxP/y*) to excitatory neurons in the forebrain postnatally, starting around postnatal day 21 (P21), with Tg(Camk2a-cre)93Kln (CamKII-cre93; EMMA 01137) results in mice that display reduced sociability in a test similar to the first phase of the three chamber test and reduced preference for the novel mouse in the habituation-dishabituation test [29]. However, another group used $Emx1^{tm1(cre)Krj}$ (Emx1-cre; JAX 005628) to delete *Mecp2* (*loxP/y*) in forebrain excitatory neurons and glia embryonically, starting around embryonic day 9.5 (E9.5), and found no significant changes in either sociability or preference for social novelty in the three chamber test [30]. Neither study reported any repetitive behaviors. Deletion of *Mecp2* (*loxP/y*) selectively in glutamatergic neurons, the most abundant neurotransmitter class of excitatory neurons, in embryos with $Slc17a6^{tm2(cre)Lowl}$ (Vglut2-cre; JAX 028863) does not impact social behavior in the partition test or nose-pokes on the hole board [31]. However, selectively expressing *Mecp2* (*loxP-stop/y*) in glutamatergic neurons with the same Cre line rescues a hypersocial phenotype on the partition test and repetitive nose-pokes on the hole board that occur in the global knockout, which suggests that restoring function to glutamatergic neurons may have network effects that subsequently increase the function of other excitatory neurotransmitters or inhibitory neurons as well [31].

On the other hand, mice that carry a deletion of another ASD candidate gene, *Mef2c* (*loxP/loxP*) with Emx1-cre spend less time interacting with the social stimulus in the first phase of the three chamber test, emit fewer USVs as pups and adults, have lower nest-building scores, and display increased repetitive jumping and fine motor movements [32]. Similarly, deletion of *Cc2d1a* (*loxP/loxP*) with Tg(CamK2a-cre)T29-1Stl (CamKIIα-cre T29-1; JAX 005359) results in mice that display reduced sociability in the first phase of the three chamber test, reduced social approach in a direct social interaction test, reduced numbers of adult USVs, and increased repetitive self-grooming (but no changes in marble-burying) [33]. Mice with *Tsc1* (*loxP/loxP*) deleted with CamKIIα-cre T29-1 also display reduced sociability in the three chamber test and increased marble-burying behavior [34].

Together, these findings indicate that disrupting the function of excitatory neurons can be sufficient to produce ASD-like behaviors, but conflicting findings between models indicate that manipulating one gene may not be generalizable to all ASDs. Therefore, studies using conditional gene expression of other ASD candidate genes are necessary to obtain a more complete understanding.

Inhibitory (GABAergic) Neurons

Although there is some evidence that the dysfunction of excitatory neurons contributes to ASD-like phenotypes, there is accumulating evidence for a role of inhibitory neurons as well. Using the line Tg(Slc32a1-cre)2.1Hzo (Viaat-cre; JAX 017535) to delete *Mecp2* (*loxP/y*) specifically in GABAergic cells in embryos results in mice that show nearly the full spectrum of phenotypes observed in the conventional knockout, including repetitive forelimb stereotypies, increased grooming, sequential head-pokes on the hole board task, decreased nest-building, and increased sociability in the partition test and the three chamber test [35]. Accordingly, selectively restoring *Mecp2* (*loxP-stop/y* and *loxP-stop/+*) to GABAergic cells with Viaat-cre rescues the hypersocial phenotype in the partition test and nest-building deficits in both male and female mice (but repetitive behaviors were not reported) [36]. Moreover, deleting *Mecp2* (*loxP/y*) in a subset of GABAergic cells in the forebrain, embryonically, with Tg(Dlx6a-cre)1Mekk (Dlx5/6-cre; JAX 008199) recapitulates the nest-building, hole board, and hypersocial phenotypes, but not grooming, indicating that some features of ASD may involve other brain regions or cell types [35].

Two of the major subclasses of GABAergic neurons are parvalbumin-positive (PV+) and somatostatin-positive (SOM+) neurons [37]. Thus, one important question is whether either or both of these cell types contribute to ASD phenotypes. Mice that lack *Mecp2* (*loxP/y*) in PV+ neurons, embryonically, created by crossing the floxed mice with $Pvalb^{tm1(cre)Arbr}$ (PV-cre; JAX 008069), display a hypersocial phenotype on the partition test, but show no differences on the hole board [38]. In contrast, mice that lack *Mecp2* (*loxP/y*) in SOM+ neurons, embryonically, created by crossing floxed mice with $Sst^{tm2.1(cre)Zjh}$ (SOM-cre; JAX 013044), display repetitive nose-pokes on the hole board, but have no social phenotype [38]. A different study found no differences in sociability in mice lacking *Mecp2* in PV+ neurons, using the same PV-cre [39].

However, another study confirmed that dysfunctional PV+ neurons contribute to social phenotypes whereas SOM+ neurons do not by heterozygous deletion of the mouse version of the gene underlying Dravet syndrome, *Scn1a* (*loxP/+*), in either type of neuron using PV-cre and SOM-cre, respectively [40]. In this case, the mice lacking *Scn1a* in PV+ neurons show decreased sociability in the three chamber test [40].

It is likely, given that the conditional knockouts restricting *Mecp2* deletion to GABAergic neurons almost completely recapitulate all of the phenotypes observed in the conventional knockout, that the pathogenesis of Rett syndrome primarily involves dysfunctional inhibitory neural networks. Some behavioral deficits have also been reported in conditional *Mecp2* knockouts where the deletion is limited to excitatory neurons, but the decrease in social behavior is distinct from the global knockout, which shows increased social interest in the partition test. It is less clear at this time whether this is generalizable to other ASDs. Therefore, more research is needed that uses conditional gene expression technology and highly penetrant ASD-causing mutations.

Other Neurotransmitter Systems

Other neurotransmitters can either be excitatory or inhibitory, depending on the action of the postsynaptic receptors that are expressed. Since the majority of neurons in the brain are either glutamatergic or GABAergic, less is known about the roles of other neurotransmitters in ASD models. However, some groups have started dissecting the roles of dopamine, serotonin, acetylcholine, and oxytocin.

Restricting the deletion of *Mecp2* (*loxP/y*) to serotonergic neurons, embryonically, with Tg(Fev-cre)1Esd (PET-1 cre; JAX 012712) results in mice that display a hyper-social phenotype on the partition test as well as increased aggression during the resident-intruder test [41]. On the other hand, selectively deleting *Mecp2* (*loxP/y*) in dopaminergic neurons, embryonically, with $Th^{tm1(cre)Te}$ (TH-cre; EMMA 00254) does not influence social behavior in these assays [41]. The PET-1 cre knockout does not have repetitive grooming or marble-burying, but these behaviors were not assessed in the TH-cre knockout [41]. This study, along with others, suggests that dysfunctional serotonergic neurons may contribute to the core symptoms of ASDs, whereas dopaminergic neurons are implicated in comorbid motor functions [42–44]. More support for the role of serotonergic neurons in ASD behaviors comes from deleting *Tsc1* (*loxP/loxP*) selectively in serotonergic neurons, embryonically, with Tg(Slc6a4-cre)ET33Gsat (Slc6a4-cre; MMRRC 031028-UCD), which results in mice with decreased sociability in the first phase of the three chamber test and increased repetitive marble-burying behaviors [34].

Recently, the cholinergic system has also been implicated in the pathogenesis of ASD with conditional gene expression in mice. Selectively deleting *Mecp2* (*loxP/y*) in cholinergic neurons, embryonically, with $Chat^{tm2(cre)Lowl}$ (Chat-cre; JAX 006410) produces mice that fail to show a preference for social novelty in the three chamber test, show decreased social investigation in a direct interaction test, and have impaired nest-building [45]. Interestingly, re-expression of *Mecp2* (*via* microinjection of an AAV) in the basal forebrain, but not in the caudate-putamen, of adult mice rescues the social deficit in the three chamber test [45]. Again, this phenotype is distinct from that in the global *Mecp2* knockout, but nonetheless provides insight into circuits underlying ASDs.

So far, the role of oxytocinergic neurons is less clear. Neither heterozygous (*loxP/+*) nor homozygous (*loxP/loxP*) deletion of *Pten* in oxytocinergic neurons, embryonically, with $Oxt^{tm1.1(cre)Dolsn}$ (Oxt-cre; JAX 024234) results in deficits in the three chamber, habituation/dishabituation, or marble-burying tests [46]. This is somewhat puzzling, given that *Pten* haploinsufficient, oxytocin knockout, and oxytocin receptor knockout mice show some overlapping impairments on these assays [47–50]. Perhaps mutations in *PTEN* and other ASD-causing mutations have more of an effect on neurons expressing the oxytocin receptor, rather than on oxytocinergic neurons. More work needs to be done to clarify the role of oxytocin and other neurotransmitters in ASDs.

The Role of the Cerebellum in ASDs

In recent years, the cerebellum has emerged as a brain region that may be implicated not only in motor impairments, but also in the core behaviors of ASDs, particularly because cerebellar Purkinje cells project to the thalamus and, for example, affect dopamine efflux in the prefrontal cortex [51]. Mice with heterozygous (*loxP/+*) or homozygous (*loxP/loxP*) deletion of *Tsc1* mostly restricted to Purkinje cells, postnatally, by means of Tg(Pcp2-cre)2Mpin (L7-cre; JAX 004146) fail to demonstrate preference for a social stimulus over a non-social stimulus or for novel social interactions over familiar ones in the three chamber test [52]. These mice also engage in elevated rates of self-grooming [52]. Similarly, mice with *Tsc2* homozygously deleted in Purkinje cells with L7-cre and heterozygously deleted in all other cell types (loxP/-) have impaired social interactions and increased rates of marble-burying [53]. Mice with *Pten* deleted (*loxP/loxP*) in Purkinje cells by L7-Cre display reduced sociability and engage in repetitive

upright scrabbling, but have reduced self-grooming [54]. Interestingly, deleting *Pten* (*loxP/loxP*) primarily in cerebellar granule cells with Tg(Gfap-cre)1Sbk (Gfap-cre) results in mice that are hyposocial in the partition test and show reduced sociability in the three chamber test, but show reduced repetitive behaviors in the marble-burying and hole board tests, and have no changes in USVs as pups [55].

Most recently, two similar studies produced opposite conclusions regarding the cerebellum's role in social behavior. Purkinje cell deletion of exon 7 of *Shank2* (*loxP/loxP*) with L7-Cre resulted in mice that failed to demonstrate a preference for sociability or for social novelty [56]. However, mice with a deletion of exons 6-7 of *Shank2* (*loxP/loxP*) with Tg(Pcp2-cre)3555Jdhu (Pcp2-cre; JAX 010536) were similar to controls on the three chamber task and had similar USVs, but had increased nose-pokes on the hole board [57]. Both groups of mice showed normal levels of self-grooming and marble-burying.

The divergent findings regarding social behavior between the two studies using *Shank2* conditional mutants have several possible explanations. One is that minor differences in the exonic deletions could disrupt the expression of different sets of Shank2 isoforms, but current knowledge regarding the structure of *Shank2* suggests this unlikely to be the case [58]. Another possibility is that the use of different Cre lines (JAX 004146 vs. JAX 010536) in the two studies may have some effect on behavior; although both Cre lines involve the same promoter, there may be differences in the spatial pattern and timing of Cre expression. The most likely explanation is that the two labs used different methods for determining behavioral phenotypes (for example, strains chosen for the stranger mice and habituation times). This last explanation is especially convincing because the wild-types in one study showed a preference for social novelty [56], whereas those in the other study did not [57], most likely indicating differences in behavioral methods.

Further investigation into the cerebellum's contributions to ASD phenotypes is clearly warranted. Moreover, the contradictory findings from similar studies underscore the need for stricter standards for behavioral methods or more side-by-side comparisons between lines of mice within investigating laboratories to make the strongest conclusions. It is unclear at this time whether, for instance, *Tsc1, Tsc2, Pten,* and *Shank2* deletions in the cerebellum have different effects on self-grooming, or whether the discrepancies between the studies are due to varying methods. It is even less clear which cerebellum-related circuitry is responsible for the ASD-like behaviors and whether there is any translational value of these findings for human patients.

Developmental Time Points Implicated through Inducible Mutations and Rescues

Clinically, ASDs are classified as a group of neurodevelopmental disorders. However, the developmental origin of ASDs has not been clearly defined. This knowledge is likely critical for effective clinical intervention. Conditional gene expression, through inducible mutations and rescues, allows researchers to determine whether ASDs are the result of disrupted development and/or ongoing neuronal dysfunction. Conditional-rescue mice also provide proof of principle for the potential of gene therapy. One attractive hypothesis for the origin of ASDs is that perturbations to synaptic development prevent patients from developing skills during a limited window of opportunity, or a critical period [59]. This would suggest that reversing the symptoms of ASD may be difficult, if not impossible. Some studies suggest that early intervention is necessary for preventing the onset of ASD-like phenotypes. However, multiple studies have also challenged the notion that ASDs represent an irreversible disruption of brain development.

Deleting *Mecp2* in adulthood (*loxP/y*) using the ubiquitously-expressed, inducible Cre line, Tg(CAG-cre/Esr1*)5Amc (CAGGS-CreER; JAX 004453) and tamoxifen administration, causes the impaired nest-building phenotype that is observed in germline knockouts [60]. Reactivating *Mecp2* in male (*loxP-stop/y*) and female (*loxP-stop/+*) model mice with the same Cre line and tamoxifen administration partially and completely rescue this nest-building impairment, respectively [61]. Neither of these studies reported any other ASD-related behaviors, nor did two other studies that reactivated *Mecp2* expression [62, 63]. However, reinstating *Ube3a* (*loxP-stop/p+*) using the same inducible Cre line fails to rescue impaired nest-building in these mice, even when tamoxifen is administered to newborn mice; only embryonic reinstatement of *Ube3a* successfully rescues this phenotype [64].

On the other hand, several ASD-related behaviors are rescued by repressing the expression of a dominant-negative form of *Nrxn1β* in excitatory neurons in adulthood using Tg(Camk2a-tTA)1Mmay (CaMKII-tTA; JAX 003010) with the administration of doxycycline, which reverses increased self-grooming and impaired sociability and preference for social novelty in the three chamber test [65]. Similarly, re-expressing normal *Shank3* isoforms by reverting an inverted portion of the gene after administering tamoxifen to adult mice (*loxP/loxP*; CAGGS-CreER) rescues increased grooming, impaired sociability in the first phase of the three chamber test, and reduced preference for social novelty in the second phase of this test [66].

While in general these studies provide some promising evidence for the reversibility of the core symptoms of

Table 2 Summary of transgenic mouse lines used for the temporospatial manipulation of ASD genes in the mouse brain.

Cre line (or "Tet-Off" line)	Commercial availability	Cells targeted	Timing of gene expression manipulation	Social communication phenotypes	Repetitive behavior phenotypes	References
CamKII-cre93	EMMA 01137	Excitatory forebrain neurons	Postnatal (~P21)	*Mecp2 (loxP/y)* ↓ Sociability* ↓ Social novelty*** NR USVs NR nest-building	*Mecp2 (loxP/y)* NR grooming NR hole board NR marble-burying	Cre line primary: [70] *Mecp2* study: [29]
CamKIIα-cre T29-1	JAX 005359	Excitatory forebrain neurons	Postnatal (~P21)	*Cc2d1a (loxP/loxP)* ↓ Sociability*, **** NR social novelty ↓ Adult USVs NR nest-building *Tsc1 (loxP/loxP)* ↓ Sociability* NR social novelty NR USVs NR nest-building	*Cc2d1a (loxP/loxP)* ↑ Grooming NR hole board ↔ Marble-burying *Tsc1 (loxP/loxP)* NR grooming NR hole board ↑ Marble-burying	Cre line primary: [20] *Cc2d1a* study: [33] *Tsc1* study: [34]
Emx1-cre	JAX 005628	Excitatory forebrain neurons and glia	Embryonic	*Mecp2 (loxP/y)* ↔ Sociability* ↔ Social novelty* NR USVs NR nest-building *Mef2c (loxP/loxP)* ↓ Sociability* NR social novelty ↓ Pup, adult USVs ↓ Nest-building	*Mecp2 (loxP/y)* NR grooming NR hole board NR marble-burying *Mef2c (loxP/loxP)* NR grooming NR hole board NR marble-burying	Cre line primary: [71] *Mecp2* study: [30] *Mef2c* study: [32]
Vglut2-cre	JAX 028863	All glutamatergic neurons	Embryonic	*Mecp2 (loxP/y)* ↔ Sociability** NR social novelty NR USVs NR nest-building *Mecp2 (loxP-stop/y)* ↑ Sociability** resc. NR social novelty NR USVs NR nest-building	*Mecp2 (loxP/y)* NR grooming ↔ Hole board NR marble-burying *Mecp2 (loxP-stop/y)* NR grooming ↑ Hole board resc. NR marble-burying	Cre line primary: [72] *Mecp2* study: [31]

Table 2 continued

Cre line (or "Tet-Off" line)	Commercial availability	Cells targeted	Timing of gene expression manipulation	Social communication phenotypes	Repetitive behavior phenotypes	References
Viaat-cre	JAX 017535	All GABAergic neurons	Embryonic	*Mecp2 (loxP/y)* ↑ Sociability*, ** NR social novelty NR USVs ↓ Nest-building *Mecp2 (loxP-stop/y)* ↑ Sociability** resc. NR social novelty NR USVs ↓ Nest-building resc.	*Mecp2 (loxP/y)* ↑ Grooming ↑ Hole board NR marble-burying *Mecp2 (loxP-stop/y)* NR grooming NR hole board NR marble-burying	Cre line primary: [35] *Mecp2* deletion study: [35] *Mecp2* rescue study: [36]
Dlx5/6-cre	JAX 008199	Subset of GABAergic neurons in forebrain	Embryonic	*Mecp2 (loxP/y)* ↑ Sociability*, ** NR social novelty NR USVs ↓ Nest-building	*Mecp2 (loxP/y)* ↔ Grooming ↑ Hole board NR marble-burying	Cre line primary: [73] *Mecp2* study: [35]
PV-cre	JAX 008069	PV+ GABAergic neurons	Embryonic	*Mecp2 (loxP/y)* ↑ Sociability** ↔ Sociability* NR social novelty NR USVs NR nest-building *Scn1a (loxP/+)* ↓ Sociability* NR social novelty NR USVs NR nest-building	*Mecp2 (loxP/y)* NR grooming ↔ Hole board NR marble-burying *Scn1a (loxP/+)* NR grooming NR hole board NR marble-burying	Cre line primary: [74] *Mecp2* studies: [38, 39] *Scn1a* study: [40]
SOM-cre	JAX 013044	SOM+ GABAergic neurons	Embryonic	*Mecp2 (loxP/y)* ↔ Sociability** NR social novelty NR USVs NR nest-building *Scn1a (loxP/+)* ↔ Sociability* NR social novelty NR USVs NR nest-building	*Mecp2 (loxP/y)* NR grooming ↑ Hole board NR marble-burying *Scn1a (loxP/+)* NR grooming NR hole board NR marble-burying	Cre line primary: [75] *Mecp2* study: [38] *Scn1a* study: [40]

Table 2 continued

Cre line (or "Tet-Off" line)	Commercial availability	Cells targeted	Timing of gene expression manipulation	Social communication phenotypes	Repetitive behavior phenotypes	References
PET-1 cre	JAX 012712	All serotonergic neurons	Embryonic	*Mecp2 (loxP/y)* ↑ Sociability** ↑ Aggression**** NR social novelty NR USVs NR nest-building	*Mecp2 (loxP/y)* ↔ Grooming NR hole board ↔ Marble-burying	Cre line primary: [76] *Mecp2* study: [41]
Slc6a4-cre	MMRRC 031028-UCD	All serotonergic neurons	Embryonic	*Tsc1 (loxP/loxP)* ↓ Sociability* NR social novelty NR USVs NR nest-building	*Tsc1* (*loxP/loxP*) NR grooming NR hole board ↑ Marble-burying	Cre line primary: [77] *Tsc1* study: [34]
TH-cre	EMMA 00254	All dopaminergic neurons	Embryonic	*Mecp2 (loxP/y)* ↔ Sociability** ↔ Aggression**** NR social novelty NR USVs NR nest-building	*Mecp2 (loxP/y)* NR grooming NR hole board NR marble-burying	Cre line primary: [78] *Mecp2* study: [41]
Chat-cre	JAX 006410	All cholinergic neurons	Embryonic	*Mecp2 (loxP/y)* ↓ Sociability**** ↓ Social novelty* NR USVs ↓ Nest-building	*Mecp2 (loxP/y)* NR grooming NR hole board NR marble-burying	Cre line primary: [79] *Mecp2* study: [45]
Oxt-cre	JAX 024234	All oxytocinergic neurons	Embryonic	*Pten* (*loxP/+*) and Pten (*loxP/loxP*) ↔ Sociability*, *** ↔ Social novelty*, *** NR USVs NR nest-building	*Pten* (*loxP/+*) and Pten (*loxP/loxP*) NR grooming NR hole board ↔ Marble-burying	Cre line primary: [80] *Pten* study: [46]

Table 2 continued

Cre line (or "Tet-Off" line)	Commercial availability	Cells targeted	Timing of gene expression manipulation	Social communication phenotypes	Repetitive behavior phenotypes	References
L7-cre	JAX 004146	Cerebellar Purkinje cells	Early postnatal	*Tsc1* (*loxP/+*) and *Tsc1* (*loxP/loxP*) ↓ Sociability* ↓ Social novelty* NR USVs NR nest-building *Tsc2* (*loxP/-*) ↓ Sociability* ↓ Social novelty* NR USVs NR nest-building *Pten* (*loxP/loxP*) ↓ Sociability* NR social novelty NR USVs NR nest-building *Shank2e6-7* (*loxP/loxP*) ↓ Sociability* ↓ Social novelty* NR USVs NR nest-building	*Tsc1* (*loxP/+*) and *Tsc1* (*loxP/loxP*) ↑ Grooming NR hole board NR marble-burying *Tsc2* (*loxP/-*) NR grooming NR hole board ↑ Marble-burying *Pten* (*loxP/loxP*) ↓ Grooming NR hole board NR marble-burying *Shank2e7* (*loxP/loxP*) ↔ Grooming NR hole board ↔ Marble-burying	Cre line primary: [81] *Tsc1* study: [52] *Tsc2* study: [53] *Pten* study: [54] *Shank2* study: [56]
Pcp2-cre	JAX 010536	Cerebellar Purkinje cells	Early postnatal	*Shank2e7* (*loxP/loxP*) ↔ Sociability* ↔ Social novelty* ↔ Adult USVs NR nest-building	*Shank2e6-7* (*loxP/loxP*) ↔ Grooming ↑ Hole board ↔ Marble-burying	Cre line primary: [82] *Shank2* study: [57]
Gfap-cre	N/A	Mostly cerebellar granule cells, few hippocampal neurons	Unclear	*Pten* (*loxP/loxP*) ↓ Sociability*, ** NR social novelty ↔ Pup USVs NR nest-building	*Pten* (*loxP/loxP*) NR grooming ↓ Hole board ↓ Marble-burying	Cre line primary: [83] *Pten* study: [55]

Table 2 continued

Cre line (or "Tet-Off" line)	Commercial availability	Cells targeted	Timing of gene expression manipulation	Social communication phenotypes	Repetitive behavior phenotypes	References
CAGGS-CreER	JAX 004453	Ubiquitous	Concurrent with tamoxifen administration (P60+ for all examples)	*Mecp2* (*loxP/y*) NR sociability NR social novelty NR USVs ↓ Nest-building *Mecp2* (*loxP-stop/y*) and *Mecp2* (*loxP-stop/+*) NR sociability NR social novelty NR USVs ↓ Nest-building resc. *Ube3a (loxP-stop/p+)* NR sociability NR social novelty NR USVs ↓ Nest-building no resc. *Shank3 (loxP/loxP)* inverted ↓ Sociability* resc. ↓ Social novelty* resc. NR USVs NR nest-building	*Mecp2* (*loxP/y*) NR grooming NR hole board NR marble-burying *Mecp2* (*loxP-stop/y*) and *Mecp2* (*loxP-stop/+*) NR grooming NR hole board NR marble-burying *Ube3a (loxP-stop/p+)* NR grooming NR hole board ↓ Marble-burying no resc. *Shank3 (loxP/loxP)* inverted ↑ Grooming resc. NR hole board NR marble-burying	Cre line primary: [84] *Mecp2* deletion study: [60] *Mecp2* rescue study: [61] *Ube3a* study: [64] *Shank3* study: [66]
CaMKII-tTA	JAX 003010	Excitatory forebrain neurons	Concurrent with doxycycline administration (P60+ for this example)	*TRE-HAβnrx1ΔC* ↓ Sociability* resc. ↓ Social novelty* resc. NR USVs NR nest-building	*TRE-HAβnrx1ΔC* ↑ Grooming resc. NR hole board NR marble-burying	Tet-Off line primary: [85] *Nrxn1β* study: [65]

NR behavioral test not reported, *resc.* abnormal phenotype was rescued with genetic manipulation, *no resc.* genetic manipulation failed to rescue abnormal phenotype, *JAX* The Jackson Laboratory, *EMMA* The European Mouse Mutant Archive, *MMRRC* Mutant Mouse Resource & Research Centers.

* In the three chamber test; ** in the partition test; *** in the habituation/dishabituation test; **** in a direct social interaction test.

↑ Mutant mice have significantly elevated amounts of this behavior when compared to controls.

↓ Mutant mice have significantly decreased amounts of this behavior when compared to controls.

↔ There were no significant differences between mutants and their controls on this behavioral test.

ASDs, research using other models and additional ASD-relevant behaviors is necessary to make more definitive conclusions. An interesting and important question is whether the findings from mouse models can be translated to humans and if the reversibility of symptoms holds true.

Concluding Remarks and Future Directions

It is clear that, at least in some cases, disrupting the function of either excitatory or inhibitory neurons is sufficient to cause the core behaviors of ASD. Likewise, manipulations of either forebrain or cerebellar neurons can induce ASD-like phenotypes in mice. The study of specific regions outside of the cerebellum is one particularly important direction for future study. For example, several studies have implicated the striatum in the pathophysiology of ASDs by showing region-specific molecular and electrophysiological alterations [67, 68]. Moreover, suppression of Shank3 expression using shRNA targeted to the ventral tegmental area can induce social deficits [69]. However, specific circuits have not yet been causally linked to ASD-relevant behaviors using cell-type specific Cre lines.

The findings reviewed here (summarized in Table 2) support the value of conditional gene-expression technology to delineate the cells and circuits underlying ASDs. However, because these studies are mostly conducted in syndromic ASD models, it remains to be seen whether these findings and conclusions are unique to these specific ASD-causing mutations. Since there are inconsistencies between studies that manipulate the same gene, it may be premature to generalize the limited available evidence. Human genetics studies have supported the role of several hundreds of genes in ASDs, so one challenge is to find out whether there are shared circuit-level mechanisms among ASDs caused by different mutations or etiologies. Caution must be taken when interpreting the results from studies that utilize conditional gene expression. Too often the efficiency and specificity of the Cre lines are overestimated. Moreover, disruption of one set of cells may have downstream or compensatory effects on other cell types in the same brain region, or even in different brain regions due to axonal projections. Hopefully, the development of more precise technology for manipulating cell types and circuits will facilitate a more complete understanding of the neural underpinnings of ASDs.

Acknowledgements We would like to thank the members of the Jiang lab for their contributions to this review. S.W.H. is supported by a Weatherstone Predoctoral Fellowship from Autism Speaks. The research in the Jiang lab is supported by NIH Grants 5R01-MH098114-03, 1R21-HD077197-01, and 1R21-MH104316-01, a Duke Institute for Brain Science Incubator award, Autism Speaks, the Marcus Foundation, the Foundation for Prader-Willi Research, the Phelan-McDermid Syndrome Foundation, the Ruth. K. Broad Foundation, Roche Inc., and SAGE Inc.

References

1. Hulbert SW, Jiang YH. Monogenic mouse models of autism spectrum disorders: Common mechanisms and missing links. Neuroscience 2016, 321: 3–23.
2. Alexander AL, Lee JE, Lazar M, Boudos R, DuBray MB, Oakes TR, *et al.* Diffusion tensor imaging of the corpus callosum in Autism. Neuroimage 2007, 34: 61–73.
3. Di Martino A, Yan CG, Li Q, Denio E, Castellanos FX, Alaerts K, *et al.* The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. Mol Psychiatry 2014, 19: 659–667.
4. Sztainberg Y, Zoghbi HY. Lessons learned from studying syndromic autism spectrum disorders. Nat Neurosci 2016, 19: 1408–1417.
5. American Psychiatric Association. DSM-5 Task Force. Diagnostic and statistical manual of mental disorders: DSM-5. 5th ed. Washington, D.C.: American Psychiatric Association, 2013.
6. Silverman JL, Yang M, Lord C, Crawley JN. Behavioural phenotyping assays for mouse models of autism. Nat Rev Neurosci 2010, 11: 490–502.
7. Bey AL, Jiang YH. Overview of mouse models of autism spectrum disorders. Curr Protoc Pharmacol 2014, 66: 5.66.61–26.
8. Nadler JJ, Moy SS, Dold G, Trang D, Simmons N, Perez A, *et al.* Automated apparatus for quantitation of social approach behaviors in mice. Genes Brain Behav 2004, 3: 303–314.
9. Choleris E, Little SR, Mong JA, Puram SV, Langer R, Pfaff DW. Microparticle-based delivery of oxytocin receptor antisense DNA in the medial amygdala blocks social recognition in female mice. Proc Natl Acad Sci U S A 2007, 104: 4670–4675.
10. Kudryavtseva NN. Use of the "partition" test in behavioral and pharmacological experiments. Neurosci Behav Physiol 2003, 33: 461–471.
11. Moretti P, Bouwknecht JA, Teague R, Paylor R, Zoghbi HY. Abnormalities of social interactions and home-cage behavior in a mouse model of Rett syndrome. Hum Mol Genet 2005, 14: 205–220.
12. Terranova ML, Laviola G. Scoring of social interactions and play in mice during adolescence. Curr Protoc Toxicol 2005, Chapter 13: Unit13.10.
13. Winslow JT, Miczek KA. Habituation of aggression in mice: pharmacological evidence of catecholaminergic and serotonergic mediation. Psychopharmacology (Berl) 1983, 81: 286–291.
14. Scattoni ML, Crawley J, Ricceri L. Ultrasonic vocalizations: a tool for behavioural phenotyping of mouse models of neurodevelopmental disorders. Neurosci Biobehav Rev 2009, 33: 508–515.
15. Deacon RM. Assessing nest building in mice. Nat Protoc 2006, 1: 1117–1119.
16. Crawley JN. Translational animal models of autism and neurodevelopmental disorders. Dialogues Clin Neurosci 2012, 14: 293–305.

17. Moy SS, Nadler JJ, Poe MD, Nonneman RJ, Young NB, Koller BH, *et al.* Development of a mouse test for repetitive, restricted behaviors: relevance to autism. Behav Brain Res 2008, 188: 178–194.
18. Thomas A, Burant A, Bui N, Graham D, Yuva-Paylor LA, Paylor R. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. Psychopharmacology (Berl) 2009, 204: 361–373.
19. Gierut JJ, Jacks TE, Haigis KM. Strategies to achieve conditional gene mutation in mice. Cold Spring Harb Protoc 2014, 2014: 339–349.
20. Tsien JZ, Chen DF, Gerber D, Tom C, Mercer EH, Anderson DJ, *et al.* Subregion- and cell type-restricted gene knockout in mouse brain. Cell 1996, 87: 1317–1326.
21. Metzger D, Clifford J, Chiba H, Chambon P. Conditional site-specific recombination in mammalian cells using a ligand-dependent chimeric Cre recombinase. Proc Natl Acad Sci U S A 1995, 92: 6991–6995.
22. Sprengel R, Hasan MT. Tetracycline-controlled genetic switches. Handb Exp Pharmacol 2007: 49–72.
23. Heldt SA, Ressler KJ. The use of lentiviral vectors and Cre/loxP to investigate the function of genes in complex behaviors. Front Mol Neurosci 2009, 2: 22.
24. Semprini S, Troup TJ, Kotelevtseva N, King K, Davis JR, Mullins LJ, *et al.* Cryptic loxP sites in mammalian genomes: genome-wide distribution and relevance for the efficiency of BAC/PAC recombineering techniques. Nucleic Acids Res 2007, 35: 1402–1410.
25. Yin H, Xue W, Chen S, Bogorad RL, Benedetti E, Grompe M, *et al.* Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat Biotechnol 2014, 32: 551–553.
26. Wang J, Li X, Zhao Y, Li J, Zhou Q, Liu Z. Generation of cell-type-specific gene mutations by expressing the sgRNA of the CRISPR system from the RNA polymerase II promoters. Protein Cell 2015, 6: 689–692.
27. Fu Y, Foden JA, Khayter C, Maeder ML, Reyon D, Joung JK, *et al.* High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat Biotechnol 2013, 31: 822–826.
28. Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2003, 2: 255–267.
29. Gemelli T, Berton O, Nelson ED, Perrotti LI, Jaenisch R, Monteggia LM. Postnatal loss of methyl-CpG binding protein 2 in the forebrain is sufficient to mediate behavioral aspects of Rett syndrome in mice. Biol Psychiatry 2006, 59: 468–476.
30. Goffin D, Brodkin ES, Blendy JA, Siegel SJ, Zhou Z. Cellular origins of auditory event-related potential deficits in Rett syndrome. Nat Neurosci 2014, 17: 804–806.
31. Meng X, Wang W, Lu H, He LJ, Chen W, Chao ES, *et al.* Manipulations of MeCP2 in glutamatergic neurons highlight their contributions to Rett and other neurological disorders. Elife 2016, 5.
32. Harrington AJ, Raissi A, Rajkovich K, Berto S, Kumar J, Molinaro G, *et al.* MEF2C regulates cortical inhibitory and excitatory synapses and behaviors relevant to neurodevelopmental disorders. Elife 2016, 5.
33. Oaks AW, Zamarbide M, Tambunan DE, Santini E, Di Costanzo S, Pond HL, *et al.* Cc2d1a loss of function disrupts functional and morphological development in forebrain neurons leading to cognitive and social deficits. Cereb Cortex 2016. doi:10.1093/cercor/bhw009.
34. McMahon JJ, Yu W, Yang J, Feng H, Helm M, McMahon E, *et al.* Seizure-dependent mTOR activation in 5-HT neurons promotes autism-like behaviors in mice. Neurobiol Dis 2015, 73: 296–306.
35. Chao HT, Chen H, Samaco RC, Xue M, Chahrour M, Yoo J, *et al.* Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. Nature 2010, 468: 263–269.
36. Ure K, Lu H, Wang W, Ito-Ishida A, Wu Z, He LJ, *et al.* Restoration of Mecp2 expression in GABAergic neurons is sufficient to rescue multiple disease features in a mouse model of Rett syndrome. Elife 2016, 5.
37. Rudy B, Fishell G, Lee S, Hjerling-Leffler J. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Dev Neurobiol 2011, 71: 45–61.
38. Ito-Ishida A, Ure K, Chen H, Swann JW, Zoghbi HY. Loss of MeCP2 in parvalbumin-and somatostatin-expressing neurons in mice leads to distinct Rett syndrome-like phenotypes. Neuron 2015, 88: 651–658.
39. He LJ, Liu N, Cheng TL, Chen XJ, Li YD, Shu YS, *et al.* Conditional deletion of Mecp2 in parvalbumin-expressing GABAergic cells results in the absence of critical period plasticity. Nat Commun 2014, 5: 5036.
40. Rubinstein M, Han S, Tai C, Westenbroek RE, Hunker A, Scheuer T, *et al.* Dissecting the phenotypes of Dravet syndrome by gene deletion. Brain 2015, 138: 2219–2233.
41. Samaco RC, Mandel-Brehm C, Chao HT, Ward CS, Fyffe-Maricich SL, Ren J, *et al.* Loss of MeCP2 in aminergic neurons causes cell-autonomous defects in neurotransmitter synthesis and specific behavioral abnormalities. Proc Natl Acad Sci U S A 2009, 106: 21966–21971.
42. Riday TT, Dankoski EC, Krouse MC, Fish EW, Walsh PL, Han JE, *et al.* Pathway-specific dopaminergic deficits in a mouse model of Angelman syndrome. J Clin Invest 2012, 122: 4544–4554.
43. Mulherkar SA, Jana NR. Loss of dopaminergic neurons and resulting behavioural deficits in mouse model of Angelman syndrome. Neurobiol Dis 2010, 40: 586–592.
44. Rothwell PE, Fuccillo MV, Maxeiner S, Hayton SJ, Gokce O, Lim BK, *et al.* Autism-associated neuroligin-3 mutations commonly impair striatal circuits to boost repetitive behaviors. Cell 2014, 158: 198–212.
45. Zhang Y, Cao SX, Sun P, He HY, Yang CH, Chen XJ, *et al.* Loss of MeCP2 in cholinergic neurons causes part of RTT-like phenotypes via α7 receptor in hippocampus. Cell Res 2016, 26: 728–742.
46. Clipperton-Allen AE, Chen Y, Page DT. Autism-relevant behaviors are minimally impacted by conditional deletion of Pten in oxytocinergic neurons. Autism Res 2016, 9: 1248–1262.
47. Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, Winslow JT. Social amnesia in mice lacking the oxytocin gene. Nat Genet 2000, 25: 284–288.
48. Pobbe RL, Pearson BL, Defensor EB, Bolivar VJ, Young WS, Lee HJ, *et al.* Oxytocin receptor knockout mice display deficits in the expression of autism-related behaviors. Horm Behav 2012, 61: 436–444.
49. Lazzari VM, Becker RO, de Azevedo MS, Morris M, Rigatto K, Almeida S, *et al.* Oxytocin modulates social interaction but is not essential for sexual behavior in male mice. Behav Brain Res 2013, 244: 130–136.
50. Clipperton-Allen AE, Page DT. Pten haploinsufficient mice show broad brain overgrowth but selective impairments in autism-relevant behavioral tests. Hum Mol Genet 2014, 23: 3490–3505.
51. Mittleman G, Goldowitz D, Heck DH, Blaha CD. Cerebellar modulation of frontal cortex dopamine efflux in mice: relevance to autism and schizophrenia. Synapse 2008, 62: 544–550.
52. Tsai PT, Hull C, Chu Y, Greene-Colozzi E, Sadowski AR, Leech JM, *et al.* Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. Nature 2012, 488: 647–651.
53. Reith RM, McKenna J, Wu H, Hashmi SS, Cho SH, Dash PK, *et al.* Loss of Tsc2 in Purkinje cells is associated with autistic-like

behavior in a mouse model of tuberous sclerosis complex. Neurobiol Dis 2013, 51: 93–103.
54. Cupolillo D, Hoxha E, Faralli A, De Luca A, Rossi F, Tempia F, *et al.* Autistic-Like Traits and Cerebellar Dysfunction in Purkinje Cell PTEN Knock-Out Mice. Neuropsychopharmacology 2016, 41: 1457–1466.
55. Lugo JN, Smith GD, Arbuckle EP, White J, Holley AJ, Floruta CM, *et al.* Deletion of PTEN produces autism-like behavioral deficits and alterations in synaptic proteins. Front Mol Neurosci 2014, 7: 27.
56. Peter S, Ten Brinke MM, Stedehouder J, Reinelt CM, Wu B, Zhou H, *et al.* Dysfunctional cerebellar Purkinje cells contribute to autism-like behaviour in Shank2-deficient mice. Nat Commun 2016, 7: 12627.
57. Ha S, Lee D, Cho YS, Chung C, Yoo YE, Kim J, *et al.* Cerebellar Shank2 regulates excitatory synapse density, motor coordination, and specific repetitive and anxiety-like behaviors. J Neurosci 2016, 36: 12129–12143.
58. Jiang YH, Ehlers MD. Modeling autism by SHANK gene mutations in mice. Neuron 2013, 78: 8–27.
59. Berger JM, Rohn TT, Oxford JT. Autism as the Early Closure of a Neuroplastic Critical Period Normally Seen in Adolescence. Biol Syst Open Access 2013, 1.
60. McGraw CM, Samaco RC, Zoghbi HY. Adult neural function requires MeCP2. Science 2011, 333: 186.
61. Lang M, Wither RG, Colic S, Wu C, Monnier PP, Bardakjian BL, *et al.* Rescue of behavioral and EEG deficits in male and female Mecp2-deficient mice by delayed Mecp2 gene reactivation. Hum Mol Genet 2014, 23: 303–318.
62. Guy J, Gan J, Selfridge J, Cobb S, Bird A. Reversal of neurological defects in a mouse model of Rett syndrome. Science 2007, 315: 1143–1147.
63. Robinson L, Guy J, McKay L, Brockett E, Spike RC, Selfridge J, *et al.* Morphological and functional reversal of phenotypes in a mouse model of Rett syndrome. Brain 2012, 135: 2699–2710.
64. Silva-Santos S, van Woerden GM, Bruinsma CF, Mientjes E, Jolfaei MA, Distel B, *et al.* Ube3a reinstatement identifies distinct developmental windows in a murine Angelman syndrome model. J Clin Invest 2015, 125: 2069–2076.
65. Rabaneda LG, Robles-Lanuza E, Nieto-González JL, Scholl FG. Neurexin dysfunction in adult neurons results in autistic-like behavior in mice. Cell Rep 2014, 8: 338–346.
66. Mei Y, Monteiro P, Zhou Y, Kim JA, Gao X, Fu Z, *et al.* Adult restoration of Shank3 expression rescues selective autistic-like phenotypes. Nature 2016, 530: 481–484.
67. Fuccillo MV. Striatal circuits as a common node for autism pathophysiology. Front Neurosci 2016, 10: 27.
68. Wang X, Bey AL, Katz BM, Badea A, Kim N, David LK, *et al.* Altered mGluR5-Homer scaffolds and corticostriatal connectivity in a Shank3 complete knockout model of autism. Nat Commun 2016, 7: 11459.
69. Bariselli S, Tzanoulinou S, Glangetas C, Prévost-Solié C, Pucci L, Viguié J, *et al.* SHANK3 controls maturation of social reward circuits in the VTA. Nat Neurosci 2016, 19: 926–934.
70. Minichiello L, Korte M, Wolfer D, Kühn R, Unsicker K, Cestari V, *et al.* Essential role for TrkB receptors in hippocampus-mediated learning. Neuron 1999, 24: 401–414.
71. Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JL, Jones KR. Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. J Neurosci 2002, 22: 6309–6314.
72. Vong L, Ye C, Yang Z, Choi B, Chua S, Lowell BB. Leptin action on GABAergic neurons prevents obesity and reduces inhibitory tone to POMC neurons. Neuron 2011, 71: 142–154.
73. Monory K, Massa F, Egertová M, Eder M, Blaudzun H, Westenbroek R, *et al.* The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron 2006, 51: 455–466.
74. Hippenmeyer S, Vrieseling E, Sigrist M, Portmann T, Laengle C, Ladle DR, *et al.* A developmental switch in the response of DRG neurons to ETS transcription factor signaling. PLoS Biol 2005, 3: e159.
75. Taniguchi H, He M, Wu P, Kim S, Paik R, Sugino K, *et al.* A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. Neuron 2011, 71: 995–1013.
76. Scott MM, Wylie CJ, Lerch JK, Murphy R, Lobur K, Herlitze S, *et al.* A genetic approach to access serotonin neurons for in vivo and in vitro studies. Proc Natl Acad Sci U S A 2005, 102: 16472–16477.
77. Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, *et al.* Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. J Neurosci 2007, 27: 9817–9823.
78. Lindeberg J, Usoskin D, Bengtsson H, Gustafsson A, Kylberg A, Söderström S, *et al.* Transgenic expression of Cre recombinase from the tyrosine hydroxylase locus. Genesis 2004, 40: 67–73.
79. Rossi J, Balthasar N, Olson D, Scott M, Berglund E, Lee CE, *et al.* Melanocortin-4 receptors expressed by cholinergic neurons regulate energy balance and glucose homeostasis. Cell Metab 2011, 13: 195–204.
80. Wu Z, Xu Y, Zhu Y, Sutton AK, Zhao R, Lowell BB, *et al.* An obligate role of oxytocin neurons in diet induced energy expenditure. PLoS One 2012, 7: e45167.
81. Barski JJ, Dethleffsen K, Meyer M. Cre recombinase expression in cerebellar Purkinje cells. Genesis 2000, 28: 93–98.
82. Zhang XM, Ng AH, Tanner JA, Wu WT, Copeland NG, Jenkins NA, *et al.* Highly restricted expression of Cre recombinase in cerebellar Purkinje cells. Genesis 2004, 40: 45–51.
83. Kwon CH, Zhu X, Zhang J, Knoop LL, Tharp R, Smeyne RJ, *et al.* Pten regulates neuronal soma size: a mouse model of Lhermitte-Duclos disease. Nat Genet 2001, 29: 404–411.
84. Hayashi S, McMahon AP. Efficient recombination in diverse tissues by a tamoxifen-inducible form of Cre: a tool for temporally regulated gene activation/inactivation in the mouse. Dev Biol 2002, 244: 305–318.
85. Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER. Control of memory formation through regulated expression of a CaMKII transgene. Science 1996, 274: 1678–1683.

Neurosci. Bull. April, 2017, 33(2):219–237
DOI 10.1007/s12264-017-0118-1

www.neurosci.cn
www.springer.com/12264

REVIEW

Candidate Biomarkers in Children with Autism Spectrum Disorder: A Review of MRI Studies

Dongyun Li[1] · Hans-Otto Karnath[2,3] · Xiu Xu[1]

Received: 8 January 2017 / Accepted: 17 February 2017 / Published online: 10 March 2017

Abstract Searching for effective biomarkers is one of the most challenging tasks in the research field of Autism Spectrum Disorder (ASD). Magnetic resonance imaging (MRI) provides a non-invasive and powerful tool for investigating changes in the structure, function, maturation, connectivity, and metabolism of the brain of children with ASD. Here, we review the more recent MRI studies in young children with ASD, aiming to provide candidate biomarkers for the diagnosis of childhood ASD. The review covers structural imaging methods, diffusion tensor imaging, resting-state functional MRI, and magnetic resonance spectroscopy. Future advances in neuroimaging techniques, as well as cross-disciplinary studies and large-scale collaborations will be needed for an integrated approach linking neuroimaging, genetics, and phenotypic data to allow the discovery of new, effective biomarkers.

Keywords Autism spectrum disorder · Biomarker · Neuroimaging · Structural MRI · Diffusion tensor imaging · Resting-state functional MRI · Magnetic resonance spectroscopy · Children · Human

✉ Xiu Xu
xuxiu@shmu.edu.cn

[1] Division of Child Health Care, Children's Hospital of Fudan University, Shanghai 201102, China

[2] Division of Neuropsychology, Center of Neurology, Hertie Institute of Clinical Brain Research, University of Tübingen, 72076 Tübingen, Germany

[3] Department of Psychology, University of South Carolina, Columbia, SC 29208, USA

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that has a strong genetic basis, a heterogeneous etiology, and various clinical presentations. The core symptoms of ASD include: (1) deficits in social communication and social interaction, and (2) restricted, repetitive patterns of behavior, interests, or activities [1]. The prevalence of ASD has dramatically increased recently from 4 in 10000 to 1 in 68 children [2, 3]. Along with the soaring incidence of ASD, significant financial and emotional costs to the individuals and their families, as well as the numerous pressures on our medical, social, and political lives have been engendered [4]. Hence, determining the causes and making an accurate diagnosis as well as early and effective intervention seem crucial for society [5].

So far, the diagnosis of ASD and the selection criteria for clinical trials have been guided by the Diagnostic and Statistical Manual of Mental Disorders or behavioral diagnostic scales. The apparent disadvantage of symptom-based criteria is that a similar symptom or phenotype may arise from diverse sets of biochemical processes, especially for disorders with numerous genetic or environmental factors like ASD [6]. In the past decade, searching for genetic biomarkers has been one of the hottest spots in ASD research. Numerous related genes have been reported including *NRXN1*, *SHANK3*, *SHANK2*, *MECP2*, *SNC2A*, *CHD8*, *DYRKIA*, *POG2*, *GRIN2B*, *KATNAL2*, *NLGN3*, *NLGN4*, *CNTN4*, *CDH10*, *CDH9*, and *SEMA5A* [7–17]. Unfortunately, the genetic underpinnings of ASD are neither simple nor consistent, considering that only 10%–38% of ASD cases have been reported with known genetic deficits [18–20]. Because of this heterogeneity and complexity, interest and efforts into searching for more biomarkers and quantifiable parameters are increasing in

order to facilitate early and reliable diagnosis, as well as to subgroup patients sharing common pathophysiological underpinnings.

Magnetic resonance imaging (MRI), a non-invasive examination tool, has been widely applied to ASD populations to delineate the developmental trajectory of the brain. Major advances in structural and functional MRI techniques in the past decades have greatly enriched our understanding of neuropathological differences in ASD [19, 21, 22]. Generally, structural MRI has revealed ASD to be a disorder with general and regional brain enlargement, especially in the frontotemporal cortices, while functional MRI studies have highlighted diminished connectivity, especially between frontal-posterior regions [19, 21–25].

Here, we review recent MRI studies (since 2000) in young children with ASD, aiming to provide effective biomarkers for the diagnosis of childhood ASD. We focus on studies using structural imaging methods, structural connectivity analyses, diffusion tensor imaging (DTI), neurochemical or metabolic quantification methods, and magnetic resonance spectroscopy (MRS), as well as functional connectivity analyses with resting-state functional MRI (rs-fMRI). We do not consider task-based functional methods, since it is almost impossible to keep a young child awake and still during a functional scan. We place the emphasis on young ASD children because in adolescents and adults the altered brain structures and activities may merely reflect the social deprivation experience elicited by reduced social attention during childhood. Therefore, it is impossible to tell whether observed functional or structural differences are the cause or the result of ASD neuropathology. Another reason is based on brain plasticity. There is growing evidence that the first 3 years of life is a particularly critical developmental period for children with ASD [26, 27]. Thus, the earlier the abnormal neurodevelopmental trajectory (even in infants and toddlers) is identified, the better guided intervention strategies for ASD children can be achieved.

Structural MRI

Structural MRI analysis for neurodevelopmental disorders began to emerge in the 1990s when it focused on the neuroanatomical aspects of brain development. It has been used to measure the total brain volume and volumes of specific structures. Earlier studies used manual delineation for the gray (GM) and white matter (WM) to calculate the volumes of specific regions of interest (ROIs). With technical developments, it is now possible to use program codes to measure the volumes automatically, allowing large data sets to be processed more efficiently [28]. Based on the different analytic methods for structural data, structural MRI studies can be classified into voxel-based morphometry (VBM) and surface-based morphometry (SBM) [29]. VBM targets tissue density and usually focuses on relative GM concentration or volume, or regional volume differences of a certain tissue. SBM addresses topological features, like surface curvature and degree of folding [29, 30]. Notably, brain volume and surface curvature have been hypothesized to have dissociable developmental trajectories with, putatively, different genetic and neurodevelopmental bases [22, 31, 32]. Table 1 summarizes the findings of structural MRI studies in children.

Cortex

The most consistent observation regarding structural cortical changes in young ASD children *versus* controls is the increased total volume both in the cortical GM and WM [22, 30, 33, 34]. Increased GM volume (GMV) and WM volume (WMV) have been found in the frontal and temporal lobes and are less pronounced in the parietal and occipital lobes. For example, Carper *et al.* (2002) revealed increased frontal and temporal GMV and frontal and parietal WMV at young ages (2–4 years old) [35]. Sparks *et al.* (2002) likewise observed increased cerebral volumes in autistic children aged 4–6 years [36]. Akshoomoff *et al.* (2004) reported increased total brain volume (TBV), as well as significantly increased WMV and GMV in children of 4–6 years with low-functioning autism compared with high-functioning autism (HFA) [37]. Hazlett *et al.* (2006) investigated 51 younger autistic children and 25 typically-developing controls (aged 18–35 months). They reported increased cerebral GMV and WMV in the brains of the autistic group [38]. In a group of 1.5–5 year-old autistic children, Schumann *et al.* (2010) found no changes in the occipital lobe but enlarged cerebral GMV in the frontal, temporal, and parietal lobes, as well as in the cingulate gyrus (located in the limbic lobe) [39]. Hazlett *et al.* (2011) compared the TBV of 6-month-old high-risk infants with autistic siblings with low-risk infants without autistic family members. They found no significant difference in the cerebrum or lateral ventricle volumes, consistent with the hypothesis that the brain enlargement might be a postnatal event, occurring at $\sim$12 months of age [34]. However, Shen *et al.* (2013) identified excessive cerebrospinal fluid (CSF) over the frontal lobes at 6–9 months of age but no such difference at 12–15 and 18–24 months [40]. Recently, Gori *et al.* (2015) extracted features from GM, WM, and CSF measurements to classify autistic and control brains in 4-year-old males. They found that only GM features in different sub-regions showed up to 80% classification performance [41].

Table 1 Structural MRI studies of children with ASD.

Reference	Age range	Brain regions	Methods	Findings in ASD group
Elia *et al.* (2000) [57]	5–17 years	Corpus callosum; midbrain; cerebellar vermis	Area measurements; T-test; regression	No abnormalities in the total vermis, vermis lobules VI-VII, pons, and midbrain
Carper *et al.* (2002) [35]	2–4 years	WM and GM volumes	ROI; SPSS	↑ WMV in frontal and parietal lobes ↑ GMV in frontal and temporal lobes
Sparks *et al.* (2002) [36]	3–5 years	Cerebrum; cerebellum; amygdala; hippocampus	ROI; SPSS; ANCOVA	↑ TBV and amygdala, cerebellar, and hippocampus volume
Herbert *et al.* (2003) [51]	7–11 years	Cerebrum; cerebellum	ROI; semi-automated segmentation; SPSS; GLM	↑ TBV and total cerebellar volume
Akshoomoff *et al.* (2004) [37]	4–6 years	Cerebrum; cerebellum; cerebellar vermis; TBV	ROI; ANOVA; segmentation	Low-functioning autism: ↑ TBV and cerebral volume; ASD: ↑ TBV, cerebral and cerebellar GMV and WMV, anterior and posterior cerebellar vermis area
McAlonan *et al.* (2005) [56]	10–12 years	GM; WM regional density	VBM; BAMM; SPSS; GLM; MANCOVA	↓ GM density in frontal and parietal areas; ↓ WM density in cerebellum and left internal capsule and fornices
Hazlett *et al.* (2006) [38]	1.5–3 years	Cerebrum; cerebellum	ROI; NLMM; segmentation	↑ GMV and WMV in cerebrum
Hardan *et al.* (2006) [42]	8–13 years	Cortical thickness	SBM; Freesurfer	Total cerebral sulcal and gyral thickness; no significant difference in frontal and occipital areas
Munson *et al.* (2006) [62]	3–4 years	Cerebrum; amygdala; hippocampus	Area measurements; linear modal	↑ Right amygdala volume
Schumann *et al.* (2010) [39]	1.5–5 years	Cerebrum	ROI; SPSS; ANCOVA; segmentation	↑ GMV and WMV in cerebrum; notably in frontal, temporal, and cingulate cortices
Jiao *et al.* (2010) [44]	7–11 years	Cortex	SBM; T-test; Freesurfer	↑ Thickness in left caudal anterior cingulate cortex and left frontal pole; ↓ thickness in right entorhinal, right lateral orbitofrontal, left lateral orbitofrontal, right medial orbitofrontal, left medial orbitofrontal cortex, and right pars triangularis
Hazlett *et al.* (2011) [34]	6–7 months	Cerebrum; cerebellum	ROI; GLM; automatic segmentation; ANOVA	No significant difference in TBV, cerebral cortex, cerebellum, or lateral ventricle volumes
Shen *et al.* (2013) [40]	(Longitudinal) 6–9 months; 13–14 months; 19–21 months	Cerebrum	ROI; LMM; manual segmentation	↑ CSF over frontal lobe at 6–9 mos; ↑ total cerebral volumes at 12–15 mos
Nordahl *et al.* (2012) [63]	2–4 years	Amygdala	ROI; ANCOVA	↑ Amygdala volume at both time points
Dierker *et al.* (2015) [45]	9–12 years	Cortex	SBM; ANOVA; freesurfer	Bilateral differences in sulcal depth in the anterior-insula, frontal-operculum, and temporal-parietal junction

Table 1 continued

Reference	Age range	Brain regions	Methods	Findings in ASD group
Frazier *et al.* (2009) [64]	7–12 years	Corpus callosum	ROI; area measurement	↓ Volume of corpus callosum
Barnea-Goraly *et al.* (2014) [79]	(Longitudinal) 8–12 years; 10–14 years	Amygdala; hippocampus	Area measurement; rm-ANOVA	No difference in hippocampus of both hemispheres

WM, white matter; GM, grey matter; TBV, total brain volume; ROI, region of interest; SBM, surface-based morphometry; VBM, voxel-based morphometry; GLM, general linear models; ANOVA, analysis of variance; ANCOVA, analysis of covariance; rm-ANOVA, repeated measures ANOVA; LMM, linear mixed models; NLMM, non-linear mixed models; SPM, Freesurfer, ad SPSS are data analysis packages.

SBM studies have only been performed with older autistic children. For instance, Hardan *et al.* (2006) applied atlas-based SBM to children aged 10 years. They found no changes in the frontal or occipital lobes but increased total cerebral sulcal and gyral thicknesses in the temporal and parietal lobes [42]. Two years later, Hardan *et al.* (2009) performed follow-up scans on the same group of autistic children, and revealed decreased cortical thickness in the frontal, temporal, and occipital lobes compared to controls [43]. Moreover, another SBM study by Jiao *et al.* (2010) on 9-year-old autistic children showed decreased thickness in the right entorhinal, right lateral orbitofrontal, left lateral orbitofrontal, right medial orbitofrontal, left medial orbitofrontal cortex, and right pars triangularis, as well as increased thickness in the left caudal anterior cingulate cortex and left frontal pole [44]. More recently, Dierker *et al.* (2015) found bilateral differences in sulcal depth in the anterior-insula, frontal-operculum, and temporal-parietal junctions [45].

According to the above studies, the main differences in the cerebrum of young autistic children are the enlarged total volume of both GM and WM. The most prominent differences were found in the frontal and temporal lobes as well as precentral regions. The growing cerebral volume in early childhood indicates simultaneously decreasing GM and WM density [40]. Interestingly, the changes seem to begin in the autistic cerebrum at the age of $\sim$12 months, i.e., the cerebral enlargement does not occur earlier than 12 months of age [34, 46].

Cerebellum and Subcortical Areas

Cerebellum and Vermis

Cerebellar dysfunction may play a crucial role in the etiology of ASD [47, 48]. The cerebellum is considered to be one of the most consistent sites of abnormality in autism [49, 50]. Most morphometric studies of ASD children have reported increased total cerebellar volume [36, 51, 52] and decreased GMV in some subregions, e.g., crus I and lobules VIII and IX [53–55]. However, the results on WMV have been inconsistent. For instance, Courchesne *et al.* (2001) found a larger cerebellar WMV in 2–3 year-old autistic children [33], while McAlonan *et al.* (2005) reported decreased WM density in the cerebellum [56]. Regarding the vermis, the results are also contradictory. In older children, Elia *et al.* (2000) measured the sizes of the cerebellar areas in 22 autistic children and reported no abnormalities in the total vermis, vermis lobules VI–VII, pons, and midbrain [57]. In contrast, Akshoomoff *et al.* (2004) revealed larger cerebellar WMV and GMV and increased areas of the anterior and posterior cerebellar vermis in 6-year-old autistic children [37].

Amygdala

The amygdala has been the focus of several studies in children with ASD, given its important role in socio-emotional processing [58–61]. Sparks *et al.* (2002) investigated 29 autistic children and typically-developing controls with an average age of 3.9 years and showed bilateral enlargement of the amygdala that was proportional to the overall increase in total cerebral volume [36]. Munson *et al.* (2006) argued that the enlargement was only found in the right amygdala at the age of 3–4 years [62]. Nordahl *et al.* (2012) scanned 85 autistic children aged 37 months on average, and re-scanned 45 members of the same group one year later. They found an increased amygdala volume in both cases, confirming that the enlargement starts at $\sim$3 years [63].

Corpus Callosum

A decreased corpus callosum (CC) size in the autistic population has been consistently reported, including reductions localized in the mid-sagittal area, anterior CC, body, and posterior splenium [2, 22, 43, 64–69]. A reduced CC has been associated with reduced integration of information and slower processing [2, 68, 69]. However, to our knowledge, CC changes in the early childhood period have not yet been studied. A report by Frazier *et al.* (2012) investigated 19 autistic children with a mean age of 10.6 years and found consistent reductions in the total CC volume [65].

Basal Ganglia

The basal ganglia play an important role in cognition and motor control *via* participation in frontostriatal, thalamocortical, and limbic circuits [70–73]. In neuroimaging studies of ASD, the basal ganglia have been studied regarding their association with repetitive behaviors [74, 75]. The most consistent finding is an increased caudate volume [76, 77]. Unfortunately, most of the studies were conducted in adults or late adolescence but not in childhood.

Hippocampus

Sparks *et al.* (2002) found enlarged hippocampi in autistic children at the ages of 3–4 years [36]. Similarly, in 7.5–12.5 year-olds, Schumann *et al.* (2004) also revealed enlarged hippocampi, especially in an HFA group [78], while in a 10 year-old group, Barnea-Goraly *et al.* (2014) observed an enlargement only in the right hippocampus [79]. Interestingly, in an older group, Barnea-Goraly *et al.* (2014) reported no difference in the hippocampus of both hemispheres. These findings indicate that the hippocampus in ASD has an unbalanced growth pattern during early brain development [79].

Conclusions

The most consistent structural MRI finding is the increased growth of total cortical volume in early ASD children. A similar trend has also been demonstrated in some subcortical brain regions (e.g., amygdala and hippocampus) and the cerebellum. Interestingly, studies comparing ASD adolescents or adults with controls did not find such differences and have even reported decreased TBV in ASD patients [35, 80]. Thus, it seems as if brain development during early childhood in ASD is more voluminous than in a typically-developing brain, especially in the frontal and temporal lobes, followed by a possibly reduced volumetric capacity of the brain after adolescence [81, 82].

Diffusion Tensor Imaging (DTI)

Molecular studies have demonstrated dysmaturation of the WM characterized by microstructural changes or disorganization in the brains of autistic populations [83, 84]. It has been reported that synaptogenesis is altered in children with ASD, affecting myelination, and thus compromising WM integrity [85]. DTI, as a non-invasive tool, has been applied in the last decade to developing brains to study both the local connectivity and WM tracts as well as fasciculi that connect regions and lobes [2, 86–90]. Metrics such as fractional anisotropy (FA), and mean diffusivity (MD) have been used to measure the directionality and the amount of diffusion, respectively, in a particular ROI or at the level of individual voxels. The most commonly-used DTI methods are tractography, voxel-wise analysis, and tract-based spatial statistics (TBSS). Table 2 summarizes the findings of DTI studies in children.

Tractography

The majority of tractography studies have found reduced FA and increased MD, indicating more WM microstructural disorganization in the frontal and temporal lobes or dominant tracts. For example, Sundaram *et al.* (2008) applied deterministic tractography to analyze WM abnormalities in the frontal lobe and checked for short-range connectivity changes in ASD children. They reported higher MD in the whole frontal lobe as well as reduced FA for short-range fibers in ASD children [91]. Hong *et al.* (2011) also used deterministic tractography to examine connectivity in the CC in HFA children. They revealed decreased WM density in the anterior third of the CC, as well as higher MD and a lower fiber number in the anterior

Table 2 Diffusion tensor imaging studies of ASD children.

Reference	Age range	Brain regions	Methods	Findings in ASD group
Sundaram *et al.* (2008) [91]	2–7 years	Association fibers in frontal lobes	DT; ROI; MANCOVA; DTIstudio	↑ MD in short and long-range fibers; ↓ FA in short-range fibers
Hong *et al.* (2011) [92]	7–11 years	Corpus callosum (CC)	DT; ROI; WM density and volume; FSL; SPSS	↓ WM density in the anterior third of the CC; ↑ MD in the anterior third of the CC
Nagae *et al.* (2012) [93]	7–18 years (+/-language impairment)	Superior longitudinal fasciculus (SLF); corticospinal tract (CST)	DT; GLM; SPSS; DTIstudio	↑ MD in CST (ASD without language impairment); ↑ MD in left SLF (ASD with language impairment)
Wolff *et al.* (2012) [94]	(Longitudinal) 6–7 months; 12–13 months; 23–25 months	Global main tracts	DT; ROI; DTIprep; SAS	↑ FA in CC body, left fornix, inferior longitudinal fasciculus, uncinate fasciculus (6 mo); no difference in FA (12 mo); ↓ FA in left anterior internal capsule and anterior thalamic radiation (24 mo)
Nair *et al.* (2013) [95]	9–17 years	Cortex; thalamus	PT; ROI; FSL; T-test	↑ MD in thalamo-cortical connectivity; negative correlation between fronto-thalamic FA and ADOS score
Joseph *et al.* (2014) [96]	4–6 years	Arcuate fasciculus	PT; ANOVA; FSL	↓ RD of arcuate fasciculus
Cheung *et al.* (2009) [97]	6–14 years	Global white matter	VBM; SPM; GLM; SPSS	↓ FA in bilateral prefrontal and temporal regions; ↑ FA in SLF and left occipital lobe
Ke *et al.* (2009) [98]	6–11 years	Regional frontal and temporal gyri	VBM; SPM	↓ WM density in right frontal and left parietal lobe; ↓ FA in frontal left temporal lobe
Barnea-Goraly *et al.* (2010) [99]	9–14 years	Regional white matter	VBM; FSL	↓ FA in frontal, temporal and parietal lobes
Poustka *et al.* (2012) [100]	8–12 years	Fornix; SLF; uncinate fasciculus; CC	VBM; DT; SPM	↓ FA in the uncinate fasciculus and right SLF; negative correlation between FA and severity of ASD symptoms
Peterson *et al.* (2015) [101]	9–12 years	Global white matter	ROI; ANCOVA; LDDMM	↑ MD through left hemisphere, especially outer-zone of cortical WM
Kumar *et al.* (2010) [106]	2–9 years	Global white matter tracts	TBSS; DT; ANOVA; FSL	↓ FA in right uncinate fasciculus, right cingulum, and CC; ↑ MD in right arcuate fasciculus
Weinstein *et al.* (2011) [107]	2–4 years	Global white matter tracts	TBSS; DT; FSL	↑ FA in CC, left SLF and bilateral cingulum
Jou *et al.* (2011) [88]	7–15 years	Global white matter tracts	TBSS; FSL; SPSS	↓ FA in general association and projection tracts

Table 2 continued

Reference	Age range	Brain regions	Methods	Findings in ASD group
Shukla *et al.* (2010) [85]	12–13 years	CC; internal capsule; middle cerebellar peduncle	TBSS; ROI; VBM; FSL; SPM	↓ FA ↑ MD for global white matter tracts
Walker *et al.* (2012) [108]	3–7 years	Global white matter tracts	TBSS; FSL	↓ FA in various areas; ↑ MD in posterior brain regions

WM, white matter; GM, grey matter; RD, radial diffusivity; FA, fractional anisotropy; MD, mean diffusivity; ROI, region of interest; TBSS, tract-based spatial statistics; DT, deterministic tractography; PT, probabilistic tractography; GLM, general linear models; ANOVA, analysis of variances; LDDMM, large deformation diffeomorphic metric mapping; VBM, voxel-based morphometry; SPM, FSL, DTIstudio, DTIprep, SPSS, and SAS are data analysis packages.

third of the transcallosal fiber tracts [92]. Using deterministic tractography, Nagae *et al.* (2012) revealed higher MD in the temporal portion of the left superior longitudinal fasciculus in ASD children with language impairment, as well as a significant negative correlation between MD and language score [93]. Wolff *et al.* (2012) investigated WM fiber tracts in a group of high-risk infants from 6 to 24 months old. Higher FA values were found in the fornix, uncinate fasciculus, and inferior longitudinal fasciculus at 6 months, whereas no such differences were observed at 12 months [94]. Nair *et al.* (2013) used both fMRI and probabilistic DTI tractography to assess the integrity of thalamo-cortical connectivity in children and adolescents with ASD. They reported increased MD in the thalamo-cortical connections, and decreased functional connectivity in the thalamo-cortical circuitry, as well as a negative correlation between the fronto-thalamic FA and the social and total Autism Diagnostic Observation Schedule score [95]. More recently, Joseph *et al.* (2014) applied both structural MRI and probabilistic DTI tractography to investigate the anatomo-behavioral relationship addressing language ability in young ASD children. In the ASD children, they found no difference in GM asymmetries but decreased leftward volume and radial diffusivity of the arcuate fasciculus [96].

Voxel-Wise Analysis

Voxel-wise analysis is used to find areas of significant difference and to overcome possible user bias. Cheung *et al.* (2009) reported reduced FA in bilateral prefrontal and temporal regions as well as the frontal striato-temporal and posterior brain pathways that are associated with communication and social reciprocity impairment and repetitive behavior [97]. Ke *et al.* (2009) investigated the WM abnormalities in a group of Chinese HFA children. The voxel-wise whole-brain analysis of FA showed decreased WM density in the right frontal lobe, left parietal lobe, and right anterior cingulate [98]. Barnea-Goraly *et al.* (2010) found reduced FA in age-matched unaffected siblings as compared to children with ASD, suggesting that this may be a potential marker of genetic risk [99]. Poustka *et al.* (2012) revealed decreased FA values in the uncinate fasciculus and right superior longitudinal fasciculus as well as a negative correlation between the FA values of the affected fiber tracts and the severity of ASD symptoms [100]. A more recent study by Peterson *et al.* (2015) found widespread increases in MD in many regions of the left hemisphere in children with ASD as compared to typically-developing children [101], supporting the left hemispheric abnormality or atypical hemispheric dominance that has been hypothesized in ASD for the past three decades [102–104]. Peterson *et al.* (2015) used an atlas-based ROI analysis in HFA children and found significantly increased

MD of the outer-zone cortical WM, suggesting hypomyelination and increased short-range cortico-cortical connections which might be due to the early WM overgrowth [101].

Tract-Based Spatial Statistics (TBSS)

A further innovative method of DTI analysis is TBSS [105]. This method provides advantages over traditional voxel-wise analysis, since it does not require smoothing and allows for a higher spatial comparability. A mean FA skeleton is built and thresholded to exclude areas of high inter-individual variability. Each individual's FA map is then projected onto the skeleton to collect standard voxel-wise FA statistics across all the individuals. Kumar *et al.* (2010) combined deterministic tractography and TBSS to investigate the CC region in 5-year-old ASD children. They found lower FA, higher MD, larger numbers of streamlines and voxels, and longer streamlines as well as correlation between macrostructural changes in the uncinate fasciculus and the score on the Gilliam Autism Rating Scale [106]. Weinstein *et al.* (2011) reported increased FA within the genu and body of the CC, left superior longitudinal fasciculus, and right and left cingulum in a group of very young ASD children using TBSS. The tractography revealed that the increased FA was concentrated in the mid-body of the CC and in the left cingulum [107]. Jou *et al.* (2011) found reduced FA in the forceps minor, inferior fronto-occipital fasciculus, and superior longitudinal fasciculus [88]. Shukla *et al.* (2011) demonstrated reduced FA and higher MD in the CC, anterior and posterior limbs of the internal capsule, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, superior longitudinal fasciculus, cingulum, anterior thalamic radiation, and corticospinal tract [85]. Similarly, Walker *et al.* (2012) revealed decreased FA values in various brain regions and increased MD in some tracts [108].

Conclusions

Studies using voxel-wise analysis have revealed decreased FA and increased MD in the major WM tracts of ASD children. However, the voxel-wise analysis of DTI has methodological disadvantages such as the dependency on the size of the smoothing kernel and the hindrance of confident conclusions [30]. TBSS overcomes these flaws and has been used in the majority of more recent and ongoing tractography studies. Predominantly the latter have revealed reduced FA and higher diffusivity in the main tracts in ASD children, including the uncinate fasciculus, arcuate fasciculus, cingulum bundle, inferior longitudinal fasciculus, and inferior fronto-occipital fasciculus.

Resting-State Functional Magnetic Resonance Imaging

Brain connectomics is a relatively new field of research that maps the brain's large-scale functional networks "at rest". Correlated and anti-correlated fMRI signals are measured without performing specific tasks. These signals imply a functional connectivity that reflects structural connectivity [109, 110]. They can be used to explore both the spatial and temporal deviations of the topology of atypical neurodevelopmental processes [111]. Table 3 summarizes the findings of rs-fMRI studies in children.

In an attempt to elucidate putative neurobiological underpinning mechanisms, Just *et al.* (2004) proposed the "hypoconnectivity theory of ASD", claiming that the deficit of integration of neuronal information in ASD might be associated with an overall under-functioning of the brain's integrative circuitry [112]. However, Courchesne and Pierce (2005) suggested that the developmental trajectory for functional brain connectivity in ASD individuals is characterized by both an early local hyperconnectivity and a long-distance hypoconnectivity of the prefrontal cortex based on the findings of an increased short-range (local) connectivity within the frontal lobe but a decreased degree of functional long-range (global) connectivity with the rest of the brain [113]. Di Martino *et al.* (2011) found only hyperconnectivity in the superior temporal gyrus, insula, and brainstem areas [114]. Moreover, Lynch *et al.* (2013) and Uddin *et al.* (2013) both demonstrated increased connectivity in the cingulate cortex and inferior and superior frontal gyri, as well as other specific areas [115, 116]. However, other childhood ASD studies mostly revealed reduced long-range connectivities across different brain regions [95, 117–119]. Thus, it has been suggested that the increased local connectivity in ASD children results from overcompensation for the reduced long-range connectivity [95].

^{1}H-MRS Metabolite Spectrum

Neurochemicals are involved in cortical activity as well as in the metabolic processes in the brain. Each metabolite has a unique chemical shift which acts as a signature that is used for the quantification of that specific metabolite. The most common method of metabolite quantification *in vivo* is through the proton resonance of hydrogen (^{1}H) atoms [120]. ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive imaging technique that estimates specific chemical metabolites [121]. Previous studies mainly used ^{1}H-MRS to quantify creatine and phosphocreatine (Cr+PCr), a measure of cellular energy metabolism [122]; N-acetylaspartate (NAA), a marker of neuronal density and activity [123]; choline-

Table 3 Resting-state functional MRI studies in ASD children.

Reference	Age range (years)	Seed brain regions	Findings in ASD group
Di Martino *et al.* (2011) [114]	7–13	Striatal regions (caudate, putamen)	↑ Connectivity in striatal-cortical circuitry
			↑ Striatal functional connectivity with the pons
			↑ Connectivity of brainstem area, with bilateral insular regions
Lynch *et al.* (2013) [115]	7–12	Precuneus	↓ Connectivity to cuneus, caudate, and thalamic nuclei
		Posterior cingulate cortex	↑ Connectivity to medial and anterolateral temporal cortex, lingual gyrus, posterior parahippocampal gyrus, temporal pole, entorhinal cortex, and perirhinal cortex within the anterior aspect of the medial temporal lobe
		Retrosplenial cortex	↑ Connectivity to inferior frontal and middle frontal gyrus, dorsal medial prefrontal cortex, posterior insular cortex, lingual gyrus, posterior parahippocampal gyrus, temporal pole, posterior superior temporal sulcus, and anterior supramarginal gyrus
Uddin *et al.* (2013) [116]	7–12	Anterior cingulate cortex	↑ Connectivity to superior frontal gyrus, thalamus, and bilateral insular cortex
		Precuneus	↑ Connectivity to posterior cingulate cortex and left angular gyrus
		Superior temporal gyrus	↑ Connectivity to middle temporal gyrus
		Postcentral gyrus	↑ Connectivity to precentral gyrus, left posterior insular cortex, and thalamus
		Lateral occipital cortex	↑ Connectivity to intracalcarine cortex, and occipital pole
Wiggins *et al.* (2011) 119]	10–18	Right superior frontal gyrus	↓ Connectivity to posterior superior frontal gyrus
Rudie *et al.* (2012) [118]	10–15 (+/-MET mutation)	Posterior cingulate cortex	↓ Connectivity of overall default mode network
			↓ Connectivity to medial prefrontal cortex in MET-homozygous
			↓ Connectivity to medial prefrontal cortex within ASD group
Abrams *et al.* (2013) [117]	8–12	Posterior superior temporal sulcus	↓ Connectivity of bilateral ventral tegmental area, nucleus accumbens, putamen of basal ganglia, ventromedial prefrontal cortex, left caudate, anterior insular cortex, and orbitofrontal cortex
Nair *et al.* (2013) [95]	9–17	Right thalamus	↑ Connectivity to temporal areas
		Thalamus	↓ Connectivity to prefrontal, parietal-occipital, and somatosensory cortical regions

containing compounds, a measure primarily reflecting the constituents of cell membranes [122]; and glutamine/glutamate/gamma-aminobutyric acid (GABA) ("Glx"). Recent ^{1}H-MRS studies in ASD patients showed generally decreased NAA [124, 125], Cr+PCr, choline, and Glx [125], as well as increased Glx in adults [126]. However, previous studies also exhibited some inconsistencies, such as decreased [127–131], unchanged, or increased NAA in ASD children compared with controls [127, 132–135]. In the following, we categorize the recent ^{1}H-MRS studies in young individuals with ASD according to the four main detectable chemical metabolites: Cr+PCr, NAA, choline, and Glx. Table 4 summarizes the findings of MRS studies in children.

Table 4 MRS studies in ASD children.

Reference	Age range (years)	Cr+PCr	NAA	Cho	Glx	Findings in ASD group
Friedman *et al.* (2003) [129]	3–4	√	√	√		↓ Cr+PCr in frontal, parietal, temporal, occipital and thalamus
						↓ NAA in bilateral frontal, parietal, and cingulate areas, and in right superior temporal gyrus and left putamen
						↓ Cho in cortical areas, temporal lobes, and thalamus
DeVito *et al.* (2007) [128]	6–17	√	√	√	√	↓ Cr+PCr in GM of frontal, temporal, occipital cortices and cerebellum
						↓ Cho in cortical areas, temporal lobes, and thalamus
						↓ Glx in GM of frontal, occipital, and temporal regions as well as cerebellum
Hardan *et al.* (2008) [130]	8–15	√	√		√	↓ Cr+PCr in bilateral thalamus
						↓ Cho in cortical areas, temporal lobes, and thalamus
						↑ Glx of thalamus is associated with abnormal sensory sensitivity and deficits in body movement modulation
Corrigan *et al.* (2013) [138]	3–4	√	√	√	√	↓ Cr+PCr in GM and WM generally
						↓ Cho in cortical areas, temporal lobes, and thalamus generally
Levitt *et al.* (2003) [131]	5–16	√	√	√		↓ Cr+PCr in frontal, parietal, temporal, occipital, and thalamus
						↓ NAA in bilateral frontal and parietal areas, and in the left caudate
						↓ Cho in cortical areas, temporal lobes, and thalamus
						↑ Cho in caudate, anterior cingulate cortex and hippocampus-amygdala complex
Friedman *et al.* (2006) [144]	3–4	√	√	√	√	↓ NAA in both GM and WM generally
						↓ Cho in cortical areas, temporal lobes, and thalamus
Corrigan *et al.* (2013) [138]	3–4	√	√	√	√	↓ NAA in both GM and WM in cortical regions
						↓ Glx in both cortical GM and WM generally
Fujii *et al.* (2010) [145]	2–13	√	√	√		↓ NAA/Cr+PCr in anterior cingulate cortex and a deficit in executive functions
Fayed *et al.* (2005) [178]	2–10		√	√		↓ Cho in both GM and WM of cortical areas, temporal lobes and thalamus
Vasconcelos *et al.* (2008) [148]	6–10	√	√	√	√	↓ Cr+PCr in cerebellum and striatum
						↓ NAA in frontal areas
						↑ Cho in caudate, anterior cingulate cortex, and hippocampus-amygdala complex
Gabis *et al.* (2008) [146]	8–14		√	√		↓ NAA in Hippocampus-amygdala complex
						↑ Cho in caudate, anterior cingulate cortex, and hippocampus-amygdala complex
Doyle-Thomas *et al.* (2014) [151]	7–18	√	√	√	√	↓ Glx/Cr+PCr in cerebellum and ↑ Glx/Cr+PCr in putamen
						↓ NAA/Cr+PCr in thalamus and ↑ NAA/Cr+PCr in caudate

Cho, Choline; Cr+PCr, creatine and phosphocreatine; Glx, glutamine,glutamate and GABA; GM, grey matter; NAA, N-acetylaspartate; WM, white matter.

Creatine and Phosphocreatine

In ^{1}H-MRS, Cr and PCr are quantified together [136]. They play a crucial role in the adenosine triphosphate (ATP) and adenosine diphosphate (ADP) energy-transfer process. ATP results from oxidative phosphorylation in neuronal and glial mitochondria and glycolysis in the cytosol. A phosphate bond is released from ATP through the enzyme creatine kinase to store energy. The free phosphate bond then binds with Cr to form PCr. When cellular

mitochondria require energy, ADP and a third phosphate bond are resynthesized to ATP via oxidative phosphorylation [122, 136, 137]. Cr is highly-expressed in the mitochondria of neurons and is crucial for cellular energy production and in the maintenance of cortical homeostasis [122, 137].

In children with ASD, reduced Cr+PCr levels have been reported across the cortical regions [128–130, 138]. Turner and Gant (2014) reviewed the biochemistry of Cr and revealed an association between reduced Cr+PCr and ASD-like behaviors, such as abnormal learning skills, intellectual disability, and repetitive and compulsive behaviors. This correlation could be due to delayed or impaired axon growth during brain development, in which Cr+PCr are crucial elements [139]. However, no correlation between Cr+PCr levels and autistic symptom severity has been found in children with ASD [140]. Moreover, cortical Cr+PCr is commonly considered to be stable and has been extensively used as a reference for other metabolites [120, 122, 127, 136].

N-Acetylaspartate (NAA)

NAA is synthesized in the mitochondria of neurons and is catabolized in glial cells and oligodendrocytes, acting as a precursor of fatty-acids to form the myelin around axons [137]. Quantified by ^{1}H-MRS, the NAA level is considered to reflect neuronal density, integrity, and metabolism [104, 131, 136, 141]. It is well accepted that NAA is comparable across both GM and WM, while it is more abundant in WM than in GM, due to its role in transmission of action potentials [142, 143].

Research on NAA has revealed a consistent phenomenon, i.e., it is generally reduced in children with ASD. In a group of 3–4 year-old ASD children, Friedman *et al.* (2003) reported NAA reduction in the frontal, parietal, and cingulate areas of both hemispheres, as well as in the right superior temporal gyrus and left putamen, compared with typically-developing children [129]. In an older group of ASD children with a mean age of 10.4 years, Levitt *et al.* (2003) revealed similar findings of reduced NAA in both left and right frontal and parietal areas, as well as in the left caudate. They also claimed that reduced parietal axon density, marked by reduced WM NAA, is associated with deficits in eye gaze, spatial perception, and memory [131]. Comparing children with ASD and those with pervasive developmental disorder, Friedman *et al.* (2006) found an extensively decreased NAA level in the ASD group across both GM and WM [144]. Similarly, Corrigan *et al.* (2013) found a general NAA reduction across most cortical regions in both GM and WM [138]. Interestingly, Fujii *et al.* (2010) reported a relationship between a reduced NAA/Cr+PCr ratio in the anterior cingulate cortex and a deficit in executive functions. Moreover, they also found a correlation between decreased NAA/Cr+PCr and social and communication disabilities [145].

Choline

Choline is synthesized in the liver and is essential for the synthesis of the neurotransmitter acetylcholine. Choline-containing compounds along with membrane phospholipids make up 40% of myelin. In ^{1}H-MRS, the choline level is highest in the WM [120]. The cortical choline levels indicate the equilibrium of neuronal cellular membrane phospholipid metabolism [120, 137, 146, 147].

In children with ASD, choline-containing compounds are generally decreased in cortical areas, the temporal lobes, and thalamus [128–131, 138], indicating a deficit in membrane phospholipid turnover. However, studies have also reported increased choline levels in ASD children in different brain regions, e.g. caudate, anterior cingulate cortex, and hippocampus-amygdala complex [131, 146, 148]. Even though the findings are contradictory, they revealed a similar phenomenon, that ASD children have neural maturation different from typically-developing children.

Glx (Glutamate, Glutamine, and GABA)

Glx are the most abundant neurotransmitters (90% in synapses), taking up to 60%–80% of the glucose oxidation and energy consumption in cortical neurons [149, 150]. They are most concentrated in GM and play essential roles in neural migration, differentiation, and plasticity [125, 132, 136, 140]. In ASD children, De Vito *et al.* (2007) reported extensive reduction of Glx in the GM of frontal, occipital, and temporal regions as well as the cerebellum [128]. Corrigan *et al.* (2013) reported a general decrease in Glx in both cortical GM and WM [138]. Harden *et al.* (2008) pointed out that abnormal sensory sensitivity and deficits in body movement modulation are associated with increased thalamic Glx [130]. In addition, Doyle-Thomas *et al.* (2014) demonstrated that an increased thalamic Glx level is associated with disabled social interaction [151].

MRI and Genetics

As outlined in the introduction, ASD has a strong genetic basis. Biological studies have revealed that many ASD-related genes influence the formation of neuronal circuits [21, 152, 153]. However, little is known about how these genes affect brain structure and function. As reviewed above, altered brain structures and functions have

continuously been reported in ASD with a satisfying amount of consistency. Since brain structure and function are heritable [21, 154–156], gene-related imaging may serve to demonstrate the possible neural mechanisms through which phenotypic heterogeneity arises from genetic heterogeneity in ASD [21].

One innovative gene-related imaging study has compared ASD children carrying positively testable ASD-related gene(s) with typically-developing controls [118]. In particular, the authors were interested in the MET (Met Receptor Tyrosine) gene. They combined fMRI, rs-fMRI, and DTI in a large sample of ASD children ($n = 164$) with MET gene classification and controls. Indeed, the presence of risk alleles in ASD children had a significantly larger impact on functional connectivity than controls. In the ASD risk-allele group, the fMRI showed reduced functional connectivity from posterior cingulate to medial prefrontal cortex. DTI exhibited decreased FA in the splenium portion of the CC that connects to the posterior cingulate. Moreover, group-by-genotype interactions revealed more deactivation of the posterior cingulate, medial prefrontal, and primary auditory cortices, lower functional connectivity of these regions, and lower WM integrity in tracts connecting these regions (splenium, cingulum, and superior and inferior longitudinal fasciculus). Investigating the impact of another ASD-related gene (CNTNAP2; contactin-associated protein-like 2), Scott-Van Zeeland *et al.* (2010) revealed that risk-allele-carrying ASD children showed a pattern of diffusely increased functional connectivity between the frontal cortex and temporal lobes, while typically-developing controls exhibited a clear connectivity between frontal cortex- and language-related cortical regions [157].

According to these limited findings, we can only speculate that ASD-related genes may contribute to atypical connectivity and development. More studies combining genetic information and multimodal neuroimaging data are needed to understand the relationship between heterogeneous neuropathological phenotypes and the clinical manifestations of ASD, as well as to improve diagnostic tools and treatment strategies [158].

Support Vector Machine Technologies

The findings of MRI studies have substantially advanced our understanding of the neural mechanisms underpinning ASDs. However, the integration of neuroimaging tools into clinical practice has so far been limited, partly because it is unclear which information revealed by these tools is relevant to diagnosis and treatment decisions. Support Vector Machine (SVM) technology is a specific type of supervised machine learning that aims to classify data points by maximizing the margin between classes in a high-dimensional space [159, 160]. The optimal SVM algorithm is developed through a "training" phase in which training data are used to develop an algorithm able to discriminate between groups previously defined by the operator (e.g. patients *versus* controls). Once a so-called "decision function" or "hyperplane" is learned from the training data, it can be used to predict the class of a new test example. The interesting aspect of the SVM is that it is multivariate and takes into account inter-regional correlations. Therefore, it is extremely well-suited to take into account and test subtle differences in intra-regional correlations of brain metabolism, function, and anatomy [161, 162]. This may be of particular relevance to ASD, in which the abnormalities are usually in the development of the whole neural system rather than isolated regions.

SVMs have been widely used to aid the diagnosis of neurological disorders, such as Alzheimer's disease [163–166] and Parkinson's disease [167, 168], as well as to identify those brain areas in stroke patients that are involved in a particular (cognitive) function [169, 170]. Among ASD patients, the SVM was first applied in adults. Singh *et al.* developed a diagnostic model generated by the LPboost-based algorithm to distinguish autistic children from controls, based on voxel-wise cortical thickness and ~40,000 points for each individual; they reported 89% classification accuracy based on cross-validation [171]. Ecker *et al.* (2010) applied SVM classifiers to investigate the predictive value of whole-brain structural volumetric changes in ASD, and obtained 81% classification accuracy based on cross-validation [172]. More recently, Ecker *et al.* (2013) pooled regional WM and GM volumes in ASD patients using SVMs and classified ASD patients with a high true positive rate [173]. As for ASD children, the number of reports is relatively limited. In a group of ASD children (mean age 9.2 ± 2.1 years), Jiao *et al.* (2010) used 4 different machine-learning techniques (including SVMs) to generate diagnostic models from both the VBM and SBM results. They reported a better classification performance for the thickness-based data than those based on regional volumes [44]. Lately, Jin *et al.* (2015) used an SVM classifier to identify high-risk ASD infants from WM tracts and whole-brain connectivity. Their proposed function achieved an accuracy of 76% and an area of 0.8 under the receiver operating characteristic curve [174].

To conclude, SVM technology is extremely promising in its contribution to an accurate and valid diagnosis of ASD. However, for ASD in children, the application of SVM is still at the beginning. More studies are needed to produce reliable diagnostic models based on imaging data.

Table 5 Summary of main MRI findings.

MRI approaches	Findings
Structural MRI	*Cortex*
	Increased total GM and WM volumes
	Increased GM and WM volumes in frontal and temporal areas
	Increased cingulate cortex
	Atypical variation of cortical thickness in frontal, temporal, and parietal lobes
	Cerebellum and subcortical areas
	Cerebellum
	Increased total volume
	Increased GM volume
	Decreased WM density
	Amygdala
	Increased volumes in younger children bilaterally
	Trajectory of development of amygdala follows overall trajectory of TBV
	Corpus callosum
	Decreased overall size
	Increased regional volume
	Basal ganglia
	Increased caudate volume
	Atypical shapes of the structures
	Hippocampus
	Increased size of hippocampi in young children
	Enlargement located especially on the right side
	No difference between sides in older children
Diffusion tensor imaging	Decreased FA in the whole brain, frontal lobe, arcuate fasciculus, across the entire CC, and in anterior thalamic radiation
	Increased FA in arcuate fasciculus and in CC in young children
	Increased MD in whole brain, frontal and temporal lobes, and across the entire CC
Resting-state fMRI	Altered functional connectivity in the default mode network
	Hyper-connectivity in striatal-cortical circuitry, precuneus, cingulate cortex, and temporal-frontal circuity
	Under-connectivity in anterior-posterior connections
Magnetic resonance spectroscopy	Decreased NAA levels in general GM and WM, especially in frontal, temporal, cingulate, and caudate areas
	Decreased Cr+PCr levels in general GM and WM, especially in frontal, parietal, temporal, occipital cortex, and thalamus
	Decreased choline levels in cortical areas, temporal lobes, and thalamus
	Increased choline levels in caudate, anterior cingulate cortex, and hippocampus-amygdala complex
	Decreased Glx in GM of frontal, occipital, temporal cortex, and cerebellar regions
	Increased Glx in thalamus and putamen

Summary and Future Direction

The main findings of MRI studies in children are summarized in Table 5.

Young ASD children exhibit differences in brain morphometry, neurochemical components, and structural and functional connectivity. So far, a diverse set of potential biomarkers including genetic, biochemical, morphological, hormonal, immunological, neuropathological, neuropsychological, and behavioral biomarkers have been identified. However, for most of these markers it is not yet clear if they are contributing factors to the development of ASD or are a result of another underlying abnormality. Longitudinal studies – especially covering very young children with ASD – may help answer this question.

It should be kept in mind that MRI techniques are limited to a certain level of spatial and temporal resolution. If this level is not precise enough to visualize the synaptic or neuronal abnormalities where the core features or heterogeneity of ASD arises neuroimaging may ultimately not be the best tool for parsing these differences. However, in combination with post-mortem tissue analysis or animal models, neuroimaging studies

have the potential to provide a critical intermediate step between the genetic basis and the phenotype. Such approaches allow us to deconstruct the conceptions of ASD deeply to where they can be grounded in biology [87, 175].

While most of the studies reviewed here are still characterized by methodological variability and relatively small sample sizes, studies with pooled data samples and homogeneous analytical approaches are needed. Large-scale collaboration networks of that type are ABIDE ("Autism Brain Imaging Data Exchange") [114, 176, 177] and NDAR ("National Database for Autism Research"). They combine imaging data from multiple sites and integrate both genetic and behavioral data. These projects will allow researchers to achieve sufficient power to detect true brain-gene-behavior relationships [21]. In general, multi-site and multi-modal studies based on large patient groups is one of the key strategies to increase the probability of the discovery of new effective biomarkers in ASD and to comprehensively characterize the clinical, behavioral, and cognitive symptoms of this disorder.

Along with the growing numbers of gene-related MRI studies, the application of multimodal MRI scanning and collaboration networks, as well as advanced analytical methods are needed. Techniques, such as a machine learning classifier or prediction, may serve to identify patterns of biomarkers across different modalities, relevant to clinical diagnosis and genotype, severity rating, prognosis, and/or response to treatment. Last but not least, further large-sample studies on ASD children are needed to search for reliable biomarker patterns and diagnostic models based on imaging data.

References

1. American Psychiatric Publishing. Autism spectrum disorder fact sheet. Bethesda, MD: NINDS, 2016.
2. Alexander AL, Lee JE, Lazar M, Boudos R, DuBray MB, Oakes TR, *et al.* Diffusion tensor imaging of the corpus callosum in Autism. Neuroimage 2007, 34: 61–73.
3. Christensen DL, Baio J, Van Naarden Braun K, Bilder D, Charles J, Constantino JN, *et al.* Prevalence and characteristics of autism spectrum disorder among children aged 8 years-autism and developmental disabilities monitoring network, 11 sites, United States, 2012. MMWR Surveill Summ 2016, 65: 1–23.
4. Buescher AV, Cidav Z, Knapp M, Mandell DS. Costs of autism spectrum disorders in the United Kingdom and the United States. JAMA Pediatr 2014, 168: 721–728.
5. Fein D, Barton M, Eigsti IM, Kelley E, Naigles L, Schultz RT, *et al.* Optimal outcome in individuals with a history of autism. J Child Psychol Psychiatry 2013, 54: 195–205.
6. Goldani AA, Downs SR, Widjaja F, Lawton B, Hendren RL. Biomarkers in autism. Front Psychiatry 2014, 5: 100.
7. El-Fishawy P, State MW. The genetics of autism: key issues, recent findings, and clinical implications. Psychiatr Clin N Am 2010, 33: 83–105.
8. Griswold AJ, Ma D, Cukier HN, Nations LD, Schmidt MA, Chung RH, *et al.* Evaluation of copy number variations reveals novel candidate genes in autism spectrum disorder-associated pathways. Hum Mol Genet 2012, 21: 3513–3523.
9. Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, *et al.* De novo gene disruptions in children on the autistic spectrum. Neuron 2012, 74: 285–299.
10. Lee TL, Raygada MJ, Rennert OM. Integrative gene network analysis provides novel regulatory relationships, genetic contributions and susceptible targets in autism spectrum disorders. Gene 2012, 496: 88–96.
11. Ma D, Salyakina D, Jaworski JM, Konidari I, Whitehead PL, Andersen AN, *et al.* A genome-wide association study of autism reveals a common novel risk locus at 5p14.1. Ann Hum Genet 2009, 73: 263–273.
12. Murdoch JD, State MW. Recent developments in the genetics of autism spectrum disorders. Curr Opin Genet Dev 2013, 23: 310–315.
13. Ronald A, Butcher LM, Docherty S, Davis OS, Schalkwyk LC, Craig IW, *et al.* A genome-wide association study of social and non-social autistic-like traits in the general population using pooled DNA, 500 K SNP microarrays and both community and diagnosed autism replication samples. Behav Genet 2010, 40: 31–45.
14. Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, *et al.* De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 2012, 485: 237–241.
15. State MW, Levitt P. The conundrums of understanding genetic risks for autism spectrum disorders. Nat Neurosci 2011, 14: 1499–1506.
16. Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, *et al.* Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature 2009, 459: 528–533.
17. Weiss LA, Arking DE, Gene Discovery Project of Johns Hopkins & the Autism Consortium, Daly MJ, Chakravarti A. A genome-wide linkage and association scan reveals novel loci for autism. Nature 2009, 461: 802–808.
18. Persico AM, Napolioni V. Autism genetics. Behav Brain Res 2013, 251: 95–112.
19. Ruggeri B, Sarkans U, Schumann G, Persico AM. Biomarkers in autism spectrum disorder: the old and the new. Psychopharmacology 2014, 231: 1201–1216.
20. Zafeiriou DI, Ververi A, Dafoulis V, Kalyva E, Vargiami E. Autism spectrum disorders: the quest for genetic syndromes. Am J Med Genet B Neuropsychiatr Genet 2013, 162B: 327–366.
21. Hernandez LM, Rudie JD, Green SA, Bookheimer S, Dapretto M. Neural signatures of autism spectrum disorders: insights into brain network dynamics. Neuropsychopharmacology 2015, 40: 171–189.
22. Mahajan R, Mostofsky SH. Neuroimaging endophenotypes in autism spectrum disorder. CNS Spectr 2015, 20: 412–426.
23. Lenroot RK, Yeung PK. Heterogeneity within autism spectrum disorders: what have we learned from neuroimaging studies? Front Hum Neurosci 2013, 7: 733.
24. Ha S, Sohn IJ, Kim N, Sim HJ, Cheon KA. Characteristics of brains in autism spectrum disorder: structure, function and

connectivity across the lifespan. Exp Neurobiol 2015, 24: 273–284.
25. Yerys BE, Herrington JD. Multimodal imaging in autism: an early review of comprehensive neural circuit characterization. Curr Psychiatry Rep 2014, 16: 496.
26. Sacrey LA, Bennett JA, Zwaigenbaum L. Early infant development and intervention for autism spectrum disorder. J Child Neurol 2015, 30: 1921–1929.
27. Zwaigenbaum L, Bauman ML, Stone WL, Yirmiya N, Estes A, Hansen RL, *et al.* Early identification of autism spectrum disorder: recommendations for practice and research. Pediatrics 2015, 136 Suppl. 1: S10–40.
28. Hernandez-Garcia L, Buschkuehl M. Advances in longitudinal MRI diagnostic tests. Expert Opin Med Diagn 2012, 6: 309–321.
29. Chen R, Jiao Y, Herskovits EH. Structural MRI in autism spectrum disorder. Pediatr Res 2011, 69: 63R–68R.
30. Ismail MM, Keynton RS, Mostapha MM, ElTanboly AH, Casanova MF, Gimel'farb GL, *et al.* Studying autism spectrum disorder with structural and diffusion magnetic resonance imaging: a survey. Front Hum Neurosci 2016, 10: 211.
31. Goldman-Rakic PS. Development of cortical circuitry and cognitive function. Child Dev 1987, 58: 601–622.
32. Panizzon MS, Fennema-Notestine C, Eyler LT, Jernigan TL, Prom-Wormley E, Neale M, *et al.* Distinct genetic influences on cortical surface area and cortical thickness. Cereb Cortex 2009, 19: 2728–2735.
33. Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD, *et al.* Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. Neurology 2001, 57: 245–254.
34. Hazlett HC, Poe MD, Gerig G, Styner M, Chappell C, Smith RG, *et al.* Early brain overgrowth in autism associated with an increase in cortical surface area before age 2 years. Arch Gen Psychiatry 2011, 68: 467–476.
35. Carper RA, Moses P, Tigue ZD, Courchesne E. Cerebral lobes in autism: early hyperplasia and abnormal age effects. Neuroimage 2002, 16: 1038–1051.
36. Sparks BF, Friedman SD, Shaw DW, Aylward EH, Echelard D, Artru AA, *et al.* Brain structural abnormalities in young children with autism spectrum disorder. Neurology 2002, 59: 184–192.
37. Akshoomoff N, Lord C, Lincoln AJ, Courchesne RY, Carper RA, Townsend J, *et al.* Outcome classification of preschool children with autism spectrum disorders using MRI brain measures. J Am Acad Child Adolesc Psychiatry 2004, 43: 349–357.
38. Hazlett HC, Poe MD, Gerig G, Smith RG, Piven J. Cortical gray and white brain tissue volume in adolescents and adults with autism. Biol Psychiatry 2006, 59: 1–6.
39. Schumann CM, Bloss CS, Barnes CC, Wideman GM, Carper RA, Akshoomoff N, *et al.* Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. J Neurosci 2010, 30: 4419–4427.
40. Shen MD, Nordahl CW, Young GS, Wootton-Gorges SL, Lee A, Liston SE, *et al.* Early brain enlargement and elevated extra-axial fluid in infants who develop autism spectrum disorder. Brain 2013, 136: 2825–2835.
41. Gori I, Giuliano A, Muratori F, Saviozzi I, Oliva P, Tancredi R, *et al.* Gray matter alterations in young children with autism spectrum disorders: comparing morphometry at the voxel and regional level. J Neuroimaging 2015, 25: 866–874.
42. Hardan AY, Muddasani S, Vemulapalli M, Keshavan MS, Minshew NJ. An MRI study of increased cortical thickness in autism. Am J Psychiatry 2006, 163: 1290–1292.
43. Hardan AY, Libove RA, Keshavan MS, Melhem NM, Minshew NJ. A preliminary longitudinal magnetic resonance imaging study of brain volume and cortical thickness in autism. Biol Psychiatry 2009, 66: 320–326.
44. Jiao Y, Chen R, Ke X, Chu K, Lu Z, Herskovits EH. Predictive models of autism spectrum disorder based on brain regional cortical thickness. Neuroimage 2010, 50: 589–599.
45. Dierker DL, Feczko E, Pruett JR, Jr., Petersen SE, Schlaggar BL, Constantino JN, *et al.* Analysis of cortical shape in children with simplex autism. Cereb Cortex 2015, 25: 1042–1051.
46. Chenn A, Walsh CA. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science 2002, 297: 365–369.
47. Becker EB, Stoodley CJ. Autism spectrum disorder and the cerebellum. Int Rev Neurobiol 2013, 113: 1–34.
48. Wang SS, Kloth AD, Badura A. The cerebellum, sensitive periods, and autism. Neuron 2014, 83: 518–532.
49. Allen G. The cerebellum in autism. Clin.Neuropsychiatry 2005, 2: 321–337.
50. Fatemi SH, Aldinger KA, Ashwood P, Bauman ML, Blaha CD, Blatt GJ, *et al.* Consensus paper: pathological role of the cerebellum in autism. Cerebellum 2012, 11: 777–807.
51. Herbert MR, Ziegler DA, Deutsch CK, O'Brien LM, Lange N, Bakardjiev A, *et al.* Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. Brain 2003, 126: 1182–1192.
52. Stanfield AC, McIntosh AM, Spencer MD, Philip R, Gaur S, Lawrie SM. Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. Eur Psychiatry 2008, 23: 289–299.
53. Duerden EG, Mak-Fan KM, Taylor MJ, Roberts SW. Regional differences in grey and white matter in children and adults with autism spectrum disorders: an activation likelihood estimate (ALE) meta-analysis. Autism Res 2012, 5: 49–66.
54. Stoodley CJ. Distinct regions of the cerebellum show gray matter decreases in autism, ADHD, and developmental dyslexia. Front Syst Neurosci 2014, 8: 92.
55. Yu KK, Cheung C, Chua SE, McAlonan GM. Can Asperger syndrome be distinguished from autism? An anatomic likelihood meta-analysis of MRI studies. J Psychiatry Neurosci 2011, 36: 412–421.
56. McAlonan GM, Cheung V, Cheung C, Suckling J, Lam GY, Tai KS, *et al.* Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism. Brain 2005, 128: 268–276.
57. Elia M, Ferri R, Musumeci SA, Panerai S, Bottitta M, Scuderi C. Clinical correlates of brain morphometric features of subjects with low-functioning autistic disorder. J Child Neurol 2000, 15: 504–508.
58. Baron-Cohen S, Ring HA, Bullmore ET, Wheelwright S, Ashwin C, Williams SC. The amygdala theory of autism. Neurosci Biobehav Rev 2000, 24: 355–364.
59. Howard MA, Cowell PE, Boucher J, Broks P, Mayes A, Farrant A, *et al.* Convergent neuroanatomical and behavioural evidence of an amygdala hypothesis of autism. Neuroreport 2000, 11: 2931–2935.
60. Schultz RT. Developmental deficits in social perception in autism: the role of the amygdala and fusiform face area. Int J Dev Neurosci 2005, 23: 125–141.
61. Sweeten TL, Posey DJ, Shekhar A, McDougle CJ. The amygdala and related structures in the pathophysiology of autism. Pharmacol Biochem Behav 2002, 71: 449–455.
62. Munson J, Dawson G, Abbott R, Faja S, Webb SJ, Friedman SD, *et al.* Amygdalar volume and behavioral development in autism. Arch Gen Psychiatry 2006, 63: 686–693.
63. Nordahl CW, Scholz R, Yang X, Buonocore MH, Simon T, Rogers S, *et al.* Increased rate of amygdala growth in children aged 2–4 years with autism spectrum disorders: a longitudinal study. Arch Gen Psychiatry 2012, 69: 53–61.

64. Frazier TW, Hardan AY. A meta-analysis of the corpus callosum in autism. Biol Psychiatry 2009, 66: 935–941.
65. Frazier TW, Keshavan MS, Minshew NJ, Hardan AY. A two-year longitudinal MRI study of the corpus callosum in autism. J Autism Dev Disord 2012, 42: 2312–2322.
66. Freitag CM, Luders E, Hulst HE, Narr KL, Thompson PM, Toga AW, *et al.* Total brain volume and corpus callosum size in medication-naive adolescents and young adults with autism spectrum disorder. Biol Psychiatry 2009, 66: 316–319.
67. Hardan AY, Minshew NJ, Keshavan MS. Corpus callosum size in autism. Neurology 2000, 55: 1033–1036.
68. Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ. Functional and anatomical cortical underconnectivity in autism: evidence from an FMRI study of an executive function task and corpus callosum morphometry. Cereb Cortex 2007, 17: 951–961.
69. Mason RA, Williams DL, Kana RK, Minshew N, Just MA. Theory of Mind disruption and recruitment of the right hemisphere during narrative comprehension in autism. Neuropsychologia 2008, 46: 269–280.
70. Bostan AC, Dum RP, Strick PL. Cerebellar networks with the cerebral cortex and basal ganglia. Trends Cogn Sci 2013, 17: 241–254.
71. Middleton FA, Strick PL. Basal ganglia and cerebellar loops: motor and cognitive circuits. Brain Res Brain Res Rev 2000, 31: 236–250.
72. Middleton FA, Strick PL. Basal ganglia output and cognition: evidence from anatomical, behavioral, and clinical studies. Brain Cogn 2000, 42: 183–200.
73. Utter AA, Basso MA. The basal ganglia: an overview of circuits and function. Neurosci Biobehav Rev 2008, 32: 333–342.
74. Estes A, Shaw DW, Sparks BF, Friedman S, Giedd JN, Dawson G, *et al.* Basal ganglia morphometry and repetitive behavior in young children with autism spectrum disorder. Autism Res 2011, 4: 212–220.
75. Hollander E, Anagnostou E, Chaplin W, Esposito K, Haznedar MM, Licalzi E, *et al.* Striatal volume on magnetic resonance imaging and repetitive behaviors in autism. Biol Psychiatry 2005, 58: 226–232.
76. Sears LL, Vest C, Mohamed S, Bailey J, Ranson BJ, Piven J. An MRI study of the basal ganglia in autism. Prog Neuropsychopharmacol Biol Psychiatry 1999, 23: 613–624.
77. Williams CA, Dagli A, Battaglia A. Genetic disorders associated with macrocephaly. Am J Med Genet A 2008, 146A: 2023–2037.
78. Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, Buonocore MH, *et al.* The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. J Neurosci 2004, 24: 6392–6401.
79. Barnea-Goraly N, Frazier TW, Piacenza L, Minshew NJ, Keshavan MS, Reiss AL, *et al.* A preliminary longitudinal volumetric MRI study of amygdala and hippocampal volumes in autism. Prog Neuropsychopharmacol Biol Psychiatry 2014, 48: 124–128.
80. Nordahl CW, Lange N, Li DD, Barnett LA, Lee A, Buonocore MH, *et al.* Brain enlargement is associated with regression in preschool-age boys with autism spectrum disorders. Proc Natl Acad Sci USA 2011, 108: 20195–20200.
81. Courchesne E, Campbell K, Solso S. Brain growth across the life span in autism: age-specific changes in anatomical pathology. Brain Res 2011, 1380: 138–145.
82. Lange N, Travers BG, Bigler ED, Prigge MB, Froehlich AL, Nielsen JA, *et al.* Longitudinal volumetric brain changes in autism spectrum disorder ages 6–35 years. Autism Res 2015, 8: 82–93.
83. Casanova MF, Buxhoeveden DP, Brown C. Clinical and macroscopic correlates of minicolumnar pathology in autism. J Child Neurol 2002, 17: 692–695.
84. Casanova MF, van Kooten IA, Switala AE, van Engeland H, Heinsen H, Steinbusch HW, *et al.* Minicolumnar abnormalities in autism. Acta Neuropathol 2006, 112: 287–303.
85. Shukla DK, Keehn B, Muller RA. Tract-specific analyses of diffusion tensor imaging show widespread white matter compromise in autism spectrum disorder. J Child Psychol Psychiatry 2011, 52: 286–295.
86. Catani M. Diffusion tensor magnetic resonance imaging tractography in cognitive disorders. Curr Opin Neurol 2006, 19: 599–606.
87. Chanraud S, Zahr N, Sullivan EV, Pfefferbaum A. MR diffusion tensor imaging: a window into white matter integrity of the working brain. Neuropsychol Rev 2010, 20: 209–225.
88. Jou RJ, Jackowski AP, Papademetris X, Rajeevan N, Staib LH, Volkmar FR. Diffusion tensor imaging in autism spectrum disorders: preliminary evidence of abnormal neural connectivity. Aust NZ J Psychiatry 2011, 45: 153-162.
89. Mukherjee P, McKinstry RC. Diffusion tensor imaging and tractography of human brain development. Neuroimaging Clin N Am 2006, 16: 19–43, vii.
90. White T, Nelson M, Lim KO. Diffusion tensor imaging in psychiatric disorders. Top Magn Reson Imaging 2008, 19: 97–109.
91. Sundaram SK, Kumar A, Makki MI, Behen ME, Chugani HT, Chugani DC. Diffusion tensor imaging of frontal lobe in autism spectrum disorder. Cereb Cortex 2008, 18: 2659–2665.
92. Hong S, Ke X, Tang T, Hang Y, Chu K, Huang H, *et al.* Detecting abnormalities of corpus callosum connectivity in autism using magnetic resonance imaging and diffusion tensor tractography. Psychiatry Res 2011, 194: 333–339.
93. Nagae LM, Zarnow DM, Blaskey L, Dell J, Khan SY, Qasmieh S, *et al.* Elevated mean diffusivity in the left hemisphere superior longitudinal fasciculus in autism spectrum disorders increases with more profound language impairment. AJNR Am J Neuroradiol 2012, 33: 1720–1725.
94. Wolff JJ, Gu H, Gerig G, Elison JT, Styner M, Gouttard S, *et al.* Differences in white matter fiber tract development present from 6 to 24 months in infants with autism. Am J Psychiatry 2012, 169: 589–600.
95. Nair A, Treiber JM, Shukla DK, Shih P, Muller RA. Impaired thalamocortical connectivity in autism spectrum disorder: a study of functional and anatomical connectivity. Brain 2013, 136: 1942–1955.
96. Joseph RM, Fricker Z, Fenoglio A, Lindgren KA, Knaus TA, Tager-Flusberg H. Structural asymmetries of language-related gray and white matter and their relationship to language function in young children with ASD. Brain Imaging Behav 2014, 8: 60–72.
97. Cheung C, Chua SE, Cheung V, Khong PL, Tai KS, Wong TK, *et al.* White matter fractional anisotrophy differences and correlates of diagnostic symptoms in autism. J Child Psychol Psychiatry 2009, 50: 1102–1112.
98. Ke X, Tang T, Hong S, Hang Y, Zou B, Li H, *et al.* White matter impairments in autism, evidence from voxel-based morphometry and diffusion tensor imaging. Brain Res 2009, 1265: 171–177.
99. Barnea-Goraly N, Lotspeich LJ, Reiss AL. Similar white matter aberrations in children with autism and their unaffected siblings: a diffusion tensor imaging study using tract-based spatial statistics. Arch Gen Psychiatry 2010, 67: 1052–1060.
100. Poustka L, Jennen-Steinmetz C, Henze R, Vomstein K, Haffner J, Sieltjes B. Fronto-temporal disconnectivity and symptom

severity in children with autism spectrum disorder. World J Biol Psychiatry 2012, 13: 269–280.
101. Peterson D, Mahajan R, Crocetti D, Mejia A, Mostofsky S. Left-hemispheric microstructural abnormalities in children with high-functioning autism spectrum disorder. Autism Res 2015, 8: 61–72.
102. Dawson G, Meltzoff AN, Osterling J, Rinaldi J. Neuropsychological correlates of early symptoms of autism. Child Dev 1998, 69: 1276–1285.
103. Escalante-Mead PR, Minshew NJ, Sweeney JA. Abnormal brain lateralization in high-functioning autism. J Autism Dev Disord 2003, 33: 539–543.
104. Kleinhans NM, Muller RA, Cohen DN, Courchesne E. Atypical functional lateralization of language in autism spectrum disorders. Brain Res 2008, 1221: 115–125.
105. Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, *et al.* Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage 2006, 31: 1487–1505.
106. Kumar A, Sundaram SK, Sivaswamy L, Behen ME, Makki MI, Ager J, *et al.* Alterations in frontal lobe tracts and corpus callosum in young children with autism spectrum disorder. Cereb Cortex 2010, 20: 2103–2113.
107. Weinstein M, Ben-Sira L, Levy Y, Zachor DA, Ben Itzhak E, Artzi M, *et al.* Abnormal white matter integrity in young children with autism. Hum Brain Mapp 2011, 32: 534–543.
108. Walker L, Gozzi M, Lenroot R, Thurm A, Behseta B, Swedo S, *et al.* Diffusion tensor imaging in young children with autism: biological effects and potential confounds. Biol Psychiatry 2012, 72: 1043–1051.
109. Mevel K, Fransson P. The functional brain connectome of the child and autism spectrum disorders. Acta Paediatr 2016, 105: 1024–1035.
110. Rane P, Cochran D, Hodge SM, Haselgrove C, Kennedy DN, Frazier JA. Connectivity in autism: a review of MRI connectivity studies. Harv Rev Psychiatry 2015, 23: 223–244.
111. Fox PT, Friston KJ. Distributed processing; distributed functions? Neuroimage 2012, 61: 407–426.
112. Just MA, Cherkassky VL, Keller TA, Minshew NJ. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain 2004, 127: 1811–1821.
113. Courchesne E, Pierce K. Brain overgrowth in autism during a critical time in development: implications for frontal pyramidal neuron and interneuron development and connectivity. Int J Dev Neurosci 2005, 23: 153–170.
114. Di Martino A, Kelly C, Grzadzinski R, Zuo XN, Mennes M, Mairena MA, *et al.* Aberrant striatal functional connectivity in children with autism. Biol Psychiatry 2011, 69: 847–856.
115. Lynch CJ, Uddin LQ, Supekar K, Khouzam A, Phillips J, Menon V. Default mode network in childhood autism: posteromedial cortex heterogeneity and relationship with social deficits. Biol Psychiatry 2013, 74: 212–219.
116. Uddin LQ, Supekar K, Lynch CJ, Khouzam A, Phillips J, Feinstein C, *et al.* Salience network-based classification and prediction of symptom severity in children with autism. JAMA Psychiatry 2013, 70: 869–879.
117. Abrams DA, Lynch CJ, Cheng KM, Phillips J, Supekar K, Ryali S, *et al.* Underconnectivity between voice-selective cortex and reward circuitry in children with autism. Proc Natl Acad Sci USA 2013, 110: 12060–12065.
118. Rudie JD, Hernandez LM, Brown JA, Beck-Pancer D, Colich NL, Gorrindo P, *et al.* Autism-associated promoter variant in MET impacts functional and structural brain networks. Neuron 2012, 75: 904–915.
119. Wiggins JL, Peltier SJ, Ashinoff S, Weng SJ, Carrasco M, Welsh RC, *et al.* Using a self-organizing map algorithm to detect age-related changes in functional connectivity during rest in autism spectrum disorders. Brain Res 2011, 1380: 187–197.
120. Bertholdo D, Watcharakorn A, Castillo M. Brain proton magnetic resonance spectroscopy: introduction and overview. Neuroimaging Clin N Am 2013, 23: 359–380.
121. Kato T, Inubushi T, Kato N. Magnetic resonance spectroscopy in affective disorders. J Neuropsychiatry Clin Neurosci 1998, 10: 133–147.
122. Ross B, Bluml S. Magnetic resonance spectroscopy of the human brain. Anat Rec 2001, 265: 54–84.
123. Clarke CE, Lowry M, Horsman A. Unchanged basal ganglia N-acetylaspartate and glutamate in idiopathic Parkinson's disease measured by proton magnetic resonance spectroscopy. Mov Disord 1997, 12: 297–301.
124. Aoki Y, Kasai K, Yamasue H. Age-related change in brain metabolite abnormalities in autism: a meta-analysis of proton magnetic resonance spectroscopy studies. Transl Psychiatry 2012, 2: e69.
125. Baruth JM, Wall CA, Patterson MC, Port JD. Proton magnetic resonance spectroscopy as a probe into the pathophysiology of autism spectrum disorders (ASD): a review. Autism Res 2013, 6: 119–133.
126. Naaijen J, Lythgoe DJ, Amiri H, Buitelaar JK, Glennon JC. Fronto-striatal glutamatergic compounds in compulsive and impulsive syndromes: a review of magnetic resonance spectroscopy studies. Neurosci Biobehav Rev 2015, 52: 74–88.
127. Brown MS, Singel D, Hepburn S, Rojas DC. Increased glutamate concentration in the auditory cortex of persons with autism and first-degree relatives: a ^{1}H-MRS study. Autism Res 2013, 6: 1–10.
128. DeVito TJ, Drost DJ, Neufeld RW, Rajakumar N, Pavlosky W, Williamson P, *et al.* Evidence for cortical dysfunction in autism: a proton magnetic resonance spectroscopic imaging study. Biol Psychiatry 2007, 61: 465–473.
129. Friedman SD, Shaw DW, Artru AA, Richards TL, Gardner J, Dawson G, *et al.* Regional brain chemical alterations in young children with autism spectrum disorder. Neurology 2003, 60: 100–107.
130. Hardan AY, Minshew NJ, Melhem NM, Srihari S, Jo B, Bansal R, *et al.* An MRI and proton spectroscopy study of the thalamus in children with autism. Psychiatry Res 2008, 163: 97–105.
131. Levitt JG, O'Neill J, Blanton RE, Smalley S, Fadale D, McCracken JT, *et al.* Proton magnetic resonance spectroscopic imaging of the brain in childhood autism. Biol Psychiatry 2003, 54: 1355–1366.
132. Bejjani A, O'Neill J, Kim JA, Frew AJ, Yee VW, Ly R, *et al.* Elevated glutamatergic compounds in pregenual anterior cingulate in pediatric autism spectrum disorder demonstrated by 1H MRS and 1H MRSI. PLoS One 2012, 7: e38786.
133. Murphy DG, Critchley HD, Schmitz N, McAlonan G, Van Amelsvoort T, Robertson D, *et al.* Asperger syndrome: a proton magnetic resonance spectroscopy study of brain. Arch Gen Psychiatry 2002, 59: 885–891.
134. O'Brien FM, Page L, O'Gorman RL, Bolton P, Sharma A, Baird G, *et al.* Maturation of limbic regions in Asperger syndrome: a preliminary study using proton magnetic resonance spectroscopy and structural magnetic resonance imaging. Psychiatry Res 2010, 184: 77–85.
135. Oner O, Devrimci-Ozguven H, Oktem F, Yagmurlu B, Baskak B, Munir KM. Proton MR spectroscopy: higher right anterior cingulate N-acetylaspartate/choline ratio in Asperger syndrome compared with healthy controls. AJNR Am J Neuroradiol 2007, 28: 1494–1498.

136. Kousi E, Tsougos I, Eftychia K. Proton magnetic resonance spectroscopy of the central nervous system. In: Fountas K (Ed.). Novel Frontiers of Advanced Neuroimaging. InTech, 2013, pp 19–50.
137. Rae CD. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. Neurochem Res 2014, 39: 1–36.
138. Corrigan NM, Shaw DW, Estes AM, Richards TL, Munson J, Friedman SD, *et al.* Atypical developmental patterns of brain chemistry in children with autism spectrum disorder. JAMA Psychiatry 2013, 70: 964–974.
139. Turner CE, Gant N. Chapter 2.2—the biochemistry of creatine. Mag Reson Spectrosc 2014: 91–103.
140. Page LA, Daly E, Schmitz N, Simmons A, Toal F, Deeley Q, *et al. In vivo* 1H-magnetic resonance spectroscopy study of amygdala-hippocampal and parietal regions in autism. Am J Psychiatry 2006, 163: 2189–2192.
141. Chugani DC, Sundram BS, Behen M, Lee ML, Moore GJ. Evidence of altered energy metabolism in autistic children. Prog Neuropsychopharmacol Biol Psychiatry 1999, 23: 635–641.
142. Ford TC, Crewther DP. A comprehensive review of the ^{1}H-MRS metabolite spectrum in autism spectrum disorder. Front Mol Neurosci 2016, 9: 14.
143. Pouwels PJ, Frahm J. Regional metabolite concentrations in human brain as determined by quantitative localized proton MRS. Magn Reson Med 1998, 39: 53–60.
144. Friedman SD, Shaw DW, Artru AA, Dawson G, Petropoulos H, Dager SR. Gray and white matter brain chemistry in young children with autism. Arch Gen Psychiatry 2006, 63: 786–794.
145. Fujii E, Mori K, Miyazaki M, Hashimoto T, Harada M, Kagami S. Function of the frontal lobe in autistic individuals: a proton magnetic resonance spectroscopic study. J Med Invest 2010, 57: 35–44.
146. Gabis L, Wei H, Azizian A, DeVincent C, Tudorica A, Kesner-Baruch Y, *et al.* 1H-magnetic resonance spectroscopy markers of cognitive and language ability in clinical subtypes of autism spectrum disorders. J Child Neurol 2008, 23: 766–774.
147. Suzuki K, Nishimura K, Sugihara G, Nakamura K, Tsuchiya KJ, Matsumoto K, *et al.* Metabolite alterations in the hippocampus of high-functioning adult subjects with autism. Int J Neuropsychopharmacol 2010, 13: 529–534.
148. Vasconcelos MM, Brito AR, Domingues RC, da Cruz LC, Jr., Gasparetto EL, Werner J, Jr., *et al.* Proton magnetic resonance spectroscopy in school-aged autistic children. J Neuroimaging 2008, 18: 288–295.
149. Brady S Seigel G, Albers RW, *et al.* Basic Neurochemistry: Principles of Molecular, Cellular and Medical Neurobiology. Cambridge: Elsevier Academic Press, 2011.
150. Rothman DL, Behar KL, Hyder F, Shulman RG. *In vivo* NMR studies of the glutamate neurotransmitter flux and neuroenergetics: implications for brain function. Annu Rev Physiol 2003, 65: 401–427.
151. Doyle-Thomas KA, Card D, Soorya LV, Wang AT, Fan J, Anagnostou E. Metabolic mapping of deep brain structures and associations with symptomatology in autism spectrum disorders. Res Autism Spectr Disord 2014, 8: 44–51.
152. Parikshak NN, Luo R, Zhang A, Won H, Lowe JK, Chandran V, *et al.* Integrative functional genomic analyses implicate specific molecular pathways and circuits in autism. Cell 2013, 155: 1008–1021.
153. Won H, Mah W, Kim E. Autism spectrum disorder causes, mechanisms, and treatments: focus on neuronal synapses. Front Mol Neurosci 2013, 6: 19.
154. Chiang MC, McMahon KL, de Zubicaray GI, Martin NG, Hickie I, Toga AW, *et al.* Genetics of white matter development: a DTI study of 705 twins and their siblings aged 12–29. Neuroimage 2011, 54: 2308–2317.
155. Fornito A, Zalesky A, Bassett DS, Meunier D, Ellison-Wright I, Yucel M, *et al.* Genetic influences on cost-efficient organization of human cortical functional networks. J Neurosci 2011, 31: 3261–3270.
156. Glahn DC, Winkler AM, Kochunov P, Almasy L, Duggirala R, Carless MA, *et al.* Genetic control over the resting brain. Proc Natl Acad Sci USA 2010, 107: 1223–1228.
157. Scott-Van Zeeland AA, Abrahams BS, Alvarez-Retuerto AI, Sonnenblick LI, Rudie JD, Ghahremani D, *et al.* Altered functional connectivity in frontal lobe circuits is associated with variation in the autism risk gene CNTNAP2. Sci Transl Med 2010, 2: 56ra80.
158. Fox MD, Greicius M. Clinical applications of resting state functional connectivity. Front Syst Neurosci 2010, 4: 19.
159. Pereira F, Mitchell T, Botvinick M. Machine learning classifiers and fMRI: a tutorial overview. Neuroimage 2009, 45: S199–209.
160. Vapnik VN. The Nature of Statistical Learning Theory. New York: Springer, 1995.
161. Koshino H, Carpenter PA, Minshew NJ, Cherkassky VL, Keller TA, Just MA. Functional connectivity in an fMRI working memory task in high-functioning autism. Neuroimage 2005, 24: 810–821.
162. McAlonan GM, Daly E, Kumari V, Critchley HD, van Amelsvoort T, Suckling J, *et al.* Brain anatomy and sensorimotor gating in Asperger's syndrome. Brain 2002, 125: 1594–1606.
163. Davatzikos C, Fan Y, Wu X, Shen D, Resnick SM. Detection of prodromal Alzheimer's disease via pattern classification of magnetic resonance imaging. Neurobiol Aging 2008, 29: 514–523.
164. Guo Y, Wu G, Commander LA, Szary S, Jewells V, Lin W, *et al.* Segmenting hippocampus from infant brains by sparse patch matching with deep-learned features. Med Image Comput Comput Assist Interv 2014, 17: 308–315.
165. Zhang D, Wang Y, Zhou L, Yuan H, Shen D, Alzheimer's disease neuroimaging I. Multimodal classification of Alzheimer's disease and mild cognitive impairment. Neuroimage 2011, 55: 856–867.
166. Zhu D, Li K, Terry DP, Puente AN, Wang L, Shen D, *et al.* Connectome-scale assessments of structural and functional connectivity in MCI. Hum Brain Mapp 2014, 35: 2911–2923.
167. Haller S, Badoud S, Nguyen D, Garibotto V, Lovblad KO, Burkhard PR. Individual detection of patients with Parkinson disease using support vector machine analysis of diffusion tensor imaging data: initial results. AJNR Am J Neuroradiol 2012, 33: 2123–2128.
168. Pan S IS, Warwick K, Aziz TZ. Parkinson's disease tremor classification—a comparison between support vector machines and neural networks. Expert Syst Appl. 2012, 39: 10764–10771.
169. Smith DV, Clithero JA, Rorden C, Karnath HO. Decoding the anatomical network of spatial attention. Proc Natl Acad Sci USA 2013, 110: 1518–1523.
170. Zhang Y, Kimberg DY, Coslett HB, Schwartz MF, Wang Z. Multivariate lesion-symptom mapping using support vector regression. Hum Brain Mapp 2014, 35: 5861–5876.
171. Singh V, Mukherjee L, Chung MK. Cortical surface thickness as a classifier: boosting for autism classification. Med Image Comput Comput Assist Interv 2008, 11: 999–1007.
172. Ecker C, Rocha-Rego V, Johnston P, Mourao-Miranda J, Marquand A, Daly EM, *et al.* Investigating the predictive value of whole-brain structural MR scans in autism: a pattern classification approach. Neuroimage 2010, 49: 44–56.
173. Ecker C, Ginestet C, Feng Y, Johnston P, Lombardo MV, Lai MC, *et al.* Brain surface anatomy in adults with autism: the relationship between surface area, cortical thickness, and autistic symptoms. JAMA Psychiatry 2013, 70: 59–70.

174. Jin Y, Wee CY, Shi F, Thung KH, Ni D, Yap PT, *et al.* Identification of infants at high-risk for autism spectrum disorder using multiparameter multiscale white matter connectivity networks. Hum Brain Mapp 2015, 36: 4880–4896.
175. Georgiades S Szatmari P, Boyle M. Importance of studying heterogeneity in autism. Neuropsychiatry 2013, 3: 123–125.
176. Di Martino A, Yan CG, Li Q, Denio E, Castellanos FX, Alaerts K, *et al.* The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. Mol Psychiatry 2014, 19: 659–667.
177. Di Martino A, Castellanos FX, Anderson J, Alaerts K, Assaf M, Behrmann Deen D, *et al.* The Autism Brain Imaging Data Exchange (ABIDE) Consortium: Open Sharing of Autism Resting State fMRI Data. Beijing: Human Brain Mapping Edition:18, 2012.
178. Fayed N, Modrego PJ. Comparative study of cerebral white matter in autism and attention-deficit/hyperactivity disorder by means of magnetic resonance spectroscopy. Acad Radiol 2005, 12: 566–569.

Neurosci. Bull. April, 2017, 33(2):238–246
DOI 10.1007/s12264-017-0120-7

www.neurosci.cn
www.springer.com/12264

REVIEW

Genes Related to Oxytocin and Arginine-Vasopressin Pathways: Associations with Autism Spectrum Disorders

Rong Zhang[1,2,3] · Hong-Feng Zhang[4] · Ji-Sheng Han[1,2,3] · Song-Ping Han[1,2,3]

Received: 8 December 2016 / Accepted: 17 February 2017 / Published online: 10 March 2017

Abstract Autism spectrum disorder (ASD) is a highly heritable neurodevelopmental disorders characterized by impaired social interactions, communication deficits, and repetitive behavior. Although the mechanisms underlying its etiology and manifestations are poorly understood, several lines of evidence from rodent and human studies suggest involvement of the evolutionarily highly-conserved oxytocin (OXT) and arginine-vasopressin (AVP), as these neuropeptides modulate various aspects of mammalian social behavior. As far as we know, there is no comprehensive review of the roles of the OXT and AVP systems in the development of ASD from the genetic aspect. In this review, we summarize the current knowledge regarding associations between ASD and single-nucleotide variants of the human OXT-AVP pathway genes *OXT*, *AVP*, AVP receptor 1a (*AVPR1a*), OXT receptor (*OXTR*), the oxytocinase/vasopressinase (*LNPEP*), and ADP-ribosyl cyclase (*CD38*).

Keywords Oxytocin · Arginine-vasopressin · Single-nucleotide polymorphisms · Autism spectrum disorder

Introduction

Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorders involving deficits in social interaction and social communication, as well as the presence of restricted interests and repetitive and stereotypic patterns of behavior. The estimated prevalence of ASD based on the 2014 National Health Interview Survey was 2.24%, more than three-fold increase since 2000 [1]. The rapid increase of ASD cases has stimulated research in recent decades. However, the etiology of ASD remains obscure, partly because of its etiological heterogeneity. Rather than a single causative factor, the combined effects and interplay between genetic heritability and environmental risk factors may be more important in the etiology of ASD. However, it is generally accepted that the etiology can, at least, be partly explained by genetic studies [2]. Specifically, studies in twins have shown a high concordance among homozygous twins (70%–90% [3]), which is much lower in discordant twins [4, 5]. The risk for a newborn child is >10-fold higher if a previous sibling has an ASD [6]. Family-based association testing (FBAT) and population-based case-control tests have increased knowledge about the genetic causes of ASD. Known variants conferring susceptibility include single-nucleotide variants, short insertions and deletions, and genomic copy-number variants [3]. Based on studies using quantitative molecular genetic techniques, the proportion of ASD explained by common

Rong Zhang and Hong-Feng Zhang have contributed equally to this review.

✉ Rong Zhang
zhangrong@bjmu.edu.cn

1 Neuroscience Research Institute, Peking University, Beijing 100191, China

2 Department of Neurobiology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China

3 Key Laboratory for Neuroscience, Ministry of Education/ National Health and Family Planning Commission, Peking University, Beijing 100191, China

4 Fujian Provincial Key Laboratory of Neurodegenerative Disease and Aging Research, Institute of Neuroscience, College of Medicine, Xiamen University, Xiamen 361005, China

genotyped single-nucleotide polymorphisms (SNPs) is estimated to be 17%–60% [7]. Therefore, the contribution of common variants to ASD is important and should not be neglected.

Oxytocin (OXT) and arginine vasopressin (AVP) are closely-related nonapeptides that only differ in two amino-acids and originate from separate genes derived from the duplication of a common ancestral gene [8]. In the vertebrate brain, both OXT and AVP are mainly synthesized in the paraventricular and supraoptic nuclei and processed along the axonal projections to the posterior lobe of the pituitary, where they are stored in secretory vesicles and released into the peripheral circulation. Besides, they are also released from dendrites and somata within the brain. In addition, these neurons project directly to other brain regions including the amygdala, striatum, hippocampus, bed nucleus of the stria terminalis, and the suprachiasmatic nucleus [9]. Recently, they have become increasingly attractive as potential therapeutic targets in the context of ASD research due to their regulatory roles in social preference, social behaviors, and recognition, as revealed by studies in both humans [9] and rodents (reviewed by Lukas and Neumann [10]). OXT and AVP function as "social factors" in the brain via binding to their corresponding receptors: the OXT receptor (OXTR) and AVP receptor 1A (AVPR1A). Evidence suggests that malfunction of these receptors is involved in the pathogenesis of ASD [11, 12]. CD38 is a nicotinamide adenine dinucleotide ectoenzyme that plays a role in hormone secretion and cell proliferation, differentiation, and migration [13]. Interestingly, this protein is highly expressed in the brain, plays an obligatory role in the central release of OXT [14] and is relevant to the development of ASD [15].

In this review, we focus on the associations between ASD and polymorphisms of genes encoding the elements of the OXT-AVP neuronal pathways OXT (*OXT/neurophysin-I*) and AVP (*AVP/neurophysin-II*), their receptors (*OXTR* and *AVPR1a*), *CD38*, and oxytocinase/vasopressinase (*LNPEP*), a peptidase responsible for the degradation of OXT and AVP into shorter peptides [16] (summarized in Table 1).

OXT and *AVP*

The human *OXT-neurophysin I* (*NPI*) and *AVP-neurophysin II* (*NPII*) loci are closely linked at chromosome 20p13, separated by only 12 kb of intergenic sequence, and are oppositely transcribed [17]. This type of genomic arrangement could result from the duplication of a common ancestral gene followed by the inversion of one of them [18]. The *OXT-NPI* gene encoding the OXT prepropeptide consists of three exons: the first encodes several peptides including a translocator signal, the nonapeptide hormone, the tripeptide processing signal, and the first 9 residues of neurophysin; the second encodes the central part of neurophysin; and the third exon encodes the C-terminal region of neurophysin [19]. The OXT prepropeptide undergoes cleavage and other modifications as it is transported along the axon to the terminals. The mature products OXT and its carrier molecule neurophysin I, are provisionally stored in the axon terminals until neural inputs elicit their release. *AVP-NPII* has almost the same gene structure and post-translational processing as *OXT-NPI* [20].

A linkage study by Allen-Brady and colleagues provisionally identified a susceptibility locus for ASD near the *OXT-NPI* gene region that met the genome-wide significance criteria [21]. In addition, Ebstein *et al*. reported nominal associations between ASD and *OXT* rs6133010, as well as the haplotypes in 170 individuals with ASD [22]. At the behavioral level, investigators found an association between *OXT* rs2770378 and autism-like traits including language impairment and restricted behaviors in females with ASD [23]. In a study of ASD and hormonal genes, two SNPs in the *OXT-NPI* gene region were examined and a single SNP, rs2740204, was associated with stereotyped behavior but not overall diagnosis in the 177 probands with ASD [24]. A recent study has also shown that various SNPs (including rs6084258, rs6133010, and rs2740204) near the *OXT* and *AVP* genes are associated with a diagnosis of ASD, social behaviors, restricted and repetitive behaviors, and intelligence quotient (IQ), as well as plasma OXT level [25].

Interestingly, in healthy individuals, polymorphisms near or within the *OXT* gene are also associated with phenotypes of brain function in social interactions such as empathy [26], maternal behaviors (breast-feeding [27] and maternal vocalization [28]) and social anxiety [29].

LNPEP

The OXT and AVP peptides have a half-life of ~20 min in cerebrospinal fluid [30] and 3 min in plasma [31]. When released centrally they are degraded within brain tissue by LNPEP, also referred to as placental leucine aminopeptidase, which preferentially degrades OXT and is thus regarded as an oxytocinase [16]. The enzyme also effectively degrades vasopressin and angiotensin III. LNPEP is detectable in various brain regions including the basal ganglia, cerebral cortex, and cerebellum [32]. In these regions, immunoreactive staining of LNPEP is specific for neurons, and not non-neuronal cells [32].

As far as we know, there is only one published study on *LNPEP* variants. The investigators found that the SNPs rs18059 and rs4869317 are associated with 28-day mortality in patients with septic shock. Moreover, the

Table 1 Polymorphisms of genes encoding elements of the OXT and AVP pathways that are associated with ASD and autistic symptoms.

Genes	Year	Design	Sample size	Ethnicity	Significant polymorphism	Refs.
OXT	2009	Family	149 families	Israeli	rs6133010	[22]
	2014		1771 children	Swedish	rs2770378	[23]
	2016	Family	156 families	Not specified	rs6084258, rs6133010 and rs2740204	[25]
OXTR	2005	Family	195 families	Han Chinese	rs2254298, rs53576	[35]
	2007	Family	57 families	Caucasian	rs2254298	[37]
	2008	Family	133 families	Israeli	rs2268494, rs1042778	[38]
	2010	Family	215 families	Japanese	No	[39]
	2010	Case-control	280 cases, 440 controls	Japanese	rs237887, rs2264891, rs2254298, rs2268495	[39]
	2010	Family	199 families	Caucasian	No	[44]
	2010	Family	100 families	Caucasian	rs2270465	[45]
	2011	Family	1238 families	Caucasian	rs2268493, rs1042778, rs7632287	[43]
	2013	Case-control	132 cases, 248 controls	Japanese	rs35062132-G	[42]
	2014	Case-control	76 cases, 99 controls	Swiss	rs2254298, rs53576	[36]
	2014	Case-control	118 cases, 412 controls	Caucasian	rs2268493	[41]
	2015		105 cases	Japanese	28 variants	[46]
	2015 (a meta-analysis)	Family and case-control	2525 families, 454 cases, 595 control	Han Chinese, Israeli, Caucasian, Japanese	rs7632287, rs237887, rs2268491, and rs2254298	[11]
	2016	Family	175 families	German	rs237889-A	[40]
AVPR1a	2002	Family	115 families	Caucasian, African- and Asian-American	RS3	[72]
	2004	Family	65 families	Not specified	RS1 and RS3	[12]
	2006	Family	116 families	Not specified	Haplotype RS1-RS3-AVR	[73]
	2010	Family	148 families	Korean	RS1 and RS3	[74]
	2011	Family	177 families	Irish	RS1 (short alleles), rs11174815	[75]
	2015	Family	205 families	Finnish	RS1 (short alleles), Haplotype rs7307997-rs1042615, and RS3-rs1042615	[76]
AVPR1b	2016	Family	207 families	Caucasian, African- and Asian-American	rs35369693 and rs28632197	[78]
CD38	2010	Family	104 families	Caucasian	rs6449197, rs3796863	[66]
	2010	Family	170 families	Israeli	rs3796863, rs3796878, rs3796867, rs4516711, rs10805347, rs1803404, rs1130169	[15]
	2010	Family	188 families	Japanese	–	[66]
	2014		1771 children	Swedish	rs6449182	[23]

OXT, oxytocin; *OXTR*, oxytocin receptor; *AVPR1a*, AVP receptor 1a; *AVPR1b*, AVP receptor 1b; *CD38*, cyclic ADP ribose hydrolase; RS1 and RS3, promoter microsatellites of *AVPR1a*.

rs4869317 TT genotype is associated with increased plasma vasopressin clearance [33]. Although there has been no direct evidence for the involvement of LNPEP in altered human behavioral phenotypes, we speculate that this aminopeptidase may play a regulatory role in human social behaviors via influencing the central OXT and/or AVP levels and perhaps is a target for drug intervention in some disorders with social defects, such as ASD.

OXTR

In the brain, OXT regulates a variety of social behaviors via binding to its sole receptor OXTR in various regions. The *OXTR* gene is present in a single copy in the human genome and has been mapped to the gene locus 3p25-3p26.2. The gene spans 17 kb, contains 3 introns and 4 exons [34], and encodes a 389-amino-acid polypeptide

belonging to class I of the G protein-coupled receptor family [18].

OXT as a genetic risk factor for ASD is also supported by linkage analysis and disease association with common variants in *OXTR*. In a study involving Han Chinese individuals, Wu *et al*. [35] used the FBAT and found a significant genetic association between ASD and two *OXTR* SNPs, rs2254298 and rs53576. A number of haplotypes constructed with two, three, or four markers, particularly those involving rs53576, were significantly linked to ASD [35]. Nyffeler *et al*. [36] also found similar associations in a Caucasian population with high-functioning autism. Jacob *et al*. [37] replicated the study of Wu *et al*. in a Caucasian sample with a strictly-defined autistic disorder. Interestingly, the SNP rs2254298 but not rs53576 was found to be associated with ASD. Moreover, over-transmission of the G-allele to probands with ASD was reported, which was inconsistent with a previous study in a Han Chinese population. Lerer *et al*. [38] conducted a comprehensive study examining all the tagged SNPs across the *OXTR* gene region. As expected, significant associations were found for single SNPs and haplotype with ASD. Notably, these polymorphisms of *OXTR* showed significant associations with IQ and the Vineland Adaptive Behavior Scales for ASD. In a Japanese population, Liu *et al*. [39] analyzed 11 *OXTR* SNPs but did not detect any significant signal in the FBAT test. However, case-control analysis revealed significant associations between four SNPs and ASD. The most significantly associated SNP was rs2254298 with "A" as the risk allele [39]. This result was similar to those in a Han Chinese population, but in contrast to the observations in Caucasians. The ethnic difference in the linkage disequilibrium structure between Asian and Caucasian populations may contribute to the difference in the role of *OXTR* polymorphisms in ASD in the two populations. A recent meta-analysis of 16 *OXTR* SNPs including 3941 individuals with ASD from 11 independent samples [11] revealed associations between ASD and the *OXTR* SNPs rs7632287, rs237887, rs2268491, and rs2254298. *OXTR* was also associated with ASD in a gene-based test. These results are the most comprehensive examination of the association of common *OXTR* variants with ASD to date. Furthermore, Kranz *et al*. [40] tested two additional *OXTR* SNPs (rs237889 and rs237897) for association with ASD in German cohorts and found nominal over-transmission for the minor A allele of variant rs237889G>A. Di Napoli *et al*. [41] focused on Asperger Syndrome, a subgroup of ASD, and discovered a significant association with rs2268493 in *OXTR*. Ma *et al*. [42] reported that the G allele of variant rs35062132C>G was correlated with an increased likelihood of ASD. Further cell experiments showed that rs35062132C>G accelerates OXT-induced receptor internalization and recycling, indicating a functional variant.

However, *OXT* SNPs were not always associated with ASD in the association studies, especially when adjustment was made for multiple comparisons. Campbell *et al*. [43] examined 25 genetic markers spanning the *OXTR* locus in a relatively large American sample, and an association of the three markers rs7632287, rs2268493, and rs1042778 was found. However, all the significant associations disappeared after correction for multiple testing. Similarly, in a combined sample from Ireland, the UK, and Portugal, the findings of Wu *et al*. [35] and Jacob *et al*. [37] were not replicated, with no marker survived for association with ASD [44]. In addition, Wermter *et al*. [45] genotyped 22 SNPs in the *OXTR* genomic region in 100 families with high-functioning and atypical ASD, and found no association after correction for multiple comparisons.

Research focusing on epigenetic modifications and rare variations of the *OXTR* may provide additional evidence for a role of this gene in ASD. In 105 ASD individuals from Japan, investigators identified 28 novel variants including potential functional variants in the intron region and one rare mis-sense variant (R150S) [46]. Gregory *et al*. [47] examined copy number variations and epigenetic changes in the *OXTR* gene, and interestingly revealed that a genomic deletion containing the *OXTR* gene was present in an autistic proband. DNA methylation analysis indicated that the promoter region of *OXTR* is hypermethylated in independent datasets of individuals with autism as compared to control samples, in both peripheral blood mononuclear cells and temporal cortex. In healthy adults, *OXTR* methylation has been associated with activity in the dorsal anterior cingulate cortex and temporal parietal junction, regions strongly associated with social perception [48].

In healthy populations, SNPs across the human *OXTR* gene have been associated with pair-bonding behaviors [49], parenting [50, 51], face-recognition skills [52, 53], and emotional and cognitive empathy [54, 55]. Neuroimaging studies have shown that carriers of the *OXTR* rs53576 AA allele have a smaller volume and reduced functional connectivity of the hypothalamus [56, 57], and GG homozygotes have an increased local volume in the left hippocampus and amygdala [58], which indicates an association between *OXTR* genetic variation and structural and functional variability in brain regions relevant to social cognition. In addition, rs53576 GG homozygotes are more responsive to intranasal OXT administration. For example, OXT administration increases preference for infants' faces [59] and social cooperation [60] among rs53576 GG homozygotes but not in A allele carriers. The most plausible mechanism by which *OXTR* SNPs influence the

effects of OXT is through altering expression of the OXTR. In prairie voles, one non-coding polymorphism in the *Oxtr* (SNP2) explains the variance in OXTR expression in particular brain regions [61]. Specifically, T-allele genotypes of SNP2 have double the OXTR density in the nucleus accumbens than CC littermates.

CD38

Further evidence for an important role of the OXT system in ASD comes from studies on CD38, a transmembrane protein involved in OXT release in the brain [62] and in the critical regulation of social behavior [14, 63]. *Cd38*-knockout mice show severe social deficits (i.e., amnesia of conspecifics) and have been discussed as a rodent model of ASD [64, 65]. In individuals with ASD, two SNPs of *CD38* (rs6449197 and rs3796863) have been associated with high-functioning autism in the US population [66]. These findings were partially confirmed in Israeli participants [15], but not in Japanese cases [66]. For the rs3796863 SNP, ASD patients carrying the CC genotype are characterized by more severe symptoms, such as restricted, repetitive, and stereotyped patterns of behavior, than those carrying the A allele [66].

In healthy populations, individuals homozygous for the CC allele on *CD38* rs3796863 show a lower level of peripheral OXT than CA/AA carriers [67, 68]. When exposed to social stimuli, healthy men with the CC allele show slower reaction-times and higher activation of the left fusiform gyrus [69], an area widely discussed in ASD research. At the behavioral level, parents with high-risk alleles have been shown to touch their infants less during a free-play session, and low-risk *CD38* alleles predict longer durations of parent-infant gaze synchrony [67].

Besides the SNPs, a mis-sense mutation (4693C>T) of *CD38* has been found in 0.6%–4.6% of a Japanese population and was associated with ASD in a case-control study [66]. Partial deletion of *CD38* has also been reported in a patient with autism and asthma [70]. Furthermore, autistic individuals also show low expression of CD38 in lymphoblastoid cells (LBCs) [15]. In LBCs, treatment with all-*trans* retinoic acid (a known inducer of CD38 [69]) reverses CD38 mRNA expression [71]. Such a demonstration may provide *in vitro* "proof of principle" that CD38 is a potential target in the clinical treatment of ASD.

AVPR1a

In contrast to only one form of OXTR, there are three subtypes of AVPR, AVPR1a, AVPR1b, and AVPR2, which are all G-protein-coupled receptors. Of those, AVPR1a is predominantly expressed in the brain and is the most strongly implicated in neuropsychiatric phenotypes. Therefore, in this section, we mainly summarize associations between polymorphisms of *AVPR1a* and ASD.

Various studies have established possible associations between polymorphisms in the promoter region of the *AVPR1a* gene and autism phenotypes. The human *AVPR1a* promoter region contains two microsatellite repeats, RS1 and RS3, in the 5′ flanking region. Of these, RS3 is a complex repeat located 3625 bp upstream of the transcription start site, and RS1 is a $(GATA)_n$ repeat located 553 bp upstream of the start site [9]. The first genetic study of *AVPR1a* and human behavior was conducted by Kim *et al*. [72], who showed a nominally significant transmission disequilibrium between an *AVPR1a* microsatellite (RS3) and ASD, but this association was not significant after Bonferroni correction. Later, Wassink *et al*. [12] also found significant disequilibrium with both RS1 and RS3 but in cases with less severe impairment of language. More recently, Yirmiya *et al*. [73] failed to find associations of specific *AVPR1a* alleles with ASD, but significant associations of haplotypes consisting of RS1, RS3, and an intronic microsatellite (AVR). In addition, significant associations have been reported between these three microsatellite haplotypes and social phenotypes of ASD. Another study genotyped 148 Korean trios (a family with parents and a child) and also found evidence for associations between *AVPR1a* microsatellites (RS1 and RS3) and ASD [74]. In a study of an Irish population, a weak association was found between short alleles of RS1 and the SNP rs11174815 and ASD [75]. Recently, a Finnish study analyzed the association of three microsatellites (RS1, RS3, and AVR) and 12 tagged SNPs in the promoter and coding regions of *AVPR1a*, and found that the best association was located in RS1 [76]. Promoter analysis predicted one potential binding site for MEF2C (myocyte enhancer factor 2C) at RS1, which may be involved in autistic behavior [77]. In addition, the *AVPR1b* SNPs rs35369693 and rs28632197 have been associated with ASD, and the significance remained after correction for multiple comparisons [78]. This was the first study reporting associations between *AVPR1b* SNPs and ASD.

These findings provide evidence for a contribution of genetic polymorphisms of *AVPR1a* to the risk for ASD, which is further supported by the social impairment found in mice lacking functional *Avpr1a* [79]. Interestingly, microsatellite repeats are also found upstream of *Avpr1a* in prairie voles, a commonly-used animal model for affiliative social behavior related to neuropeptide signaling [80]. In this type of animal, microsatellite length causes intraspecific variation in *Avpr1a* expression and, consequently, social behavioral traits [81].

In individuals who have developed normally, long *AVPR1a* RS3 repeats are associated with higher expression of hippocampal *AVPR1a* [82] than in those carrying short

RS3 repeats. In addition, longer alleles of RS3 are associated with a higher level of economic altruism [82] and a greater level of prepulse inhibition [83], which is an indicator of social cognition. Moreover, polymorphisms of RS3 are also linked to adulthood social interaction [84], pair-bonding [85], trust behavior [86], and non-clinical autism spectrum phenotypes [87] in healthy individuals.

OXTR Gene Polymorphisms and Efficacy of OXT Administration

Since OXT is closely associated with a series of social behaviors, the neuropeptide is regarded as a potential agent for ASD treatment [9, 88–93]. Accumulating evidence has suggested that exogenous OXT administration is beneficial for the remission of autistic symptoms by improving cooperation and a sense of trust [94], as well as enhancing social responsiveness [95, 96] and social reciprocity [97, 98]. However, several studies failed to replicate the beneficial clinical effects of OXT on ASD [99, 100]. We speculate that these inconsistent findings may be at least partly associated with genetic polymorphisms of *OXTR*. Because intranasally-administered OXT is considered to act through the OXTR [18] and the latter contains several dozen SNPs, the administered OXT would not be expected to have a pharmacological effect if there is a loss-of-function mutation in *OXTR*. Therefore, the efficacy of OXT administration might differ according to *OXTR* gene polymorphisms.

Animal studies have suggested that some *OXTR* SNPs contribute to individual differences in OXTR expression, but only in particular brain regions [61]. A single-dose study in healthy volunteers showed that *OXTR* gene polymorphisms alter the sensitivity to reward-relevant features and/or their aversive properties in infants [59] and also influence the improvement of neural responses associated with social cooperation [60]. With long-term OXT administration, ASD patients carrying a T-allele at rs6791619 of the *OXTR* show improved Clinical Global Impression-Improvement scores, providing direct evidence that *OXTR* SNPs are associated with the efficacy of OXT treatment [101]. Therefore, besides the regimen (e.g., dosage and number of administrations per day), participant characteristics including their genetic background are also important factors that need to be considered in clinical trials of OXT administration [102].

Conclusions and Perspectives

In the current review, we summarize the key findings on associations between ASD and genetic polymorphisms of five genes that are key players in the architecture of the OXT-AVP neural pathways. We suggest that targeting elements of the OXT and AVP pathways is a potentially fruitful approach for drug discovery as well as a source of potential biomarkers for the early diagnosis of social disorders, especially ASD.

Animal studies suggest that epigenetic markers, including methylation and histone acetylation of the *OXTR*, are important in regulating the *OXTR* and *AVPR1a* genes [103, 104]. Notably, failure to examine the epigenetic modulation of OXT-pathway genes may be one reason for the lack of conclusive findings in a recent meta-analysis of *OXTR* rs53576 and rs2254298 [105]. Further investigations need to focus on not only the functional significance of *OXTR* SNPs but also potential epigenetic mechanisms, which will allow stronger and more comprehensive conclusions as to whether disruptions in oxytocinergic signaling contribute to a risk for ASD or are associated with variability in social deficiency in ASD.

Acknowledgements This review was supported by a fund from University of Ulm-Peking University Health Science Center Joint Center for Neuroscience (BMU20160563).

References

1. Zablotsky B, Black LI, Maenner MJ, Schieve LA, Blumberg SJ. Estimated prevalence of autism and other developmental disabilities following questionnaire changes in the 2014 national health interview survey. Natl Health Stat Report 2015: 1–20.
2. Hens K, Peeters H, Dierickx K. The ethics of complexity. Genetics and autism, a literature review. Am J Med Genet B Neuropsychiatr Genet 2016, 171B: 305–316.
3. Ronemus M, Iossifov I, Levy D, Wigler M. The role of de novo mutations in the genetics of autism spectrum disorders. Nat Rev Genet 2014, 15: 133–141.
4. Tchaconas A, Adesman A. Autism spectrum disorders: a pediatric overview and update. Curr Opin Pediatr 2013, 25: 130–144.
5. Szatmari P. Is autism, at least in part, a disorder of fetal programming? Arch Gen Psychiatry 2011, 68: 1091–1092.
6. Constantino JN, Zhang Y, Frazier T, Abbacchi AM, Law P. Sibling recurrence and the genetic epidemiology of autism. Am J Psychiatry 2010, 167: 1349–1356.
7. Gratten J, Wray NR, Keller MC, Visscher PM. Large-scale genomics unveils the genetic architecture of psychiatric disorders. Nat Neurosci 2014, 17: 782–790.
8. Donaldson ZR, Young LJ. Oxytocin, vasopressin, and the neurogenetics of sociality. Science 2008, 322: 900–904.
9. Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M. Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. Nat Rev Neurosci 2011, 12: 524–538.

10. Lukas M, Neumann ID. Oxytocin and vasopressin in rodent behaviors related to social dysfunctions in autism spectrum disorders. Behav Brain Res 2013, 251: 85–94.
11. LoParo D, Waldman ID. The oxytocin receptor gene (OXTR) is associated with autism spectrum disorder: a meta-analysis. Mol Psychiatry 2015, 20: 640–646.
12. Wassink TH, Piven J, Vieland VJ, Pietila J, Goedken RJ, Folstein SE, *et al*. Examination of AVPR1a as an autism susceptibility gene. Mol Psychiatry 2004, 9: 968–972.
13. Malavasi F, Deaglio S, Funaro A, Ferrero E, Horenstein AL, Ortolan E, *et al*. Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. Physiol Rev 2008, 88: 841–886.
14. Jin D, Liu HX, Hirai H, Torashima T, Nagai T, Lopatina O, *et al*. CD38 is critical for social behaviour by regulating oxytocin secretion. Nature 2007, 446: 41–45.
15. Lerer E, Levi S, Israel S, Yaari M, Nemanov L, Mankuta D, *et al*. Low CD38 expression in lymphoblastoid cells and haplotypes are both associated with autism in a family-based study. Autism Res 2010, 3: 293–302.
16. Tsujimoto M, Hattori A. The oxytocinase subfamily of M1 aminopeptidases. Biochim Biophys Acta 2005, 1751: 9–18.
17. Rao VV, Loffler C, Battey J, Hansmann I. The human gene for oxytocin-neurophysin I (OXT) is physically mapped to chromosome 20p13 by in situ hybridization. Cytogenet Cell Genet 1992, 61: 271–273.
18. Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. Physiol Rev 2001, 81: 629–683.
19. Feldman R, Monakhov M, Pratt M, Ebstein RP. Oxytocin pathway genes: evolutionary ancient system impacting on human affiliation, sociality, and psychopathology. Biol Psychiatry 2016, 79: 174–184.
20. Ruppert S, Scherer G, Schutz G. Recent gene conversion involving bovine vasopressin and oxytocin precursor genes suggested by nucleotide sequence. Nature 1984, 308: 554–557.
21. Allen-Brady K, Miller J, Matsunami N, Stevens J, Block H, Farley M, *et al*. A high-density SNP genome-wide linkage scan in a large autism extended pedigree. Mol Psychiatry 2009, 14: 590–600.
22. Ebstein RP, Israel S, Lerer E, Uzefovsky F, Shalev I, Gritsenko I, *et al*. Arginine vasopressin and oxytocin modulate human social behavior. Ann N Y Acad Sci 2009, 1167: 87–102.
23. Hovey D, Zettergren A, Jonsson L, Melke J, Anckarsater H, Lichtenstein P, *et al*. Associations between oxytocin-related genes and autistic-like traits. Soc Neurosci 2014, 9: 378–386.
24. Yrigollen CM, Han SS, Kochetkova A, Babitz T, Chang JT, Volkmar FR, *et al*. Genes controlling affiliative behavior as candidate genes for autism. Biol Psychiatry 2008, 63: 911–916.
25. Francis SM, Kistner-Griffin E, Yan Z, Guter S, Cook EH, Jacob S. Variants in adjacent oxytocin/vasopressin gene region and associations with ASD diagnosis and other autism related endophenotypes. Front Neurosci 2016, 10: 195.
26. Shamay-Tsoory SG. The neural bases for empathy. Neuroscientist 2011, 17: 18–24.
27. Jonas W, Mileva-Seitz V, Girard AW, Bisceglia R, Kennedy JL, Sokolowski M, *et al*. Genetic variation in oxytocin rs2740210 and early adversity associated with postpartum depression and breastfeeding duration. Genes Brain Behav 2013, 12: 681–694.
28. Mileva-Seitz V, Steiner M, Atkinson L, Meaney MJ, Levitan R, Kennedy JL, *et al*. Interaction between oxytocin genotypes and early experience predicts quality of mothering and postpartum mood. PLoS One 2013, 8: e61443.
29. Love TM, Enoch MA, Hodgkinson CA, Pecina M, Mickey B, Koeppe RA, *et al*. Oxytocin gene polymorphisms influence human dopaminergic function in a sex-dependent manner. Biol Psychiatry 2012, 72: 198–206.
30. Ludwig M, Leng G. Dendritic peptide release and peptide-dependent behaviours. Nat Rev Neurosci 2006, 7: 126–136.
31. Gazis D. Plasma half-lives of vasopressin and oxytocin analogs after iv injection in rats. Proc Soc Exp Biol Med 1978, 158: 663–665.
32. Matsumoto H, Nagasaka T, Hattori A, Rogi T, Tsuruoka N, Mizutani S, *et al*. Expression of placental leucine aminopeptidase/oxytocinase in neuronal cells and its action on neuronal peptides. Eur J Biochem 2001, 268: 3259–3266.
33. Nakada TA, Russell JA, Wellman H, Boyd JH, Nakada E, Thain KR, *et al*. Leucyl/cystinyl aminopeptidase gene variants in septic shock. Chest 2011, 139: 1042–1049.
34. Inoue T, Kimura T, Azuma C, Inazawa J, Takemura M, Kikuchi T, *et al*. Structural organization of the human oxytocin receptor gene. J Biol Chem 1994, 269: 32451–32456.
35. Wu S, Jia M, Ruan Y, Liu J, Guo Y, Shuang M, *et al*. Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. Biol Psychiatry 2005, 58: 74–77.
36. Nyffeler J, Walitza S, Bobrowski E, Gundelfinger R, Grunblatt E. Association study in siblings and case-controls of serotonin- and oxytocin-related genes with high functioning autism. J Mol Psychiatry 2014, 2: 1.
37. Jacob S, Brune CW, Carter CS, Leventhal BL, Lord C, Cook EH, Jr. Association of the oxytocin receptor gene (OXTR) in Caucasian children and adolescents with autism. Neurosci Lett 2007, 417: 6–9.
38. Lerer E, Levi S, Salomon S, Darvasi A, Yirmiya N, Ebstein RP. Association between the oxytocin receptor (OXTR) gene and autism: relationship to Vineland Adaptive Behavior Scales and cognition. Mol Psychiatry 2008, 13: 980–988.
39. Liu X, Kawamura Y, Shimada T, Otowa T, Koishi S, Sugiyama T, *et al*. Association of the oxytocin receptor (OXTR) gene polymorphisms with autism spectrum disorder (ASD) in the Japanese population. J Hum Genet 2010, 55: 137–141.
40. Kranz TM, Kopp M, Waltes R, Sachse M, Duketis E, Jarczok TA, *et al*. Meta-analysis and association of two common polymorphisms of the human oxytocin receptor gene in autism spectrum disorder. Autism Res 2016, 9: 1036–1045.
41. Di Napoli A, Warrier V, Baron-Cohen S, Chakrabarti B. Genetic variation in the oxytocin receptor (OXTR) gene is associated with Asperger Syndrome. Mol Autism 2014, 5: 48.
42. Ma WJ, Hashii M, Munesue T, Hayashi K, Yagi K, Yamagishi M, *et al*. Non-synonymous single-nucleotide variations of the human oxytocin receptor gene and autism spectrum disorders: a case-control study in a Japanese population and functional analysis. Mol Autism 2013, 4: 22.
43. Campbell DB, Datta D, Jones ST, Batey Lee E, Sutcliffe JS, Hammock EA, *et al*. Association of oxytocin receptor (OXTR) gene variants with multiple phenotype domains of autism spectrum disorder. J Neurodev Disord 2011, 3: 101–112.
44. Tansey KE, Brookes KJ, Hill MJ, Cochrane LE, Gill M, Skuse D, *et al*. Oxytocin receptor (OXTR) does not play a major role in the aetiology of autism: genetic and molecular studies. Neurosci Lett 2010, 474: 163–167.
45. Wermter AK, Kamp-Becker I, Hesse P, Schulte-Korne G, Strauch K, Remschmidt H. Evidence for the involvement of genetic variation in the oxytocin receptor gene (OXTR) in the etiology of autistic disorders on high-functioning level. Am J Med Genet B Neuropsychiatr Genet 2010, 153B: 629–639.
46. Liu X, Kawashima M, Miyagawa T, Otowa T, Latt KZ, Thiri M, *et al*. Novel rare variations of the oxytocin receptor (OXTR) gene in autism spectrum disorder individuals. Hum Genome Var 2015, 2: 15024.
47. Gregory SG, Connelly JJ, Towers AJ, Johnson J, Biscocho D, Markunas CA, *et al*. Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. BMC Med 2009, 7: 62.

48. Puglia MH, Lillard TS, Morris JP, Connelly JJ. Epigenetic modification of the oxytocin receptor gene influences the perception of anger and fear in the human brain. Proc Natl Acad Sci U S A 2015, 112: 3308–3313.
49. Walum H, Lichtenstein P, Neiderhiser JM, Reiss D, Ganiban JM, Spotts EL, *et al*. Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. Biol Psychiatry 2012, 71: 419–426.
50. Rilling JK, Young LJ. The biology of mammalian parenting and its effect on offspring social development. Science 2014, 345: 771–776.
51. Klahr AM, Klump K, Burt SA. A constructive replication of the association between the oxytocin receptor genotype and parenting. J Fam Psychol 2015, 29: 91–99.
52. Skuse DH, Lori A, Cubells JF, Lee I, Conneely KN, Puura K, *et al*. Common polymorphism in the oxytocin receptor gene (OXTR) is associated with human social recognition skills. Proc Natl Acad Sci U S A 2014, 111: 1987–1992.
53. Westberg L, Henningsson S, Zettergren A, Svard J, Hovey D, Lin T, *et al*. Variation in the oxytocin receptor gene is associated with face recognition and its neural correlates. Front Behav Neurosci 2016, 10: 178.
54. Uzefovsky F, Shalev I, Israel S, Edelman S, Raz Y, Mankuta D, *et al*. Oxytocin receptor and vasopressin receptor 1a genes are respectively associated with emotional and cognitive empathy. Horm Behav 2015, 67: 60–65.
55. Weisman O, Pelphrey KA, Leckman JF, Feldman R, Lu Y, Chong A, *et al*. The association between 2D:4D ratio and cognitive empathy is contingent on a common polymorphism in the oxytocin receptor gene (OXTR rs53576). Psychoneuroendocrinology 2015, 58: 23–32.
56. Tost H, Kolachana B, Hakimi S, Lemaitre H, Verchinski BA, Mattay VS, *et al*. A common allele in the oxytocin receptor gene (OXTR) impacts prosocial temperament and human hypothalamic-limbic structure and function. Proc Natl Acad Sci U S A 2010, 107: 13936–13941.
57. Wang J, Qin W, Liu B, Wang D, Zhang Y, Jiang T, *et al*. Variant in OXTR gene and functional connectivity of the hypothalamus in normal subjects. Neuroimage 2013, 81: 199–204.
58. Schneider-Hassloff H, Straube B, Jansen A, Nuscheler B, Wemken G, Witt SH, *et al*. Oxytocin receptor polymorphism and childhood social experiences shape adult personality, brain structure and neural correlates of mentalizing. Neuroimage 2016, 134: 671–684.
59. Marsh AA, Yu HH, Pine DS, Gorodetsky EK, Goldman D, Blair RJ. The influence of oxytocin administration on responses to infant faces and potential moderation by OXTR genotype. Psychopharmacology (Berl) 2012, 224: 469–476.
60. Feng C, Lori A, Waldman ID, Binder EB, Haroon E, Rilling JK. A common oxytocin receptor gene (OXTR) polymorphism modulates intranasal oxytocin effects on the neural response to social cooperation in humans. Genes Brain Behav 2015, 14: 516–525.
61. King LB, Walum H, Inoue K, Eyrich NW, Young LJ. Variation in the oxytocin receptor gene predicts brain region-specific expression and social attachment. Biol Psychiatry 2016, 80: 160–169.
62. Lopatina O, Liu HX, Amina S, Hashii M, Higashida H. Oxytocin-induced elevation of ADP-ribosyl cyclase activity, cyclic ADP-ribose or Ca(2+) concentrations is involved in autoregulation of oxytocin secretion in the hypothalamus and posterior pituitary in male mice. Neuropharmacology 2010, 58: 50–55.
63. Liu HX, Lopatina O, Higashida C, Tsuji T, Kato I, Takasawa S, *et al*. Locomotor activity, ultrasonic vocalization and oxytocin levels in infant CD38 knockout mice. Neurosci Lett 2008, 448: 67–70.
64. Higashida H, Yokoyama S, Kikuchi M, Munesue T. CD38 and its role in oxytocin secretion and social behavior. Horm Behav 2012, 61: 351–358.
65. Higashida H, Yokoyama S, Munesue T, Kikuchi M, Minabe Y, Lopatina O. CD38 gene knockout juvenile mice: a model of oxytocin signal defects in autism. Biol Pharm Bull 2011, 34: 1369–1372.
66. Munesue T, Yokoyama S, Nakamura K, Anitha A, Yamada K, Hayashi K, *et al*. Two genetic variants of CD38 in subjects with autism spectrum disorder and controls. Neurosci Res 2010, 67: 181–191.
67. Feldman R, Zagoory-Sharon O, Weisman O, Schneiderman I, Gordon I, Maoz R, *et al*. Sensitive parenting is associated with plasma oxytocin and polymorphisms in the OXTR and CD38 genes. Biol Psychiatry 2012, 72: 175–181.
68. Feldman R, Gordon I, Influs M, Gutbir T, Ebstein RP. Parental oxytocin and early caregiving jointly shape children's oxytocin response and social reciprocity. Neuropsychopharmacology 2013, 38: 1154–1162.
69. Ferrero E, Malavasi F. A natural history of the human CD38 gene. In: Lee HC (Ed.). Cyclic ADP-Ribose and NAADP. Structures, Metabolism and Functions. Dordrecht: Kluwer, 2002: 65–79.
70. Ceroni F, Sagar A, Simpson NH, Gawthrope AJ, Newbury DF, Pinto D, *et al*. A deletion involving CD38 and BST1 results in a fusion transcript in a patient with autism and asthma. Autism Res 2014, 7: 254–263.
71. Riebold M, Mankuta D, Lerer E, Israel S, Zhong S, Nemanov L, *et al*. All-trans retinoic acid upregulates reduced CD38 transcription in lymphoblastoid cell lines from Autism spectrum disorder. Mol Med 2011, 17: 799–806.
72. Kim SJ, Young LJ, Gonen D, Veenstra-VanderWeele J, Courchesne R, Courchesne E, *et al*. Transmission disequilibrium testing of arginine vasopressin receptor 1A (AVPR1A) polymorphisms in autism. Mol Psychiatry 2002, 7: 503–507.
73. Yirmiya N, Rosenberg C, Levi S, Salomon S, Shulman C, Nemanov L, *et al*. Association between the arginine vasopressin 1a receptor (AVPR1a) gene and autism in a family-based study: mediation by socialization skills. Mol Psychiatry 2006, 11: 488–494.
74. Yang SY, Cho SC, Yoo HJ, Cho IH, Park M, Yoe J, *et al*. Family-based association study of microsatellites in the 5′ flanking region of AVPR1A with autism spectrum disorder in the Korean population. Psychiatry Res 2010, 178: 199–201.
75. Tansey KE, Hill MJ, Cochrane LE, Gill M, Anney RJ, Gallagher L. Functionality of promoter microsatellites of arginine vasopressin receptor 1A (AVPR1A): implications for autism. Mol Autism 2011, 2: 3.
76. Kantojarvi K, Oikkonen J, Kotala I, Kallela J, Vanhala R, Onkamo P, *et al*. Association and promoter analysis of AVPR1A in finnish autism families. Autism Res 2015, 8: 634–639.
77. Barbosa AC, Kim MS, Ertunc M, Adachi M, Nelson ED, McAnally J, *et al*. MEF2C, a transcription factor that facilitates learning and memory by negative regulation of synapse numbers and function. Proc Natl Acad Sci U S A 2008, 105: 9391–9396.
78. Francis SM, Kim SJ, Kistner-Griffin E, Guter S, Cook EH, Jacob S. ASD and genetic associations with receptors for oxytocin and vasopressin-AVPR1A, AVPR1B, and OXTR. Front Neurosci 2016, 10: 516.
79. Bielsky IF, Hu SB, Szegda KL, Westphal H, Young LJ. Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice. Neuropsychopharmacology 2004, 29: 483–493.

80. Mabry KE, Streatfeild CA, Keane B, Solomon NG. avpr1a length polymorphism is not associated with either social or genetic monogamy in free-living prairie voles. Anim Behav 2011, 81: 11–18.
81. Hammock EA, Young LJ. Microsatellite instability generates diversity in brain and sociobehavioral traits. Science 2005, 308: 1630–1634.
82. Knafo A, Israel S, Darvasi A, Bachner-Melman R, Uzefovsky F, Cohen L, *et al*. Individual differences in allocation of funds in the dictator game associated with length of the arginine vasopressin 1a receptor RS3 promoter region and correlation between RS3 length and hippocampal mRNA. Genes Brain Behav 2008, 7: 266–275.
83. Levin R, Heresco-Levy U, Bachner-Melman R, Israel S, Shalev I, Ebstein RP. Association between arginine vasopressin 1a receptor (AVPR1a) promoter region polymorphisms and prepulse inhibition. Psychoneuroendocrinology 2009, 34: 901–908.
84. Liu JJ, Lou F, Lavebratt C, Forsell Y. Impact of childhood adversity and vasopressin receptor 1a variation on social interaction in adulthood: a cross-sectional study. PLoS One 2015, 10: e0136436.
85. Walum H, Westberg L, Henningsson S, Neiderhiser JM, Reiss D, Igl W, *et al*. Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. Proc Natl Acad Sci U S A 2008, 105: 14153–14156.
86. Krueger F, Parasuraman R, Iyengar V, Thornburg M, Weel J, Lin M, *et al*. Oxytocin receptor genetic variation promotes human trust behavior. Front Hum Neurosci 2012, 6: 4.
87. Procyshyn TL, Hurd PL, Crespi BJ. Association testing of vasopressin receptor 1a microsatellite polymorphisms in nonclinical autism spectrum phenotypes. Autism Res 2016. doi:10.1002/aur.1716.
88. Neumann ID, Slattery DA. Oxytocin in General Anxiety and Social Fear: A Translational Approach. Biol Psychiatry 2016, 79: 213–221.
89. Anagnostou E, Soorya L, Brian J, Dupuis A, Mankad D, Smile S, *et al*. Intranasal oxytocin in the treatment of autism spectrum disorders: a review of literature and early safety and efficacy data in youth. Brain Res 2014, 1580: 188–198.
90. Guastella AJ, Hickie IB. Oxytocin treatment, circuitry and autism: a critical review of the literature placing oxytocin into the autism context. Biol Psychiatry 2016, 79: 234–242.
91. Gumley A, Braehler C, Macbeth A. A meta-analysis and theoretical critique of oxytocin and psychosis: prospects for attachment and compassion in promoting recovery. Br J Clin Psychol 2014, 53: 42–61.
92. Bartz JA, Hollander E. Oxytocin and experimental therapeutics in autism spectrum disorders. Prog Brain Res 2008, 170: 451–462.
93. Macdonald K, Macdonald TM. The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans. Harv Rev Psychiatry 2010, 18: 1–21.
94. Andari E, Duhamel JR, Zalla T, Herbrecht E, Leboyer M, Sirigu A. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. Proc Natl Acad Sci U S A 2010, 107: 4389–4394.
95. Yatawara CJ, Einfeld SL, Hickie IB, Davenport TA, Guastella AJ. The effect of oxytocin nasal spray on social interaction deficits observed in young children with autism: a randomized clinical crossover trial. Mol Psychiatry 2016, 21: 1225–1231.
96. Kosaka H, Munesue T, Ishitobi M, Asano M, Omori M, Sato M, *et al*. Long-term oxytocin administration improves social behaviors in a girl with autistic disorder. BMC Psychiatry 2012, 12: 110.
97. Watanabe T, Kuroda M, Kuwabara H, Aoki Y, Iwashiro N, Tatsunobu N, *et al*. Clinical and neural effects of six-week administration of oxytocin on core symptoms of autism. Brain 2015, 138: 3400–3412.
98. Gordon I, Jack A, Pretzsch CM, Vander Wyk B, Leckman JF, Feldman R, *et al*. Intranasal oxytocin enhances connectivity in the neural circuitry supporting social motivation and social perception in children with autism. Sci Rep 2016, 6: 35054.
99. Dadds MR, MacDonald E, Cauchi A, Williams K, Levy F, Brennan J. Nasal oxytocin for social deficits in childhood autism: a randomized controlled trial. J Autism Dev Disord 2014, 44: 521–531.
100. Guastella AJ, Gray KM, Rinehart NJ, Alvares GA, Tonge BJ, Hickie IB, *et al*. The effects of a course of intranasal oxytocin on social behaviors in youth diagnosed with autism spectrum disorders: a randomized controlled trial. J Child Psychol Psychiatry 2015, 56: 444–452.
101. Kosaka H, Okamoto Y, Munesue T, Yamasue H, Inohara K, Fujioka T, *et al*. Oxytocin efficacy is modulated by dosage and oxytocin receptor genotype in young adults with high-functioning autism: a 24-week randomized clinical trial. Transl Psychiatry 2016, 6: e872.
102. Okamoto Y, Ishitobi M, Wada Y, Kosaka H. The potential of nasal oxytocin administration for remediation of autism spectrum disorders. CNS Neurol Disord Drug Targets 2016, 15: 564–577.
103. Wang H, Duclot F, Liu Y, Wang Z, Kabbaj M. Histone deacetylase inhibitors facilitate partner preference formation in female prairie voles. Nat Neurosci 2013, 16: 919–924.
104. Harony-Nicolas H, Mamrut S, Brodsky L, Shahar-Gold H, Barki-Harrington L, Wagner S. Brain region-specific methylation in the promoter of the murine oxytocin receptor gene is involved in its expression regulation. Psychoneuroendocrinology 2014, 39: 121–131.
105. Bakermans-Kranenburg MJ, van Ijzendoorn MH. A sociability gene? Meta-analysis of oxytocin receptor genotype effects in humans. Psychiatr Genet 2014, 24: 45–51.

Neurosci. Bull. April, 2017, 33(2):247–250
DOI 10.1007/s12264-017-0115-4

www.neurosci.cn
www.springer.com/12264

REVIEW

Non-human Primate Models for Brain Disorders – Towards Genetic Manipulations *via* Innovative Technology

Zilong Qiu[1] · **Xiao Li**[1]

Received: 25 January 2017 / Accepted: 17 February 2017 / Published online: 1 March 2017

Abstract Modeling brain disorders has always been one of the key tasks in neurobiological studies. A wide range of organisms including worms, fruit flies, zebrafish, and rodents have been used for modeling brain disorders. However, whether complicated neurological and psychiatric symptoms can be faithfully mimicked in animals is still debatable. In this review, we discuss key findings using non-human primates to address the neural mechanisms underlying stress and anxiety behaviors, as well as technical advances for establishing genetically-engineered non-human primate models of autism spectrum disorders and other disorders. Considering the close evolutionary connections and similarity of brain structures between non-human primates and humans, together with the rapid progress in genome-editing technology, non-human primates will be indispensable for pathophysiological studies and exploring potential therapeutic methods for treating brain disorders.

Keywords Non-human primates · Brain disorders · Genome editing · Autism · Neurological disorders · Psychiatric disorders

Introduction

Psychiatric disorders are a group of mental illnesses without detectable pathological symptoms such as neuronal death. They include schizophrenia, depression, bipolar disorder, and autism spectrum disorders (ASDs). The onset of most psychiatric disorders is normally in adulthood. However, the onset of ASDs is during the first 3 years of life. Therefore, ASDs are also considered to be developmental disorders, possibly caused by disruption of neural development leading to abnormal social behaviors and other symptoms [1].

With recent advances in human genetics and neurobiological studies, ASDs have been found to include both genetic and environmental components. Particularly, mechanistic studies in animal models have provided tremendous insights into how genetic and environmental factors contribute to neural development and underlie the proper functioning of cognitive and social behaviors. A wide range of animal models including worms, fruit flies, zebrafish, and rodents have been used to study neural development and disorders. However, whether complicated neurological and psychiatric symptoms can be faithfully mimicked in animals other than primates is still debatable. In this review, we discuss technical advances for studying the neural mechanisms underlying ASDs and other brain disorders using non-human primates as animal models. Considering the close evolutionary connections and similarity of brain structures in non-human primates and humans, together with the rapid progress of genome-editing technology, non-human primates will be indispensable in pathophysiological studies and potential interventions for psychiatric disorders [2].

Pharmacological and Environmental Approaches

The general approaches used in rodents for modeling psychiatric disorders, such as chronic restraint stress, chronic unpredictable mild stress, and chronic social defeat stress, are efficient for inducing anxiety-like behaviors for

✉ Zilong Qiu
zqiu@ion.ac.cn

[1] Institute of Neuroscience, Key Laboratory of Primate Neurobiology, State Key Laboratory of Neuroscience, Center for Excellence in Brain Science and Intelligence Technology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

various periods of time. However, for ethical reasons, most of these approaches are not practical in non-human primates. Under these circumstances, researchers have designed mild stress models, in which monkeys are subjected to gentle stresses. Kalin and colleagues have established various stress models in monkeys, followed by assessment of physiological and neurochemical parameters [3]. After being stared by humans, infant monkeys appear to exhibit anxiety-like activity such as aggressive barking. Infant monkeys make distress calls when separated from their mothers. Investigators then found, using pharmacological approaches, that the opiate and GABA systems play different roles in regulating these behaviors, suggesting roles of these neuromodulators in emotional behaviors in primates [3].

With regard to the close relationships between monkeys and humans, whether complex environmental factors can be applied to monkeys is a critical question to address. Hu and colleagues have designed a paradigm in which maternal separation paradigms are mimicked in monkeys [4]. They investigated the impact of maternal separation in early life on social behaviors during adolescence, as well as levels of stress hormones. Importantly, they found that maternal separation during early life dramatically influences social behaviors and stress levels, consistent with previous studies in humans. Further mechanistic studies using these monkeys may elucidate the underlying circuit basis of how early life stress affects behaviors during adulthood. The classical methods of studying the neural mechanisms underlying psychiatric disorders have been nicely summarized by Suomi [5] and Watson and Platt [6].

Genetic Approaches Based on Viral Transduction

Over the years, genetic approaches have been widely applied in model organisms, such as worms, fruit flies, and rodents, to establish models of brain disorders. Due to the difficulty of handling germline cells and the unavailability of embryonic stem cells, genetic engineering in monkeys did not become available until 2001 [7]. In this seminal work, Chan and colleagues infected rhesus monkey (*Macaca mulatta*) oocytes with a retrovirus harboring the green fluorescence protein (GFP) gene, followed by *in vitro* fertilization (IVF) with sperm. After transplantation of fertilized eggs into surrogate monkeys, researchers successfully acquired transgenic monkeys carrying the GFP gene. This pioneering work launched a new era of genetic engineering in non-human primates.

Taking advantage of the high efficiency of lentiviral infection, Li, Chan, and colleagues at Emory University used lentivirus harboring the disease-causing Huntingtin (HTT) mutant gene to infect rhesus monkey oocytes [8]. After IVF, investigators successfully obtained several transgenic monkeys carrying the HTT mutant gene. Remarkably, the monkeys carrying the mutant gene showed clear signs of neuronal death in the brain, which is barely detectable in rodent models of Huntington disease. Thus, for the first time, a non-human primate model of a human brain disorder exhibited the unique advantage of mimicking the neuropathological symptoms, which are difficult to find in rodent models.

Another pioneering work in genetic engineering for non-human primates was done in marmosets (*Callithrix jacchus*). Sasaki and colleagues also used lentiviral-based methods to produce transgenic marmosets carrying GFP transgenes [9]. Importantly, they obtained a second generation carrying the transgene, taking advantage of the shorter reproduction in marmosets than macaques, and indicating the reliability of transgenic monkeys as animal models.

Given the availability of lentiviral transduction methods, a series of transgenic monkeys have been produced during the last several years to model various brain disorders. In order to make monkey models of Parkinson disease, Li and colleagues produced transgenic monkeys expressing the mutant form of α-synuclein (A53T) [10]. Although the α-synuclein (A53T) transgenic monkeys have not yet shown Parkinsonism-like symptoms, such as tremor and degeneration of dopaminergic neurons, various cognitive defects and neuropathology in the brain have been identified, suggesting that α-synuclein (A53T) causes pathological changes in the monkey brain [11].

Due to the long reproductive cycle of macaques (typically reaching sexual maturation at 4–5 years of age), obtaining a second generation is time-consuming and costly. In recent work on making monkey models of ASDs, Qiu and colleagues used lentivirus-based transgenic methods to produce transgenic monkeys with the autism gene *MECP2* and examined social and motor behaviors associated with social interaction and cognition. They found that *MECP2* transgenic monkeys indeed exhibit abnormal behaviors, including repetitive locomotor activity, elevated anxiety levels, and defects in social interactions, which nicely mimic ASDs in human patients [12]. Remarkably, investigators applied xenograft methods to transplant testicular tissues from one sexually immature transgenic monkey into nude mice. After 9 months, the testicular tissues became mature and the mature sperm were collected for IVF. Second-generation transgenic monkeys were obtained ~3.5 years after the birth of the first-generation transgenic monkeys, dramatically shortening the time for acquiring a second generation [12]. This work provides a framework in which monkey models of brain disorders can be established.

Genetic Approaches Based on Genome-Editing Methods

With the emergence of the CRISPR-Cas9 and TALEN genome-editing methods from 2011, genetic engineering in non-human primates became feasible and relatively easy. The landmark work was done by Niu and colleagues, who injected Cas9 mRNA along with single-guide RNA (sgRNA) into the fertilized eggs of cynomolgus monkeys (*Macaca fascicularis*) and transplanted them into surrogate monkeys [13]. Among the newborn monkeys, researchers identified genomic mutations in the sgRNA-targeting sites and the cellular effects of genetic deletions. However, due to the prolonged effective period of CRISPR/Cas9, the mutant monkeys are normally mosaics with various mutations, which also differ across tissues. The mosaicism in the offspring monkeys interferes with the interpretation of the consequences of genetic deletions caused by CRISPR/Cas9.

In another study, Chen and colleagues used CRISPR/Cas9 to target the dystrophin gene, mutation of which leads to Duchenne muscular dystrophy (DMD) [14]. Although the genetic mutation was clearly identified in sgRNA-targeted sites of the monkey DMD gene, whether the DMD mutation leads to behavioral abnormalities in monkeys is yet to be determined.

To model Rett syndrome, a developmental disorder, investigators used TALEN-based genome-editing to target the *MECP2* (methyl-CpG binding protein 2) gene in cynomolgus monkeys [15, 16], and reported that TALEN is also effective in inducing genomic mutations in the monkey genome and yield offspring carrying mutations in the *MECP2* gene. However, whether genomic mutation of *MECP2* leads to behavioral changes similar to human Rett syndrome remains to be determined [15].

Connectomic View of the Psychiatric Brain

The rapid progress of genetic engineering in non-human primates provides researchers with a wide platform to study primate brains at an unimaginable scale. It is likely that brain imaging and cognitive studies using genetically-engineered monkeys will be available soon, and will provide causal links between genes and brain structure in the non-human primate. An elegant example is the work by Wang and colleagues, who investigated the effects of ketamine on the functional brain network using magnetic resonance imaging [17].

Although ketamine, a non-competitive N-methyl-D-aspartate receptor antagonist, has rapid and prolonged antidepressant effects in depressed patients after a single dose, the underlying mechanisms by which ketamine affects brain structure in patients are unknown. Given the lack of models of depression in non-human primates, researchers took another approach by giving rhesus monkeys a single dose of ketamine, and examining the functional connectivity of the brain using magnetic resonance imaging. They found that ketamine treatment leads to large-scale downregulation of functional connectivity, which is precisely opposite to the brain network in depressed patients, suggesting that ketamine has antidepressant effects by affecting the global brain network. This work remarkably illustrates the value of cutting-edge technology such as brain imaging in studying the neural mechanisms underlying brain disorders.

Concluding Remarks

We are in an era when multiple disciplines have interacted to elucidate the mechanisms of complicated human brain disorders. Given the complexity of the brain, it has always been difficult to recapitulate the core symptoms of human brain disorders in animal models. With the rapid development of genetic-engineering technology, such as lentiviral transduction and genome-editing, it is foreseen that non-human primates will play a crucial role as animal models in the near future. It is worthwhile noting that current therapeutic methods for human brain disorders such as deep brain stimulation and transcranial magnetic stimulation will also need to be tested in non-human primates prior to human trials. Therefore, non-human primate models of brain disorders are also indispensable for translational studies. Together with the technical advances in brain imaging and non-invasive neural modulation methods such as ultrasonic stimulation, non-human primates will play more competitive roles in modeling brain disorders and serve as a platform on which researchers can seek potential therapies.

Acknowledgements This review was supported by the Chinese Academy of Sciences Strategic Priority Research Program (XDB02050400) and the National Natural Science Foundation of China (91432111).

References

1. Thapar A, Cooper M, Rutter M. Neurodevelopmental disorders. Lancet Psychiatry 2016. doi: 10.1016/S2215-0366(16)30376-5.

2. de la Torre-Ubieta L, Won HJ, Stein JL, Geschwind DH. Advancing the understanding of autism disease mechanisms through genetics. Nat Med 2016, 22: 345–361.
3. Kalin NH, Shelton SE. Defensive behaviors in infant rhesus-monkeys - environmental cues and neurochemical regulation. Science 1989, 243: 1718–1721.
4. Feng X, Wang L, Yang S, Qin D, Wang J, Li C, *et al.* Maternal separation produces lasting changes in cortisol and behavior in rhesus monkeys. Proc Natl Acad Sci USA 2011, 108: 14312–14317.
5. Suomi SJ. Risk, resilience, and gene x environment interactions in rhesus monkeys. Ann NY Acad Sci 2006, 1094: 52–62.
6. Watson KK, Platt ML. Of mice and monkeys: using non-human primate models to bridge mouse- and human-based investigations of autism spectrum disorders. J Neurodev Disord 2012, 4: 21.
7. Chan AWS, Chong KY, Martinovich C, Simerly C, Schatten G. Transgenic monkeys produced by retroviral gene transfer into mature oocytes. Science 2001, 291: 309–312.
8. Yang SH, Cheng PH, Banta H, Piotrowska-Nitsche K, Yang JJ, Cheng ECH, *et al.* Towards a transgenic model of Huntington's disease in a non-human primate. Nature 2008, 453: 921–924.
9. Sasaki E, Suemizu H, Shimada A, Hanazawa K, Oiwa R, Kamioka M, *et al.* Generation of transgenic non-human primates with germline transmission. Nature 2009, 459: 523–527.
10. Niu YY, Guo XY, Chen YC, Wang CE, Gao JQ, Yang WL, *et al.* Early Parkinson's disease symptoms in alpha-synuclein transgenic monkeys. Hum Mol Genet 2015, 24: 2308–2317.
11. Yang WL, Wang GH, Wang CE, Guo XY, Yin P, Gao JQ, *et al.* Mutant alpha-synuclein causes age-dependent neuropathology in monkey brain. J Neurosci 2015, 35: 8345–8358.
12. Liu Z, Li X, Zhang JT, Cai YJ, Cheng TL, Cheng C, *et al.* Autism-like behaviours and germline transmission in transgenic monkeys overexpressing MeCP2. Nature 2016, 530: 98–102.
13. Niu YY, Shen B, Cui YQ, Chen YC, Wang JY, Wang L, *et al.* Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell 2014, 156: 836–843.
14. Chen YC, Zheng YH, Kang Y, Yang WL, Niu YY, Guo XY, *et al.* Functional disruption of the dystrophin gene in rhesus monkey using CRISPR/Cas9. Hum Mol Genet 2015, 24: 3764–3774.
15. Liu HL, Chen YC, Niu YY, Zhang KS, Kang Y, Ge WH, *et al.* TALEN-mediated gene mutagenesis in rhesus and cynomolgus monkeys. Cell Stem Cell 2014, 14: 323–328.
16. Liu Z, Zhou X, Zhu Y, Chen ZF, Yu B, Wang Y, *et al.* Generation of a monkey with MECP2 mutations by TALEN-based gene targeting. Neurosci Bull 2014, 30: 381–386.
17. Lv Q, Yang LQ, Li GL, Wang ZW, Shen ZM, Yu WW, *et al.* Large-scale persistent network reconfiguration induced by ketamine in anesthetized monkeys: relevance to mood disorders. Biol Psychiatry 2016, 79: 765–775.

Neurosci. Bull. April, 2017, 33(2):251–254
DOI 10.1007/s12264-017-0119-0

www.neurosci.cn
www.springer.com/12264

LETTER TO THE EDITOR

Identification of the Genetic Cause for Childhood Disintegrative Disorder by Whole-Exome Sequencing

Zhu Wen[1] · Tian-Lin Cheng[2] · Da-Zhi Yin[2] · Shi-Bang Sun[1] · Zheng Wang[2] · Shun-Ying Yu[1] · Yi Zhang[3,4] · Zilong Qiu[2] · Ya-Song Du[1]

Received: 26 January 2017 / Accepted: 20 February 2017 / Published online: 10 March 2017

Dear Editor,

Childhood Disintegrative Disorder (CDD), also known as Heller's syndrome and disintegrative psychosis, is a rare progressive neurological disorder, characterized by a late onset (>2 years of age) and regression of language, social function, and motor skills [1]. Based on 4 surveys of CDD from different countries, the pooled estimate for the prevalence of CDD is 0.17/10,000, 600 times less prevalent than autism [2].

CDD is characterized by an onset between 3 and 4 years of age after a period of apparently normal development; a severe regression with the progressive loss or marked impairment of spoken language, loss of play, loss of social skills, and loss of bowel and bladder control; a prodromal period of behavioral disruption with extreme agitation, fearfulness, and possible hallucinations, and a poor outcome with severe intellectual deterioration.

CDD was first described by Thomas Heller in 1908. However, the definition has continued evolving over the past century, perhaps because of the rarity and lack of explanation for the condition. Initially, CDD was considered strictly a medical disorder and was believed to have identifiable medical causes. However, no specific medical or neurological cause has been found to account for all occurrences of the disorder by investigators who reviewed the reported cases [3]. Should CDD be considered a distinct diagnosis? Under the proposed DSM-5 revisions, all pervasive developmental disorders, including CDD, will be subsumed under the single diagnostic category of autism spectrum disorders. The rationale for this is the similarity between these disorders, as it is now thought that their symptoms place them on a continuum with autism. Thus, CDD is also considered a low-functioning form of autistic spectrum disorder [3].

Interestingly, mucopolysaccharidosis III (MPS III) is a rare genetic disease characterized by progressive cognitive decline and severe hyperactivity, with an onset between 2 and 6 years of age. Moreover, patients are often initially misdiagnosed as autism spectrum disorders, idiopathic developmental delay, attention deficit/hyperactivity disorder, or combinations of these, placing them at risk for unnecessary testing and treatments [4, 5]. Clinically, MPS III is composed of four different subtypes, each of which is caused by a deficiency in a different enzyme in the catabolic pathway of heparan sulfate, a type of glycosaminoglycan (GAG). All four subtypes are inherited in an autosomal recessive pattern. Collectively, the reported incidence of MPS III varies between 0.28 and 4.1 per 100,000 live births [6].

Electronic supplementary material The online version of this article (doi:10.1007/s12264-017-0119-0) contains supplementary material, which is available to authorized users.

✉ Yi Zhang
zy@eulertechnology.com

✉ Zilong Qiu
zqiu@ion.ac.cn

✉ Ya-Song Du
yasongdu@163.com

[1] Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China

[2] Institute of Neuroscience, Center for Excellence in Brain Science and Intelligence Technology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

[3] School of Life Sciences, Peking University, Beijing 100871, China

[4] Euler Genomics, Beijing 102206, China

Here we report the case of a sibling presenting with CDD. First, two brothers were seen by a pediatric psychiatrist at the Outpatient Clinic of the Department of Child and Adolescent Psychiatry at the Shanghai Mental Health Center. They had both gradually lost language and life skills after the age of 4 years. According to their parents, the elder brother (K) is 10 years old, the younger brother (Z) is 8 years old, and both were born in Henan Province, China. They showed almost normal pervasive development before they were 4 years old. K was able to pronounce his first words ("mama", "papa") at 10 months and could walk at 12 months. He was able to use idiomatic phrases to communication when he was 2 years old. Z started to speak in single words ("mama", "papa") at 7 months, walk at 10 months, and use idiomatic phrases after 1 year. However, after reaching 4 years of age they both quickly regressed in terms of language, cognition, and social skills and behavior. K became incontinent and nonverbal after 5 years and started to communicate by screaming and crying without appropriate eye contact or facial expressions. He lost all ability to communicate at the age of 6. The general development of the younger brother, Z, was almost the same as his brother's. After Z was 4 years old, he experienced social and learning problems. He ceased to show initiative in communications and merely responded to simple questions without making eye contact. Z became incontinent and showed intellectual deficiency after 5 years old. At the age of 10, when visiting a child psychiatrist for the second time, Z was only able to phonate single words, fuzzily.

The medical history of Z included intermittent convulsions, difficulty sleeping involving waking up and sitting on the bed 4–10 times per night, frequent ear infections, and diarrhea. K showed facial dysmorphias and gastrointestinal symptoms. On physical examination, the patients were conscious but apathetic and not in acute distress. The neurological examination was unremarkable. Their lungs were clear. K's heart rate was 84 beats per minute, and Z's was 80.

Clinical scales, including the Autism Diagnostic Interview-Revised (ADI-R), the Child Autism Rating Scale (CARS), and the Autism Behavior Checklist (ABC), were used to assess the general condition of each brother. In the ADI-R, the present scores of K and Z in the domain of reciprocal social interaction were 35 and 34, respectively (cutoff = 10). At the age of 4, the scores of reciprocal social interaction were 9 (K) and 2 (Z). The present scores of communication (verbal total) were 19 (K) and 25 (Z) (cutoff = 8), but they were 11 (K) and 1 (Z) at the age of 4. In the area of repetitive behaviors and stereotyped patterns, their scores were 0 (K) and 10 (Z) as of this work, and 5 (K) and 1 (Z) at the age of 4 (cutoff = 3) (Table S1). K's CARS score was 60 (full marks) as of this work and 57 as of 2 years ago. Z's CARS scores were 59 as of this work and 40 as of 2 years ago, indicating severe autistic symptoms of K and Z (Table S2). The general scores of K and Z in the ABC were >67, indicating that the deterioration had continued quickly over the past 2 years (Table S3).

The particular family history and disease burden (Fig. 1A) strongly suggested an inherited disease with recessive, *de novo* X-linked or germline mutations. Therefore, whole-exome sequencing was performed on peripheral blood samples from the four-member family. One mutation, Chr17:78188885 A>G, SGSH (N-sulphoglucosamine sulphohydrolase) F101S, was found to fit the homozygous-recessive genetic model. The mutation was unique to the family. It was not found in any public database or in any of the in-house Chinese controls (12500). Both parents were heterozygous SGSH F101S carriers, and both offspring were homozygous SGSH F101S mutants (Fig. 1B). The mutation, located in the dimerization surface of the SGSH protein, may negate enzymatic function (Fig. 1C).

To further identify abnormalities in the brain structure of the patients carrying *SGSH* mutations, magnetic resonance imaging (MRI) was performed on the elder brother, K, with T2WI and DTI. K's gray matter had become thinner, with a larger sulcus in the bilateral frontal lobes and temporal lobes than in the occipital lobe. K's supratentorial ventricles showed remarkable dilatation (Fig. 1D, E). An FA map based on DTI showed that the white matter fibers in K's brain appeared sparse and degenerated, especially in the corpus callosum (Fig. 1F, G), while the healthy parents appeared normal under brain imaging examination (Fig. S1, S2).

Biochemical and metabolic assessments of MPS IIIA were performed to determine whether these brothers with homozygous *SGSH* mutations had any defects in glycol-metabolism. First, urine samples from both brothers underwent a color change by reaction with a cationic dye on treated filter paper, and excess GAG was noted in the brothers' urine samples. In analytical electrophoresis tests, K's sample showed bands of GAG excretion at dermatan sulfate and heparan sulfate (HS), and Z's showed bands at chondroitin sulfate and HS areas. The results of enzyme activity assays showed that the activity of N-sulfoglucosamine sulfohydrolase had downgraded to 7.7 nmol/g/h (normal cutoff ≥119.6 nmol/g/h), but other enzymes were normal.

SGSH encodes N-sulfoglucosamine sulfohydrolase (MIM: 605270), and mutations hamper the degradation of heparan sulfate, which causes MPS IIIA, a rare autosomal-recessive inherited metabolic disease. The symptoms of MPS IIIA are caused by enzyme deficiency, preventing a necessary metabolic step in the degradation of heparan sulfate. In patients carrying loss-of-function mutations of

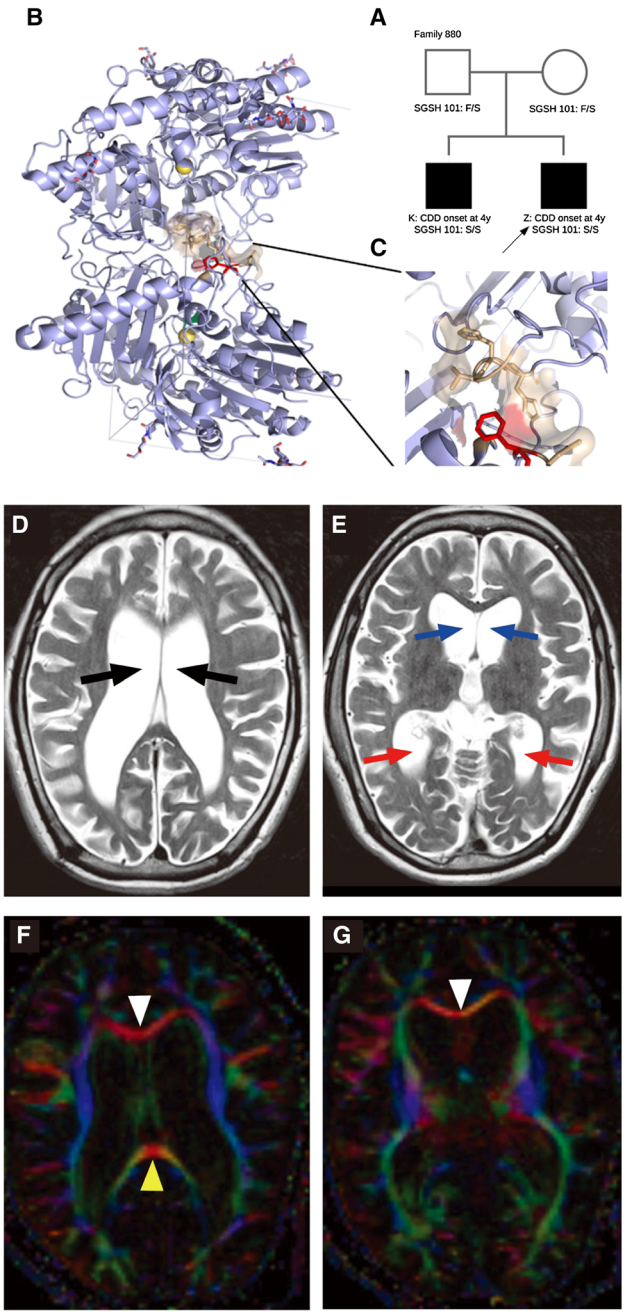


Fig. 1 Genetic genogram, structure of protein SGSH, and images of the brain of patient K. **A** Genetic genogram. **B, C** Protein structure and Sanger sequencing for mutation sites in the SGSH gene. The F101 residue is shown in *red*, interacting residues from the other part of the enzyme dimer are shown in *yellow*. **D** Cerebral atrophy and ventriculomegaly (*arrows*) are shown on T2WI. **E** *Blue* and *red arrows* indicate anterior and posterior horns of the lateral ventricle. **F, G** Fractional anisotropy map showing a thin corpus callosum genu (*white triangle*) and splenium (*yellow triangle*).

SGSH, undegraded heparan sulfate accumulates in the central nervous system, where heparan sulfate in lysosomes leads to severe neurodegeneration [5, 7].

The *SGSH* mutation c.302T>C (p.F101S) has not been previously entered into the Human Gene Mutation Database (HGMD Professional 2015.12) (http://www.hgmd.cf.ac.uk/ac/index.php), which contains 118 mutations (including 91 missense/nonsense mutations) from MPS IIIA patients. Thus, the SGSH F101S mutation is considered to be a new recessive allele for MPS IIIA and CDD.

Due to its rarity, there have been few neuroimaging studies of CDD cases. The MRI data of a girl (8 years old) affected with MPS IIIA showed a thin corpus callosum in the posterior area [8]. In our patient K, the corpus callosum was thinner than usual and showed severe degeneration. In addition, the girl showed hyperintensity in the sub-cortical area and hippocampus on T2WI images and the imaging was not clear [8]. However in patients with MPS, abnormal brain structures are frequently observed under MRI and CT: Virchow-Robin perivascular spaces, white matter abnormalities, and ventriculomegaly [9].

From the viewpoint of symptomatology, the clinical features of patients K and Z corresponded to the diagnostic standards of CDD. Surprisingly, the child psychiatrist did not realize that they were affected by MPS IIIA before the results of whole-exome sequencing and biochemical and metabolic assessments became available. One study reported that when 21 children with *SGSH* gene mutations and enzyme deficiency were assessed using the Autism Diagnostic Observation Schedule, 13 met the criteria for autism. Among them, social and emotional abnormalities were most frequent, but repetitive behaviors and restricted interests were largely absent, which is consistent with the ADI-R results of the sibling [4]. Children affected by MPS IIIA may show autistic behaviors which lead to a misdiagnosis of ASD by psychiatrists or pediatricians [6, 10].

Although the pathogenetic mechanism of CDD is still not clear, a few reports have suggested a link between MPS IIIA and CDD/ASD [4]. According to previous reports, MPS III has an incidence of 1% among autistic patients, but the true rate might be lower [11]. A recent study screened 778 Turkish patients with ASD for inherited metabolic abnormalities by examination of urinary GAG and homocysteine levels. In that work, 300 of the patients whose metabolic and physical examinations met the study's criteria were enrolled, and among them, one patient was diagnosed with MPS III [12].

In summary, this work describes a newly-discovered disease-causing mutation, F101S, in the *SGSH* gene, mutations of which impair the degradation of heparan sulfate and classically cause MPS IIIA. This case provides a possible genetic explanation for the specific symptoms of patients with CDD. It is worth noting that pediatric psychiatrists should be aware of potential metabolic diseases, particularly when a patient has a strongly-inherited

tendency or specific regressive pattern. Genetic screening and biochemical tests are very effective to clarify the diagnosis.

Acknowledgements This work was supported by the Chinese Academy of Sciences Strategic Priority Research Program, China (XDB02050400), the National Natural Science Foundation of China (91432111), and the Shanghai Second Medical University—Institute of Neuroscience Research Center for Brain Disorders, China (2015NKX005). We gratefully acknowledge the participation of the brothers with CDD and their parents, without whom this study could not have been done. We also acknowledge members of the laboratories of Drs. Qiu Z, Du Y, and Wang Z for valuable discussions.

References

1. Homan KJ, Mellon MW, Houlihan D, Katusic MZ. Brief report: childhood disintegrative disorder: a brief examination of eight case studies. J Autism Dev Disord 2011, 41: 497–504.
2. Fombonne E. Prevalence of childhood disintegrative disorder. Autism 2002, 6:149–157.
3. Hendry CN. Childhood disintegrative disorder: should it be considered a distinct diagnosis? Clin Psychol Rev 2000, 20: 77–90.
4. Rumsey RK, Rudser K, Delaney K, Potegal M, Whitley CB, Shapiro E. Acquired autistic behaviors in children with mucopolysaccharidosis type IIIA. J Pediatr 2014, 164: 1147–1151.e1.
5. Wijburg FA, Węgrzyn G, Burton BK, Tylki-Szymańska A. Mucopolysaccharidosis type III (Sanfilippo syndrome) and misdiagnosis of idiopathic developmental delay, attention deficit/hyperactivity disorder or autism spectrum disorder. Acta Paediatr Int J Paediatr 2013, 102: 462–470.
6. Valstar MJ, Ruijter GJG, van Diggelen OP, Poorthuis BJ, Wijburg FA. Sanfilippo syndrome: A mini-review. J Inherit Metab Dis 2008, 31: 240–252.
7. Fedele AO. Sanfilippo syndrome: Causes, consequences, and treatments. Appl Clin Genet 2015, 8: 269–281.
8. Philippe A, Craus Y, Rio M, Bahi-Buisson N, Boddaert N, Malan V, *et al.* Case report: an unexpected link between partial deletion of the SHANK3 gene and Heller's dementia infantilis, a rare subtype of autism spectrum disorder. BMC Psychiatry 2015, 15: 256.
9. Calleja Gero ML, González Gutiérrez-Solana L, López Marín L, López Pino MA, Fournier Del Castillo C, Duat Rodríguez A. Neuroimaging findings in patient series with mucopolysaccharidosis. Neurologia 2012, 27: 407–413.
10. de Bildt A, Sytema S, Zander E, Bölte S, Sturm H, Yirmiya N, *et al.* Autism diagnostic interview-revised (ADI-R) algorithms for toddlers and young preschoolers: application in a non-US sample of 1,104 children. J Autism Dev Disord 2015, 45: 2076–2091.
11. Ritvo ER, Mason-Brothers A, Freeman BJ, Pingree C, Jenson WR, McMahon WM, *et al.* The UCLA-university of utah epidemiologic survey of autism: the etiologic role of rare diseases. Am J Psychiatry 1990, 147: 1614–1621.
12. Kiykim E, Zeybek CA, Zubarioglu T, Cansever S, Yalcinkaya C, Soyucen E, *et al.* Inherited metabolic disorders in Turkish patients with autism spectrum disorders. Autism Res 2016, 9: 217–223.

Neuroscience Bulletin

Neuroscience Bulletin

Copyright Information

For Authors

As soon as an article is accepted for publication, authors will be requested to assign copyright of the article (or to grant exclusive publication and dissemination rights) to the publisher (respective the owner if other than Springer). This will ensure the widest possible protection and dissemination of information under copyright laws.

More information about copyright regulations for this journal is available at www.springer.com/12264

For Readers

While the advice and information in this journal is believed to be true and accurate at the date of its publication, neither the authors, the editors, nor the publisher can accept any legal responsibility for any errors or omissions that may have been made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

All articles published in this journal are protected by copyright, which covers the exclusive rights to reproduce and distribute the article (e.g., as offprints), as well as all translation rights. No material published in this journal may be reproduced photographically or stored on microfilm, in electronic data bases, on video disks, etc., without first obtaining written permission from the publisher (respective the copyright owner if other than Springer). The use of general descriptive names, trade names, trademarks, etc., in this publication, even if not specifically identified, does not imply that these names are not protected by the relevant laws and regulations.

Springer has partnered with Copyright Clearance Center's RightsLink service to offer a variety of options for reusing Springer content. For permission to reuse our content please locate the material that you wish to use on link.springer.com or on springerimages.com and click on the permissions link or go to copyright.com and enter the title of the publication that you wish to use. For assistance in placing a permission request, Copyright Clearance Center can be contacted directly via phone: +1-855-239-3415, fax: +1-978-646-8600, or e-mail: info@copyright.com

Journal Website

www.springer.com/12264
Electronic edition: link.springer.com/journal/12264

Subscription Information

Volume 33 (6 issues) will be published in 2017.

ISSN: 1673-7067 print
ISSN: 1995-8218 electronic

For information on subscription rates please contact Springer Customer Service Center: customerservice@springer.com

The Americas (North, South, Central America and the Caribbean)
Springer Journal Fulfillment, 233 Spring Street, New York, NY 10013-1578, USA,
Tel.: 800-SPRINGER (777-4643); 212-460-1500 (outside North America)

Outside the Americas
Springer Customer Service Center GmbH, Tiergartenstr. 15, 69121 Heidelberg, Germany
Tel.: +49-6221-345-4303

Advertisements

E-mail contact: advertising@springer.com or anzeigen@springer.com (Germany)

Disclaimer

Springer publishes advertisements in this journal in reliance upon the responsibility of the advertiser to comply with all legal requirements relating to the marketing and sale of products or services advertised. Springer and the editors are not responsible for claims made in the advertisements published in the journal. The appearance of advertisements in Springer publications does not constitute endorsement, implied or intended, of the product advertised or the claims made for it by the advertiser.

Office of Publication

Springer Science+Business Media B.V., Van Godewijckstraat 30, 3311 GX Dordrecht, The Netherlands