

Variants in the promoter region of *CYP7A1* are associated with neuromyelitis optica but not with multiple sclerosis in the Han Chinese population

Gui-Xian Zhao^{1,5,#}, Ying Liu^{1,2,#}, Zhen-Xin Li¹, Chuan-Zhen Lv¹, Anthony Traboulsee³, A. Dossa Sadovnick^{3,4}, Zhi-Ying Wu¹

¹Department of Neurology and Institute of Neurology, Huashan Hospital, Institute of Brain Science and State Key Laboratory of Medical Neurobiology, Shanghai Medical College, Fudan University, Shanghai 200040, China

²Department of Neurology and Institute of Neurology, First Affiliated Hospital, Fujian Medical University, Fuzhou 350005, China

³Faculty of Medicine, Division of Neurology, University of British Columbia, Vancouver, Canada

⁴Department of Medical Genetics, University of British Columbia, Vancouver, Canada

⁵Shanghai Key Laboratory of Signaling and Disease Research, Shanghai 200040, China

#These authors contributed equally to this work.

Corresponding author: Zhi-Ying Wu. E-mail: zhiyingwu@fudan.edu.cn

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ABSTRACT

Multiple sclerosis (MS) and neuromyelitis optica (NMO) are common autoimmune demyelinating disorders of the central nervous system. The exact etiology of each remains unclear. *CYP7A1* was reported to be associated with NMO in Korean patients, but this is yet to be confirmed in other populations. In this study, we used Sanger sequencing to detect SNPs in the promoter region of *CYP7A1* in a population consisting of unrelated patients and controls from the Han Chinese population (129 MS; 89 NMO; 325 controls). Two known SNPs, –204A>C (rs3808607) and –469T>C (rs3824260), and a novel SNP (–208G>C) were identified in the 5'-UTR of *CYP7A1*. The –204A>C was in complete linkage with –469T>C and both were associated with NMO but not with MS. Results suggest that the *CYP7A1* allele was associated with NMO. NMO and MS have different genetic risk factors. This further supports the emerging evidence that MS and NMO are distinct disorders.

Keywords: multiple sclerosis; neuromyelitis optica;

CYP7A1; association; Chinese

INTRODUCTION

Multiple sclerosis (MS) is the most common autoimmune demyelinating disorder of the central nervous system (CNS). The exact etiology remains unclear although genes, the environment and interactions thereof play significant roles^[1,2]. The diagnosis of MS depends on clinical and paraclinical features including magnetic resonance imaging (MRI)^[3]. No specific diagnostic biomarker has been identified to date^[3]. Similarly, neuromyelitis optica (NMO) is an inflammatory demyelinating disease of the CNS previously distinguished from MS by having severe attacks of optic neuritis and transverse myelitis with longitudinally extensive lesions on spinal MRI but with a brain MRI often characterized by very few lesions^[4,5]. Morbidity for NMO greatly exceeds that for MS^[6]. Despite advances in diagnostic criteria^[3,7], MS and NMO have overlapping clinical manifestations, such as age of onset, female predilection, optic neuritis, and spinal syndrome. Thus, in the clinical setting, it can be difficult to differentiate them, especially if the patient is anti-aquaporin-4 (AQP4) antibody

negative. Diagnostic accuracy is critical with respect to therapies that can influence the prognosis^[4,5,8].

MS is relatively frequent in Caucasians and relatively infrequent in Asians. However, NMO is relatively common in Asians but rare in Caucasians. We hypothesized that this difference may result from genetic factors^[9,10]. To date, little is known about the genetic factors that may distinguish NMO from MS. The genetic component of MS susceptibility is complex and has been studied for several decades. Although several genes have been implicated, the greatest impact is from the human leukocyte antigen (HLA) DRB1*1501 (6p21.1-21.3) genotype^[11]. However, the genetics of NMO susceptibility remain largely unknown. Recently, the interleukin 17 (IL-17) and HLA-DPB1*0501 allele were reported to be associated with Asian NMO patients positive for the NMO-IgG antibody^[12-17], and there was a suggestion that the emergence of anti-AQP4 antibody is reinforced by the presence of the HLA-DPB*0501 and IL-17 allele^[13,15]. In addition, *CYP7A1* was reported to be associated with Korean NMO patients^[18].

Cholesterol 7 α -hydroxylase, which is encoded by *CYP7A1*, is a rate-limiting enzyme for cholesterol catabolism and bile-acid synthesis involved in cholesterol homeostasis^[19-22]. The human *CYP7A1* gene, consisting of 6 exons, is located on chromosome 8 (8q11-q12)^[23]. Here, we assessed the association between the SNPs in the promoter region of *CYP7A1* and Han Chinese patients diagnosed with either MS or NMO compared to controls.

METHODS

Subjects

The study protocol was approved by the Ethics Committee of Huashan Hospital, a general-service hospital in Shanghai. Informed consent was obtained from the participants or their legal surrogates prior to inclusion in the study. Unrelated Han Chinese patients (MS or NMO) and controls (individuals with no history of autoimmune disease) from southern China were recruited from Huashan Hospital. MS patients were diagnosed according to the 2005 McDonald Criteria^[24] and NMO patients were diagnosed according to the revised 2006 Wingerchuk Criteria^[25]. Medical history, demographic information and diagnosis were independently reviewed by two senior neurologists.

Serology included autoimmune antibodies (ANA, ENA, dsDNA, ANCA, and AMA); complement and NMO-IgG were tested. All samples from MS and NMO patients were tested for IgG-Index but this was not done for controls. Genomic DNA was extracted from peripheral EDTA blood with a TIANamp Blood DNA kit (Tiangen Biotech, Beijing) for all patients and controls.

Genotyping of SNPs in the Promoter of *CYP7A1* Using Sanger Sequencing

The primers (forward: 5'-GGATGTTAGGTGAGTAACATG-3' and reverse: 5'-AGCATGCTGCTATAGCAATCC-3') were designed to amplify the 5'-untranslated region (UTR) and intron-exon boundary of exon 1 of *CYP7A1*. The PCR product (662 bp) was generated using a GeneAmp PCR system 9600 (Applied Biosystems, Foster City, CA). Amplified products were purified and direct sequencing of DNA performed using an ABI 3730 Automated DNA Sequencer (Applied Biosystems)^[26]. Sequences were compared with the genomic DNA sequence of *CYP7A1* (NCBI Sequence Viewer NM_000780.3), and nucleotide changes were numbered corresponding to their position in *CYP7A1*.

Statistical Analysis

The Hardy-Weinberg equilibrium of the SNPs rs3808607 and rs3824260 in the promoter region of *CYP7A1* was determined for all participants using the χ^2 test (df = 1). Genotype distributions and allele frequencies in MS and NMO patients were compared to controls, also by the χ^2 test. Unconditional logistic regression was used to calculate the odds ratios (ORs) and 95% confidence intervals (95% CIs) as estimates of relative risk for the single-locus genotypes, with adjustment for age and sex. All statistical analyses were performed using STATA software (version 9.2; College Station, TX). The criterion for a significant difference was $P < 0.05$.

RESULTS

We recruited 129 unrelated MS patients, 89 unrelated NMO patients, and 325 controls who attended either Huashan Hospital or the First Affiliated Hospital from November 1, 2007 to July 31, 2011. The participants' demographic data are shown in Table S1. Anti-AQP4 antibody was tested in

41 NMO patients, and 20 (48.8%) were positive, a lower proportion than has been reported in previous studies. Consistent with previous reports, there were no antibody-positive MS patients^[27].

Two known SNPs [–204A>C (rs3808607) and –469T>C (rs3824260)] and a novel SNP (–208G>C) were identified in the 5'-UTR of *CYP7A1*. Their chromatograms are shown in Figure S1. A heterozygous –208G>C was found in one female NMO patient and one female control and was considered to represent a rare polymorphism. The –204A>C was in complete linkage with –469T>C; the C allele of –204A>C with the T allele of –469 T>C; and the A allele of –204A>C with the C allele of –469T>C, resulting in 3 possible linkages: CC–TT, AA–CC and AC–CT (Fig. S2). Thus, –204A>C was chosen for the subsequent statistical analyses and was under Hardy-Weinberg equilibrium in the MS patients, NMO patients and controls ($P > 0.05$) (Table S2).

The genotype and allele distribution of –204A>C and the corresponding logistic regression analyses are shown in Tables 1 and 2. The frequency of the AA genotype did not differ significantly for MS patients, NMO patients and controls. However, NMO patients had a higher frequency of the AC+CC genotype than controls ($P = 0.031$). No differences were found when comparing NMO and MS patients ($P = 0.057$) and MS patients and controls ($P = 0.919$). NMO patients had a higher frequency of the –204A>C A allele than either controls ($P = 0.034$) or MS patients ($P = 0.039$). No difference was found between MS patients and controls ($P = 0.659$).

DISCUSSION

Similar to MS, NMO is a common autoimmune demyelinating disorder of the CNS. Although genes, the environment and their interactions play significant roles in the pathogenesis of the disease, no clear etiology has been established yet^[10]. As reported previously, the genetic component of MS susceptibility is complex and has been studied for several decades, and NMO is presumably a polygenetic condition, like MS. However, the genetics of NMO susceptibility remain largely unknown. It is important to find a risk gene that may be helpful in revealing the etiology of NMO.

Recently, *CYP7A1* was reported to be associated with Korean NMO patients^[18]. Cholesterol 7 α -hydroxylase, which is encoded by *CYP7A1*, is a member

of the cytochrome P450 superfamily of enzymes and is the first rate-limiting enzyme in the bile-acid synthesis pathway. A previous study identified several cell-specific enhancer elements between –432 and –220 in the promoter region of *CYP7A1*, including the functional binding sites for hepatocyte nuclear factor-3 (HNF-3), HNF-4, and a ubiquitous transcription factor, the activity of which is controlled in part by HNF-3^[28]. Deletion of the segment from –213 to –91 reduces the promoter activity by 40%^[22], and this defect in *CYP7A1* may play a pathogenic role in cholesterol gallstone disease^[29]. The activity of *CYP7A1* may also be affected by –204A>C and –469T>C, by influencing its transcription rate^[22].

In the present study, we found that these two polymorphisms are tightly linked. The CC genotype of –204A>C but not –469T>C confers a higher risk for gallbladder cancer (GBC) in a North Indian population^[30], indicating that these two SNPs are not tightly linked to GBC. Possible explanations include (1) the allele distribution may vary among different ethnic groups, or (2) methodological differences. Restriction fragment length polymorphism was used in the GBC study^[30] and the more sensitive direct DNA sequencing in the present study.

Association between –204A>C and NMO patients was found in the present study, replicating the Korean findings^[18]. These results indicate that MS and NMO have a different genetic basis, which is consistent with the emerging understanding of their distinct clinical, pathogenic and laboratory features. The association between the *CYP7A1* polymorphisms and NMO or MS patients should be further studied in more samples and different ethnic and geographic groups such as Canadian Chinese and Canadian Caucasians. Although there is no direct or identifiable pathogenic role for this gene in NMO, *CYP7A1* may be related to NMO through vitamin D like MS, or its activity may be regulated by gender and hormonal status and then affects NMO through cholesterol metabolism, but there is no evidence for this^[31,32]. Therefore, further exploration of this gene region for other candidates that play an important role in the susceptibility/pathogenesis of NMO is warranted.

SUPPLEMENTAL DATA

Supplemental data include two figures and two tables and can be

Table 1. Genotype and allele distribution of –204A>C (rs3808607)

rs3808607	Control	NMO		MS	
Total	325 (%)	89 (%)	<i>P</i> 1	129 (%)	<i>P</i> 2
AA	98 (30.2)	37 (41.6)	$\chi^2 = 4.593$	38 (29.5)	$\chi^2 = 0.2248$
AC	173 (53.2)	42 (47.2)	<i>P</i> = 0.1006	66 (51.2)	<i>P</i> = 0.6354
CC	54 (16.6)	10 (11.2)		25 (19.4)	
A	369 (56.8)	116 (65.2)	$\chi^2 = 4.063$	142 (55.0)	$\chi^2 = 0.4955$
C	281 (43.2)	62 (34.8)	<i>P</i> = 0.0438	116 (45.0)	<i>P</i> = 0.7805

*P*1: NMO vs Control; *P*2: MS vs Control.

Table 2. Logistic regression analysis of rs3808607 (–204A>C) within the *CYP7A1* promoter

Comparison	Genotypes / Alleles [#]	No. (frequency) Patients	Control	Logistic regression [*] OR (95% CI)	<i>P</i>
All patients	AA	75 (34.4)	98 (30.2)	Reference	
vs controls	AC	108 (49.5)	173 (53.2)	0.80 (0.53–1.19)	0.262
	CC	35 (16.1)	54 (16.6)	0.86 (0.50–1.47)	0.572
	AC+CC	143 (65.6)	227 (69.9)	0.81 (0.55–1.18)	0.227
	A	258 (59.2)	369 (56.8)	Reference	
	C	178 (40.8)	281 (43.2)	0.90 (0.69–1.71)	0.434
NMO vs	AA	37 (41.6)	98 (30.2)	Reference	
controls	AC	42 (47.2)	173 (53.2)	0.59 (0.34–1.02)	0.059
	CC	10 (11.2)	54 (16.6)	0.47 (0.21–1.07)	0.072
	AC+CC	52 (58.4)	227 (69.9)	0.56 (0.33–0.95)	0.031
	A	116 (65.2)	369 (56.8)	Reference	
	C	62 (34.8)	281 (43.2)	0.66 (0.45–0.99)	0.034
MS vs	AA	38 (29.5)	98 (30.2)	Reference	
controls	AC	66 (51.2)	173 (53.2)	0.98 (0.61–1.57)	0.918
	CC	25 (19.4)	54 (16.6)	1.18 (0.64–2.17)	0.599
	AC+CC	91 (70.5)	227 (69.9)	1.02 (0.65–1.61)	0.919
	A	142 (55.0)	369 (56.8)	Reference	
	C	116 (45.0)	281 (43.2)	1.07 (0.79–1.45)	0.659
NMO vs MS	AA	37 (41.6)	38 (29.5)	Reference	
	AC	42 (47.2)	66 (51.2)	1.63 (0.87–3.02)	0.124
	CC	10 (11.2)	25 (19.4)	2.37 (0.97–5.77)	0.058
	AC+CC	52 (58.43)	91 (70.5)	1.77 (0.98–3.20)	0.057
	A	116 (65.2)	142 (55.0)	Reference	
	C	62 (34.8)	116 (45.0)	1.56 (1.02–2.38)	0.039

[#]The common genotype (AA) and common allele ("A" allele) were used as the reference genotype. ^{*}Adjusted for age and sex. *P* values from unconditional logistic regression analyses.

found online at <http://www.neurosci.cn/epData.asp?id=100>.

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REFERENCES

- [1] Martin R, McFarland HF, McFarlin DE. Immunological aspects of demyelinating diseases. *Annu Rev Immunol* 1992, 10: 153–187.
- [2] Steinman L. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell* 1996, 85: 299–302.
- [3] Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, *et al.* Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011, 69: 292–302.
- [4] Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. *N Engl J Med* 2000, 343: 938–952.
- [5] Kira J. Neuromyelitis optica and opticospinal multiple sclerosis: Mechanisms and pathogenesis. *Pathophysiology* 2011, 18: 69–79.
- [6] Argyriou AA, Makris N. Neuromyelitis optica: a distinct demyelinating disease of the central nervous system. *Acta Neurol Scand* 2008, 118: 209–217.
- [7] Sellner J, Boggild M, Clanet M, Hintzen RQ, Illes Z, Montalban X, *et al.* EFNS guidelines on diagnosis and management of neuromyelitis optica. *Eur J Neurol* 2010, 17: 1019–1032.
- [8] Wingerchuk DM, Hogancamp WF, O'Brien PC, Weinshenker BG. The clinical course of neuromyelitis optica (Devic's syndrome). *Neurology* 1999, 53: 1107–1114.
- [9] Wingerchuk DM, Lucchinetti CF. Comparative immunopathogenesis of acute disseminated encephalomyelitis, neuromyelitis optica, and multiple sclerosis. *Curr Opin Neurol* 2007, 20: 343–350.
- [10] Eckstein C, Saidha S, Levy M. A differential diagnosis of central nervous system demyelination: beyond multiple sclerosis. *J Neurol* 2012, 259: 801–816.
- [11] International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium 2, Sawcer S, Hellenthal G, Pirinen M, Spencer CC, *et al.* Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 2011, 476: 214–219.
- [12] Yang KL, Chen SP, Shyr MH, Lin PY. High-resolution human leukocyte antigen (HLA) haplotypes and linkage disequilibrium of HLA-B and -C and HLA-DRB1 and -DQB1 alleles in a Taiwanese population. *Hum Immunol* 2009, 70: 269–276.
- [13] Wang H, Dai Y, Qiu W, Zhong X, Wu A, Wang Y, *et al.* HLA-DPB1 0501 is associated with susceptibility to anti-aquaporin-4 antibodies positive neuromyelitis optica in southern Han Chinese. *J Neuroimmunol* 2011, 233: 181–184.
- [14] Matiello M, Schaefer-Klein J, Brum DG, Atkinson EJ, Kantarci OH, Weinshenker BG, *et al.* HLA-DRB1*1501 tagging rs3135388 polymorphism is not associated with neuromyelitis optica. *Mult Scler* 2010, 16: 981–984.
- [15] Wang H, Zhong X, Wang K, Qiu W, Li J, Dai Y, *et al.* Interleukin 17 gene polymorphism is associated with anti-aquaporin 4 antibody-positive neuromyelitis optica in the Southern Han Chinese—a case control study. *J Neurol Sci* 2012, 314: 26–28.
- [16] Matsushita T, Matsuoka T, Isobe N, Kawano Y, Minohara M, Shi N, *et al.* Association of the HLA-DPB1*0501 allele with anti-aquaporin-4 antibody positivity in Japanese patients with idiopathic central nervous system demyelinating disorders. *Tissue Antigens* 2009, 73: 171–176.
- [17] Asgari N, Nielsen C, Stenager E, Kyvik KO, Lillevang ST. HLA, PTPN22 and PD-1 associations as markers of autoimmunity in neuromyelitis optica. *Mult Scler* 2012, 18: 23–30.
- [18] Kim HJ, Park HY, Kim E, Lee KS, Kim KK, Choi BO, *et al.* Common CYP7A1 promoter polymorphism associated with risk of neuromyelitis optica. *Neurobiol Dis* 2010, 37: 349–355.
- [19] Russell DW, Setchell KD. Bile acid biosynthesis. *Biochemistry* 1992, 31: 4737–4749.
- [20] Couture P, Otvos JD, Cupples LA, Wilson PW, Schaefer EJ, Ordovas JM. Association of the A-204C polymorphism in the cholesterol 7 α -hydroxylase gene with variations in plasma low density lipoprotein cholesterol levels in the Framingham Offspring Study. *J Lipid Res* 1999, 40: 1883–1889.
- [21] Hofman MK, Princen HM, Zwinderman AH, Jukema JW. Genetic variation in the rate-limiting enzyme in cholesterol catabolism (cholesterol 7 α -hydroxylase) influences the progression of atherosclerosis and risk of new clinical events. *Clin Sci (Lond)* 2005, 108: 539–545.
- [22] Cooper AD, Chen J, Botelho-Yetkinler MJ, Cao Y, Taniguchi T, Levy-Wilson B. Characterization of hepatic-specific regulatory elements in the promoter region of the human cholesterol 7 α -hydroxylase gene. *J Biol Chem* 1997, 272: 3444–3452.

- [23] Karam WG, Chiang JY. Polymorphisms of human cholesterol 7 α -hydroxylase. *Biochem Biophys Res Commun* 1992, 185: 588–595.
- [24] Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, *et al.* Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol* 2005, 58: 840–846.
- [25] Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology* 2006, 66: 1485–1489.
- [26] Wu ZY, Zhao GX, Chen WJ, Wang N, Wan B, Lin MT, *et al.* Mutation analysis of 218 Chinese patients with Wilson disease revealed no correlation between the canine copper toxicosis gene MURR1 and Wilson disease. *J Mol Med (Berl)* 2006, 84: 438–442.
- [27] Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, *et al.* A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* 2004, 364: 2106–2112.
- [28] Molowa DT, Chen WS, Cimis GM, Tan CP. Transcriptional regulation of the human cholesterol 7 α -hydroxylase gene. *Biochemistry* 1992, 31: 2539–2544.
- [29] Dixit M, Choudhuri G, Mittal B. Association of lipoprotein receptor, receptor-associated protein, and metabolizing enzyme gene polymorphisms with gallstone disease: A case-control study. *Hepatol Res* 2006, 36: 61–69.
- [30] Srivastava A, Choudhuri G, Mittal B. *CYP7A1* (-204 A>C; rs3808607 and -469 T>C; rs3824260) promoter polymorphisms and risk of gallbladder cancer in North Indian population. *Metabolism* 2010, 59: 767–773.
- [31] Orton SM, Morris AP, Herrera BM, Ramagopalan SV, Lincoln MR, Chao MJ, *et al.* Evidence for genetic regulation of vitamin D status in twins with multiple sclerosis. *Am J Clin Nutr* 2008, 88: 441–447.
- [32] Roy S, Freake HC, Fernandez ML. Gender and hormonal status affect the regulation of hepatic cholesterol 7 α -hydroxylase activity and mRNA abundance by dietary soluble fiber in the guinea pig. *Atherosclerosis* 2002, 163: 29–37.