NIH Public Access
Author Manuscript
Neuroscience. Author manuscript; available in PMC 2014 October 15.

Published in final edited form as:
Neuroscience. 2013 September 17; 0: 585–593. doi:10.1016/j.neuroscience.2013.06.056.

Embryonic disruption of the candidate dyslexia susceptibility gene homologue Kiaa0319-like results in neuronal migration disorders

MP Platt[1,*], **WT Adler**[1,*], **AJ Mehlhorn**[1,*], **GC Johnson**[1], **KA Wright**[1], **RT Choi**[2], **WH Tsang**[2], **MW Poon**[2], **SY Yeung**[2], **MMY Waye**[2], **AM Galaburda**[1], and **GD Rosen**[1]

[1]Department of Neurology, Beth Israel Deaconess Medical Center, Boston, MA, USA

[2]School of Biomedical Sciences & School of Life Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong, China

Abstract

Developmental dyslexia, the most common childhood learning disorder, is highly heritable, and recent studies have identified *KIAA0319-Like* (*KIAA0319L*) as a candidate dyslexia susceptibility gene at the 1p36-34 (DYX8) locus. In this experiment, we investigated the anatomical effects of knocking down this gene during rat corticogenesis. Cortical progenitor cells were transfected using *in utero* electroporation on embryonic day (E) 15.5 with plasmids encoding either: (1) *Kiaa0319l* shRNA, (2) an expression construct for human *KIAA0319L*, (3) *Kiaa0319l* shRNA + KIAA0319L expression construct (rescue), or (4) controls (scrambled *Kiaa0319l* shRNA or empty expression vector). Mothers were injected with BrdU at either E13.5, E15.5, or E17.5. Disruption of Kiaa0319l function (by knockdown, overexpression, or rescue) resulted in the formation of large nodular periventricular heterotopia in approximately 25% of the rats, and these heterotopia can be seen as early as postnatal day 1. Only a small subset of heterotopic neurons had been transfected, indicating non-cell autonomous effects of the transfection. Most heterotopic neurons were generated in mid- to late-gestation, and laminar markers suggest that they were destined for upper cortical laminæ. Finally, we found that transfected neurons in the cerebral cortex were located in their expected laminæ. These results indicate that *KIAA0319L* is the fourth of four candidate dyslexia susceptibility genes that is involved in neuronal migration, which supports the association of abnormal neuronal migration with developmental dyslexia.

Keywords

Developmental Dyslexia; Neuronal Migration; Cerebral cortex; *In Utero* Electroporation; RNAi

---

© 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

Corresponding Author: Glenn D. Rosen, Department of Neurology, E/CLS-643, Beth Israel Deaconess Medical Center, 330 Brookline Ave., Boston, MA 02215, grosen@bidmc.harvard.edu, P: +1-617-735-2871, F: +1-617-735-2870.
*Authors contributed equally to this manuscript

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

1. INTRODUCTION

Developmental dyslexia, the most common childhood learning disorder, is highly heritable (Peterson and Pennington, 2012). At present, nine loci have been associated with developmental dyslexia, including those on Chromosomes (Chrs) 1p36-34 (DYX8), 2p16 (DYX3), 3p13-11q (DYX5), 6q11-16 (DYX4), 6p21-22 (DYX2), 11p15 (DYX7), 12p14, 15q21 (DYX1), 18p11-q12 (DYX6) (see Scerri and Schulte-Korne, 2010, Skiba et al., 2011, Peterson and Pennington, 2012 for reviews). The DYX8 region on Chrs 1p34-36 was originally identified by linkage analysis (Grigorenko et al., 2001), and was subsequently confirmed in a set of 100 families (Tzenova et al., 2004). A candidate gene, *KIAA0319-Like* (*KIAA0319L)*, was identified in this interval, which has shown strong associations with deficits in spelling, phonemic awareness, rapid naming of colors and objects, single-word reading, and lifetime dyslexia diagnosis (Couto et al., 2008). This gene is of particular interest because of its similarity to *KIAA0319*, a candidate dyslexia susceptibility gene (CDSG) previously identified at the DYX2 locus (Francks et al., 2004, Cope et al., 2005, Harold et al., 2006, Paracchini et al., 2006, Paracchini et al., 2008, Zou et al., 2012).

Studies of post-mortem dyslexic brains revealed that these brains had cerebrocortical neuronal migration disorders ranging from small heterotopia to focal microgyria (Galaburda and Kemper, 1979, Galaburda et al., 1985, Humphreys et al., 1990). More recently, MRI studies have found an association between more severe neuronal migration anomalies (periventricular nodular heterotopia) and developmental dyslexia (Chang et al., 2005, Chang et al., 2007). Further, all CDSGs thus far examined have been shown to function as neuronal migration genes, including *DYX1C1* (Wang et al., 2006), *DCDC2* (Meng et al., 2005), *ROBO1* (Hannula-Jouppi et al., 2005, Andrews et al., 2006, Andrews et al., 2008, Gonda et al., 2012) and *KIAA0319* (Paracchini et al., 2006). Specifically, embryonic knockdown of CDSG homologue function in rats using interference RNA (RNAi) disrupts neuronal migration, as evidenced by the presence of white matter heterotopia (*Dyx1c1* and *Kiaa0319*) and “overmigration” of neurons past their expected laminar location in the cerebral cortex (*Dyx1c1* and *Dcdc2*, Wang et al., 2006, Rosen et al., 2007, Burbridge et al., 2008, Peschansky et al., 2010, Currier et al., 2011). Taken together, these results suggest that neuronal migration errors may contribute to the developmental dyslexia phenotype.

Although the specific function of *KIAA0319L* is unknown, it has been shown to interact with Nogo receptor 1, an axon guidance protein (Poon et al., 2011a) that is similar to *ROBO1*, a CDSG on the DYX5 locus (Nopola-Hemmi et al., 2001, Hannula-Jouppi et al., 2005), which has been shown to modulate axon guidance and neuronal migration (Wong et al., 2001, Hivert et al., 2002, Gonda et al., 2012). In the mouse brain, *Kiaa0319l* is expressed in both astrocytes and neurons, most strongly in the olfactory bulb, hippocampus, and neocortex (Poon et al., 2011b). Because of its similarity to *KIAA0319*, we investigated this gene’s function using *in utero* electroporation to transfect a subset of embryonic neuronal progenitor cells with plasmids containing either small hairpin RNA (shRNA) targeted against *Kiaa0319l* or expression constructs *in vivo*. We found that disrupting *Kiaa0319l* function interferes with neuronal migration leading to the formation of periventricular nodular heterotopia. These heterotopia contain late generated neurons destined for the upper neocortical laminæ. Interestingly, they also contain large numbers of untransfected neurons,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

some of which are GABAergic, suggesting that non-cell autonomous effects, too, are involved in the formation of these heterotopia.

2. EXPERIMENTAL PROCEDURES

2.1. *In utero* electroporation

All procedures were approved by the Institutional Animal Care and Use Committee at Beth Israel Deaconess Medical Center. Pregnant Wistar rats (Charles River, Wilmington, MA, USA) were assigned to one of three experimental conditions: *Kiaa0319l* shRNA, KIAA0319L expression, or rescue (*Kiaa0319l* shRNA + KIAA0319L expression). Within each litter, pups randomly received an experimental treatment or a control electroporation (a scrambled version of the *Kiaa0319l* shRNA, empty expression construct, or *Kiaa0319l* shRNA respectively). *In utero* electroporations were performed at embryonic day (E) 15.5 as previously described (Bai et al., 2003; Burbridge et al., 2008; Peschansky et al., 2009). Experimental constructs were co-transfected with mRFP, while the control constructs were co-transfected with eGFP. The concentrations of enhanced green fluorescent protein (eGFP) and monomeric red fluorescent protein (mRFP) plasmids were 0.75 μg/μL, and the shRNA and expression construct concentrations used were 1.5 μg/μL.

2.2. Plasmids

For the *Kiaa0319l* shRNA condition, plasmids encoding shRNA (prKLshr4) and plasmids encoding mRFP (pCAGGS-RFP) were co-transfected. Littermates were co-transfected with a plasmid encoding a scrambled version of the shRNA (pKLsh1 Scram) along with a plasmid encoding eGFP (pCAGGS-eGFP). In the expression condition, pups were co-transfected with plasmid encoding human KIAA0319L (PWP1KL) and mRFP, while their littermates were co-transfected with an empty version of the expression construct (PWP1) and eGFP. In the rescue condition, subjects were co-transfected with *Kiaa0319l* shRNA, the KIAA0319L expression construct, and mRFP plasmids, while their littermates received the *Kiaa0319l* shRNA and eGFP plasmids. Previous research indicates that co-transfection is highly efficient (Rosen et al., 2007).

2.3. BrdU injection

Pregnant rats were anesthetized with isoflurane (5%) and i.p. injected with 50 mg/kg of 5-bromo-2-deoxyuridine (BrdU; Sigma Aldrich, St Louis, MO, USA, 10 mg/ml solution) at either E13.5, E15.5, or E17.5.

2.4. Histology

Animals were sacrificed at embryonic day E19.5 or postnatal day (P) 1, P5, P10, or P21. P10 and P21 rats were deeply anesthetized (Ketamine/Xylazine 10:1, 100 mg/ml), sacrificed, and fixed by transcardial perfusion with 0.9% saline followed by 4% paraformaldehyde (PFA). Brains were extracted, post-fixed in PFA for 24 h, and cryoprotected, first in 10% and then 30% sucrose phosphate buffer. Tissue was sectioned frozen at 40 μm on a sliding microtome. Sections were stored in series of every tenth section in phosphate buffer containing 0.02% sodium azide as a preservative. One series was then mounted on a slide and visualized under fluorescence for the presence of eGFP or mRFP. After fluorescence

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

screening, this slide was used for Nissl staining with Thionin. Pups aged P1 and P5 were anesthetized on ice, then sacrificed by transcardial perfusion as described above. Brains were extracted and post-fixed for 48 h, then cryoprotected as above, and sectioned at 18 μm on a cryostat (Leica CM1900, Leica Microsystems, Buffalo Grove, IL, USA). Six series of every tenth section were saved on positively charged slides (Fisher Scientific, Waltham, MA, USA). One series was visualized under fluorescence for the presence of eGFP or mRFP. One series was dried in a vacuum desiccator overnight, then immediately stained for Nissl substance with Thionin. A cohort of animals was sacrificed at E19.5 by decapitation after Cesarean section. Heads were immersion-fixed for 24 h in PFA, at which point brains were extracted and placed in fresh PFA for 24 h, then cryoprotected in sucrose phosphate buffers as described above, and sectioned on a cryostat as described above.

2.4.1. Immunohistochemistry—Immunoperoxidase activity was detected using 3,3-diaminobenzidine (DAB, Vector Labs) according to ABC protocols. One series adjacent to the Nissl-stained series was used for the immunohistochemical detection of eGFP (AB3080, Millipore Corp., Billerica, MA, USA, 1:1800) or mRFP (18-732-292379, Genway Biotech, San Diego, CA, USA, 1:5000). Adjacent series were stained for the laminar markers CUX1 (sc-13024, Santa Cruz Biotechnology, Santa Cruz, CA, USA, 1:1000) or FOXP2 (sc-21069, Santa Cruz Biotechnology, 1:50). Primary antibodies were tagged with biotinylated secondary antibodies (Vector Labs, all 1:200). Immunohistochemically processed tissue was mounted, counterstained with Methyl Green/Alcian Blue, and coverslipped with Permount mounting medium (Fisher Scientific, Waltham, MA, USA).

Immunofluorescence for co-labeling of transfected cells with BrdU and CUX1 was performed on tissue containing heterotopia. Primary antibodies (347580, Becton Dickinson, San Jose, CA, USA, and sc-13024, Santa Cruz Biotechnology, respectively) were tagged with fluorescent secondary antibodies (A11008 and A11012, Life Technologies INVITROGEN, Carlsbad, CA, USA for CUX1; 610-146-003, Rockland Immunochemicals, Gilbertsville, PA, USA for BrdU staining). GABAergic markers were detected with primary antibodies (Calretinin - AB1550 and Parvalbumin - MAB1572, Millipore, Billerica, MA, USA) and tagged with the same secondary antibodies as above. Fluorescent slides were coverslipped with VectaShield Hard Set (H-1400, Vector Labs).

2.5. Image processing

Fluorescent images were obtained on a confocal microscope (Zeiss LSM 510 Meta, Carl Zeiss, Inc., Thornwood, NY, USA). Photomicrographs were adjusted for exposure and sharpened (unsharp mask filter) using Adobe Photoshop (Adobe Inc., San Jose, CA, USA). Some images were acquired using the Virtual Slice Module of Neurolucida. Image montages were created in Adobe Illustrator.

2.6. Analysis

2.6.1. Qualitative Analysis—All analysis was performed with the experimenter blind to the subject condition. Nissl-stained series were examined for the presence of cortical and/or hippocampal anatomical malformations, including periventricular heterotopia. In a similar

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

manner, sections stained for BrdU and laminar markers were examined, and locations of labeled neurons recorded.

2.6.2. Quantitative analysis—For each brain, four sections were chosen from the fluorescent protein immuno-stained series. Care was taken to ensure that the four sections were architectonically similar across the different series. The locations of immunohistochemically stained neurons were charted using the program Neurolucida. The charted sections were then analyzed in a custom Matlab (Mathworks, Natick, MA, USA) program (Peschansky et al., 2010), which determined the location of each marked neuron as a percentage of cortical depth, with 0% corresponding to the white matter/subcortical plate boundary and 100% representing the pial surface. The region analyzed in Matlab was limited to the somatosensory cortex. We determined the mean percentage distance traveled into the neocortex for each group. Results were analyzed by analysis of variance with Fisher Least Significant Difference post-hoc tests.

3. RESULTS

There were no qualitative or quantitative differences between animals transfected with scrambled shRNA and those transfected with the empty KIAA0319L expression vector. For the qualitative analysis we report only on the scrambled shRNA. For the quantitative analysis, we pool the results from these two groups.

3.1. Disruption of Kiaa0319l affects neuronal migration

We initially evaluated the position of transfected neurons 4 days post transfection (E19.5). Most of the neurons transfected with scrambled shRNA had exited the ventricular zone (VZ) and subventricular zone (SVZ) and had entered the cortical plate (CP) with only a few neurons in transit in the intermediate zone (IZ; Fig 1A). By contrast, there were two distinct populations of neurons transfected with *Kiaa0319l* shRNA: One population was located in the VZ and SVZ, whereas the neurons in the other group had fully penetrated the CP (Fig 1B). Neurons transfected with the KIAA0319L expression construct were mostly found in the cortical plate, although there was a population in the SVZ and VZ, albeit smaller than that of the *Kiaa0319l* shRNA (Fig 1C). Most of the neurons co-transfected with *Kiaa0319l* shRNA and the KIAA0319L expression construct (rescue) were found in the CP, but there were also collections of transfected cells in the VZ and SVZ (Fig 1D). Taken together, these results suggest that disrupting Kiaa0319l function by either knocking it down or overexpressing it disrupts neuronal migration, at least in the initial period after transfection.

Examination of the position of transfected neurons at P21 supported a role for Kiaa0319l in neuronal migration. Specifically, we found periventricular nodular heterotopia (PVNH) in 4/16 P21 rats embryonically transfected with *Kiaa0319l* shRNA, 2/14 rats transfected with KIAA0319L expression construct, and 2/4 rats in the rescue condition. In contrast, there was no evidence of PVNH in either the scrambled shRNA (0/8) or the empty overexpression (0/7) group (Fig 2A). PVNH extend 300–700 μm mediolaterally and 1000–3000 μm rostrocaudally (Fig 2B–I). In most cases, these malformations also include migration anomalies in the hippocampus (Fig 2G–I), with heterotopic neurons in the *stratum radiatum*.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

PVNH were seen as early as P1 (Fig 2J,K), indicating that this disruption begins before the end of neuronal migration to the cerebral cortex.

3.2. Neurons in periventricular nodular heterotopia are born in mid- to late-gestation and destined for the upper cortical laminae

PVNH contained transfected and non-transfected neurons (Fig 3A,B,E,F), thus documenting non-cell autonomous effects of embryonic disruption of Kiaa0319l. In order to assess the original destination of the neurons in PVNH, we stained for the layer 2–4 marker CUX1 (Nieto et al., 2004) and the layer 6 marker FOXP2 (Keays et al., 2007). We found that the majority of neurons in PVNH were CUX1+, and none were FOXP2+ (Fig 3C,D,G,H), which indicated that the heterotopic neurons were destined for the upper cortical laminæ, and were presumably born during mid- to late-gestation.

In order to confirm the birthdates of these neurons, we injected BrdU into pregnant mothers either 2 days before transfection (E13.5), the day of transfection (E15.5), or 2 days following transfection (E17.5). As expected, there were no BrdU+ neurons following E13.5 injections, supporting the notion that neurons born prior to the transfection should be absent from PVNH (Fig 4A). In contrast, injection of BrdU at E15.5 resulted in large numbers of BrdU+ neurons in PVNH. Interestingly, although there were some BrdU+ neurons that were also transfected, there was also a substantial population of BrdU+ neurons that was not transfected, as well as transfected neurons that were not BrdU+ (Fig 4B). This again confirmed the presence of non-cell autonomous effects of embryonic transfection. There were fewer E17.5 BrdU+ neurons in PVNH when compared to E15.5 injections, and some of these neurons showed transfection, but most did not (Fig 4C).

We further analyzed the composition of the non-transfected neurons in PVNH by immunohistochemically staining sections for the GABAergic markers parvalbumin and calretinin. Because GABAergic neurons are generated outside the ventricular zone (in the medial ganglionic eminence), we did not expect that they would be transfected, and the presence of GABAergic neurons in these PVNH would therefore provide further evidence of non-cell autonomous effects. In fact, we found both parvalbumin+ and calretinin+ neurons scattered throughout these PVNH, and as expected, none of these neurons showed evidence of transfection (Fig 5).

3.3. There is no overmigration phenotype following disruption of Kiaa0319l

We quantified the positions of transfected neurons that migrated into the cerebral cortex from each expression condition (shRNA, overexpression construct, rescue, and control conditions). There were no differences among the groups, and therefore no overmigration phenotype (Fig 6). These findings—heterotopic collections of neurons and no overmigration —are similar to those found following *Kiaa0319* shRNA transfection.

4. DISCUSSION

In this study, we investigated the effects of embryonic disruption of the candidate dyslexia gene homologue *Kiaa0319l* on neuronal migration to the cerebral cortex. We hypothesized that, because of its structural similarity to the previously investigated candidate dyslexia

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

susceptibility gene *KIAA0319*, knockdown and overexpressing this gene would yield similar results (Paracchini et al., 2006, Peschansky et al., 2010). Specifically, we hypothesized that embryonic knockdown of *Kiaa0319l* would result in white matter heterotopia, but no measurable disruption of the position of transfected neurons in the cerebral cortex. In fact, we found that *in utero* electroporation of plasmids encoding *Kiaa0319l* shRNA resulted in much larger periventricular heterotopia than those seen following transfection with *Kiaa0319* shRNA, and that overexpression of the KIAA0319L protein also resulted in periventricular heterotopia, the latter not found with KIAA0319 overexpression (Peschansky et al., 2010). However, as with Kiaa0319 knockdown or overexpression, transfection with *Kiaa0319l* shRNA did not alter the laminar positions of neurons in the cerebral cortex (Peschansky et al., 2010).

KIAA0319L on Chr 1p34.2 was first proposed as a candidate dyslexia susceptibility gene by Couto and colleagues (2008), based on its homology (61%) with *KIAA0319* on Chr 6p22.3-p22.2. These authors found a modestly significant association of one single nucleotide polymorphism (SNP) and haplotype with reading disability as a categorical trait, as well as with quantitative measures of single word-reading and rapid naming of objects and colors. The function of this gene has not been completely characterized, although there is evidence that KIAA0319L interacts with the axon guidance protein Nogo receptor 1 (NgR1). This finding, combined with the fact that these proteins interacted in cortical neurons *in vitro*, suggested that *KIAA0319L* may participate in axonal guidance in the cerebral cortex (Poon et al., 2011a). Subsequent work in mice found that *Kiaa0319L* was localized to layers 4 and 6 of cerebral cortex, but also to most of the hippocampus, the molecular layer (and occasional Purkinje cells) of the cerebellum, thalamus, and olfactory bulb (Poon et al., 2011b). The current experiments suggest that KIAA0319L also plays a role in neuronal migration. That one gene can have a role in both axon outgrowth and neuronal migration is not unprecedented. As an example, the candidate dyslexia susceptibility gene *ROBO1* has been well characterized as being important for axonal outgrowth, especially in the development of the corpus callosum (Long et al., 2004), but also has a role in neuronal migration to the cortex (Andrews et al., 2006, Andrews et al., 2008, Gonda et al., 2012).

Immunohistochemical stains revealed that PVNH contain populations of neurons that are CUX1+ but not FOXP2+, indicating that these neurons would normally be destined for the upper cortical laminæ. Moreover, BrdU birthdating confirmed that neurons in the PVNH were born when these upper laminæ neurons were generated in mid- to late-gestation. These results are identical to what was found following embryonic knockdown of the CDSGs *Dyx1c1* and *Kiaa0319* (Rosen et al., 2007, Peschansky et al., 2010). However, there are differences: Heterotopia from the Kiaa0319l manipulations are much larger and extend from the subcortical white matter to the ventricular surface (Rosen et al., 2007, Peschansky et al., 2010). Further, overexpressing KIAA0319L resulted in PVNH, whereas we found no effects of CDSG overexpression in any previous experiments. Although there is no evidence linking *KIAA0319L* mutations to PVNH in humans, previous work has shown that individuals with PVNH, similar to the type seen here, are associated with an increased incidence of developmental dyslexia (Chang et al., 2005, Chang et al., 2007).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Another discrepancy between the current study and previous publications is the relatively low incidence of heterotopia in the present experiment when compared with those following transfection with *Kiaa0319* shRNA. Specifically, we found that the incidence of PVNH following *in utero* electroporation of shRNA targeted against *Kiaa0319l* was approximately 25%. In comparison, we found that approximately half of the *Kiaa0319* shRNA-transfected animals had heterotopia (Peschansky et al., 2010). On the other hand, the incidence in the current experiment identically matches that following embryonic knockdown of *Dyx1c1* (Rosen et al., 2007). We do not yet know why the incidence of malformations is variable among CDSGs, but we hypothesize that it may be related to the regions of the brain that were transfected. We observed, for example, a higher incidence of PVNH following transfections in rostral dorsal cortex (rostral somatosensory, motor) than in transfections performed more caudally and ventrally (caudal somatosensory cortex, temporal cortex).

In previous studies, animals that had been embryonically transfected with shRNA targeted against either *Dyx1c1* or *Dcdc2* had transfected neurons that migrated into the cerebral cortex past their expected laminar location (Rosen et al., 2007, Burbridge et al., 2008). In contrast, transfection with *Kiaa0319* shRNA did not show any differential between the positions of transfected neurons and those transfected with scrambled shRNA (Peschansky et al., 2010). The results from the current experiment suggest that the homology between *Kiaa0319* and *Kiaa0319l* extends to the overmigration phenotype.

A concern for experiments involving transfection of shRNA for the purposes of reducing a targeted gene's expression is that of off-target effects (Bridge et al., 2003, Sledz et al., 2003, LoTurco et al., 2009). These are commonly controlled for by "rescue" experiments, whereby shRNA of the targeted gene is transfected along with vectors expressing the protein normally produced by that gene. In order for these rescue experiments to work, however, any phenotype related to shRNA transfection must not be present following transfection of the expression construct alone. In previous studies of the effects of embryonic knockdown of CDSGs, we met these conditions—transfection with plasmids containing shRNA targeted against the CDSG resulted in phenotypes (heterotopia and/or overmigration) that were not seen following transfection with the CDSG expression construct. In the design of the current experiment, we attempted to control for the effects of off-target effects by transfecting embryonic rats with both *Kiaa0319l* shRNA and KIAA0319L expression vector, and found that half of the four surviving animals had PVNH. Interpretation of these results is complicated by the fact that transfection with *either Kiaa0319l* shRNA or KIAA0319L expression resulted in PVNH (see above). We cannot, therefore, definitively exclude the possibility of off-target effects in this case, but consider it unlikely given that disruption of Kiaa0319l (by either knockdown or overexpression) results in large heterotopia not seen in previous experiments using *in utero* electroporation.

4.1. Conclusions

Kiaa0319l is the fourth of four CDSGs where disruption of gene expression under comparable experimental conditions results in neuronal migration disorders. This supports the association of abnormal neuronal migration with developmental dyslexia. Whether this association reflects a causal role for some of the behavioral phenotypes that have been seen

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

with these genetic manipulations remains to be further studied. Future experiments designed to test the effects of Kiaa0319l disruption on behavioral paradigms previously demonstrated to be affected in similar preparations (e.g., Threlkeld et al., 2009, Szalkowski et al., 2011, Szalkowski et al., 2012) could prove instructive in this regard.

Acknowledgments

The research was supported, in part, by P01 HD057853. RTCi was supported by the DREAM programme of Chinese University of Hong Kong (CUHK). MWP and WHT were supported by graduate studentships from CUHK.

ABBREVIATIONS

BrdU	5-bromo-2-deoxyuridine
CDSG	Candidate Dyslexia Susceptibility Gene
CP	Cortical Plate
DAB	3,3-diaminobenzidine
E	Embryonic Day
eGFP	Enhanced Green Fluorescent Protein
GABAergic	Transmitting or secreting Gamma-Aminobutyric Acid
IZ	Intermediate Zone
mRFP	Monomeric Red Fluorescent Protein
P	Postnatal Day
PFA	Paraformaldehyde (4%)
PVNH	Periventricular Nodular Heterotopia
RNAi	Interference RNA
shRNA	Small Hairpin RNA
SVZ	Subventricular Zone
VZ	Ventricular Zone

References

Andrews W, Barber M, Hernadez-Miranda LR, Xian J, Rakic S, Sundaresan V, Rabbitts TH, Pannell R, Rabbitts P, Thompson H, Erskine L, Murakami F, Parnavelas JG. The role of Slit-Robo signaling in the generation, migration and morphological differentiation of cortical interneurons. Dev Biol. 2008; 313:648–658. [PubMed: 18054781]

Andrews W, Liapi A, Plachez C, Camurri L, Zhang J, Mori S, Murakami F, Parnavelas JG, Sundaresan V, Richards LJ. Robo1 regulates the development of major axon tracts and interneuron migration in the forebrain. Development. 2006; 133:2243–2252. [PubMed: 16690755]

Bridge AJ, Pebernard S, Ducraux A, Nicoulaz AL, Iggo R. Induction of an interferon response by RNAi vectors in mammalian cells. Nat Genet. 2003; 34:263–264. [PubMed: 12796781]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Burbridge T, Wang Y, Volz A, Peschansky V, Lisann L, Galaburda AM, LoTurco JJ, Rosen GD. Postnatal analysis of the effect of embryonic knockdown and overexpression of candidate dyslexia susceptibility gene *Dcdc2*. Neuroscience. 2008; 152:723–733. [PubMed: 18313856]

Chang BS, Katzir T, Liu T, Corriveau K, Barzillai M, Apse KA, Bodell A, Hackney D, Alsop D, Wong S, Walsh CA. A structural basis for reading fluency: White matter defects in a genetic brain malformation. Neurology. 2007; 69:2146–2154. [PubMed: 18056578]

Chang BS, Ly J, Appignani B, Bodell A, Apse KA, Ravenscroft RS, Sheen VL, Doherty MJ, Hackney DB, O'Connor M, Galaburda AM, Walsh CA. Reading impairment in the neuronal migration disorder of periventricular nodular heterotopia. Neurology. 2005; 64:799–803. [PubMed: 15753412]

Cope N, Harold D, Hill G, Moskvina V, Stevenson J, Holmans P, Owen MJ, O'Donovan MC, Williams J. Strong evidence that KIAA0319 on chromosome 6p is a susceptibility gene for developmental dyslexia. Am J Hum Genet. 2005; 76:581–591. [PubMed: 15717286]

Couto JM, Gomez L, Wigg K, Cate-Carter T, Archibald J, Anderson B, Tannock R, Kerr EN, Lovett MW, Humphries T, Barr CL. The KIAA0319-like (KIAA0319L) gene on chromosome 1p34 as a candidate for reading disabilities. J Neurogenet. 2008; 22:295–313. [PubMed: 19085271]

Currier TA, Etchegaray MA, Haight JL, Galaburda AM, Rosen GD. The effects of embryonic knockdown of the candidate dyslexia susceptibility gene homologue *Dyx1c1* on the distribution of GABAergic neurons in the cerebral cortex. Neuroscience. 2011; 172:535–546. [PubMed: 21070838]

Francks C, Paracchini S, Smith SD, Richardson AJ, Scerri TS, Cardon LR, Marlow AJ, MacPhie IL, Walter J, Pennington BF, Fisher SE, Olson RK, DeFries JC, Stein JF, Monaco AP. 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. 2004; 75:1046–1058. [PubMed: 15514892]

Galaburda AM, Kemper TL. Cytoarchitectonic abnormalities in developmental dyslexia; a case study. Ann Neurol. 1979; 6:94–100. [PubMed: 496415]

Galaburda AM, Sherman GF, Rosen GD, Aboitiz F, Geschwind N. Developmental dyslexia: Four consecutive cases with cortical anomalies. Ann Neurol. 1985; 18:222–233. [PubMed: 4037763]

Gonda Y, Andrews WD, Tabata H, Namba T, Parnavelas JG, Nakajima K, Kohsaka S, Hanashima C, Uchino S. Robo1 regulates the migration and laminar distribution of upper-layer pyramidal neurons of the cerebral cortex. Cereb Cortex. 2012

Grigorenko EL, Wood FB, Meyer MS, Pauls JE, Hart LA, Pauls DL. Linkage studies suggest a possible locus for developmental dyslexia on chromosome 1p. Am J Med Genet. 2001; 105:120–129. [PubMed: 11424982]

Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, Kaariainen H, Kere J. The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. PLoS Genet. 2005; 1:e50. [PubMed: 16254601]

Harold D, Paracchini S, Scerri T, Dennis M, Cope N, Hill G, Moskvina V, Walter J, Richardson AJ, Owen MJ, Stein JF, Green ED, O'Donovan MC, Williams J, Monaco AP. Further evidence that the KIAA0319 gene confers susceptibility to developmental dyslexia. Mol Psychiatry. 2006; 11:1085–1091. 1061. [PubMed: 17033633]

Hivert B, Liu Z, Chuang CY, Doherty P, Sundaresan V. Robo1 and Robo2 are homophilic binding molecules that promote axonal growth. Mol Cell Neurosci. 2002; 21:534–545. [PubMed: 12504588]

Humphreys P, Kaufmann WE, Galaburda AM. Developmental dyslexia in women: Neuropathological findings in three cases. Ann Neurol. 1990; 28:727–738. [PubMed: 2285260]

Keays DA, Tian G, Poirier K, Huang GJ, Siebold C, Cleak J, Oliver PL, Fray M, Harvey RJ, Molnar Z, Pinon MC, Dear N, Valdar W, Brown SD, Davies KE, Rawlins JN, Cowan NJ, Nolan P, Chelly J, Flint J. Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. Cell. 2007; 128:45–57. [PubMed: 17218254]

Long H, Sabatier C, Ma L, Plump A, Yuan W, Ornitz DM, Tamada A, Murakami F, Goodman CS, Tessier-Lavigne M. Conserved roles for Slit and Robo proteins in midline commissural axon guidance. Neuron. 2004; 42:213–223. [PubMed: 15091338]

LoTurco J, Manent JB, Sidiqi F. New and improved tools for in utero electroporation studies of developing cerebral cortex. Cereb Cortex. 2009; 19(Suppl 1):i120–125. [PubMed: 19395528]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Meng H, Smith SD, Hager K, Held M, Liu J, Olson RK, Pennington BF, DeFries JC, Gelernter J, O'Reilly-Pol T, Somlo S, Skudlarski P, Shaywitz SE, Shaywitz BA, Marchione K, Wang Y, Paramasivam M, LoTurco JJ, Page GP, Gruen JR. *DCDC2* is associated with reading disability and modulates neuronal development in the brain. Proc Natl Acad Sci U S A. 2005; 102:17053–17058. [PubMed: 16278297]

Nieto M, Monuki ES, Tang H, Imitola J, Haubst N, Khoury SJ, Cunningham J, Gotz M, Walsh CA. Expression of Cux-1 and Cux-2 in the subventricular zone and upper layers II-IV of the cerebral cortex. J Comp Neurol. 2004; 479:168–180. [PubMed: 15452856]

Nopola-Hemmi J, Myllyluoma B, Haltia T, Taipale M, Ollikainen V, Ahonen T, Voutilainen A, Kere J, Widen E. A dominant gene for developmental dyslexia on chromosome 3. J Med Genet. 2001; 38:658–664. [PubMed: 11584043]

Paracchini S, Steer CD, Buckingham L-L, Morris AP, Ring S, Scerri T, Stein J, Pembrey ME, Ragoussis J, Golding J, Monaco AP. Association of the KIAA0319 Dyslexia Susceptibility Gene With Reading Skills in the General Population. Am J Psychiatry. 2008; 165:1576–1584. [PubMed: 18829873]

Paracchini S, Thomas A, Castro S, Lai C, Paramasivam M, Wang Y, Keating BJ, Taylor JM, Hacking DF, Scerri T, Francks C, Richardson AJ, Wade-Martins R, Stein JF, Knight JC, Copp AJ, LoTurco JJ, Monaco AP. The chromosome 6p22 haplotype associated with dyslexia reduces the expression of KIAA0319, a novel gene involved in neuronal migration. Hum Mol Genet. 2006; 15:1659–1666. [PubMed: 16600991]

Peschansky VJ, Burbridge TJ, Volz AJ, Fiondella C, Wissner-Gross Z, Galaburda AM, Turco JJ, Rosen GD. The effect of variation in expression of the candidate dyslexia susceptibility gene homolog Kiaa0319 on neuronal migration and dendritic morphology in the rat. Cereb Cortex. 2010; 20:884–897. [PubMed: 19679544]

Peterson RL, Pennington BF. Developmental dyslexia. Lancet. 2012; 379:1997–2007. [PubMed: 22513218]

Poon MW, Tsang WH, Chan SO, Li HM, Ng HK, Waye MM. Dyslexia-associated kiaa0319-like protein interacts with axon guidance receptor nogo receptor 1. Cell Mol Neurobiol. 2011a; 31:27–35. [PubMed: 20697954]

Poon MW, Tsang WH, Waye MM, Chan SO. Distribution of Kiaa0319-like immunoreactivity in the adult mouse brain--a novel protein encoded by the putative dyslexia susceptibility gene KIAA0319-like. Histol Histopathol. 2011b; 26:953–963. [PubMed: 21692029]

Rosen GD, Bai J, Wang Y, Fiondella CG, Threlkeld SW, LoTurco JJ, Galaburda AM. Disruption of neuronal migration by targeted RNAi knockdown of *Dyx1c1* results in neocortical and hippocampal malformations. Cereb Cortex. 2007; 17:2562–2572. [PubMed: 17218481]

Scerri TS, Schulte-Korne G. Genetics of developmental dyslexia. Eur Child Adolesc Psychiatry. 2010; 19:179–197. [PubMed: 20091194]

Skiba T, Landi N, Wagner R, Grigorenko EL. In search of the perfect phenotype: an analysis of linkage and association studies of reading and reading-related processes. Behav Genet. 2011; 41:6–30. [PubMed: 21243420]

Sledz CA, Holko M, de Veer MJ, Silverman RH, Williams BR. Activation of the interferon system by short-interfering RNAs. Nature cell biology. 2003; 5:834–839.

Szalkowski CE, Fiondella CG, Galaburda AM, Rosen GD, Loturco JJ, Fitch RH. Neocortical disruption and behavioral impairments in rats following in utero RNAi of candidate dyslexia risk gene Kiaa0319. Int J Dev Neurosci. 2012; 30:293–302. [PubMed: 22326444]

Szalkowski CE, Hinman JR, Threlkeld SW, Wang Y, LePack A, Rosen GD, Chrobak JJ, LoTurco JJ, Fitch RH. Persistent spatial working memory deficits in rats following *in utero* RNAi of *Dyx1c1*. Genes Brain Behav. 2011; 10:244–252. [PubMed: 20977651]

Threlkeld SW, Hill CA, Cleary CE, Trong DT, Rosen GD, Fitch RH. Developmental learning impairments in a rodent model of nodular heterotopia. Journal of Neurodevelopmental Disorders. 2009; 1:237–250. [PubMed: 21547717]

Tzenova J, Kaplan BJ, Petryshen TL, Field LL. Confirmation of a dyslexia susceptibility locus on chromosome 1p34-p36 in a set of 100 Canadian families. Am J Med Genet. 2004; 127B:117–124. [PubMed: 15108193]

Wang Y, Paramasivam M, Thomas A, Bai J, Kaminen N, Kere J, Voskul J, Rosen G, Galaburda A, LoTurco J. Dyx1c1 functions in neuronal migration in developing neocortex. Neuroscience. 2006; 143:515–522. [PubMed: 16989952]

Wong K, Ren XR, Huang YZ, Xie Y, Liu G, Saito H, Tang H, Wen L, Brady-Kalnay SM, Mei L, Wu JY, Xiong WC, Rao Y. Signal transduction in neuronal migration: roles of GTPase activating proteins and the small GTPase Cdc42 in the Slit-Robo pathway. Cell. 2001; 107:209–221. [PubMed: 11672528]

Zou L, Chen W, Shao S, Sun Z, Zhong R, Shi J, Miao X, Song R. Genetic variant in *KIAA0319*, but not in *DYX1C1*, is associated with risk of dyslexia: An integrated meta-analysis. Am J Med Genet B Neuropsychiatr Genet. 2012; 159B:970–976. [PubMed: 23065966]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Highlights

- Disruption of Kiaa0319l function results in large nodular periventricular heterotopias
- Neurons in these heterotopias are generated in mid- to late-gestation
- Neurons in these heterotopias are destined to upper cortical laminæ
- Transfected neurons in the cerebral cortex are located in their expected laminæ.
- *KIAA0319L* is another candidate dyslexia susceptibility gene that modulates neuronal migration

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

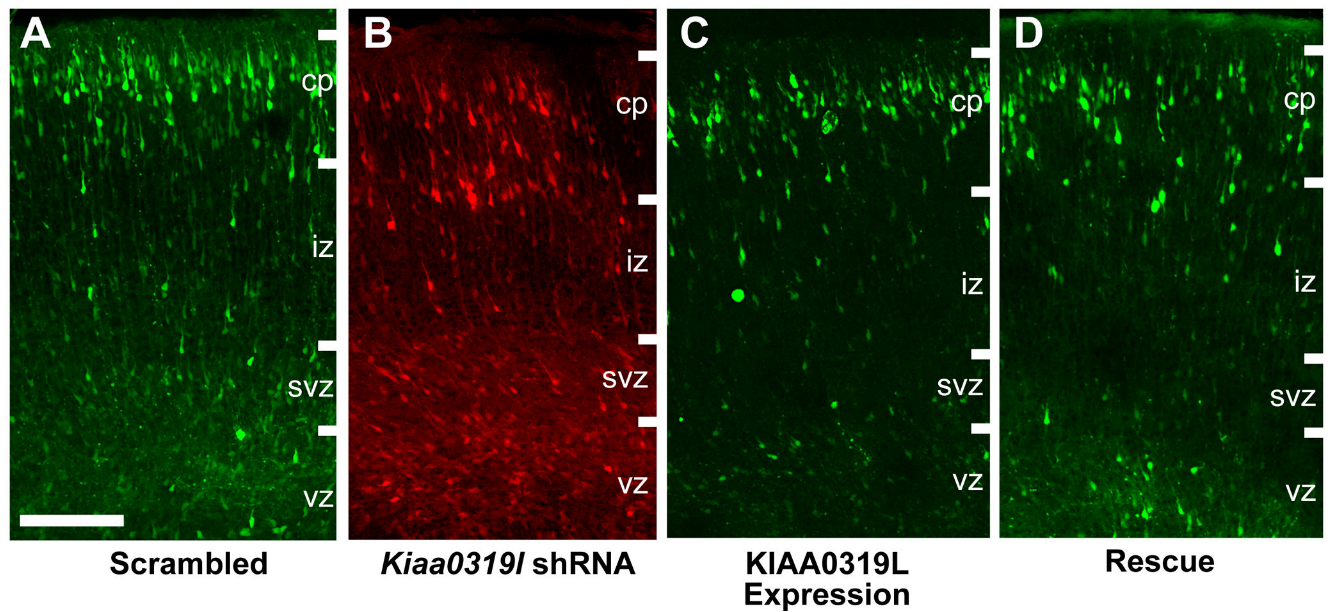

Fig 1.

Developing cortex in each condition 4 days after transfection. **A**. Most neurons transfected with scrambled shRNA have migrated to the cortical plate (CP) by E19.5. There are some migrating neurons in the intermediate zone (IZ) as well. **B**. One population of neurons transfected with shRNA targeted against *Kiaa0319l* remain in the ventricular zone (VZ) and subventricular zone (SVZ), and another have migrated to the cortical plate (CP). **C**. Most neurons transfected with the KIAA0319L expression construct migrate into the CP, although there is a smaller population locate in the SVZ and VZ. **D**. Neurons in the rescue condition (*Kiaa0319l* shRNA+ KIAA0319L Expression) have one population that have migrated to the CP, and another that remain in the SVZ and VZ. Scale = 200 μm.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

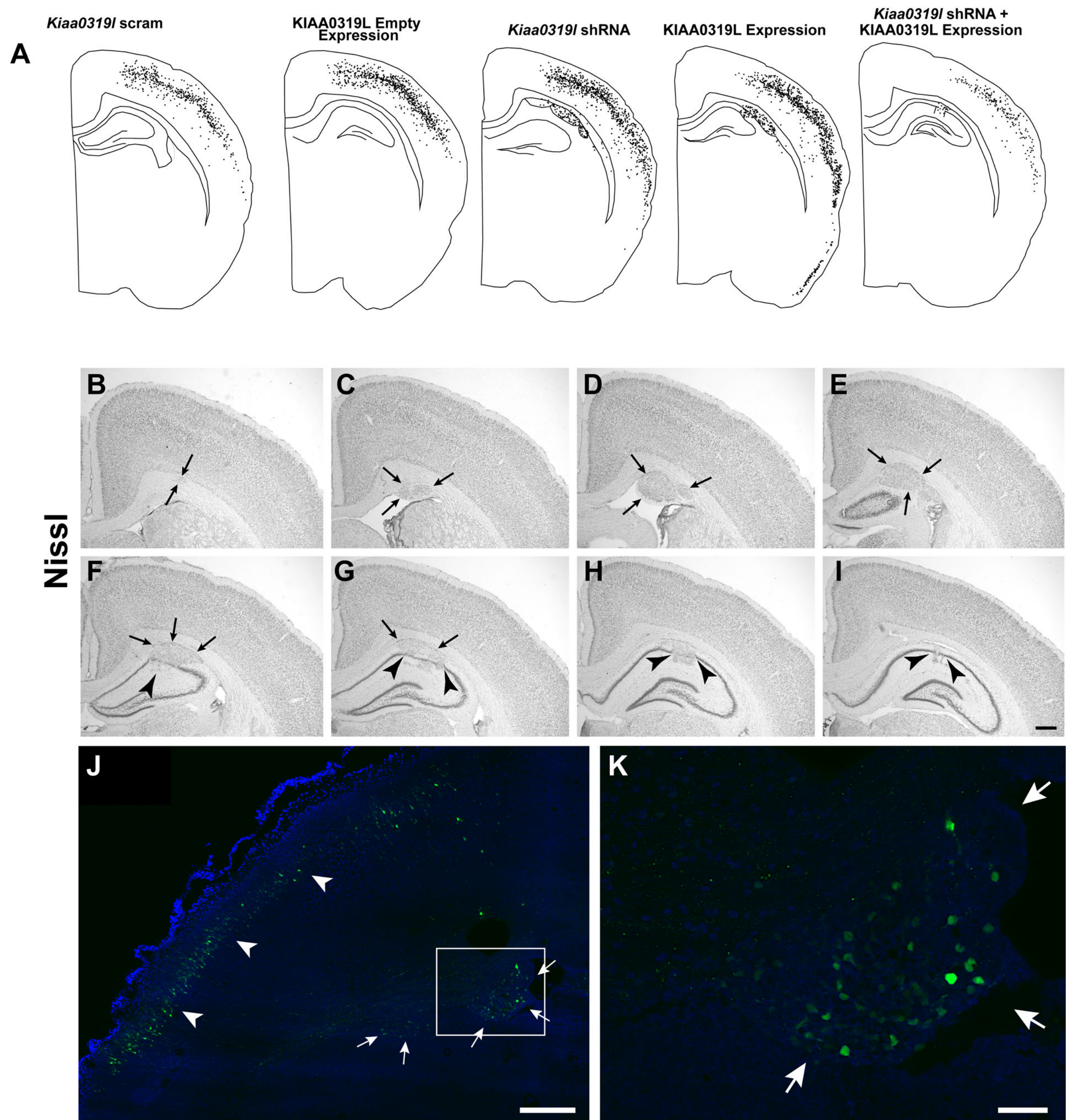

Fig 2.

Periventricular nodular heterotopia (PVNH) following disruption of *Kiaa0319l* function. A. Neurolucida tracings of transfected neurons in each of 5 conditions at P21. Transfection with scrambled *Kiaa0319l* shRNA or KIAA0319L empty expression labels neurons predominantly in the supragranular layers of the neocortex. Transfection with *Kiaa0319l* shRNA, KIAA0319L expression, or both constructs simultaneously result in PVNH and hippocampal migration abnormalities. **B–I.** Nissl stained photomicrographs from a coronally sectioned P21 rat that was embryonically transfected with *Kiaa0319l* shRNA at E15.5. Arrows denote PVNH, arrowheads denote hippocampal migration abnormality. Bar in I for

B–I = 200 μm. **J**. Photomontage of a P1 rat that was embryonically transfected with Kiaa0319l shRNA at E15.5. Arrows denote two heterotopic clusters of neurons at the border of the ventricle. Arrowheads indicate neurons that migrated to the cortical plate. Box indicates area of magnification for panel K. Bar = 1 mm **K**. High power photomicrograph of collection of transfected neurons at the border of the ventricle as seen in panel J. Bar = 200 μm.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

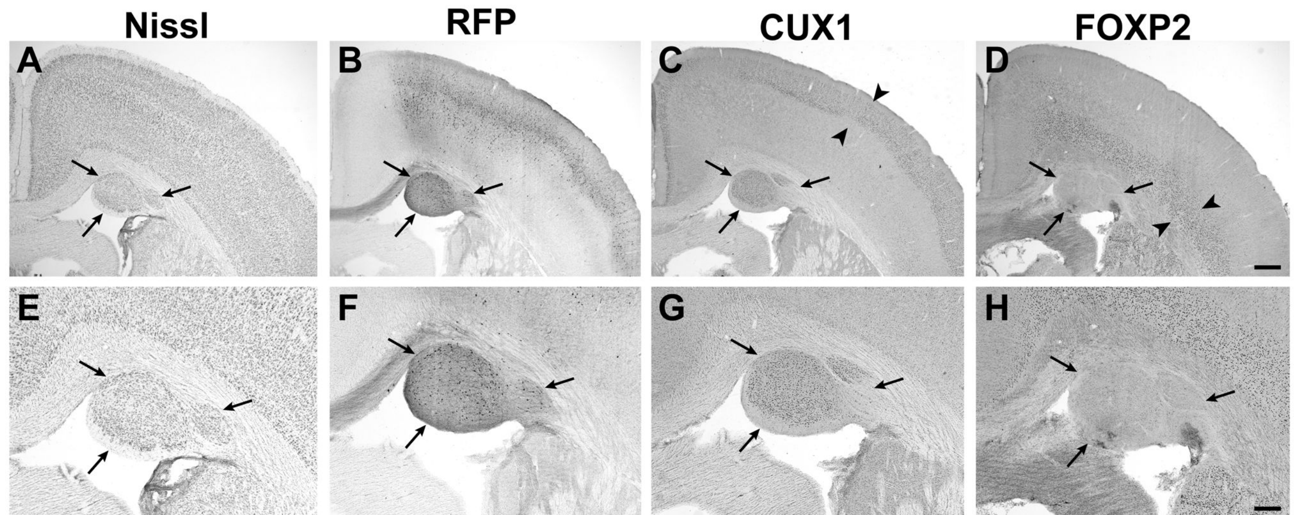

Fig 3.

Cell-autonomous and non-cell autonomous effects of embryonic transfection disruption of Kiaa0319l. **A**. Nissl stained photomicrograph (same as panel 2C) illustrating PVNH (arrows) in rat embryonically transfected with *Kiaa0319l* shRNA. **B**. Section adjacent to A stained with mRFP indicating neurons that were embryonically transfected. Examination of PVNH reveals that not all neurons in these heterotopia are transfected, indicating that there are non-cell autonomous effects of *Kiaa0319l* disruption. **C**. Section adjacent to B stained with an anti-CUX1 antibody. Arrowheads denote normal pattern of CUX1 immunoreactivity in layers 2–4 of the cerebral cortex. Most of the neurons in this heterotopic collection of neurons (arrows) are CUX1-positive, indicating that these neurons were destined for the upper cortical laminæ. D. Section adjacent to C stained with an anti-FOXP2 anitbody. Arrowheads denote normal pattern of FOXP2 immunoreactivity in layers 5–6 of the cerebral cortex. None of the heterotopic neurons (arrows) are FOXP2-positive. Bar in D = 200 μm for panels A–D. E–H are higher power photomicrographs of panels A–D, respectively. Bar in H = 100 μm for panels E–H.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

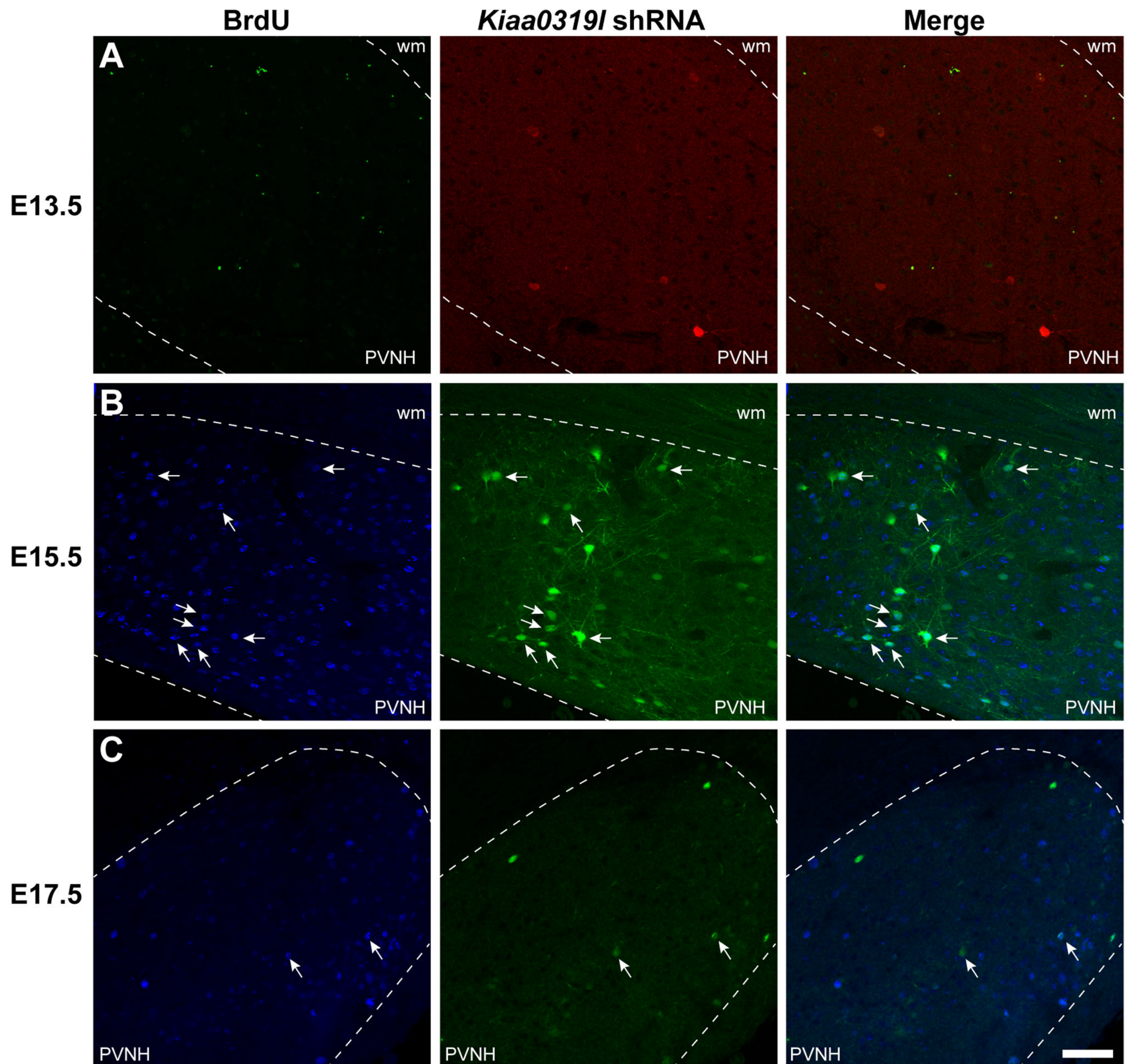

Fig 4.

Heterotopic neurons are born in mid- to late-gestation. **A**. Following injection of BrdU at E13.5, there are no BrdU+ neurons contained within PVNH (left panel) in a rat embryonically transfected with *Kiaa0319l* shRNA on E15.5. There are transfected heterotopic neurons (red) (middle panel). **B**. Injection of BrdU at E15.5 labels large numbers of BrdU+ neurons (blue) within PVNH. Middle panel illustrate transfected neurons (green). Arrows indicate BrdU+ neurons that are co-labeled with eGFP (right panel). **C**. BrdU injected at E17.5 results in a population of BrdU+ neurons (blue) within PVNH, some of which are co-labeled (arrows). Wm = white matter. Bar = 200 μm in all panels.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

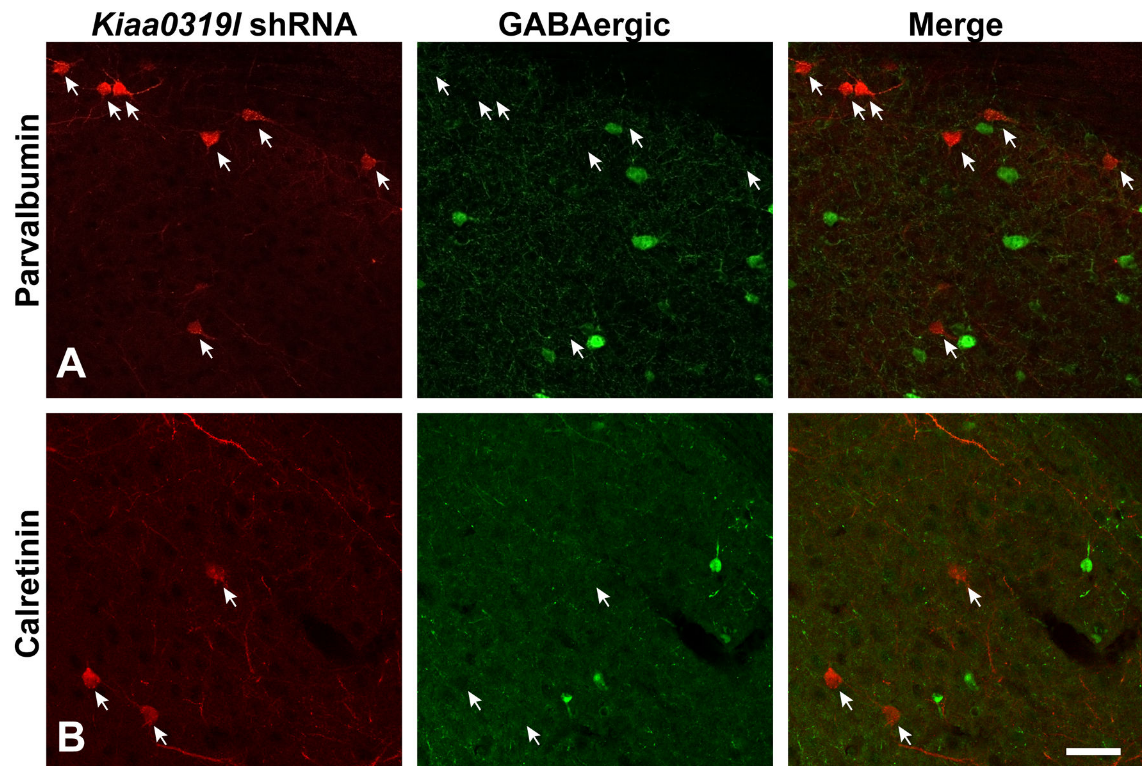

Fig 5.

GABAergic neurons in PVNH. Parvalbumin+ (A) and Calretinin+ (B) neurons located in PVNH in a rat transfected with *Kiaa0319l* shRNA. These GABAergic neurons are not co-localized with transfected neurons, indicating that there are non-cell autonomous effects of Kiaa0319l disruption.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

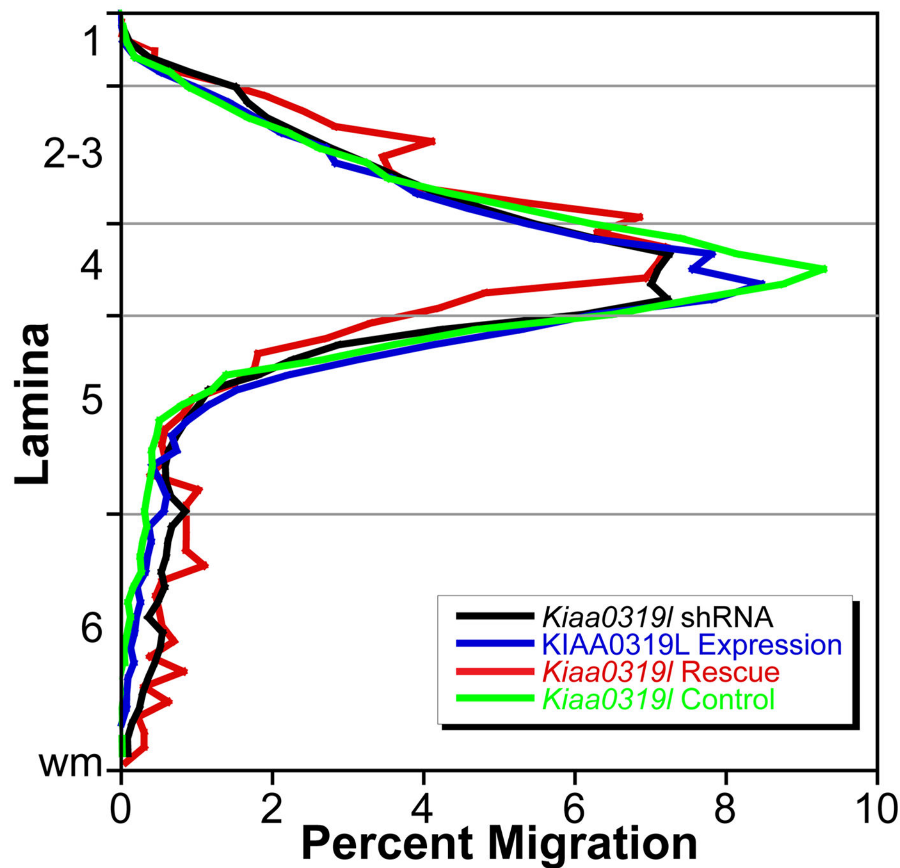

Fig 6.

Position of transfected neurons in the cerebral cortex. Line chart illustrating laminar position of transfected neurons in the cerebral cortex. X axis is the percent of migrated neurons while the Y-axis is the laminar position. There is no difference among the groups in their laminar positions in the cerebral cortex.