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·Original Article·

Developmental distribution pattern of metabotropic glutamate receptor 5 in prenatal human hippocampus

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Abstract: **Objective** Metabotropic glutamate receptor 5 (mGluR5) is concentrated in zones of active neurogenesis in the prenatal and postnatal rodent brain and plays an important role in the regulation of neurogenesis. However, little is known about mGluR5 in the prenatal human brain. Here, we aimed to explore the expression pattern and cellular distribution of mGluR5 in human fetal hippocampus. **Methods** Thirty-four human fetuses were divided into four groups according to gestational age: 9–11, 14–16, 22–24 and 32–36 weeks. The hippocampus was dissected out and prepared. The protein and mRNA expression of mGluR5 were evaluated by Western blot and immunohistochemistry or real-time PCR. The cellular distribution of mGluR5 was observed with double-labeling immunofluorescence. **Results** Both mGluR5 mRNA and protein were detected in the prenatal human hippocampus by real-time PCR and immunoblotting, and the expression levels increased gradually over time. The immunohistochemistry results were consistent with immunoblotting and showed that mGluR5 immunoreactivity was mainly present in the inner marginal zone (IMZ), hippocampal plate (HP) and ventricular zone (VZ). The double-labeling immunofluorescence showed that mGluR5 was present in neural stem cells (nestin-positive), neuroblasts (DCX-positive) and mature neurons (NeuN-positive), but not in typical astrocytes (GFAP-positive). The cells co-expressing mGluR5 and nestin were mainly located in the IMZ, HP and subplate at 11 weeks, all layers at 16 weeks, and CA1 at 24 weeks. As development proceeded, the number of mGluR5/nestin double-positive cells decreased gradually so that there were only a handful of double-labeled cells at 32 weeks. However, mGluR5/DCX double-positive cells were only found in the HP, IZ and IMZ at 11 weeks. **Conclusion** The pattern of mGluR5 expression by neural stem/progenitor cells, neuroblasts and neurons provides important anatomical evidence for the role of mGluR5 in the regulation of human hippocampal development.

Keywords: metabotropic glutamate receptor 5; neurogenesis; hippocampus; human fetus

1 Introduction

The hippocampus is part of the limbic system and plays an important role in learning and memory, emotion, and mood. In addition, the subgranular zone in the dentate gyrus (DG) is an important site of ongoing neurogenesis throughout the lifespan of humans and other mammals[1]. Many central nervous system (CNS) disorders such as neuropsychiatric diseases (depression, anxiety disorders, and schizophrenia), neurodegenerative dis-

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eases (Alzheimer's disease, Parkinson's disease), and epilepsy are thought to be associated with abnormalities of the hippocampus[2].

The development of the CNS requires the coordination of cell proliferation, migration, differentiation, synaptogenesis and apoptosis. Cell proliferation is regulated by distinct genetic programs and responses to extracellular cues such as growth factors and neurotransmitters[3,4]. Recent evidence has highlighted the importance of glutamate, a major excitatory neurotransmitter in the mammalian CNS, in neural progenitor proliferation, neuronal migration, survival, differentiation, and synaptic plasticity in the prenatal, postnatal, and adult CNS after injury or disease[5].

Glutamate acts on specialized ionotropic and metabotropic classes of receptors. Metabotropic glutamate receptors (mGluRs) belong to a G protein-coupled receptor superfamily that includes $GABA_B$ receptors, calcium (Ca^{2+})-sensing receptors, some taste receptors, and pheromone receptors. A growing body of evidence indicates that mGluRs support basic developmental processes, such as proliferation, differentiation, and the survival of neural progenitors[6]. mGluR5, a group I metabotropic glutamate receptor, is the predominant subtype expressed in zones of active neurogenesis in the prenatal and postnatal rat brain[7] and in cultured neurospheres from mouse forebrain[8]. Cell proliferation is decreased by pharmacological blockade of mGluR5 and increased by its activation. The numbers of proliferating neural progenitors are significantly reduced in the hippocampal subventricular zone (SVZ) and DG in adult mice lacking mGluR5 or treated with an mGluR5 antagonist[9,10]. In particular, the study by Castiglione suggested that mGluR5 specifically supports the survival of progenitors undergoing differentiation into neurons[8].

In the adult animal and human brains, many neurons in the hippocampus, cerebral cortex and corpus striatum express mGluR5, which is associated with synaptic transmission[11]. In addition, mGluR5 is also expressed by other cell-types in the brain, such as astrocytes and microglia[12-14]. It is unclear whether these cells express mGluR5 in human hippocampal development, especially in the late period before birth.

Although some animal studies have investigated the expression of mGluR5 and its co-localization with neural stem/precursor cells in the developing hippocampus, little is known about its expression and cellular distribution during human hippocampal development, especially the relationship between mGluR5 and neural progenitors. In the present study, we aimed to (1) monitor mGluR5 mRNA and protein expression in the hippocampus of the human fetus; and (2) determine the cellular distribution of mGluR5.

2 Materials and methods

2.1 Human brain samples A total of 45 deceased fetuses from spontaneous or medically-induced abortions were obtained after legal approval (in accordance with the legislation of China). The protocols were approved by the Ethics Board of Xi'an Jiaotong University Medical School and informed consent was obtained from each woman before collecting samples. Gestational age was estimated from the last menstrual period, as well as foot length and crown-rump length. Finally, 34 brains with no obvious abnormality were used. The samples were divided into four groups according to gestational age: 9–11 (n = 9), 14–16 (n = 10), 22–24 (n = 9) and 32–36 weeks (n = 6). Adult cerebral cortex (positive control for mGluR5 protein detection with Western blot) and cerebellum (negative control for mGluR5 protein detection with Western blot) were obtained at autopsy from four controls without a history of neurological disease. The interval between death and tissue fixation was usually <12 h.

2.2 Tissue preparation The brain was rapidly removed, microdissected, and small blocks of tissue from the right hippocampus were immediately fixed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB) at 4°C for ~3 days, then transferred to 30% sucrose in 0.1 mol/L PB and stored overnight. Coronal sections (20 μm) were cut on a cryostat (Slee Technik, Mainz, Germany) and the serial sections were mounted on gelatin-coated slides. Meanwhile, ~100 mg of tissue from the left hippocampus was immediately immersed in liquid nitrogen for protein and mRNA expression analysis.

2.3 Quantitative real-time PCR Total RNA from fetal

hippocampus was extracted using TRIzol LS reagent (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. Briefly, the freshly-isolated tissue (50 mg) was homogenized in 0.75 mL TRIzol LS using a power homogenizer (Polytron Kinematica, Lucerne, Switzerland). Samples with volumes <0.25 mL were adjusted to 0.25 mL with water, homogenized, incubated for 5 min at room temperature, and treated with 0.2 mL chloroform per 0.75 mL TRIzol LS. Sample tubes were securely capped, shaken vigorously by hand for 15 s, incubated at room temperature for 15 min, and centrifuged (12 000 g for 15 min at 4°C). The mixture separated into a lower red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase (about 70% of the volume of TRIzol LS used for homogenization) was transferred to a clean tube, mixed with 0.5 mL isopropyl alcohol to precipitate the RNA, incubated at room temperature for 10 min, and centrifuged (12 000 g for 10 min at 4°C). The supernatant was removed and the RNA pellet was washed once with 1 mL 75% ethanol, mixed by vortexing, and centrifuged (7 500 g for 5 min at 4°C). The pellet was air-dried for 10 min, dissolved in RNase-free water by passing the solution a few times through a pipette tip, incubated for 10 min at 55°C, and stored at –70°C. Reverse transcription was performed using a PrimeScript TM RT reagent kit (Takara Bio Inc., Shiga, Japan) and 500 ng RNA as the starting template. The mixture was incubated at 37°C for 15 min, and reverse transcription was inactivated by heating (85°C for 5 s).

After cDNA synthesis, the real-time quantitative RT-PCR was carried out using SYBR Premix Ex Taq TM II (Takara) and β-actin as the reference gene. The sequences of β-actin and mGluR5 primers are listed in Table 1. All reactions were performed in triplicate, had 20-μL reaction volumes, including 2 μL cDNA, 10 μL SYBR Premix EX Taq TM II, 0.8 μL each of forward and reverse primer (10 μmol/L for each primer), and 6.4 μL ddH_2O, and were incubated under the following conditions: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, 62°C for 30 s, and finally cooled to 4°C. The PCR products were separated by 1.2% agarose gel electrophoresis. The bands were then visualized under UV light after staining by ethidium bromide.

Table 1. Sequences and annealing temperatures of primers

Target gene	Gene Bank Access No.	Primer sequences	Length (bp)	T_m (°C)	Products (bp)
mGluR5	NM_000842.1	Forward 5'-CATCATCAGATCTGTGTGCGATGAA- 3'	25	62	144
		Reverse 5'-TGGCATGCCTTGCATGTGTA-3'	20		
β-actin	NM_001101	Forward 5'-TGGCACCCAGCACAATGAA-3'	19	62	186
		Reverse 5'-CTAAGTCATAGTCCGCCTAGAAGCA-3'	25		

2.4 Western blot Western blot analysis of mGluR5 in protein extracts from brain prepared according to Romano[15] was carried out as described previously[16]. Briefly, 20 μg protein per lane was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8% polyacrylamide gels). The gel was blotted onto a nitrocellulose membrane, which was then incubated with 10% nonfat powdered milk dissolved in Tris-buffered saline with Tween 20 (TBST) for 1 h to block nonspecific binding sites, incubated overnight with primary polyclonal antibody (see Table 2), washed extensively with TBST, incubated for 2 h with secondary goat anti-rabbit antibody coupled to horseradish peroxidase (1:2 000, GAR-HRP, Fisher), and visualized by enhanced chemiluminescence (ECL reagent, Amersham Biosciences, Piscataway, NJ). We performed the experiments on three different fetuses for each devel-

Table 2. Antibodies used in this study

Antibody	Host	Company	Concentration in immunohistochemistry	Concentration in Western blot
mGluR5	Rabbit	Chemicon	1:1 000	1:2 000
	Rabbit	Abcam	1:500	1:1 000
Nestin	Mouse	Chemicon	1:200	
DCX	Goat	Santa Cruz	1:100	
	Guinea pig	Chemicon	1:1 000	
NeuN	mouse	Chemicon	1:300	
GFAP	mouse	Chemicon	1:500	

opmental time point.

2.5 Immunohistochemical study of mGluR5 expression Staining for mGluR5 was performed as follows. Tissue sections were treated with 0.3% H_2O_2 for 15 min, with 2% normal goat serum in PBS for 30 min, and with primary antibodies in PBS (for concentrations, see Table 2) overnight at 4°C. After washing with PBS, slices were incubated with secondary anti-rabbit, -mouse, or -goat antibody (1:200; Vector Laboratories, Burlingame, CA) for 2 h at room temperature. After washing, slices were incubated with ABC Elite solution (Vector Laboratories), then washed in PBS, reacted with a solution of 0.012% H_2O_2 and 0.05% 3, 3-diaminobenzidine (Sigma, St. Louis, MO) in Tris buffer for 10 min. They were washed in PBS, dehydrated in a graded ethanol series, clarified, coverslipped with a xylene-based mounting medium (DPX), and viewed. Photomicrographs were taken under an Olympus BX51 microscope equipped with a Spot camera (DP71). In the negative control, primary antibodies were replaced by PBS.

2.6 Double-labeling immunofluorescence microscopy Double-labeling immunofluorescence microscopy was carried out to examine mGluR5 co-localization with neural progenitor cell markers (nestin and DCX), a neuron marker (NeuN) and an astrocyte marker (GFAP: in the early development stage, GFAP was also a progenitor marker, not exclusively labeling astrocytes) in the hippocampus. Sections were washed in 0.01 mol/L PBS and incubated overnight with primary antibodies (Table 2). After washing in PBS, they were incubated for 4–6 h at room temperature with fluorescein isothiocyanate-conjugated or tetramethyl rhodamine isothiocyanate-conjugated anti-IgG. Finally, they were incubated with 4',6-diamidino-2-phenylindole (DAPI) for 5 min to stain nuclei, washed in PBS, covered with Antifade mounting medium (Beyotime, China), and examined under an Olympus BX51 (Olympus Corp., Tokyo, Japan) fluorescence microscope. Sections incubated without primary antibody were taken as negative controls.

2.7 Image analysis and cell counting Optical density analysis and cell counting were carried out using the Image-Pro Plus system (Media Cybernetics, Silver Spring, MD) by an experimenter unaware of the identity of the groups. One group of immunostained sections (containing four samples of brain tissue) from different fetuses was digitized under a 40× objective lens. The number of positive cells and integrated optical density (IOD) were obtained in a 100 μm ×100 μm area based on the appearance of cells with Image-pro plus 5 (Media Cybernetics, USA). Three visual fields were selected randomly to count the cells in each section. Then the percentage of double-labeled cells relative to mGluR5-positive cells or nestin-positive cells was calculated.

2.8 Statistical analysis The mGluR5 protein or mRNA level at different time points was normalized to the β-actin level, and is indicated as mean ± SD. All the data were analyzed by Student's *t*-test or one-way ANOVA followed by Student–Newman–Keuls *post hoc* multiple comparisons using the SPSS 11.0 software package. A P value of <0.05 was considered to be statistically significant.

3 Results

3.1 Changes in expression of mGluR5 protein and mRNA during hippocampal development Western blots revealed a clear band (132 kDa) for mGluR5 in all except the negative group (adult cerebellum) (Fig. 1A). The difference in mGluR5 expression between gestational 11 and 36 weeks was significant (P <0.05). In all groups, real-time PCR showed a significant increase in mGluR5 mRNA expression from 11 to 32 weeks (Fig. 1B). On Western blots, the mGluR5 protein level also increased significantly (Fig. 1A), apparently in parallel with the mRNA level.

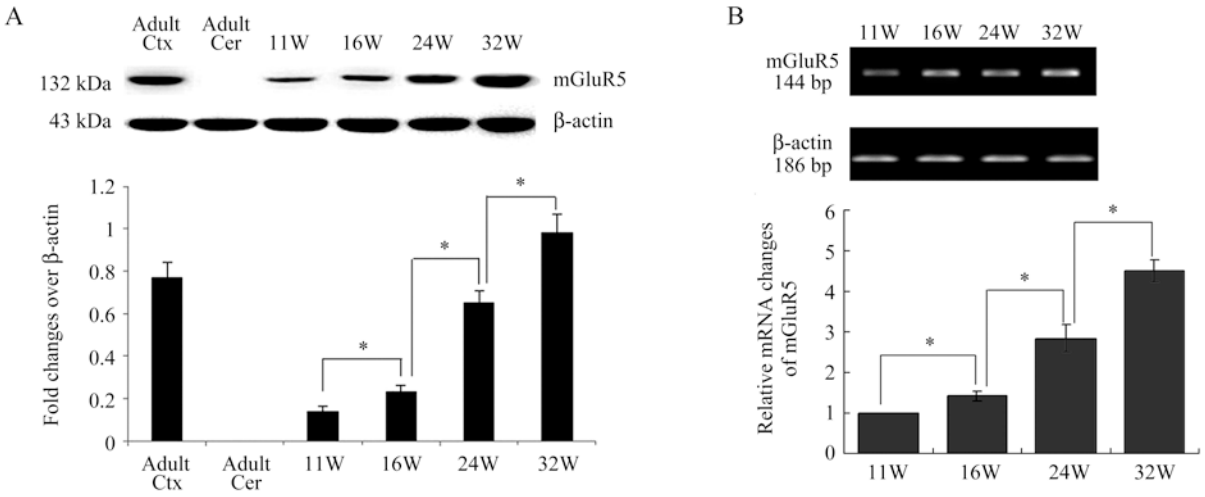

Fig. 1. Developmental changes in mGluR5 protein and mRNA. A: Statistics of Western blot show time-dependent changes of mGluR5 with significant differences between 11 and 16 weeks (W), 16 and 24 W, and 24 and 32 W. B: mGluR5 mRNA was expressed in all groups and increased significantly with the pattern of protein changes. Values are mean ± SD, *P <0.05. Ctx, cortex; Cer, cerebellum.

3.2 Distribution of mGluR5 protein during human hippocampal development In the sections negative for mGluR5 immunostaining, there were no immuno-positive cells. However, cells in the subplate, hippocampal plate (HP), and inner marginal zone (IMZ) were strongly immunostained for mGluR5 at 11 weeks (Fig. 2A, a), and the innermost 1–2 layers of cells on the ventricular surface and intermediate zone (IZ) were lightly stained. In the subplate, the reactivity was clearly on the plasma membrane surface and the tightly-packed cell bodies were round or oval and 10–15 μm in diameter. The HP had some fusiform cells and many processes extending to the outside. The immunopositive cells were similar in the IMZ and subplate.

At 16 weeks (Fig. 2B, b), mGluR5 showed strong expression in the IZ, subplate, HP, and IMZ, and weak expression in the ventricular zone (VZ). In all layers, mGluR5 immunoreactivity, especially in the IZ, was significantly higher than at 11 weeks (Fig. 2E). At 24 weeks (Fig. 2C, c), mGluR5 immunoreactivity was detectable in CA1 and CA3 [stratum oriens (SO) and stratum pyramidale (SP)] but almost undetectable in CA3 [stratum radiatum (SR)[17], stratum lacunosum-moleculare (SLM)] and the DG. At 32 weeks (Fig. 2D, d), compared with 24 weeks, mGluR5 immunostaining decreased in CA3 SO, remained unchanged in CA3 (SR, SLM), and increased in CA1 SP (3.42 times the level at 24 weeks) and DG. At the same time, mGluR5 became apparent in the granule cell layer in the DG.

3.3 Co-localization of mGluR5 with nestin, DCX, NeuN and GFAP In the negative control sections for mGluR5, nestin, DCX, NeuN and GFAP, there was no detectable fluorescence. At 11 weeks, mGluR5-nestin double-labeled

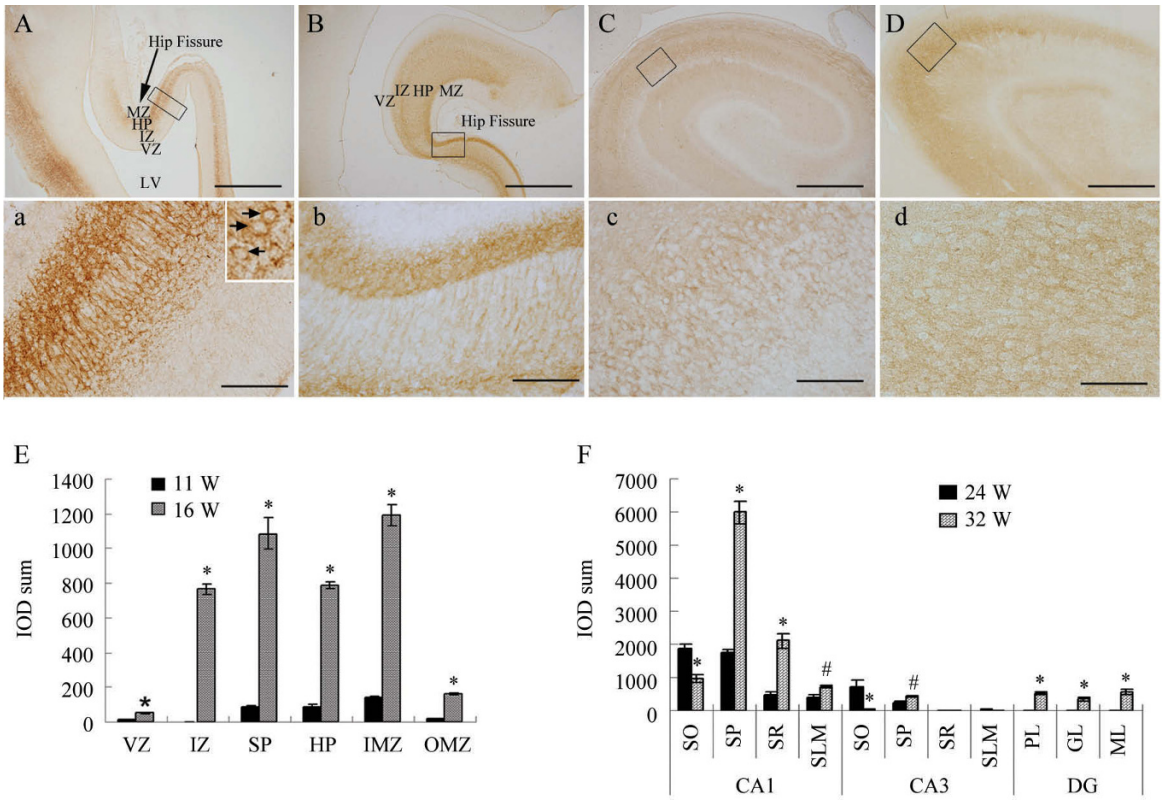

Fig. 2. Developmental profile of mGluR5 protein in fetal hippocampus. A–D are representative sections at 11, 16, 24 and 32 weeks (W) at lower magnification. a–d are higher power images of the areas enclosed by squares in A–D. A: Strong staining in IMZ, HP and subplate, slight staining in OMZ, IZ and VZ at 11 W. a: The immunoreactive cells in subplate and IMZ were round, small and compact (higher magnification in insert). Arrows indicate cells positive for mGluR5. B: The positive layers at 16 W were wider than those at 11 W. b: The IMZ also showed strong staining and the somata were round or oval. C: Almost equal staining throughout the hippocampus. c: SP of CA1 contained the most positive cell bodies. D: Staining was stronger in CA1 than in CA3 and DG. d: Positive cell bodies in SP of CA1 grew bigger. E: The IOD of every layer increased from 11 to 16 W, *P <0.01 *versus* the same layer at 11 W. F: The IOD of most layers other than SO in CA1, SO, SR and SLM in CA3 increased from 24 to 32 W. *P <0.01, $^{\#}P$ <0.05 *versus* the same layer at 24 W. Scale bars, 1 mm in A–D; 50 μm in a–d. DG, dentate gyrus; GL, granular cell layer; HP, hippocampal plate; IMZ, inner marginal zone; IZ, intermediate zone; ML, molecular layer; OMZ, outer marginal zone; PL, polymorphic layer; SO, stratum oriens; SLM, stratum lacunosum-moleculare; SR, stratum radiatum; SP in E, subplate; SP in F, stratum pyramidale; VZ, ventricular zone.

cells were frequently seen in the subplate, HP, and IMZ and the percentage of double-labeled cells relative to mGluR5-labeled cells or nestin-labeled cells was 90% or 64%, respectively (Fig. 3A–C). At this stage of development, about half of neural stem/progenitor cells expressed mGluR5. From 11 to 16 weeks, the area of double-labeled cells extended to not only the subplate, HP, and IMZ, but also the IZ and the inner part of the VZ (Fig. 3D–I; Table 3). About 78% of the nestin-positive cells and 75% of the mGluR5-positive cells were double-labeled (Table 3). At 24 weeks, the double-labeled cells resided mainly in the SO and SP of CA1, rarely in CA3 and DG (Fig. 3J–L). At 32 weeks, nestin- and mGluR5-positive cells had very different distributions. The nestin-positive cells were mainly in the DG whereas mGluR5-positive cells and their processes were mainly in the SP and SR of CA1. However, there were also a handful of double-labeled cells in the SP and SR, especially in the molecular layer and polymorphic layer of the DG (Fig. 3M–O). Notably, double-labeled cells decreased in number over time (Table 3).

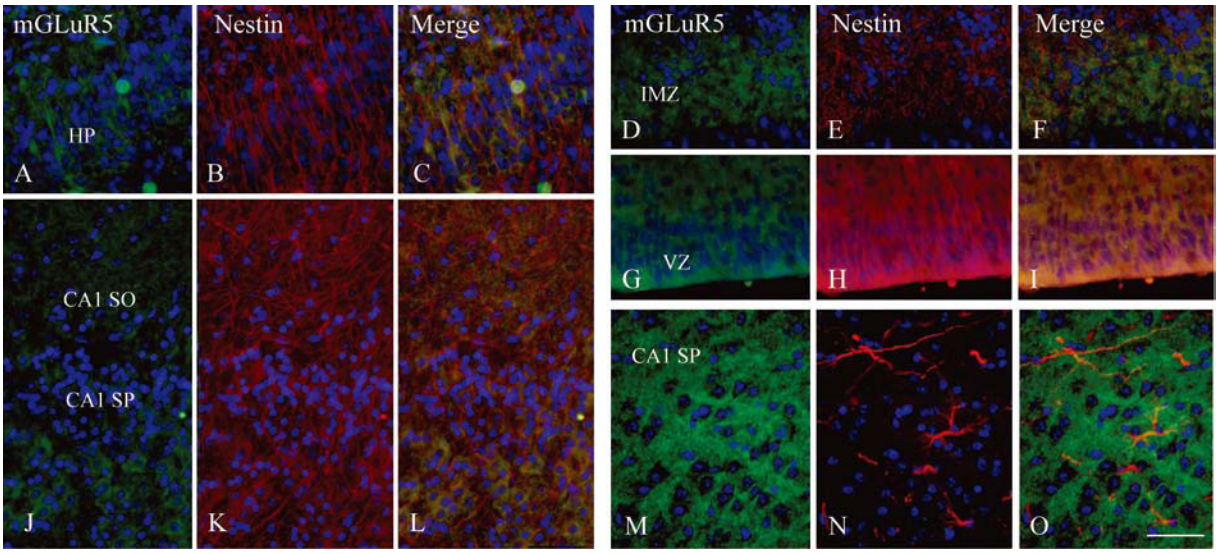

Fig. 3. Co-localization of mGluR5 (green) and nestin (red) in developing hippocampus (nuclei stained blue). A–C: At 11 weeks (W), double-labeled cells were mainly localized in subplate, HP and IMZ. At 16 W, the main layers with double-labeled cells were IMZ (D–F) and VZ (G–I). At 24 W, the double-labeled cells were found in SO and SP in CA1 (J–L). By 32 W, there were almost no double-labeled cells in the hippocampus except for a few in SP in CA1 and DG (M–O). Scale bar, 50 μm. HP, hippocampal plate; IMZ, inner marginal zone; SO, stratum oriens; SP, stratum pyramidale; VZ, ventricular zone.

Table 3. Developmental changes of percentage and number of mGluR5 and nestin double-labeled cells

	Double-labeled (% of total mGluR5$^+$)	Double-labeled (% of total nestin$^+$)	Number of double-labeled cells (number/mm^2)
11 weeks	90 ± 4	64 ± 2	2674 ± 326
16 weeks	75 ± 3*	78 ± 3*	1538 ± 143*
24 weeks	70 ± 3	60 ± 2*	651 ± 94**
32 weeks	37 ± 1 (CA1)**	53 ± 1 (CA1)	32 ± 4 (CA1)**
	78 ± 2 (DG)	28 ± 1 (DG)	461 ± 37 (DG)

*P <0.05, **P <0.01 *versus* the next younger group. The data from CA1 at 32 weeks were compared with those at 24 weeks. DG, dentate gyrus.

mGluR5 was co-localized with DCX at 11 weeks, and double-labeled cells were mainly in the HP, IZ and IMZ (Fig. 4A–C). Surprisingly, at 16, 24, and 32 weeks, no double-labeled cells were detectable, although antibodies from two companies were used (Fig. 4D–F).

In order to investigate whether neurons express mGluR5 in the developing human hippocampus, we carried out a double-labeling experiment for mGluR5 and NeuN (a marker of mature neurons). Cells double-labeled for mGluR5 and NeuN were first detected in the HP at 24 weeks and increased in number at 32 weeks. They were mainly located in the SP of CA1 and the granular cell layer of the DG (Fig. 4G–I). Moreover, the double-labeled cells appeared in the HP in an "inside-out" pattern. As for astrocytes, a few cells double-labeled for mGluR5 and GFAP were mainly found in the VZ and SVZ, while they were not detected in regions such as the hippocampal fimbria where astrocytes are known to be concentrated (Fig. 4J–L).

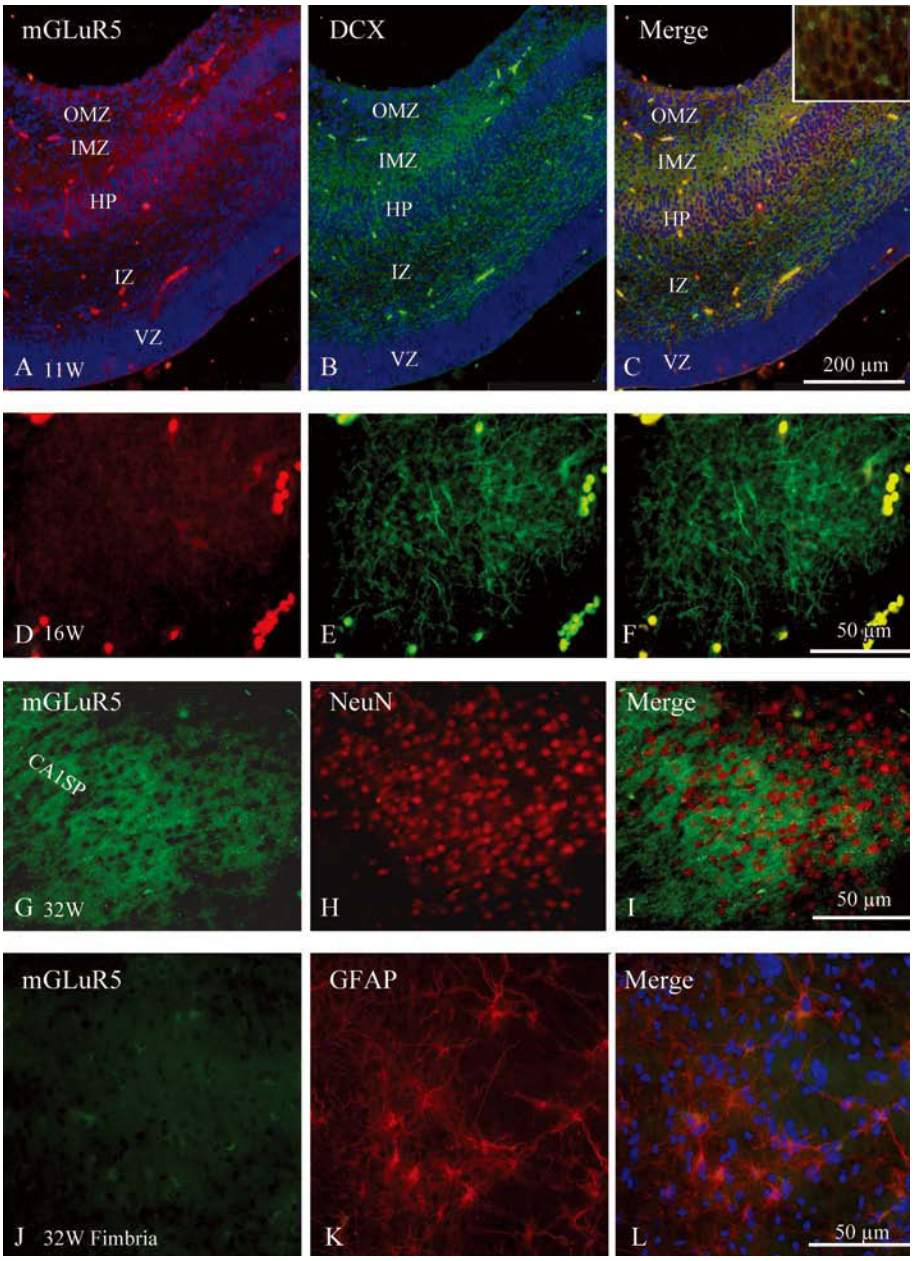

Fig. 4. Co-localization of mGluR5$^+$/DCX$^+$, mGluR5$^+$/NeuN$^+$, and mGluR5$^+$/GFAP$^+$ in hippocampus. A–C: mGluR5 (red) and DCX (green) double-labeled cells were mainly located in the HP and IMZ at 11 weeks (W) (nuclei stained blue). Insert in C is a high magnification image of double-labeled cells in the HP (nuclei not shown). Note that non-specific intense red or green fluorescence may be due to the microvasculature, including erythrocytes. Scale bar, 200 μm. D–F: However, cells double-labeled with mGluR5 and DCX were not detected after 16 W. G–I: At 32 W, mGluR5 (green) was expressed by numerous neurons (red) in CA1. J–L: mGluR5 (green) and GFAP (red) double-labeled cells were not detected in hippocampal fimbria. Scale bars, 50 μm. HP, hippocampal plate; IMZ, inner marginal zone; IZ, intermediate zone; OMZ, outer marginal zone; SP, stratum pyramidale; VZ, ventricular zone.

4 Discussion

Our main findings were that (1) both mGluR5 mRNA expression and protein levels in the hippocampus increased gradually over time; and (2) the expression of mGluR5 by neural stem/progenitor cells, neuroblasts and neurons im-

plied their participation in the regulation of neurogenesis in the developing human hippocampus.

4.1 mGluR5 expression in the fetal hippocampus mGluR5 mRNA and protein are present in the fetal hippocampus of animals. In mice, mGluR5 mRNA is detectable at E15 and protein at E18, and increases perinatally, reaching a peak in the second postnatal week and decreasing thereafter[9,15]. The profile of mGluR5 protein expression in rats is similar to that in mice. During development, mGluR5a declines while mGluR5b rises to predominate in adulthood[18,19]. At the microscopic level, mGluR5 is seen mainly in unmyelinated axons and pyramidal cell dendrites in the first two postnatal weeks[18,20], but later its presence on unmyelinated axons is markedly reduced and the receptor becomes more widely distributed on dendritic spines[18,20].

It is known that mGluR5 is expressed in neurons of adult human hippocampus, but whether it is also expressed in the prenatal hippocampus was unknown. Here, we demonstrated the presence of mGluR5 protein and mRNA in human fetal hippocampus using immunoblotting, immunohistochemistry and real-time PCR. mGluR5 (both mRNA and protein) was expressed in increasing amounts before birth and was mainly found in the hippocampal subplate, HP, and IMZ at 11 weeks, with extension to the IZ at 16 weeks and wide distribution in all layers of subregions except for CA3 SR and SLM after 24 weeks. However, mGluR5 in mice occurs mainly in the VZ at E15 and gradually spreads to the SVZ at E18. We speculate there are two possible interpretations: (1) the expression of mGluR5 in the VZ of the prenatal hippocampus in humans is too weak to be detected; or (2) strong expression of mGluR5 in the VZ occurs earlier than 11 weeks.

4.2 mGluR5 expression by neural stem/progenitor cells, neuroblasts and neurons in the prenatal hippocampus Neural stem/progenitor cells are thought to express mGluR5 because co-labeled cells are seen in the prenatal or postnatal SVZ and ependymal cell layer[7]. In our study, a few double-labeled cells (mGluR5 and nestin) were detected in the prenatal hippocampus. Meanwhile, the number of double-positive cells and the percentage relative to mGluR5-positive cells decreased over time. At the early stage of development, mGluR5 may be mainly involved in the regulation of neural stem/progenitor cells. During the middle or late stage of development, mGluR5 was mainly expressed in neurons, some astrocytes, and a few neural stem/progenitor cells, and it may participate in neurogenesis-related processes such as synaptogenesis and synaptic plasticity. Paradoxically, mGluR5 mRNA is not found in nestin-positive cells in the SVZ and subgranular zone in adult rats[21], and mGluR5-knockout mice have an almost normal hippocampus after birth[22]. Furthermore, in all stages we studied, some mGluR5-negative neural stem/progenitor cells were always present. So we speculate that mGluR5 plays a partial and stage-specific role in neural stem cell proliferation, differentiation, and survival.

Cells positive for both mGluR5 and DCX were frequently found in the HP and IMZ at 11 weeks, but rarely thereafter. In the middle and late stages of prenatal development, hippocampal neuroblasts expressed hardly any mGluR5, suggesting that it has little effect on migrating and differentiating neuroblasts. Although neurons undergoing maturation in the hippocampus strongly express mGluR5, its expression may decline or cease before neuronal maturation.

A growing body of studies shows that mGluR5 is involved in regulating neurogenesis. This role appears to be complex, in that regulation differs in different germinal zones. *In vivo* and *in vitro* studies show that mGluR5-mediated effects on striatal neuronal progenitors are restricted mainly to early cycling populations in the VZ, with little effect on secondary proliferative populations in the SVZ. In contrast to its proliferative effects in the ventral telencephalon, mGluR5 has no such effects on dorsal telencephalon-derived cortical neuroblasts[23]. Studies in our laboratory showed that mGluR5 enhances the proliferation of neural stem cells from human fetal brain along with activation of the mitogen-activated protein kinase (MAPK) signaling pathway[24].

It is widely believed that mGluR5 in mature neurons takes part in synaptogenesis, synaptic plasticity and synaptic transmission. In addition, mGluR5 can be expressed by mature astrocytes, microglia and oligodendroglia. How-

ever, López-Bendito *et al.* did not find any cells double-labeled with mGluR5 and GFAP in fetal rat brain[18]. We found no cells double-labeled with mGluR5 and GFAP in the developing hippocampus.

This study has some limitations, such as the small sample size and variance in genetic background and quality. But these limitations do not influence the conclusion that mGluR5 is expressed in the prenatal human fetal hippocampus by neural stem/progenitor cells, migrating neuronal progenitor cells and neurons, but not astrocytes. During development, the expression level of mGluR5 in neural stem/progenitor cells and the number of $mGluR5^+$/$nestin^+$ cells decrease in hippocampus, but a growing number of mature neurons expressing mGluR5 could result in an increase of mGluR5 in the whole hippocampus. Therefore, mGluR5 mainly regulates the proliferation of neural stem/progenitor cells with MAPK pathway in early development[24]. However, in late development, it mainly takes part in the synaptogenesis, synaptic plasticity and synaptic transmission. So, its regulatory role in the proliferation, differentiation, and survival of neural progenitor cells in human fetal hippocampus may be partial and stage-specific. Notably, detailed *in vitro* functional studies on the role and mechanism of action of mGluR5 in neurogenesis in humans are required to corroborate the anatomical observations reported here.

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