Histol Histopathol (2011) 26: 953-963

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Distribution of Kiaa0319-like immunoreactivity in the adult mouse brain - a novel protein encoded by the putative dyslexia susceptibility gene KIAA0319-like

Ming-Wai Poon, Wan-Hong Tsang, Mary Miu-Yee Waye and Sun-On Chan

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

Summary. Kiaa0319L is a novel protein encoded by a recently discovered gene KIAA0319-like(L) that may be associated with reading disability. Little is known about the characteristics of this protein and its distribution in the brain. We investigated here expression of this protein in adult mice, using an antibody specific for human and rodent Kiaa0319L. In the brain, Kiaa0319L was localized strongly in the olfactory bulb, and strong expression was found in other regions, including hippocampus, cerebellum, diencephalon and the cerebral cortex. Immunohistochemistry confirmed expression in these brain regions, and showed further that the protein was expressed preferentially in neurons in layer IV and VI of the neocortex, CA1 and CA2 subfields of the hippocampus and a subpopulation of neurons in CA3 and dentate gyrus. Furthermore, the protein was confined to dendrites of CA1 neurons in the stratum radiatum, but not those in the stratum oriens, and in astrocytes within the hippocampus. In the cerebellum, the protein was observed in the molecular layer and a fraction of Purkinje neurons. These findings confirmed expression of Kiaa0319L in brain regions that are involved in reading performance, supporting its possible involvement in reading disability. The specific patterns of localization in the neocortex, hippocampus and cerebellum suggest further that this protein may be related to other biological processes in a subpopulation of neurons within these regions, eg. formation and maintenance of polarity in the neuron.

Key words: Kiaa0319L, Developmental dyslexia, Cerebral cortex, Hippocampus, Cerebellum

Offprint requests to: Prof. Mary Waye, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong. e-mail: mary-waye@cuhk.edu.hk

Introduction

Developmental dyslexia or reading disability is a common neurobehavioral disorder affecting school-age children with a prevalence rate of 5-10% (Shaywitz et al., 1990). Genetic mapping has identified several genomic regions known as Dyslexia Susceptibility 1-9 (DYX1-9) that show genetic linkage and association with dyslexia. These genomic regions are distributed on different chromosomes, which include chromosome 1p (Rabin et al., 1993; Grigorenko et al., 2001) and 6p (Cardon et al., 1994; Grigorenko et al., 1997; Kaplan et al., 2002). Research so far has proposed 4 candidate genes within these loci, which include dyslexia susceptibility 1 candidate 1 (DYX1C1) (Taipale et al., 2003; Dahdouh et al., 2009), doublecortin domain-containing protein 2 (DCDC2) (Meng et al., 2005; Schumacher et al., 2005), round-about Drosophila homolog 1 (ROBO1) (Hannula-Jouppi et al., 2005) and KIAA0319 (Cope et al., 2005). Among these, KIAA0319 is one of the two best characterized genes within the 6p22 locus that have been shown to associate with reading disability. The encoded transcripts are expressed in various brain regions, including cerebral cortex, in adult and embryonic mice and in human fetuses (Paracchini et al., 2006; Velayos-Baeza et al., 2007). Moreover, interference of normal Kiaa0319 expression with shRNAs produces a failure in radial migration of neurons in the developing rat neocortex, suggesting a link of reading disability to abnormality in neuronal migration mediated by KIAA0319 (Paracchini et al., 2006; Peschansky et al., 2010).

In a recent study, a novel gene KIAA0319-like (KIAA0319L) on chromosome 1p34 (or DYX8), which shares homology to KIAA0319, has been suggested to be a functional candidate for reading disability (Couto et

al., 2008). We have previously reported that Kiaa0319L interacts with the Nogo receptor (Poon et al., 2011a) and that Kiaa0319L encodes N-glycosylated isoforms that form homo-dimers (Poon et al., 2011b). Further evidence supporting its expression in the brain and its involvement in cortical development is lacking. We investigated in this study whether the protein encoded by KIAA0319L is expressed in the adult mouse brain and whether the expression is confined to brain regions that relate to its proposed functions in reading activities, using a novel antibody that recognizes specifically the Kiaa0319L protein.

Materials and methods

Peptide design, peptide synthesis and antibody production

A synthetic peptide of 17 amino acids was designed according to the 592th-608th amino acid residues of the deduced sequence of Kiaa0319L, which correspond to the hydrophilic region of the protein, using the computer software from Cambridge Research Biochemicals (Cleveland, UK). A Cys was added to the N-terminal to assist conjugation of the peptide. The peptide sequence was searched by "BLAST" to ensure that it was specific for Kiaa0319L. The antigenic peptide was linked to keyhole limpet haemocyanin before immunizing rabbits for 8 weeks. Enzyme-linked immunosorbent assay (ELISA) was used to check for successful production of antibody in the harvest bleeds. Sera were harvested, affinity purified and used as the source of antibody (from Cambridge Research Biochemicals, UK).

Computational domain prediction and peptide design

The deduced protein sequences were analyzed by bioinformatic programmes available on ExPASy Molecular Biology Server (http://www.expasy.org). InterProScan (http://www.ebi.ac.uk/Tools/InterProScan) was used for domain search, Signal IP (http://www.cbs.dtu.dk/services/SignalP) for prediction of signal peptide cleavage sites, NetOGlyc (http://www.cbs.dtu.dk/services/NetOGlyc) for mucin type GalNAc O-glycosylation sites, NetNGlyc (http://www.cbs.dtu.dk/services/NetNGlyc) for N-Glycosylation sites, SOSUI (http://bp.nuap.nagoya-u.ac.jp/sosui) for transmembrane regions, and Clustal W2 (http://www.ebi.ac.uk/Tools/clustalw2) for protein sequence alignment. A protein sequence similarity search was performed using NCBI BLAST Search.

Animals

Adult C57BL/6 mice aged 2 months were used for Western blot analyses and immunohistochemistry. The experiment was carried out in accordance to institutional ethics guidelines for minimizing animal use and suffering and the Animal Licence Ordinance.

Western blot analyses

Western blots were performed using procedures described in our earlier study (Wang et al., 2008). Adult mice were killed by cervical dislocation and tissues from various body parts were collected in liquid nitrogen. Total protein was extracted in ice-cooled RIPA buffer with protease inhibitor cocktail (Roche, USA). Protein concentration was measured using BioRad Bradford Assay according to the manufacturer's protocol. Proteins were denatured with Lamelli sample buffer containing ß-mercaptoethanol at room temperature and boiled for 3 mins. The samples were loaded into a 6-8% gel before running for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). Proteins were trans-blotted onto a nitrocellulose membrane. The membrane was blocked in phosphate buffered saline (pH7.4) supplemented with 0.1% tween-20 and 5% skimmed milk (PBS-Tween-Milk). The primary antibodies used included: anti-Kiaa0319L (1:1000), anti-beta-actin (1:10000; Invitrogen, USA). Membranes were washed 3 times with PBS, then incubated with PBS-Tween-Milk with corresponding antibody for 1 hr at room temperature. Secondary antibodies used included: anti-rabbit IgG-HRP (1:1000; Dako, USA); anti-mouse IgG-HRP (1:1000, Dako, USA). The staining was visualized with enhanced chemiluminescence (ECL) method (GE Healthcare, UK). For each immunoblot, three replicates were done on 3 independent protein samples. Intensity of the bands was measured by the AlphaEase FC software (Apha Innotech Co, USA). Specificity of anti-Kiaa0319L antibody was verified in control preparations in which blots were incubated in primary antibody pretreated with the antigenic peptide. Two μl of peptide (1 μg/μl in PBS) was incubated with 1 μl of primary antibody at room temperature for 2 hrs prior to the incubation with membrane. Other controls were treated with similar procedures but with the addition of preimmuned rabbit serum.

Immunohistochemistry

The immunostaining procedure was described previously (Lin et al., 2002; Chung et al., 2004). Adult mice were anesthetized with an intraperitoneal injection of chloral hydrate (420 mg/kg body weight) and perfused with 4% paraformaldehyde in PBS. The brain was post-fixed overnight and sectioned at 100 μm thickness with a vibratome. The sections were briefly rinsed in PBS, blocked in 10% normal donkey serum (Jackson ImmunoResearch, USA) and 0.1% triton X-100, incubated with anti-Kiaa0319L antibody (1:100) raised in rabbit in PBS supplemented with 1% normal donkey serum and 0.1% Triton X-100 and then treated with secondary antibody (anti-rabbit FITC, 1:100; Jackson ImmunoResearch, USA) in 1% normal donkey serum and 0.1% triton X-100. Sections were mounted on glass slides and viewed under a confocal microscopy system (FV300, Olympus Co, Japan). Control

preparations were treated with the above procedures but with primary antibody preabsorbed with the antigenic peptide or with the absence of primary antibody. No obvious staining was observed in any control sections.

Double immunofluorescence staining was performed on frontal sections of the brain. In brief, sections were blocked with 10% normal donkey serum and 0.1% triton X-100, incubated in anti-Kiaa0319L (1:100) together with i) microtubule associated protein (MAP) 2a+2b (1:100; Abcam, USA) or TuJ1(1:500; Abcam, USA) for neuronal beta-III-tubulin to check for neuronal identity of the cells; ii) glial fibrillary acidic protein (or GFAP; 1:100; Abcam, USA) for glial specific expression. The control for this part of experiment was processed with the above procedure in the absence of primary antibodies.

Results

Computational analysis of protein sequence of human and mouse Kiaa0319L

Bioinformatic analyses of human Kiaa0319L NP079150.3 and NP872628.1 (from NCBI database) revealed a similarity in arrangement of domains in consensus regions of the proteins. In the conserved regions, a signal peptide was identified in the first 49 amino acids at the N-terminus, followed by a motif at N-terminus with a seven Cysteines (MANSC) domain that extended up to the 127th amino acid (Fig. 1A). Four polycystic kidney disease (PKD) domains were predicted at 409th - 498th, 504th - 594th, 600th - 688th and 694th -785th amino acid. A fibronectin III (FNIII) domain was predicted in the third and fourth PKD domains, starting from 685th-773th amino acid. At the C terminus, a transmembrane region was identified in 931st-953th amino acid. Spliced variations occurred downstream to this transmembrane region. Several N- and O-glycosylation sites were predicted on the protein.

A computational analysis was also performed on 3 isoforms of mouse Kiaa0319L: NP001030602.1, NP001030603.1 and NP598647.1. Domain alignments in these proteins are similar to those predicted in the human sequences. For example, a signal peptide was predicted in the first 49 amino acids at the N-terminus, followed by a MANSC domain (50th-127th amino acid) (Fig. 1B). The mouse proteins also contain 4 predicted PKD domains at 408th-497th, 503rd-593rd, 599th-687th and 693rd-784th amino acid, highly comparable to the locations in the deduced sequence of their human equivalence. However, no FNIII domain was predicted. A transmembrane region was found at the 929th-951st amino acid. The three isoforms differ in spliced regions downstream of the transmembrane region at the C-terminus.

Antibody design

Peptide sequence for immunogen for antibody production was chosen from a sequence in the conserved region of the Kiaa0319L protein (Fig. 1A), corresponding to the 592nd -608th amino acid residues that straddle across the second and third PKD domains of human NP079150.3. This sequence is not conserved in related molecule Kiaa0319, but is shared by the *Homo sapiens* NP872628.1, *Pan troglodytes* (XP_513307: 592nd-608th amino acid residue), *Rattus norvegicus* and *Mus musculus* (NP001030602.1, NP001030603.1 and NP598647: 591st-607th amino acid). It has one amino acid mismatch for the *Gallus gallus* (XM_417781: 323rd -339th amino acid). The peptide sequence 5'-QPENNKPPQADAGPDKE-3' was synthesized and used for immunization of rabbits.

Specificity of anti-Kiaa0319L antibody

We evaluated the specificity of Kiaa0319L antiserum harvested from the immunized rabbits using Western blots of mouse brain total lysates. The polyclonal antibody revealed a major band with molecular mass of 140kDa, and two other bands with molecular masses of 190kDa and 160kDa respectively, which are slightly greater than the predicted size of Kiaa0319L protein (115kDa) (Fig. 1C). These bands were absent in the blot that was probed with antibody pre-absorbed with the antigenic peptide (Fig. 1C), supporting that the antibody is specific for the Kiaa0319L protein.

Western blot analyses of Kiaa0319L expression

The expression of Kiaa0319L was examined in whole brain lysates of adult mice. We found no significant difference between the male and female brain ($p>0.05$, Mann-Whitney test) (Fig. 2A,B). Immunoblots of various regions of adult mouse brain showed a widespread expression in the cerebral cortex, cerebellum, hippocampus, diencephalon, brainstem, olfactory bulb and spinal cord (Fig. 2C). Quantitative analyses of the blots from three independent experiments indicated that relative to the expression in the spinal cord (which was the least), the expression was significantly strongest in the olfactory bulb, strong in hippocampus, cerebellum, diencephalon and cortex, and lower in brain stem and spinal cord (Fig. 2D).

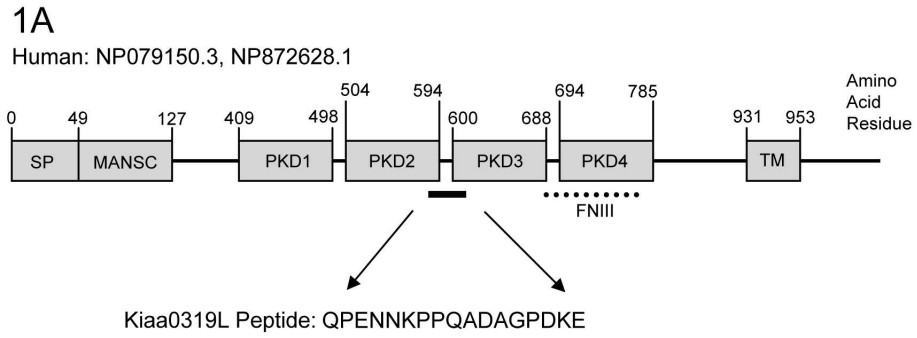
Localization of Kiaa0319L protein in the adult mouse brain

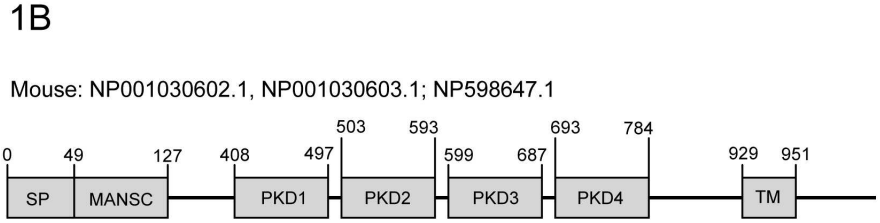
To visualize localization of Kiaa0319L protein in various brain regions, immunohistochemistry was performed on sections of adult mouse brain using our antibody. In parasaggital sections (n=3 animals), the protein was localized widely in different brain regions. The regions most intensely stained include the cerebral cortex, hippocampus, cerebellum, central gray of the midbrain and olfactory bulb (Fig. 3A). In control preparations, no detectable signal was observed in

sections of the brain stained with antibody preabsorbed with antigenic peptide (Fig. 3B) or with the absence of the primary antibody (Fig. 3C), indicating specificity of the staining. Expression of this protein in various cell types were further investigated in the cerebellum, cerebral cortex and hippocampus - regions that are known to be involved in reading tasks (Robichon et al., 2000; Leonard et al., 2001; Eckert et al., 2003; Pernet et al., 2009).

In the cerebellum, strong staining was observed in the Purkinje cell layer and the molecular layer, whilst the granule cell layer only showed a weak signal (Fig. 3D). Under a higher magnification we found widespread staining in the molecular layer, except in some small sized cells (Fig. 3E). Moreover, only a portion of the Purkinje neurons were labeled, whereas the others were devoid of labeling in both the soma and the proximal dendrites. This was confirmed by double staining of the sections with anti-Kiaa0319L and MAP2a+2b antibody. We found colocalization in about half of the Purkinje neurons in the sections examined (Fig. 3F), labeling both cytoplasm and dendrites. The other Purkinje neurons were marked only by the MAP2a+2b antibody, similar to the granular cells.

Kiaa0319L was also found widely in the cerebral cortex of adult mice. Examination of frontal sections of the cortex (n=5) at the striatal level showed distinct labeling of neurons in the cortical layers (Fig. 4A). Kiaa0319L positive neurons were observed largely in layer IV (Fig. 4C) and layer VI (Fig. 4D), whereas only





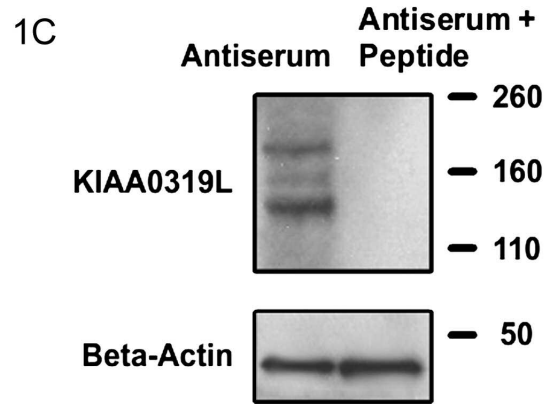
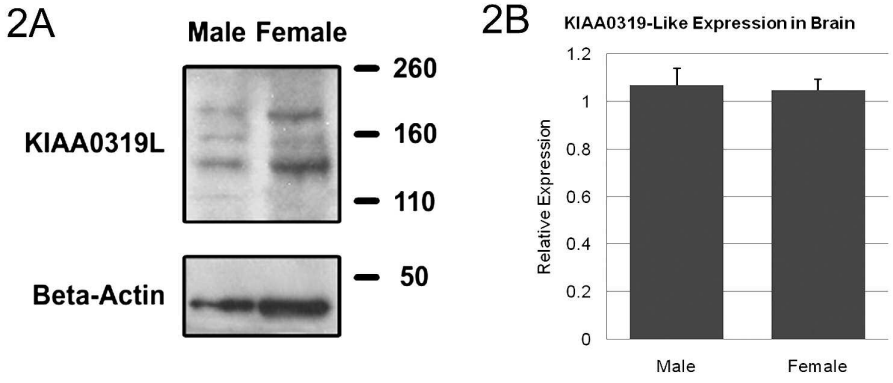
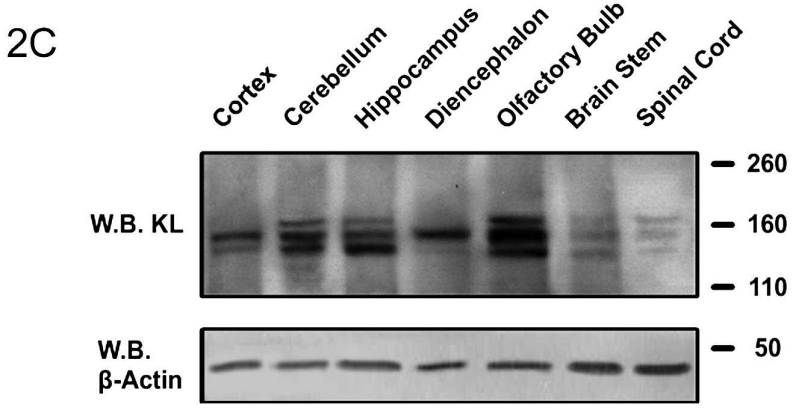


Fig. 1. The human and mouse KIAA0319-like(L) protein isoforms. **A.** Scheme of the human KIAA0319-like encoded protein isoforms NP079150.3 and NP872628.1 from NCBI database. Peptide used to raise polyclonal anti-Kiaa0319L antibody is also indicated. **B.** Scheme of the mouse KIAA0319L encoded protein isoforms NP001030602.1, NP001030603.1 and NP598647.1 from NCBI database. The domains predicted in these sequences are shown: signal peptide (SP), MANSC domain, PKD (1-4) domains and transmembrane (TM) domain. Note the fibronectin (FNIII) domain (dotted line in A) is predicted on the 3rd to 4th PKD domain in human sequence. **C.** Western blot analyses for specificity of rabbit polyclonal anti-Kiaa0319L antibody in C57 mouse brain. Bands sizes of about 190kDa, 160kDa and 140kDa were detected with the anti-Kiaa0319L antibody in the mouse brain lysate on the nitrocellulose membrane blot. These bands were not observed in blot treated with anti-Kiaa0319L pre-absorbed with the antigenic peptide.

a few were found in layer II, III and V. A number of Kiaa0319L negative neurons, characterized by the presence of proximal dendrites, were observed in these three layers (Fig. 4B,C). In Kiaa0319L expressing neurons, the staining was confined to the soma and the nucleus, and occasionally on the proximal dendrites.

In the hippocampus (n=6), Kiaa0319L was present most abundantly in cell bodies of pyramidal neurons in the stratum pyramidale in both CA1 and CA2 subfields (Fig. 5A,B). However, in CA3 only a small fraction of neurons expressed this protein (Fig. 5C). Examination of labeling in CA1 showed that the staining was confined to the soma as well as to the dendrites of pyramidal neurons in the stratum radiatum (Fig. 5A), but not to those in the stratum oriens. This polarized expression pattern was further confirmed in sections of CA1 stained





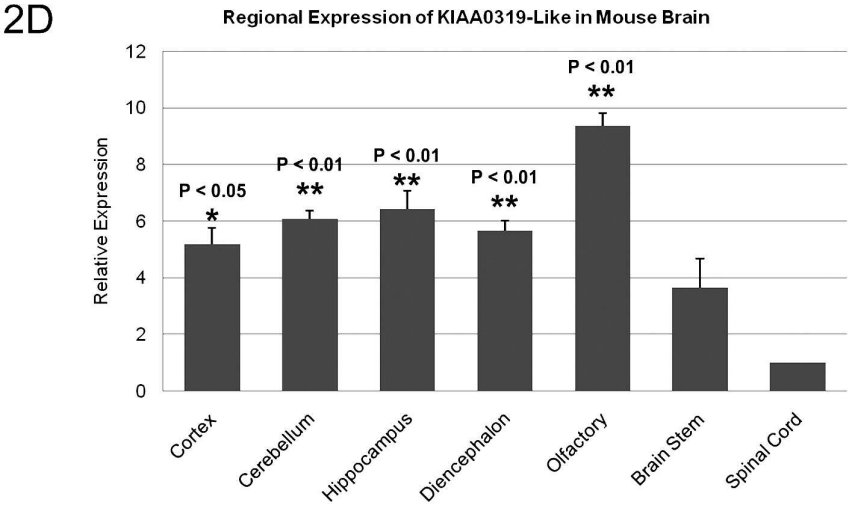


Fig. 2. Expression profiles of Kiaa0319L protein in the adult mouse brain. **A-B.** Western blots of total brain lysates revealed 3 bands of about 140kDa, 160kDa, 190kDa corresponding to Kiaa0319L. Quantitative measurements showed no significant difference ($p>0.05$) in relative expression of Kiaa0319L (ratio of Kiaa0319L/beta-actin) between male and female animals. **C-D.** Analyses of the protein profiles showed expression in different regions of the brain. Measurements of relative expression (ratio of reading in a brain region/that in spinal cord) from 3 independent experiments showed the highest level in olfactory bulb, a high level of expression in hippocampus, cerebelum, diencephalon ($p<0.01$) and cortex ($p<0.05$), and a lowest expression in brain stem, when compared to that of spinal cord. The error bars correspond to the standard error of the means (SEM).

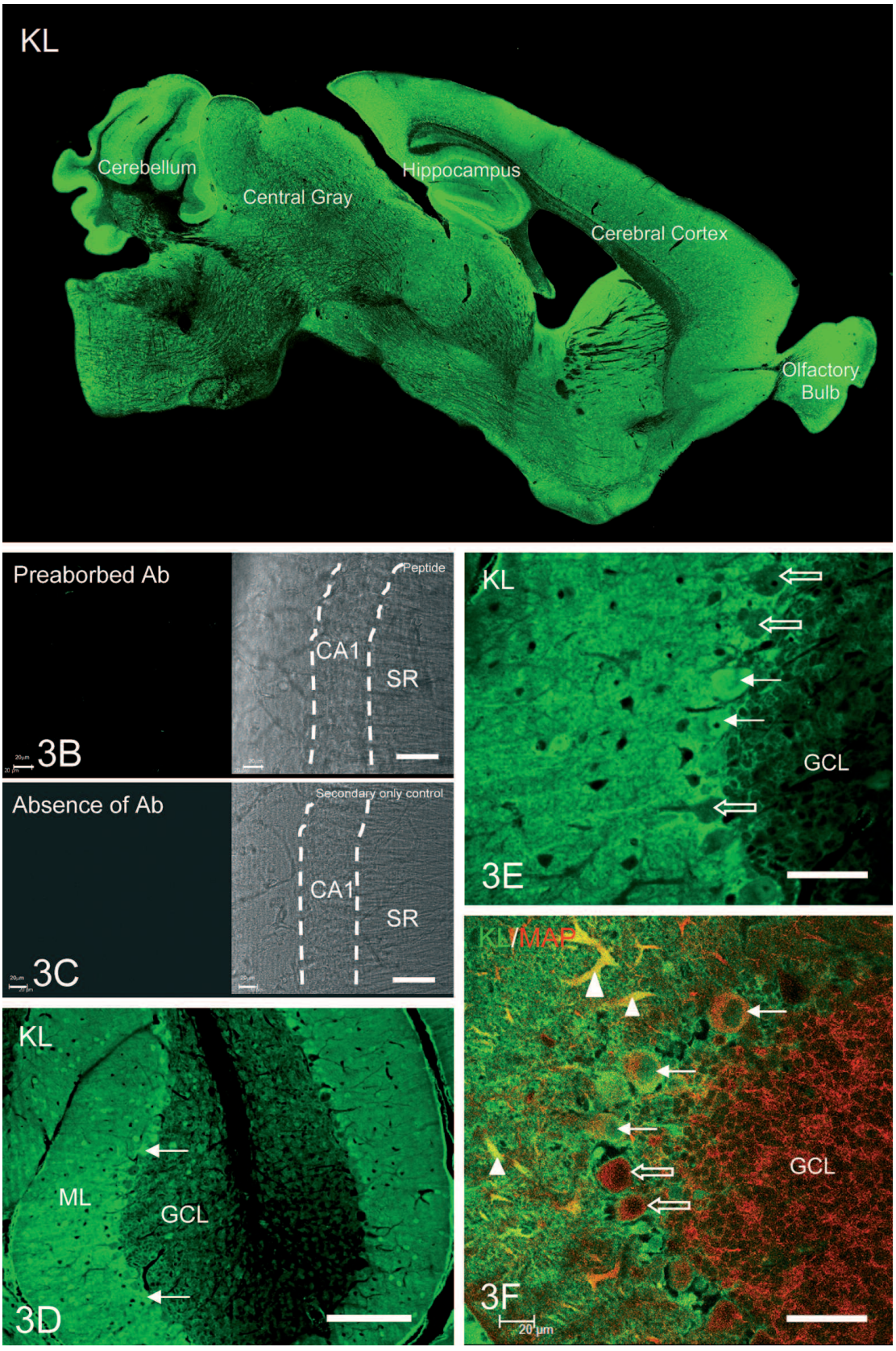


Fig. 3. Localization of Kiaa0319L in the adult mouse brain. **A.** A montage confocal image showing distribution of Kiaa0319L protein in a parasagittal section of the brain. Note that the staining was widespread, with strongest signals at the olfactory bulb, strong signals at the hippocampus, cerebellum, diencephalon and cortex, and lowest signals at brain stem and spinal cord. **B-C.** No detectable signal was observed in sections stained with antigen preabsorbed antibody or absence of the Kiaa0319L antibody. Fluorescence images are on the left; transmitted light image of the same area is on the right. CA1: CA1 subfield of hippocampus; SR: stratum radiatum. **D.** In sagittal sections of the cerebellum, immunostaining was observed largely in the molecular layer (ML) and the Purkinje cell layer (arrows). Only a basal level of staining was detected in the granule cell layer (GCL). **E.** Only a portion of the Purkinje neurons were immunoreactive for Kiaa0319L (solid arrows), others were devoid of any staining (empty arrows). **F.** Double label study showed a colocalization of Kiaa0319L with MAP2a+2b in some Purkinje neurons (solid arrows) and their dendrites (arrowheads) whilst there were a number of neurons that were stained only by MAP2a+2b antibody (empty arrows). Scale bars: A, 1 mm; B-D, 50 μm; E, 25 μm; F, 20 μm.

with Kiaa0319L antibody and TuJ1, which showed colocalization in the soma and proximal segment of dendrites in the stratum radiatum (Fig. 5E). In the dentate gyrus, neurons in the granule cell layer were also labeled with Kiaa0319L antibody but the labeling was confined to the outer part of this cell layer adjacent to the molecular layer; inner parts of the layer were devoid of labeling (Fig. 5D). A number of astrocyte-like cells that are immunoreactive to Kiaa0319L were found in the CA regions and the dentate gyrus (Fig. 5A, C and D). To determine the identity of these cells, hippocampal sections (n=3) were stained with anti-Kiaa0319L and anti-GFAP. Results showed that Kiaa0319L protein was localized in GFAP positive astrocytes that disperse within the stratum oriens and stratum radiatum in the CA1 region, but not in those in the pyramidal neuronal layer (Fig. 5F).

Discussion

In this study, we have characterized localization of Kiaa0319L, a protein associated potentially with reading disability, in various brain regions of adult mice, using a novel antibody raised in rabbit, which binds specifically to this protein. The major findings are: 1) Western blotting showed abundant expression of Kiaa0319L in brain regions that are involved in reading activities, particularly in the cerebral cortex, hippocampus and diencephalon; 2) immunocytochemistry revealed a selective expression in a subpopulation of neurons

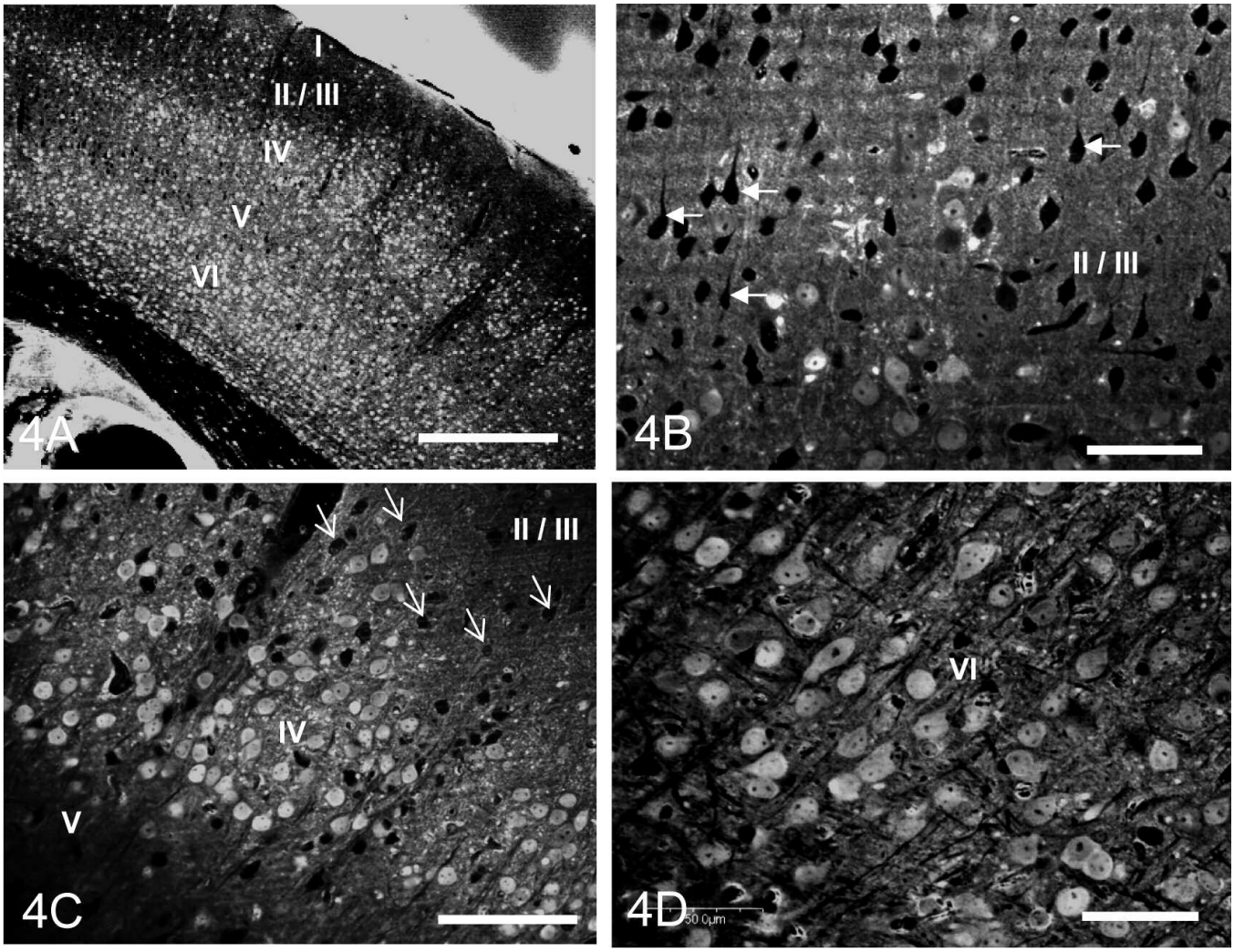


Fig. 4. Localization of Kiaa0319L in the cerebral cortex. **A.** In frontal section of the cerebral cortex, Kiaa0319L was expressed by neurons largely in layer IV and VI. **B.** Only a small number of neurons in layer II and III were labelled by the antibody. Many neurons, characterized by the presence of proximal dendrites, were devoid of labeling (arrows). **C.** Neurons in layer IV were labelled strongly by Kiaa0319L antibody, but those in layer II and III were largely unlabelled (arrows). **D.** The labeling was localized in the cytoplasm and nucleus in layer VI neurons. Scale bars: A, 500 μm; B, D, 50 μm; C, 100 μm.

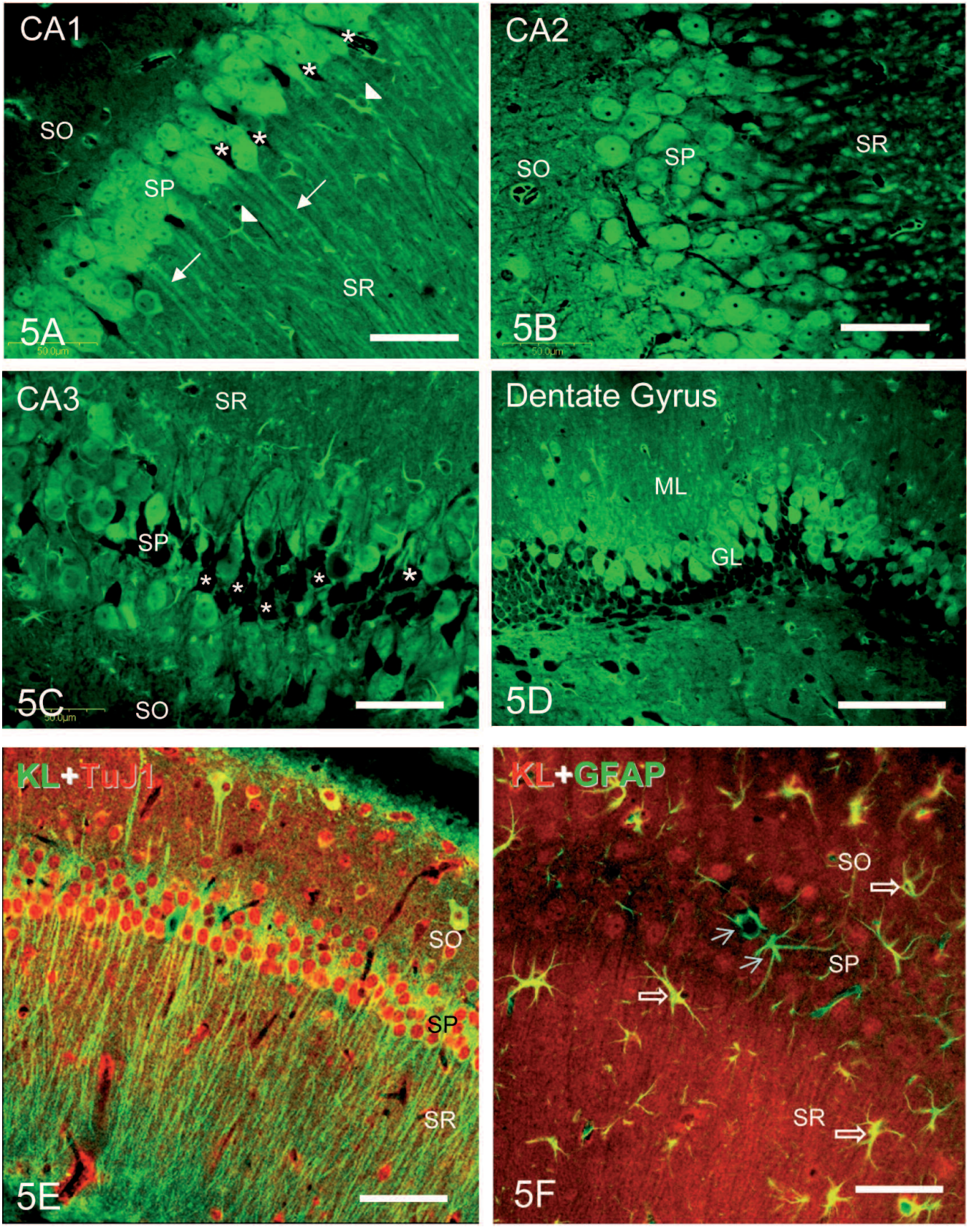

CA1
SO
SP
SR
5A
CA2
SO
SP
SR
5B
CA3
SR
SP
SO
5C
Dentate Gyrus
ML
GL
5D
KL+TuJ1
SO
SP
SR
5E
KL+GFAP
SO
SP
SR
5F

Fig. 5. Expression of Kiaa0319L protein in the mouse hippocampus. **A.** In frontal sections of the hippocampus, intense staining was found in the pyramidal cell layer (SP) in CA1 subfields. The labeling was confined to the soma and dendrites (arrows) in the stratum radiatum (SR), but not in the dendrites in the stratum oriens (SO). Some unlabelled cells were observed in the pyramidal cell layer (asterisks). Labeling was also observed in glial-like cells (arrowheads). **B.** A similar pattern of labeling was found in pyramidal neurons in CA2 subfield. **C.** In CA3, there was an increase in pyramidal neurons that lack Kiaa0319L expression (asterisk). **D.** In the dentate gyrus, the protein was localized on the part of the granule cell layer adjacent to the molecular layer (ML). **E.** Double labelling of CA1 subfield revealed colocalization of Kiaa0319L in the soma and proximal dendrites of pyramidal neurons (marked by TuJ1) in stratum radiatum. **F.** Colocalization studies of the CA1 region showed intense expression of Kiaa0319L in GFAP positive astrocytes (empty arrows) located in the stratum radiatum and stratum oriens. Astrocytes in the pyramidal cell layer were not labelled by the Kiaa0319L antibody (arrows). Scale bars: A-D, F, 50 μm; E, 100 μm.

within a particular brain region; for example, the protein is preferentially expressed in neurons at layer IV and VI of the cerebral cortex, in the molecular and Purkinje cell layer of the cerebellum, and pyramidal neurons in CA1 and CA2 in the hippocampus; 3) the protein is selectively expressed in dendrites of CA1 pyramidal neurons in the stratum radiatum but not the stratum oriens; and 4) the expression is not restricted to neurons but is also present in astrocytes in the hippocampus.

From domain prediction of human, mouse and rat Kiaa0319L, all deduced sequences share the MANSC domain, 4 PKD domain clusters and a transmembrane region at the C terminus. These domain sequences are highly conserved among these species and are similar to the deduced sequence of Kiaa0319 protein. A minor difference is that Kiaa0319 contains 5 PKD domains instead of 4 in Kiaa0319L, and some of its spliced forms lack the transmembrane region (Velayos-Baeza et al., 2007). The MANSC domain is involved in protein binding (Guo et al., 2004) and the PKD domains have been shown to form a homophilic interaction that mediates cell-cell adhesion involving polycystin-1 (Ibraghimov-Beskrovnaya et al., 2000; Streets et al., 2003). It is argued that the MANSC domain and/or PKD domains may mediate interaction with receptor proteins present on other cells, which may underlie migration of neurons along glial processes during early stages of neocortical development (Paracchini et al., 2006; Velayos-Baeza et al., 2007). Since Kiaa0319L shares such a high homology to Kiaa0319 in domain arrangement, it is possible that it may also play a part in the regulation of neuronal migration during early brain development, particularly in the cerebral cortex, hippocampus and olfactory bulb, where a high level of expression is observed. We are currently determining whether this protein is expressed abundantly in these regions during development.

The antibody was raised against the conserved region within the PKD domain clusters of human Kiaa0319L protein, which is identical in sequence to the corresponding regions in the chimpanzee, rat and mouse, suggesting that the antibody should be able to recognize the protein in these species. Western blotting using this antibody shows bands at 140 kDa, 160 kDa and 190 kDa, which is larger than the expected size (115 kDa) of Kiaa0319L. The staining is abolished by pre-absorption of the antibody with antigenic peptide, supporting that this antibody is specific for the Kiaa0319L protein. This is further supported by staining of brain sections with preabsorbed antibody or in the absence of the primary antibody, which reveals no detectable signal in regions that are stained otherwise intensely with the Kiaa0319L antibody. The differences in molecular sizes are unlikely to be caused by polymerization because the increases are not a multiple of the protein size, but are probably caused by glycosylation, which is predicted by the existence of several N- and O- glycosylation sites. Glycosylation is also reported for the dyslexia-associated gene KIAA0319, which has been shown to encode highly N- and O-glycosylated plasma membrane and secreted isoforms (Ohtsubo and Marth, 2006; Velayos-Baeza et al., 2008).

In this study, we have shown that Kiaa0319L is widely expressed in the adult mouse brain, and is particularly enriched in the cerebral cortex, hippocampus, cerebellum, olfactory bulb and diencephalon, and with lower expression in the brain stem and spinal cord. This pattern of expression is similar to that of its homolog Kiaa0319 in adult and embryonic mice, with localization of its transcripts in these regions (Paracchini et al., 2006). A similar pattern of expression was also reported in the human brain using RT-PCR technique (Meng et al., 2005). Expression studies have reported localization of transcripts of other dyslexia associated genes, DCDC2 and DYX1C1, to the cerebral cortex, hippocampus, cerebellum and hypothalamus in the human brain (Taipale et al., 2003; Meng et al., 2005; Schumacher et al., 2005), lending support to the possible contribution of Kiaa0319L in the development of brain regions that relate to reading ability.

Within these brain regions we note that not all neurons express the Kiaa0319L protein, and that this protein is expressed not only in neurons but also in astrocytes. For example, the protein is localized largely in layer IV and VI in the cerebral cortex, but is rare in those at other layers. In the cerebellum, the protein is found in the molecular layer and Purkinje cell layer, but at a basal level in the granule cell layer; within the population of Purkinje neurons, only a portion expresses this protein. Another example is at the hippocampus, where most neurons in the CA1 and CA2 subfields are immunoreactive to Kiaa0319L, but those in CA3 are largely negative. Furthermore, only a fraction of granule

cells in the dentate gyrus expresses Kiaa0319L. These results indicate that Kiaa0319L is not expressed unanimously throughout the population of neurons in these brain regions. Whether it is related to functions specific to a subpopulation of neurons in these regions remains to be elucidated. In addition to a neuronal localization, the protein is also found in astrocytes in the hippocampus. The presence of Kiaa0319L suggests that the protein may be synthesized by the astrocytes, as a glial expression of another dyslexia associated gene DYX1C1is also reported in the human brain (Taipale et al., 2003). However, it is also possible that the protein is released from the surrounding neurons and binds to the surface of the astrocyte, as suggested by the findings in human embryonic kidney 293T cells, that its related homolog, Kiaa0319, exists in several secreted forms resulting from proteolytic cleavages of the extracellular domain (Velayos-Baeza et al., 2010). Another interesting finding is that Kiaa0319L is localized preferentially to dendrites of pyramidal neurons in the stratum radiatum of the CA1 subfield, which could lead to the hypothesis that this protein may be involved in determination and maintenance of polarity and dendritic morphology in these neurons. Similar functions have been reported recently in the rat cerebral cortex transfected embryonically with Kiaa0319 (Peschansky et al., 2010), in which the apical but not basal dendrites exhibit exaggerated growth with knockdown by KIAA0319 shRNA, and that this effect is rescued by overexpression of the protein. Whether KIAA0319L plays similar roles in the hippocampal neurons remains to be determined in future experimentation.

Acknowledgements. This work was partially supported by grants from the Research Grant Council of the Hong Kong Special Administrative Region (Project No. CUHK461709 to SOC) and donation from the Croucher Foundation (to MMYW).

References

Cardon L.R., Smith S.D., Fulker D.W., Kimberling W.J., Pennington B.F. and DeFries J.C. (1994). Quantitative trait locus for reading disability on chromosome 6. Science 266, 276-279.

Chung K.Y., Leung K.M., Lin C.C., Tam K.C., Hao Y.L., Taylor J.S.H. and Chan S.O. (2004). Regionally specific expression of L1 and sialylated NCAM in the retinofugal pathway of mouse embryos. J. Comp. Neurol. 471, 482-498.

Cope N., Harold D., Hill G., Moskvina V., Stevenson J., Holmans P., Owen M.J., O'Donovan M.C. and Williams J. (2005). Strong evidence that KIAA0319 on chromosome 6p is a susceptibility gene for developmental dyslexia. Am. J. Hum. Genet. 76, 581-591.

Couto J.M., Gomez L., Wigg K., Cate-Carter T., Archibald J., Anderson B., Tannock R., Kerr E.N., Lovett M.W., Humphries T. and Barr C.L. (2008). The KIAA0319-like (KIAA0319L) Gene on chromosome 1p34 as a candidate for reading disabilities. J. Neurogenet. 22, 295-313.

Dahdouh F., Anthoni H., Tapia-Páez I., Peyrard-Janvid M., Schulte-Körne G., Warnke A., Remschmidt H., Ziegler A., Kere J., Müller-Myhsok B., Nöthen M.M., Schumacher J. and Zucchelli M. (2009). Further evidence for DYX1C1 as a susceptibility factor for dyslexia. Psychiatric Genet. 19, 59-63.

Eckert M.A., Leonard C.M., Richards T.L., Aylward E.H., Thomson J. and Berninger V.W. (2003). Anatomical correlates of dyslexia: frontal and cerebellar findings. Brain 126, 482-494.

Grigorenko E.L., Wood F.B., Meyer M.S., Hart L.A., Speed W.C., Shuster A. and Pauls D.L. (1997). Susceptibility loci for distinct components of developmental dyslexia on chromosomes 6 and 15. Am. J. Hum. Genet. 60, 27-39.

Grigorenko E.L., Wood F.B., Meyer M.S., Pauls J.E., Hart L.A. and Pauls D.L. (2001). Linkage studies suggest a possible locus for developmental dyslexia on chromosome 1p. Am. J. Med. Genet. 105, 120-129.

Guo J., Chen S., Huang C., Chen L., Studholme D.J., Zhao S. and Yu L. (2004). MANSC: a seven-cysteine-containing domain present in animal membrane and extracellular proteins. Trends Biochem. Sci. 29, 172-174.

Hannula-Jouppi K., Kaminen-Ahola N., Taipale M., Eklund R., Nopola-Hemmi J., Kääriäinen H and Kere J. (2005). The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. PLoS Genet. 1, e50.

Ibraghimov-Beskrovnaya O., Bukanov N.O., Donohue L.C., Dackowski W.R., Klinger K.W. and Landes G.M. (2000). Strong homophilic interactions of the Ig-like domains of polycystin-1, the protein product of an autosomal dominant polycystic kidney disease gene, PKD1. Hum. Mol. Genet. 9, 1641-1649.

Kaplan D.E., Gayán J., Ahn J., Won T.W., Pauls D., Olson R.K., DeFries J.C., Wood F., Pennington B.F., Page G.P., Smith S.D. and Gruen J.R. (2002). Evidence for linkage and association with reading disability on 6p21.3-22. Am. J. Hum. Genet. 70, 1287-1298.

Leonard C.M., Eckert M.A., Lombardino L.J., Oakland T., Kranzler J., Mohr C.M., Wayne M.K. and Freeman A. (2001). Anatomical risk factors for phonological dyslexia. Cerebral Cortex 11, 148-157.

Lin L., Taylor J.S.H. and Chan S.O. (2002). Changes in expression of fibroblast growth factor receptors during development of the mouse retinofugal pathway. J. Comp. Neurol. 451, 22-32.

Meng H., Smith S.D., Hager K., Held M., Liu J., Olson R.K., Pennington B.F., DeFries J.C., Gelernter J., O'Reilly-Pol T., Somlo S., Skudlarski P., Shaywitz S.E., Shaywitz B.A., Marchione K., Wang Y., Paramasivam M., LoTurco J.J., Page G.P. and Gruen J.R. (2005). DCDC2 is associated with reading disability and modulates neuronal development in the brain. Proc. Natl. Acad. Sci. USA 102. 17053-17058.

Ohtsubo K. and Marth J.D. (2006). Glycosylation in cellular mechanisms of health and disease. Cell 126, 855-867.

Paracchini S., Thomas A., Castro S., Lai C., Paramasivam M., Wang Y., Keating B.J., Taylor J.M., Hacking D.F., Scerri T., Francks C., Richardson A.J., Wade-Martins R., Stein J.F., Knight J.C., Copp A.J., Loturco J. and Monaco A.P. (2006). The chromosome 6p22 haplotype associated with dyslexia reduces the expression of KIAA0319, a novel gene involved in neuronal migration. Hum. Mol. Genet. 15, 1659-1666.

Pernet C., Poline J., Demonet J. and Rousselet G. (2009). Brain classification reveals the right cerebellum as the best biomarker of dyslexia. BMC Neurosci. 10, 67.

Peschansky V.J., Burbridge T.J., Volz A.J., Fiondella C., Wissner-Gross Z., Galaburda A.M., Lo Turco J.J. and Rosen G.D. (2010). The effect of variation in expression of the candidate dyslexia

susceptibility gene homolog Kiaa0319 on neuronal migration and dendritic morphology in the rat. Cerebral Cortex 20, 884-897.

Poon M.W., Tsang W.H., Chan S.O., Li H.M., Ng H.K. and Waye M.M.Y. (2011a). Dyslexia-associated kiaa0319-like protein interacts with axon guidance receptor nogo receptor 1. Cell Mol. Neurobiol. 31, 27-35.

Poon M.W., Chan H.L., Lim K.P. and Waye M.M.Y. (2011b). The dyslexia candidate gene kiaa0319L encodes N-glycosylated isoforms that form homo-dimmers. J. Biochem. Mol. Biol. (in press).

Rabin M., Wen X.L., Hepburn M., Lubs H.A., Feldman E. and Duara R. (1993). Suggestive linkage of developmental dyslexia to chromosome 1p34-36. Lancet 342, 178.

Robichon F, Levrier O., Farnarier P. and Habib M. (2000). Developmental dyslexia: atypical cortical asymmetries and functional significance. Eur. J. Neurol. 7, 35-46.

Schumacher J., Anthoni H., Dahdouh F., König I.R., Hillmer A.M., Kluck N., Manthey M., Plume E., Warnke A., Remschmidt H., Hülsmann J., Cichon S., Lindgren C.M., Propping P., Zucchelli M., Ziegler A., Peyrard-Janvid M., Schulte-Körne G., Nöthen M.M. and Kere J. (2005). Strong genetic evidence of DCDC2 as a susceptibility gene for dyslexia. Am. J. Hum. Genet. 78, 52-62.

Shaywitz S.E., Shaywitz G.A., Fletcher J.M. and Estabar M.D. (1990). Prevalence of reading disability in boys and girls. JAMA 264, 998-1002.

Streets A.J., Newby L.J., O'Hare M.J., Bukanov N.O., Ibraghimov-Beskrovnaya O. and Ong A.C. (2003). Functional analysis of PKD1 transgenic lines reveals a direct role for polycystin-1 in mediating cell-cell adhesion. J. Am. Soc. Nephrol. 14, 1804-1815.

Taipale M., Kaminen N., Nopola-Hemmi J., Haltia T., Myllyluoma B., Lyytinen H., Muller K., Kaaranen M., Lindsberg P.J., Hannula-Jouppi K. and Kere J. (2003). A candidate gene for developmental dyslexia encodes a nuclear tetratricopeptide repeat domain protein dynamically regulated in brain. Proc. Natl. Acad. Sci. USA 100, 11553-11558.

Velayos-Baeza A., Toma C., da Roza S., Paracchini S. and Monaco A.P. (2007). Alternative splicing in the dyslexia-associated gene KIAA0319. Mamm. Genome 18, 627-634.

Velayos-Baeza A., Toma C., Paracchini S. and Monaco A.P. (2008). The dyslexia-associated gene KIAA0319 encodes highly N- and O-glycosylated plasma membrane and secreted isoforms. Hum. Mol. Genet. 17, 859-871.

Velayos-Baeza A., Levecque C., Kobayashi K, Holloway Z.G. and Manaco A.P. (2010). The dyslexia-associated KIAA0319 protein undergoes proteolytic processing with {gamma}-secretase-independent intramembrane cleavage. J. Biol. Chem. 51, 40148-40162.

Wang J., Chan C.K., Taylor J.S.H. and Chan S.O. (2008). Localization of Nogo and its receptor in the optic pathway of mouse embryos. J. Neurosci. Res. 86, 1721-1733.

Accepted January 17, 2010