

The chromosome 6p22 haplotype associated with dyslexia reduces the expression of *KIAA0319*, a novel gene involved in neuronal migration

Silvia Paracchini¹, Ankur Thomas², Sandra Castro³, Cecilia Lai^{3,4}, Murugan Paramasivam², Yu Wang², Brendan J. Keating¹, Jennifer M. Taylor¹, Douglas F. Hacking⁵, Thomas Scerri¹, Clyde Francks¹, Alex J. Richardson⁶, Richard Wade-Martins¹, John F. Stein⁶, Julian C. Knight¹, Andrew J. Copp³, Joseph LoTurco² and Anthony P. Monaco^{1,*}

¹Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK,

²Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06268-4156, USA, ³Neural Development Unit, Institute of Child Health, University College London, London WC1N 1EH, UK, ⁴Wolfson Institute for Biomedical Research, University College London, The Cruciform Building, Gower Street, London WC1E 6BT, UK,

⁵Division of Molecular Medicine, Walter and Eliza Hall Institute, 1G, Royal Parade, Parkville, VIC, 3050, Australia and

⁶Department of Physiology, University of Oxford, Parks Road, Oxford OX1 3PT, UK

Received February 14, 2006; Revised and Accepted March 28, 2006

Dyslexia is one of the most prevalent childhood cognitive disorders, affecting ~5% of school-age children. We have recently identified a risk haplotype associated with dyslexia on chromosome 6p22.2 which spans the *TTRAP* gene and portions of *THEM2* and *KIAA0319*. Here we show that in the presence of the risk haplotype, the expression of the *KIAA0319* gene is reduced but the expression of the other two genes remains unaffected. Using *in situ* hybridization, we detect a very distinct expression pattern of the *KIAA0319* gene in the developing cerebral neocortex of mouse and human fetuses. Moreover, interference with rat *Kiaa0319* expression *in utero* leads to impaired neuronal migration in the developing cerebral neocortex. These data suggest a direct link between a specific genetic background and a biological mechanism leading to the development of dyslexia: the risk haplotype on chromosome 6p22.2 down-regulates the *KIAA0319* gene which is required for neuronal migration during the formation of the cerebral neocortex.

INTRODUCTION

Dyslexia [reading disability (RD)] is a specific difficulty with learning to read, in the absence of sensory or neurological impairments and despite an appropriate social, intellectual and educational environment. Although the neurological and cognitive basis of RD are yet to be elucidated, the strong genetic aetiology of this disorder is widely accepted. RD is highly familial and heritable, and most likely results from the interactions between multiple genetic and environmental risk factors (1–3).

Linkage studies have identified several genomic regions that may harbour susceptibility loci for RD including the regions on chromosomes 2, 3, 6, 15 and 18 (1,2). The linkage on chromosome 6p21.3-23 is the most consistently replicated, and is supported by evidence found in five separate samples (1,2,4–9).

We have recently shown that a 77 kb region of strong inter-marker linkage disequilibrium on chromosome 6p22.2 was associated with RD in two large samples of nuclear families from the UK and the US (10). This chromosomal region encompasses the first four exons of the *KIAA0319* gene, the

*To whom correspondence should be addressed. Tel: +44 01865287503; Fax: +44 01865287650; Email: anthony.monaco@well.ox.ac.uk

© The Author 2006. Published by Oxford University Press. All rights reserved.

The online version of this article has been published under an open access model. Users are entitled to use, reproduce, disseminate, or display the open access version of this article for non-commercial purposes provided that: the original authorship is properly and fully attributed; the Journal and Oxford University Press are attributed as the original place of publication with the correct citation details given; if an article is subsequently reproduced or disseminated not in its entirety but only in part or as a derivative work this must be clearly indicated. For commercial re-use, please contact: journals.permissions@oxfordjournals.org

entire *TTRAP* gene and the regulatory sequences of the *THEM2* gene. Another study in a completely independent sample found evidence of association in the same small region (11). We found that three single nucleotide polymorphisms (SNPs; rs4504469, rs2038137 and rs2143340) described the most common haplotypes across the region (10). The 1-1-2 haplotype (where 1 is the major allele and 2 is the minor allele of each SNP) was significantly associated with RD in both the UK and US samples. Mutation screening of all the exons and predicted promoters of the three genes failed to detect any obvious functional mutation on this haplotype that would disrupt any of the three genes.

Here, we test the hypothesis that the 1-1-2 haplotype might influence transcriptional regulation of either *KIAA0319*, *TTRAP* or *THEM2*. We have used matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (12,13) to assess quantitatively relative differences of allele-specific transcripts in cell lines heterozygous for the risk haplotype. We show that the risk haplotype is associated with a reduction of the *KIAA0319* gene expression but does not affect the expression of the other two genes. We also demonstrate by *in situ* hybridization that *KIAA0319* has a very specific spatial-temporal expression pattern during brain development. Furthermore, we show that RNA interference (RNAi) with rat *Kiaa0319* leads to a significant impairment in radial neuronal migration, which is a key step for the formation of the cerebral neocortex.

RESULTS

The RD risk haplotype reduces *KIAA0319* transcription

A panel of 16 lymphoblastoid cell lines was genotyped to identify cell lines heterozygous both for the 1-1-2 haplotype and for markers to be used in the gene expression assay. We selected three cell lines which were both heterozygous for the risk haplotype and for markers located within mRNA transcripts, or in the vicinity of the transcription start site of *KIAA0319*, *TTRAP* and *THEM2* (Supplementary Material, Table S1). GM07031 and GM13051 were heterozygous for at least one marker in each of the three genes, whereas GM11879 was heterozygous only for markers within *TTRAP* and *THEM2* (Supplementary Material, Table S1). We assessed the allele-specific gene expression variations by measuring primer extension products with MALDI-TOF mass spectrometry in a quantitative fashion (12). We used cDNA as template of the primer extension when markers were located within the transcripts. Alternatively, for markers located in proximity of the transcription start sites, we used fragments of chromatin immunoprecipitated (ChIP) with a specific antibody against the phosphorylated RNA polymerase II (Pol II) for the haplotype-specific chromatin immunoprecipitation (haploChIP) assay (13). This method enables Pol II affinity for different haplotypes to be measured, providing an indirect evaluation of allele-specific transcription activity.

The markers genotyped for *TTRAP* and *THEM2* in the three cell lines showed no quantitative differences of allele-specific transcription (Fig. 1D; Supplementary Material, Table S2). The measurements were consistent across different cell lines,

replication experiments and markers, which were tested either using cDNA or ChIP templates (Supplementary Material, Table S2). In contrast, markers rs2038137 and rs2038135, which lie at the beginning of the first intron of *KIAA0319* (Fig. 1A), showed relative allele transcription ratios significantly different from one ($P < 0.01$) in both GM07031 and GM13051 (Fig. 1D; Supplementary Material, Table S2). Specifically, these data, obtained by the analysis of ChIP material, are consistent with a lower Pol II activity on the risk haplotype. As a negative control, we performed the haploChIP assay on three cell lines (GM07047, GM07050, GM07051) that are heterozygous for rs2038137 and rs2038135 but do not carry the risk haplotype. In each of these cell lines, the allele ratios were not significantly different from one, and showed the opposite trend (Fig. 1E; Supplementary Material, Table S2), ruling out the possibility that our original observation was the consequence of an assay artefact. The comparison of the mean allele ratios calculated on all the measurements across all cell lines carrying the risk haplotype ($X_{rs2038137} = 0.57 \pm 0.09$; $X_{rs2038135} = 0.73 \pm 0.1$) versus the mean allele ratios in cell lines without the risk haplotype ($X_{rs2038137} = 1.23 \pm 0.16$; $X_{rs2038135} = 1.29 \pm 0.14$), showed that the two groups of cells were highly significantly different ($P = 7 \times 10^{-16}$, rs2038137; $P = 4 \times 10^{-13}$, rs2038135).

Although we obtained a clear PCR product on ChIP template for fragments containing rs2038137 and rs2038135 in lymphoblastoid cells, indicating that RNA Pol II is binding at the *KIAA0319* promoter, we were unable to detect an RT-PCR product in these cell lines. This inconsistency could be explained, for example, by a very low level of *KIAA0319* expression in lymphoblasts. Therefore, in addition, we genotyped a panel of 13 human neuroblastoma cell lines where the *KIAA0319* transcripts were detectable. Two cell lines, SH-SY5Y and CHP-212, were heterozygous both for the risk haplotype and rs4504469, a *KIAA0319* coding SNP located within the 77 kb genomic region associated with RD. Therefore, it was possible to analyse the rs4504469 SNP using cDNA templates. The allele ratios were significantly different from one in both cell lines ($P = 0.0001$, SH-SY5Y; $P = 0.00004$, CHP-212) indicating again a lower amount of transcript from the risk haplotype compared with non-risk haplotypes (Fig. 1E; Supplementary Material, Table S2). The mean allele ratio across both the cell lines ($X_{rs4504469} = 0.65 \pm 0.13$) was significantly different ($P = 7 \times 10^{-7}$) from the mean allele ratio ($X_{rs4504469} = 1.13 \pm 0.2$) observed in three cell lines (H4, Kelly, SK-N-AS) that were heterozygous for rs4504469 but did not carry the risk haplotype. We observed analogous results when we used a probe for the primer extension reaction targeting the reverse cDNA strand (Fig. 1E). Again, the mean allele ratio in cell lines with the risk haplotype ($X_{rs4504469_r} = 0.59 \pm 0.15$) was significantly different ($P = 0.001$) from the mean observed in cell lines that did not carry the risk haplotype ($X_{rs4504469_r} = 1.04 \pm 0.16$). Therefore, as for the results obtained with markers rs2038137 and rs2038135 in the lymphoblastoid cell lines, the effect on gene expression cannot be detected by simply looking at the individual SNPs but it depends on the haplotypic background.

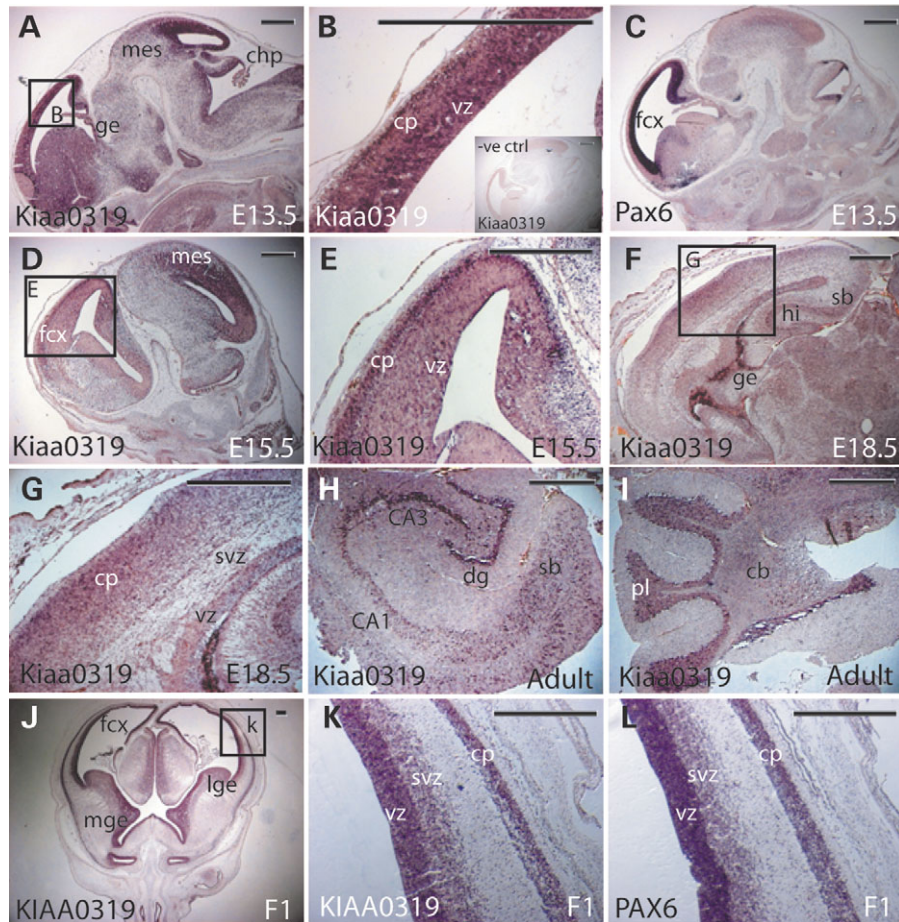


Figure 2. *KIAA0319* is expressed in the cerebral neocortex, ganglionic eminence, hippocampus, midbrain and cerebellum of the developing mouse and human brain. *In situ* hybridization of (A–C) E13.5, (D, E) E15.5, (F, G) E18.5 and (H, I) adult mouse brain and of (J–L) fetal week 1 (57 days post-fertilization) human head. Probes are: (A, B, D–K) *KIAA0319* antisense and (C, L) *Pax6* as a positive control. *KIAA0319* sense-strand negative control (inset in B) shows lack of non-specific hybridization. Boxed areas in (A), (D), (F) and (J) are shown at higher magnification in (B), (E), (G) and (K), respectively. Section planes: (A–E) sagittal or parasagittal; (F, G) coronal; (H–L) transverse. Abbreviations: CA1, CA3, regions of hippocampus; cb, cerebellum; chp, choroid plexus; dg, dentate gyrus; fcx, frontal neocortex; ge, ganglionic eminence; hi, hippocampus; lge, lateral ganglionic eminence; mes, mesencephalon; mge, medial ganglionic eminence; pl, Purkinje cell layer; sb, subiculum. Scale bars: 0.5 mm.

KIAA0319 expression in the developing brain

KIAA0319 has been reported to exhibit relatively specific expression in the brain compared with *TTRAP* and *THEM2*, which have more ubiquitous expression profiles (14) (<http://symatlas.gnf.org/SymAtlas/>). In order to study in greater detail the expression of *KIAA0319* in the developing brain, we performed *in situ* hybridization on sections of mouse and human brains at various developmental stages. In the mouse at embryonic day (E) 13.5, when neuronal migration is underway, low intensity expression of *Kiaa0319* was detected in the frontal neocortex, ganglionic eminence, mesencephalon and cerebellum (Fig. 2A, B). *Pax6* served as a positive control for gene expression in the neocortex (Fig. 2C). A sense-strand negative control probe showed a lack of non-specific hybridization (Fig. 2B, inset). The thalamus that expresses *Pax6* strongly, was not found to express *Kiaa0319* at this stage (data not shown). At E15.5, expression of *Kiaa0319* in the developing mouse cerebral neocortex and mesencephalon was more intense

(Fig. 2D, E) and, by E18.5, when neuronal migration is largely completed (15,16), *Kiaa0319* was expressed prominently in the cortical plate (CP) and in the remnant of the ventricular zone (VZ), with less intense expression in the subventricular zone (SVZ) (Fig. 2F, G). In the adult mouse brain, intense expression of *Kiaa0319* was detected in the CA3 region of the hippocampus and dentate gyrus (Fig. 2H), and in the Purkinje cell layer of the adult mouse cerebellum (Fig. 2I).

In situ hybridization on sections of an early fetal human brain (57 days post-fertilisation) revealed a similar pattern of *KIAA0319* expression as in the mouse fetal brain. Particularly intense expression was seen in the developing cerebral neocortex and ganglionic eminence (Fig. 2J, K), where intensity of *KIAA0319* expression was comparable to that of the positive control *PAX6* (Fig. 2L). At this stage of human development, neuronal migration is well underway in the fetal neocortex and *KIAA0319* expression was prominent in the developing CP and in the VZ, with less intense expression in the SVZ (Fig. 2K).

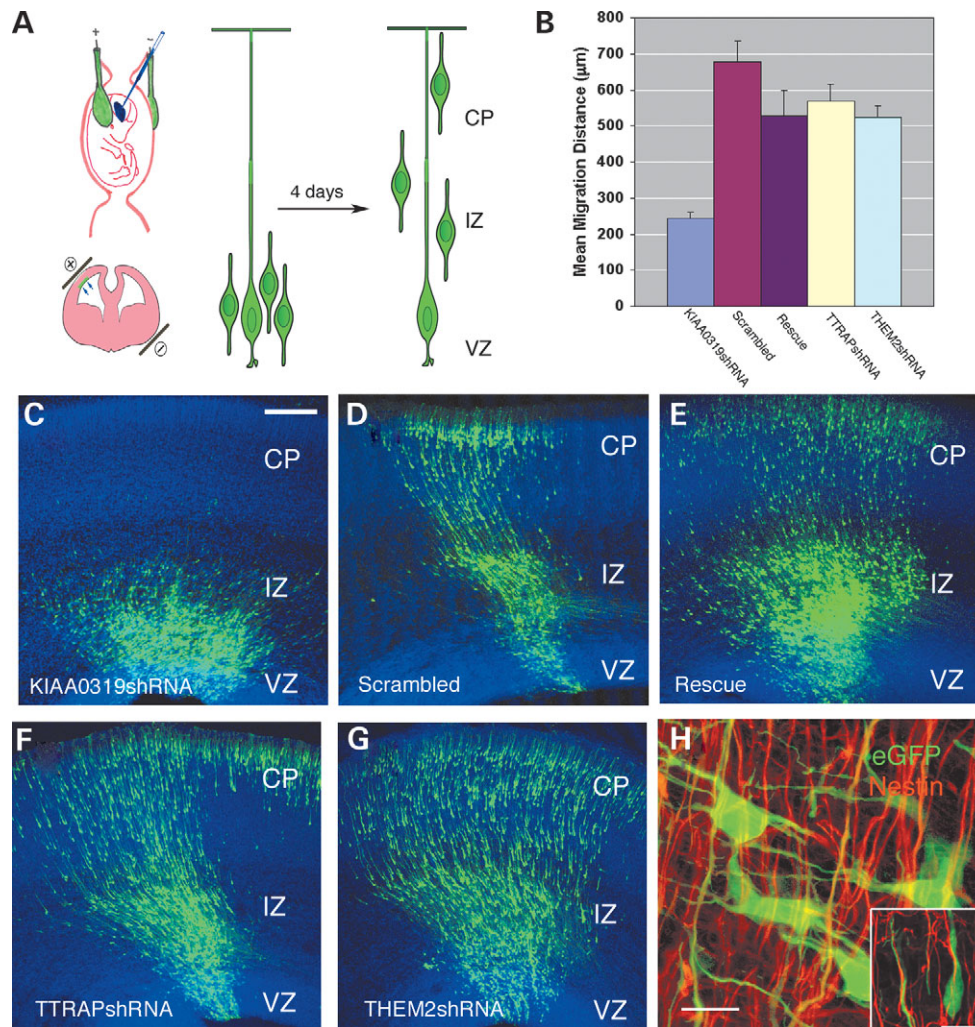


Figure 3. Interference with KIAA0319 disrupts radial migration in the developing rat neocortex. (A) Depiction of *in utero* electroporation assay for neuronal migration in fetal neocortex. Combinations of plasmids coding for shRNAs, eGFP and KIAA0319 were injected into the ventricles of the E14 rat forebrain, in order to transfect neuronal progenitor cells at the VZ surface. Over the course of 4 days, neurons migrated radially towards the IZ and CP. (B) Average distance migrated by neurons 4 days following co-transfection of eGFP and one of the following five plasmids: (i) KIAA0319shRNA; (ii) shRNA containing scrambled sequence of a KIAA0319shRNA vector; (iii) KIAA0319shRNA co-transfected with a KIAA0319 expression plasmid (rescue experiment); (iv) TTRAPshRNA; (v) THEM2shRNA. (C–G) Images showing positions of populations of transfected neurons with one of the five plasmid combinations, as described above. (H) Relationship between radial glial fibres in the IZ labelled with an antibody to nestin (red) and cells transfected with eGFP and KIAA0319shRNA. The inset at the bottom right corner shows typical parallel arrangements between migrating neurons and radial glial fibres. Scale bars: 150 µm in C–G, and 15 µm in H.

In summary, the spatial-temporal expression pattern of *KIAA0319* in the developing mouse and human fetal brain is suggestive of a role in neuronal migration during formation of the cerebral neocortex.

RNAi of *Kiaa0319* disrupts neuronal migration

Our *in situ* hybridization expression data indicated that *KIAA0319* is expressed in the developing cerebral neocortex when neurogenesis and neuronal migration are underway. To test whether *KIAA0319* is involved in neuronal migration we used *in utero* RNAi in developing rat neocortex (Fig. 3A) (17). This method has been used previously to successfully study the cellular roles of genes initially shown to be important for neuronal migration through identification of mutations

that cause neuronal migration disruption and malformation of the human cerebral cortex (17–19).

Plasmids encoding short hairpin RNA (shRNA) directed against the coding sequence of *Kiaa0319* were co-electroporated with an eGFP expression plasmid. Cells are initially transfected and labelled at the VZ surface and then over the course of several days migrate out towards the pial surface. We determined the progress of migration by measuring the distance of eGFP-positive (green fluorescent protein) cells from the VZ surface (Fig. 3A). Similar to shRNA vectors targeting known migration control genes, *LIS1* and *DCX* (18,19), we found that shRNA vectors targeting *Kiaa0319* significantly reduced the distance migrated by neurons (Fig. 3C) that remained within the VZ and intermediate zone (IZ) (Fig. 3C).

In order to test for the specificity of the shRNAs targeting *Kiaa0319*, we co-transfected the shRNA vector with a plasmid encoding rat KIAA0319. Although the transfection of KIAA0319 expression plasmid had no effect on migration when transfected alone (data not shown), it blocked or rescued the impairment in migration caused by transfection of shRNA against *Kiaa0319* (Fig. 3E). The rescue is demonstrated by the presence of eGFP-positive neurons within the CP (Fig. 3E), and indicates that the migration impairment following transfection of shRNA is indeed specific to *Kiaa0319*. Additional evidence for specificity of the shRNA against *Kiaa0319* includes the (i) absence of migration disruption by a shRNA vector with a scrambled hairpin sequence containing the same base composition as one of the *Kiaa0319* shRNA vectors (Fig. 3D), (ii) inhibition of migration by two additional shRNA vectors that target different regions of *Kiaa0319* transcript (not shown) and (iii) absence of migration disruption by shRNA vectors targeting *Them2* and *Ttrap* (Fig. 3F and G). A statistical comparison of migration observed following all transfection conditions (Fig. 3B) showed that *Kiaa0319* shRNA resulted in significantly shorter migration distances from the VZ than all other conditions ($n = 5$, $P < 0.001$).

In addition to reduced migration distance, RNAi of *Kiaa0319* caused a marked change in the normal morphology of migrating neurons. Normally the cellular processes of migrating neurons run parallel and are in close apposition to radial glial fibres (Fig. 3H, inset). In contrast, after RNAi of *Kiaa0319* neurons assumed a largely orthogonal orientation with respect to radial glial fibres in the IZ (Fig. 3H). Migrating neurons are known to migrate along and to be guided by radial glial fibres (20), and the loss of this relationship suggests that *KIAA0319* may be required for appropriate adhesion between migrating neurons and radial glial fibres.

DISCUSSION

We have performed a functional analysis of the rs4504469-rs2038137-rs2143340;1-1-2 haplotype that we previously found to be associated to RD (10). This haplotype covers the entire *TTRAP* gene and portions of the *KIAA0319* and *THEM2* genes. All of our results, using three different approaches, converge to support *KIAA0319* as a strong candidate at the chromosome 6p locus for RD susceptibility. Firstly, we have shown that the relative expression of the *KIAA0319* gene associated with the 1-1-2 risk haplotype is ~40% lower. Second, *in situ* hybridization analysis shows that *KIAA0319* has a distinct spatial-temporal pattern of expression in fetal mouse and human brains. In particular, *KIAA0319* is expressed in the developing cerebral neocortex when neuronal migration takes place. Finally, using *in utero* RNAi we show that *Kiaa0319* expression in neurons is essential for their radially directed migration towards the CP.

Neuronal migration is a key step in the development of the neocortex, which provides the foundation for higher cognitive functions. During radial neuronal migration, newborn neurons move orthogonally from the VZ to the outer surface of the brain along radially oriented glial fibres (21). When *Kiaa0319* expression was suppressed by RNAi, the neurons failed to associate with the glial fibres, disrupting the initiation of the migration process. The predicted KIAA0319 protein

contains four polycystic kidney disease (PKD) domains that have an immunoglobulin-like fold, and were originally found in the PKD1 protein (22). PKD domains have been implicated in cell-cell adhesion processes (23), raising the possibility that the PKD domains of KIAA0319 could mediate the appropriate adhesion between neurons and the glial fibres during neuronal migration. It will be interesting to examine, in future work, whether this putative function of KIAA0319 is mediated through interactions with molecules such as astrotactin and the neuregulin-Erb4 system, which have been shown previously to participate in the adhesion of migrating neurons to radial glia (24).

Irregularities during neuronal migration have been proposed as a neurological mechanism implicated in the development of RD. The most significant contribution to the neurology of RD was the anatomical study of post-mortem brains of dyslexic individuals (25,26). Although limited to a small number of cases, this analysis showed that brains from dyslexic cases differ from control brains by the presence of cortical anomalies that resulted from abnormal neuronal migration during cortical development. In another study, subjects affected by periventricular nodular heteropia, a neuronal migration disorder, were found to be impaired in their reading skills although of otherwise normal intelligence (27). Further evidence comes from a very recent study in which dyslexic and control individuals, who had been previously analysed by positron emission tomography (PET) activation (28), also underwent brain morphological analysis (29). The study found that the cortical regions, which showed altered activation during reading tasks in the dyslexic individuals, carried structural abnormalities consistent with defective neuronal migration.

The 1-1-2 haplotype is likely to carry one or more variants responsible for down-regulation of *KIAA0319* expression. The penetrance of an aetiological variant of this type (in contrast to coding mutations, for example) is expected to be low. Indeed, in our association analysis we found that the minor allele of rs2143340 that effectively tags the 1-1-2 haplotype, had an increase of frequency (up to 28%) only in the most severe dyslexic cases, whereas it showed the same frequency of 16% in the complete set of dyslexic probands as in a control population (10). It is likely that the reduced expression of *KIAA0319* is not sufficient by itself to cause RD but that it has an impact on reading abilities only when combined with other genetic or environmental factors. Very recently, two other genes have been suggested as candidates for RD and both of them seem to play a role in the brain development (30,31). The *DCDC2* gene that contains a double-cortin homology domain has been shown as well to be involved in neuronal migration using *in utero* RNAi (31). The *ROBO1* gene (30) is a neuronal axon guidance receptor whose orthologue in *Drosophila*, the *robo* gene, has been implicated in axon and dendritic guidance across the midline (32). It is possible that the *KIAA0319* risk haplotype has an impact on reading abilities when combined with other functional variants in one of these or other genes.

In conclusion, our data show for the first time, a direct link between a specific genetic background and a biological mechanism in the neurological causation of RD. Studying other genes and proteins that interact with *KIAA0319*, or are involved in the neuronal migration process, might lead to the discovery of other components responsible for RD and

enable a better understanding of the molecular mechanisms underlying the development of cognitive functions.

MATERIALS AND METHODS

Genotyping and cell culture

We selected a panel of 16 lymphoblastoid cell lines from the Centre d'Etude du Polymorphisme Humain (CEPH) and 13 human neuroblastoma cell lines for genotyping purposes. We genotyped them for the three tagging SNPs across the region associated with RD, to identify cell lines heterozygous for the 1-1-2 haplotype. We classified the three marker haplotypes as in our collection of RD families (10). The selected lymphoblastoid cell lines were genotyped for additional 25 markers in order to identify heterozygous markers to be used in the gene expression assay. All primer sequences are available on request. We established which alleles were on the risk haplotype using the sequence of the human BAC clone RP11-195J19 as reference that completely covers our locus of interest and carries the 1-1-2 haplotype. Comparison of the lymphoblast and BAC genotypes showed a very high level of sequence identity confirming that the selected cell lines do carry the risk haplotype and not rare haplotypes. (Supplementary Material, Table S1).

Cell maintenance, preparation of cDNA and ChIP from the lymphoblastoid cell lines was as described previously except that no caesium chloride purification step was used for chromatin preparation (13,33). HaploChIP was performed as previously described using antibody versus the phosphorylated serine residues of the C-terminal domain of Pol II (Ser5, MMS-134R clone H14; Covance) (13). The neuroblastoma cell lines SH-SY5Y, CHP-212, H4, Kelly and SK-N-AS were grown according to the ECACC guidelines. We isolated total RNA using Trizol (Invitrogen) and the RNeasy kit (Qiagen). We prepared cDNA using random decamers and SuperScript™ III RNA transcriptase (Invitrogen).

Quantitative allele-specific gene expression assay

We assess quantitative allele-specific gene expression differences by using mass spectrometry (12,13). The assay included three main steps: PCR amplification, primer extension reaction and mass spectrometry analysis. The PCR was carried out on 20 ng of cDNA or ChIP extracted from ~5000 cells, depending on the marker location. The final volume of 10 μ l PCR reaction included 0.5 U Immolase *Taq* (Bioline), 0.8 mM dNTPs, 2 mM MgCl₂ and 0.2 M each primer. Conditions for the PCR were 95°C for 7 min; 14 cycles of 94°C for 30 s, 62–55°C for 30 s, 72°C for 30 s, decreasing the annealing temperature 0.5°C at each cycle; 20 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s; 72°C for 7 min. Excess of primer was removed by exonuclease I treatment. Each sample was amplified in four independent reactions and then spotted twice on a 384-well plate, leading to eight measures per sample in each experiment. Primer extension reaction and mass spectrometry analysis were performed using the MassARRAY (Sequenom) platform following the manufacturer's instruction. All primer sequences are available on request. To check for the reproducibility of the assay, we obtained two or three independent biological

replicates for the lymphoblastoid cell lines GM07031 and GM13051, respectively. The assays for the *KIAA0319* markers were repeated up to 16 times using cDNA and ChIP extracted from at least two independent cell cultures. Allele ratios in the transcript were normalized using genomic DNA (where the allele ratio is expected to be 1:1) from the same cell line. The results are given as mean \pm standard deviation (s.d.) and were calculated across all the measurements for each cell line. The allele ratios were observed to approximate a normal distribution and therefore, the significance of the result was determined using one-sample *t*-test.

RNA *in situ* hybridization

The expression pattern of *KIAA0319* was investigated by non-radioactive RNA *in situ* hybridization on paraffin wax sections, as described earlier (34). Random-bred CD1 mice provided a source of mouse fetuses (E13.5, E15.5 and E18.5) and dissected adult mouse brain. Human fetal material (stage F1; 57 days post-fertilization) was obtained from the Medical Research Council/Wellcome Trust Human Developmental Biology Resource, with full ethical approval. To produce a hybridization probe, the coding region of *Kiaa0319* was amplified by PCR using cDNA from E15 CD1 whole mouse fetuses. Primers were: (5'-3') GGGCC AAATCAGACCATCAC and (3'-5') GCTATGGCTTTGTC CACATTC, generating a 437 bp product corresponding to nucleotides 1881–2317 of mouse *Kiaa0319* mRNA (NCBI accession number: AK122246). PCR product was cloned into pGEMT-easy vector, and the insert sequence and orientation were confirmed by direct sequencing. The construct was linearized with *Sac*II and *Spe*I, transcribed with SP6 and T7 RNA polymerases to synthesize digoxigenin-labelled sense and antisense riboprobes, respectively. The Pax6 probe for *in situ* hybridization was as described earlier (35).

In utero RNAi

In utero RNAi methods were applied to fetal rats as described earlier (Fig. 3A) (17). An electroporation pulse was applied across the uterus to transfect a sub-population of cells at the VZ surface of E14 rat fetal dorsal forebrain. The embryonic neocortex was processed for confocal microscopy and the distance migrated by neurons was quantified by measuring the distance from the VZ surface to the soma of the transfected cells. A migration index is computed for each brain based on the average of cellular distances. Migration distances were determined with automated particle analyses in ImageJ. Ten shRNA vectors were used in the present study: three designed against *Kiaa0319*, three against *Them2*, three against *Ttrap* and a scrambled control vector containing the same bases as one of the *KIAA0319*shRNA plasmid but in different order. All vectors were co-transfected with 0.5 μ g of eGFP expression plasmid. For the rescue experiments 1.0 μ g *KIAA0319*shRNA was co-transfected with 3.0 μ g of pCAKIAA0319, a *KIAA0319* expression plasmid. The pCAKIAA0319 plasmid was produced by amplifying *Kiaa0319* cDNA by RT-PCR of total RNA isolated from E14 rat brain (primers; ATGGTGTCCCCACCAGGAGTAC and TTATCTGTCTTTGAGTAATAACCA). Amplicons were first cloned into

pGEMT-Easy, and then further sub-cloned into EcoRI and BglII sites of pCAGGS. Test for statistical significance of differences in migration were made of the average migration distance for different transfection conditions by ANOVA. At least five different brains and six sections were used for each condition.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

ACKNOWLEDGEMENTS

We thank Tara Caffrey for assistance in tissue culture and Emma Banfield for help with statistical analysis. This work was supported by grants to A.P.M. from the Wellcome Trust. M.P., Y.W. and J.L. are supported by grants from NIH; S.C. and A.C. are supported by the Wellcome Trust and Medical Research Council. Funding to pay the Open Access publication charges for this article was provided by the Wellcome Trust.

Conflict of Interest statement. The authors declare they have no conflict of interest.

REFERENCES

- Fisher, S.E., Francks, C., Marlow, A.J., MacPhie, I.L., Newbury, D.F., Cardon, L.R., Ishikawa-Brush, Y., Richardson, A.J., Talcott, J.B., Gayan, J. *et al.* (2002) Independent genome-wide scans identify a chromosome 18 quantitative-trait locus influencing dyslexia. *Nat. Genet.*, **30**, 86–91.
- Fisher, S.E. and DeFries, J.C. (2002) Developmental dyslexia: genetic dissection of a complex cognitive trait. *Nat. Rev. Neurosci.*, **3**, 767–780.
- DeFries, J.C., Fulker, D.W. and LaBuda, M.C. (1987) Evidence for a genetic aetiology in reading disability of twins. *Nature*, **329**, 537–539.
- 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.
- Cardon, L.R., Smith, S.D., Fulker, D.W., Kimberling, W.J., Pennington, B.F. and DeFries, J.C. (1995) Quantitative trait locus for reading disability: correction. *Science*, **268**, 1553.
- Fisher, S.E., Marlow, A.J., Lamb, J., Maestrini, E., Williams, D.F., Richardson, A.J., Weeks, D.E., Stein, J.F. and Monaco, A.P. (1999) A quantitative-trait locus on chromosome 6p influences different aspects of developmental dyslexia. *Am. J. Hum. Genet.*, **64**, 146–156.
- Gayan, J., Smith, S.D., Cherny, S.S., Cardon, L.R., Fulker, D.W., Brower, A.M., Olson, R.K., Pennington, B.F. and DeFries, J.C. (1999) Quantitative-trait locus for specific language and reading deficits on chromosome 6p. *Am. J. Hum. Genet.*, **64**, 157–164.
- 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. and Pauls, D.L. (2000) Chromosome 6p influences on different dyslexia-related cognitive processes: further confirmation. *Am. J. Hum. Genet.*, **66**, 715–723.
- Francks, C., Paracchini, S., Smith, S.D., Richardson, A.J., Scerri, T.S., Cardon, L.R., Marlow, A.J., MacPhie, I.L., Walter, J., Pennington, B.F. *et al.* (2004) A 77-kilobase region of chromosome 6p22.2 is associated with dyslexia in families from the United Kingdom and from the United States. *Am. J. Hum. Genet.*, **75**, 1046–1058.
- 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.
- Ding, C. and Cantor, C.R. (2003) A high-throughput gene expression analysis technique using competitive PCR and matrix-assisted laser desorption/ionization time-of-flight MS. *Proc. Natl Acad. Sci. USA*, **100**, 3059–3064.
- Knight, J.C., Keating, B.J., Rockett, K.A. and Kwiatkowski, D.P. (2003) *In vivo* characterization of regulatory polymorphisms by allele-specific quantification of RNA polymerase loading. *Nat. Genet.*, **33**, 469–475.
- Londin, E.R., Meng, H. and Gruen, J.R. (2003) A transcription map of the 6p22.3 reading disability locus identifying candidate genes. *BMC Genomics*, **4**, 25.
- Angevine, J.B. and Sidman, R.L. (1961) Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature*, **192**, 766–768.
- Caviness, V.S., Jr (1982) Neocortical histogenesis in normal and reeler mice: a developmental study based upon [3H]thymidine autoradiography. *Brain Res.*, **256**, 293–302.
- Bai, J., Ramos, R.L., Ackman, J.B., Thomas, A.M., Lee, R.V. and LoTurco, J.J. (2003) RNAi reveals doublecortin is required for radial migration in rat neocortex. *Nat. Neurosci.*, **6**, 1277–1283.
- Pilz, D.T., Matsumoto, N., Minnerath, S., Mills, P., Gleeson, J.G., Allen, K.M., Walsh, C.A., Barkovich, A.J., Dobyns, W.B., Ledbetter, D.H. *et al.* (1998) LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation. *Hum. Mol. Genet.*, **7**, 2029–2037.
- Tsai, J.W., Chen, Y., Kriegstein, A.R. and Vallee, R.B. (2005) LIS1 RNA interference blocks neural stem cell division, morphogenesis, and motility at multiple stages. *J. Cell. Biol.*, **170**, 935–945.
- Nadarajah, B. and Parnavelas, J.G. (2002) Modes of neuronal migration in the developing cerebral cortex. *Nat. Rev. Neurosci.*, **3**, 423–432.
- Hatten, M.E. (1999) Central nervous system neuronal migration. *Annu. Rev. Neurosci.*, **22**, 511–539.
- Bycroft, M., Bateman, A., Clarke, J., Hamill, S.J., Sandford, R., Thomas, R.L. and Chothia, C. (1999) The structure of a PKD domain from polycystin-1: implications for polycystic kidney disease. *EMBO J.*, **18**, 297–305.
- 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.
- Hatten, M.E. (2002) New directions in neuronal migration. *Science*, **297**, 1660–1663.
- Galaburda, A.M. and Kemper, T.L. (1979) Cytoarchitectonic abnormalities in developmental dyslexia: a case study. *Ann. Neurol.*, **6**, 94–100.
- Galaburda, A.M., Sherman, G.F., Rosen, G.D., Aboitiz, F. and Geschwind, N. (1985) Developmental dyslexia: four consecutive patients with cortical anomalies. *Ann. Neurol.*, **18**, 222–233.
- Chang, B.S., Ly, J., Appignani, B., Bodell, A., Apse, K.A., Ravenscroft, R.S., Sheen, V.L., Doherty, M.J., Hackney, D.B., O'Connor, M. *et al.* (2005) Reading impairment in the neuronal migration disorder of periventricular nodular heterotopia. *Neurology*, **64**, 799–803.
- Brunswick, N., Cappa, S.F., Cossu, G., Habib, M., Frith, C.D. *et al.* (2001) Dyslexia: cultural diversity and biological unity. *Science*, **291**, 2165–2167.
- Silani, G., Frith, U., Demonet, J.F., Fazio, F., Perani, D., Price, C., Frith, C.D. and Paulesu, E. (2005) Brain abnormalities underlying altered activation in dyslexia: a voxel based morphometry study. *Brain*, **128**, 2453–2461.
- Hannula-Jouppi, K., Kaminen-Ahola, N., Taipale, M., Eklund, R., Nopola-Hemmi, J., Kaariainen, H. and Kere, J. (2005) The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. *PLoS Genet.*, **1**, e50.
- 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. *et al.* (2005) DCDC2 is associated with reading disability and modulates neuronal development in the brain. *Proc. Natl Acad. Sci. USA*, **102**, 17053–17058.
- Kidd, T., Brose, K., Mitchell, K.J., Fetter, R.D., Tessier-Lavigne, M., Goodman, C.S. and Tear, G. (1998) Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. *Cell*, **92**, 205–215.
- Knight, J.C., Keating, B.J. and Kwiatkowski, D.P. (2004) Allele-specific repression of lymphotoxin-alpha by activated B cell factor-1. *Nat. Genet.*, **36**, 394–399.
- Lai, C.S., Gerrelli, D., Monaco, A.P., Fisher, S.E. and Copp, A.J. (2003) FOXP2 expression during brain development coincides with adult sites of pathology in a severe speech and language disorder. *Brain*, **126**, 2455–2462.
- Walther, C. and Gruss, P. (1991) Pax-6, a murine paired box gene, is expressed in the developing CNS. *Development*, **113**, 1435–1449.