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ORIGINAL ARTICLE

Genes, Brain and Behavior

Rapid auditory processing and medial geniculate nucleus anomalies in *Kiaa0319* knockout mice

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Abstract

Developmental dyslexia is a common neurodevelopmental disorder characterized by difficulties in reading and writing. Although underlying biological and genetic mechanisms remain unclear, anomalies in phonological processing and auditory processing have been associated with dyslexia. Several candidate risk genes have also been identified, with *KIAA0319* as a main candidate. Animal models targeting the rodent homolog (*Kiaa0319*) have been used to explore putative behavioral and anatomic anomalies, with mixed results. For example after downregulation of *Kiaa0319* expression in rats via shRNA, significant adult rapid auditory processing impairments were reported, along with cortical anomalies reflecting atypical neuronal migration. Conversely, *Kiaa0319* knockout (KO) mice were reported to have typical adult auditory processing, and no visible cortical anomalies. To address these inconsistencies, we tested *Kiaa0319* KO mice on auditory processing tasks similar to those used previously in rat shRNA knockdown studies. Subsequent neuroanatomic analyses on these same mice targeted medial geniculate nucleus (MGN), a receptive communication-related brain structure. Results confirm that *Kiaa0319* KO mice exhibit significant auditory processing impairments specific to rapid/brief stimuli, and also show significant volumetric reductions and a shift toward fewer large and smaller neurons in the MGN. The latter finding is consistent with post mortem MGN data from human dyslexic brains. Combined evidence supports a role for *KIAA0319* in the development of auditory CNS pathways subserving rapid auditory processing functions critical to the development of speech processing, language, and ultimately reading. Results affirm *KIAA0319* variation as a possible risk factor for dyslexia specifically via anomalies in central acoustic processing pathways.

KEYWORDS

auditory processing, behavior, dyslexia, gap detection, Kiaa0319, MGN, neurodevelopment, reading ability, thalamic nuclei, transgenic mouse model

1 | INTRODUCTION

Developmental dyslexia is characterized by difficulties in reading and writing ability that cannot be explained by intellectual impairment, socioeconomic status, primary sensory impairments (i.e., blindness or deafness), or other comorbid conditions.[1,2] Dyslexia is considered a common neurodevelopmental disorder, and is estimated to impact nearly 7% of the population,[3] with males affected over females by about two-fold.[4,5] Individuals diagnosed with dyslexia often exhibit underlying "domain-general" impairments (e.g., auditory, visual and/or memory processing deficits), and these core functional disabilities are thought to contribute directly to the higher-order language-based phenotype. They include phonological impairments,[6,7] visuospatial/visual attention impairments,[8–11] and working memory impairments.[12,13] Auditory temporal processing impairments have also been consistently reported in individuals with developmental dyslexia, suggesting that disruptions to reading may arise from a temporal processing deficit in the auditory, as well as the visual, domain.[14–19] Because auditory temporal processing is crucial to early speech perception and language development/acquisition as a whole, problems resulting from poor rapid acoustic discrimination could contribute to phonemic and/or phonologic difficulties associated with developmental dsylexia.[20–24] The current study was designed to explore the impact of an identified dyslexia risk gene—*KIAA0319*—on this developmental association through characterization of rapid auditory processing indices (e.g., gap detection thresholds[25]) in a genetically engineered mouse model.

From a genetic perspective, the etiology of dyslexia remains poorly understood, but it is clear that there is a strong contribution.[26] For example, Vogler et al.,[27] reported that among families with parents affected by reading difficulties, the probability of children developing a reading disability was quadrupled. Twin studies also support the heritability of dyslexia,[28,29] and more recent linkage studies and genome-wide association studies have highlighted multiple candidate dyslexia susceptibility genes. These include *KIAA0319*, as well as *DYX1C1* and *DCDC2* (see References 30–33 for review). *KIAA0319* has been particularly well validated as a dyslexia candidate gene in multiple independent association studies,[34–36] and has been associated with various other language- and communication-related neurodevelopmental disorders,[37,38] although not all studies confirm the association. Functionally, *KIAA0319* encodes for transmembrane protein KIAA0319.[39] While it remains unclear how disruptions in expression ultimately impact reading, the gene product can localize to plasma membranes and undergo proteolytic processing, with yet unclear function. Possibilities include a role in cell signaling, gene expression and/or cell–cell adhesion.[39–41]

While the function of *KIAA0319* (and other dyslexia-risk genes) continue to be studied, the core neurodevelopmental features underlying reading disability also remain poorly defined. One of the most prominent, yet controversial theories, is the neuronal migration hypothesis, which suggests that atypical neuronal migration is a leading contributor to the behavioral impairments seen in dyslexia. Galaburda and Kemper,[42] followed by Galaburda et al.,[43] were the first to identify post mortem cortical neuronal migration abnormalities in dyslexic individuals. Subsequent research has explored the role of specific dyslexia-risk genes in neuronal migration. *KIAA0319* was of particular interest in this context given associations with cell signaling and cell–cell adhesion. To better understand the role of *KIAA0319*, and possible intermediary links to neuronal migration, genetic knockdowns of the rodent homolog (*Kiaa0319*) were developed using embryonic transfection with small hairpin RNAs (shRNAs). Paracchini et al.[35] found that when *Kiaa0319* was downregulated via embryonic shRNA in rats, radial neuronal migration was impaired, resulting in subsequent white matter heterotopias (see also Reference 44). These findings were replicated by Peschansky et al.,[45] who reported anomalous neuronal migration in *Kiaa0319* shRNA rats, and abnormal dendritic growth/differentiation.

Behavioral phenotyping of *Kiaa0319* shRNA rats has also been used to explore how disruption of this gene affects "domain-general" functions associated with dyslexia (e.g., short-term working memory, spatial learning and complex auditory processing). Szalkowski et al.,[46] found that *Kiaa0319* shRNA rats had significantly impaired rapid auditory processing ability, with no deficits in radial-arm maze learning and memory (in contrast to auditory processing *and* learning/memory deficits seen in rats with shRNA for another dyslexia-risk gene, *Dyx1c1*[47,48]). This suggested anomalous expression of KIAA0319 could contribute to neural abnormalities in auditory processing functions crucial to phonology, language development and reading.

In counter-point to the neuronal migration hypothesis,[48] reported that memory deficits observed in rats with shRNA for *Dyx1c1* were identical for rats with *no* observable cortical migrational abnormalities, as compared with those *with* malformations. This suggested that visible cortical migrational anomalies may be co-occurring but *not* causal to functional dyslexia-related deficits. Further support came from a 2017 study of an engineered *Kiaa0319* mouse model,[49] which showed no evidence of neuronal migration anomalies in *Kiaa0319* knockout mice. This study also reported a lack of auditory processing anomalies in *Kiaa0319* KO mice using a paradigm that interrogated differences in optimal processing time (10–800 ms) for a fixed duration acoustic pre-pulse inhibition cue of 20 ms. However, this paradigm differed substantially from the one used in Szalokowski et al.,[46] which tested thresholds for detection of a silent gap of varying duration (2–100 ms). Concomitant evidence from Guidi et al.[50] showed that mice with double knock-outs for *Kiaa0319* (from the line used in Reference 49) crossed with a *Kiaa0319L* (aka *AU040320*) knockout *did* show abnormalities on a behavioral gap-in-noise detection task (following a silent-gap detection paradigm more closely aligned with[46]). However, these authors did not detect gap-detection deficits in the single-gene *Kiaa0319* KO. They also showed anomalous recordings of click-evoked auditory brainstem responses (ABRs), with suprathreshold deficits in wave III amplitude in *Kiaa0319L* KO mice, and more general deficits in double KOs. Moreover, they confirmed an absence of cortical laminar abnormalitites in *Kiaa0319* KO mice, again suggesting a neurobehavioral pathway to dyslexia outside of atypical neuronal migration.

In the current study we sought to interrogate the behavioral and neuroanatomical consequences of early *Kiaa0319* disruption using the KO mice developed by Martinez-Garay et al.,[49] and Guidi et al.[50] and following the behavioral methods of Szalkowski et al.[46] Our evaluations included a battery of rapid auditory processing tasks modeled on acoustic processing deficits seen in individuals with dyslexia ([14–18] for review). Tasks were explicitly designed to target the "auditory temporal processing" hypothesis of dyslexia, which purports that early defects in acoustic and phonetic discrimination can derail the trajectory of language and reading development.[17] This theory is supported by robust evidence that behavioral and neuroimaging measures of auditory temporal processing in infants strongly predict language outcomes.[20,51,52] A second aim was to perform histological assessments on the thalamic nucleus subserving auditory processing (medial geniculate nucleus; MGN), to evaluate the impact of KIAA0319 protein dysregulation on the CNS. Our overall goal was to build on the findings of Martinez-Garay et al.[49] using auditory processing tasks that were successful in detecting deficits in other rodent models of candidate dyslexia susceptibility genes (e.g., *Dcdc2*,[53]; *Dyx1c1*,[54]), as well as to identify neuroanatomical correlates of any auditory processing impairments that might be observed.

We hypothesized that *Kiaa0319* mice would show: (1) a lack of noticeable cortical lamination abnormality[49]; (2) functional anomalies specific to rapid acoustic processing; and (3) anatomic (MGN) acoustic anomalies. Results were expected to provide insight to links between *KIAA0319* disruption and the skills required for typical reading ability, and offer important comparison to phenotypes from other mouse models of dyslexia-risk genes (e.g., *Kiaa0319* vs. *Dcdc2* vs. *Dyx1c1*), as well as *Kiaa0319* knockdown in rats.

TABLE 1 A summary of the behavioral and histological assessments conducted

Task	Age	Function
Normal single tone (8 kHz)	P65	Baseline prepulse inhibition and hearing ability
Embedded tone 100 at 10.5 kHz	P68–P72	Assessment of frequency- and temporally-driven auditory processing ability
Embedded tone 10 at 10.5 kHz	P75–P79	Assessment of temporally-driven auditory processing ability
Silent gap 300	P82–P86	
Silent gap 100	P89–P93	Assessment of frequency-driven auditory processing ability
Pitch discrimination at 10.5 kHz	P96–P100	
Normal single tone (40 kHz)	P101	Baseline prepulse inhibition and hearing ability
Embedded tone 100 at 40 kHz	P103–P105	Assessment of frequency- and temporally-driven auditory processing ability
Pitch discrimination at 40 kHz	P110–P113	Assessment of frequency-driven auditory processing ability
Histological assessments	P120	Histological assessments of MGN development

2 | METHODS

2.1 | Subjects

C57BL/6J-*D130043K22Rik*$^{tm1b(KOMP)Wtsi}$ mice,[49] also described as *Kiaa0319-NZ* and here referred to as *Kiaa0319*$^{-/-}$ or *Kiaa0319* KO, were used. Frozen embryos were thawed and transferred to C57BL/6J carrier-mothers at the Center for Mouse Genome Modification (CMGM), UConn Health (Farmington, CT). Resulting *Kiaa0319* KO offspring were crossed in adulthood with wildtype C57BL/6J mice from The Jackson Laboratory (Bar Harbor, ME; stock number: 000664) to generate an F1 generation. From the F1 generation, non-sibling, heterozygous–heterozygous breeding pairs (*Kiaa0319*$^{+/-}$ × *Kiaa0319*$^{+/-}$) were used to generate matched within-litter wildtype (WT), heterozygous (HT), and homozygous (KO) offspring (F2 generation). Two F2 generation batches were created – batch F2a was generated first, with the F2b generation following 10 weeks later. Both F2a and F2b were generated from the same F2 breeders (i.e., F1 heterozygous breeding pairs), and were tested identically. Subjects were genotyped via ear-punch as previously described.[49]

Experimental batch 1 (i.e., F2a) consisted of four (4) WT males, seven (7) KO males, seven (7) WT females, and seven (7) KO females, while the second experimental batch (F2b) contained five (5) WT males, four (4) KO males, five (5) WT females, and two (2) KO females (total: $n = 9$ WT males; 12 WT females; 11 KO males; 9 KO females). Subjects were provided food and water ad lib and were single-housed after postnatal day (P) 30 in standard Plexiglass mouse cages. All mice were kept on a 12:12 h light/dark cycle, with experimentation occurring during the light cycle. Testing order was randomized prior to the start of experimentation, and experimenters remained blind to genotype. All testing procedures occurred in compliance with National Institutes of Health and approved by the University of Connecticut's Institutional Animal Care and Use Committee (IACUC). See Table 1 for a summary of experimental procedures.

2.2 | Rapid auditory processing (postnatal (P) 65)

2.2.1 | Modified prepulse inhibition paradigm

Starting at a post-puberty age (P65), subjects underwent testing for rapid auditory processing ability using a modified prepulse inhibition paradigm capable of dissociating cue detection ability and reflex startle response from learned associations (see References 25,55 for review). For all auditory processing tasks, subjects were placed on load-cell platforms (Med Associates, St. Albans, VT), which measure the subject's motor reflex (i.e., downward force exerted on the pad),

and allowed each subject to freely roam the open and opaque Plexiglass chamber (20.5 cm × 21.5 cm × 30.5 cm). During the testing session, an auditory cue (generated via RPvdsEx software and a RZ6 multifunction processor (Tucker Davis Technologies, Alachua, FL) was pseudorandomly presented before a 50 ms, 105 dB white noise burst (1000–10,000 Hz; startle eliciting stimulus [SES]). If the subject was able to detect the auditory cue, the force measured by the load-cell platform was reduced as a result of an attenuated acoustic startle response (ASR). The ASR to the SES for each subject was measured when the cue was both present and absent, and within a testing session there was an even distribution of cued and uncued trials. An "attenuation score" was calculated to quantify the reduction in ASR (and therefore cue detection) by comparing the mean startle amplitude on cued trails to the mean startle amplitude on uncued trials. Lower attenuation scores indicate better/enhanced performance, while higher attenuation scores indicate similarities in ASR between cued and uncued trials. ASR measurements were recorded using a Biopac MP150 acquisition system and Acqknowledge 4.1 software (Biopac Systems, Goleta, CA), and calculated using the formula:

$$Attenuation\ Score = \frac{mean\ cued\ ASR}{mean\ uncued\ ASR}\ x\ 100$$

2.2.2 | Normal single tone (P65, P101)

Prior to evaluation on complex auditory processing tasks, subjects were tested on normal single tone (NST) to establish baseline hearing ability and prepulse inhibition, as well as to confirm typical motor (startle) reflexes. Here, subjects were presented with a 50 ms, 75 dB, 8000 Hz pure tone auditory cue, which occurred 50 ms before the white-noise SES (called "NST 8 kHz"). Testing consisted of one testing session with 104 trials, and an even distribution of cued and uncued trials (52 cued/52 uncued; presented in pseudorandom order). Trials occurred between 16 and 24 s apart. Attenuation scores were calculated from this task and used as a covariate on more complex auditory processing tasks, to eliminate bias from individual hearing differences. An ultrasonic version of this task was also used at P101—all subsequent ultrasonic tasks were implemented to account for the higher-frequency mouse audiogram.[56] The trial distribution, cue duration, cue volume and inter-trial interval was identical to NST 8 kHz. However, the cue frequency was changed to 40,000 Hz (NST 40 kHz).

2.2.3 | Embedded tone (P68–72; P75–79; P103–105)

Embedded tone (EBT) can evaluate a subject's ability to detect a subtle change in cue duration and cue frequency during the presentation of a constant, pure-tone background. For all cued trials, a 75 dB, 5600 Hz tone that varied in duration was presented instead of the otherwise constant 75 dB, 10,500 Hz pure-tone background. There were two sub-ultrasonic versions of this task; (1) EBT 100, which contained cues that varied between 2 and 100 ms (EBT 100 at 10.5 kHz), and (2) EBT 10, which contained cues that varied between 2 and 10 ms (EBT 10 at 10.5 kHz). Additionally, an ultrasonic version of this task was implemented. Here, a 75 dB, 35,000 Hz cue that varied in duration was presented just prior to the 105 dB SES (2 ms up/down ramp from the 40,000 Hz constant background). Cue durations varied between 2 and 100 ms (EBT 100 at 40 kHz). For all versions of EBT, 300 trials occurred during each testing session, with an even distribution of cued and uncued trials. Inter-trial intervals varied between 16 and 24 s. For uncued trials, the cue was set to 0 ms in duration. Sub-ultrasonic versions of EBT were conducted over 5 consecutive days, while ultrasonic EBT was conducted over 3 consecutive days. It is important to note that during the presentation of the cue, the background frequency ramped into the stimulus frequency (2 ms onset and offset), such that only one frequency was presented at any time.

2.2.4 | Silent gap (P82–86; P89–93)

Silent gap (SG) measures a subject's ability to detect temporal changes in auditory information, and gap detection thresholds are widely accepted as an index of acoustic temporal acuity.[25] To test this, subjects were presented with a continuous white noise background (1000–10,000 Hz; 75 dB). On cued trials, a "silent" gap of variable duration occurred in a white noise background prior to the SES (100 ms cue-burst interval, fixed). Two versions of the task were used: (1) SG 300, in which the cue (gap) varied between 50 and 300 ms; and (2) SG 100, in which the cue (gap) varied between 2 and 100 ms. For both SG 300 and SG 100, each testing session consisted of 300 evenly distributed and pseudorandom trials, with inter-trial intervals varying between 16 and 24 s. Uncued trials contained no cue (i.e., a gap presented for 0 ms). Each SG task was presented for 5 consecutive days.

2.2.5 | Pitch discrimination (P96–100; P110–113)

To evaluate the ability to detect subtle changes in pitch, subjects underwent testing on Pitch discrimination (PD). This task consisted of a constant 75 dB, 10,500 Hz background tone and a 75 dB, 300 ms cue that varied in pitch. Just like EBT, the cue and the background tones were not presented simultaneously but transitioned fluidly with a 2 ms shift in pitch during the onset and offset of the cue (i.e., frequency shift). During the sub-ultrasonic version of this task (PD at 10.5 kHz), the pitch of the cue varied between 10,425 and 10,550 Hz. For the ultrasonic version of this task (PD at 40 kHz), the background tone was presented at 40.5 kHz and the pitch of the cue varied between 32,000 and 48,000 Hz. Both versions of the task included presentation of 300 even distributed cued and uncued trials, with inter-trial intervals between 16 and 24 s. Sub-ultrasonic tasks were conducted for 5 consecutive days and ultrasonic PD was conducted over 4 consecutive days.

2.3 | Histology

2.3.1 | Serial sectioning

Seven days following the completion of behavioral testing (P120), subjects were weighed and anesthetized using ketamine (100 mg/kg; Henry Schein, Melville, NY) and xylazine (15 mg/kg; Thermo Scientific, Waltham, MA) prior to undergoing a transcardial perfusion with 0.9% saline and 10% formalin (Sigma-Aldrich, St. Louis, MO). After brains were extracted following protocols outlined by Reference 57, tissue was placed in glass vials and postfixed in 10% formalin. After being allowed to postfix for at least 2 weeks, each brain was removed from formalin and serially sectioned in the coronal plane via a Leica VT1000 S vibroslicer (Leica Biosystems Inc., Buffalo Grove, IL) at 60 μm. Sectioning began at the anterior-most portion of the brain (cerebellum removed), and progressed posteriorly. Every other section was mounted to a gelatin-subbed glass slide. Tissue was stained with cresyl violet and cover-slipped with DPX mounting medium (Sigma-Aldrich, St. Louis, MO). Typical cortical lamina was clearly identified in all subjects, and no visual anomalies were observed in the cortices of any of the mice.

2.3.2 | Stereological measurements

Cresyl violet stained tissue underwent stereological analysis via Stereo Investigator (MBF Biosciences, Williston, VT) using a Zeiss Axio Imager A2 microscope (Carl Zeiss, Thornwood, NY). A stereotaxic atlas[58] was used to define the boundaries of the MGN and contours were drawn at ×2.5 magnification. The volume of the MGN was estimated via the Cavalieri Estimator probe (analyzed under ×2.5 magnification), and the Optical Fractionator probe was used to estimate neuron population (analyzed under ×100 magnification). The Nucleator probe (an MBF-supported algorithm) was used to measure average neuron area and volume. Neuron areas were hand-calculated for cells that met criteria within the boundaries of the MGN (100X) and within a defined sampling grid (225 μm × 225 μm, and a 25 μm × 25 μm counting frame), specifically including neurons with a clearly defined nucleus (excluding glia and other cell types). Areas were derived from computer-generated radial vectors aligned to the nucleus and demarcated by intersections with cell membrane boundaries. Cell volumes were reconstructed from serial sampling grids (every other section across six total sections). All measures were performed by an experienced investigator, and blind to genotype.

2.4 | Statistical analysis

All subjects were used for statistical analysis, and each Genotype (WT, $n = 21$; KO, $n = 20$) included both male and female subjects. Since no Sex*Genotype differences were found on any measure, Genotype effects are reported with pooling across Sex. For NST (8 and 40 kHz), a univariate analysis of variance (ANOVA) was conducted. NST was also used as a covariate in the analysis of more complex tasks (corresponding frequency ranges). Thus, NST 8 kHz was used as a covariate for sub-ultrasonic tasks, while NST 40 kHz was used as a covariate for ultrasonic tasks. For complex auditory processing tasks, a repeated measures ANOVA was used to ascertain Genotype differences. For sub-ultrasonic tasks, a 2 (Genotype; between-subjects variable) × 5 (Day) × 9 (Cue) repeated measures ANOVA was conducted. For EBT 100 at 40 kHz, a 2 (Genotype) × 3 (Day) × 5 (Cue) repeated measures ANOVA was conducted; and for PD at 40 kHz, a 2 (Genotype) × 4 (Day) × 5 (Cue) repeated measures ANOVA was conducted. Repeated measures ANOVAs were performed in R via the ez package.[59]

For histological assessments, 20 WT subjects and 19 KO subjects were evaluated. Genotype differences in structural volume, neuron population and average neuron area were assessed in the MGN via univariate ANOVAs. The Kolmogorov–Smirnov (K-S) test was used as a more sensitive measure of cumulative percent cell size distribution. Histological measurements were evaluated for Right MGN, Left MGN and total (overall) MGN. All ANOVAs were conducted using the car package[60] in R (v3.4.4[61]).

3 | RESULTS

3.1 | Rapid auditory processing

3.1.1 | NST

NST was administered to measure baseline hearing ability and pre-pulse inhibition at sub-ultrasonic (NST 8 kHz) and ultrasonic (NST 40 kHz) frequencies. We found no significant effect of Genotype on NST 8 kHz ($F[1, 39] = 1.66$, $p > 0.05$; Figure 1A), nor on NST 40 kHz ($F[1, 39] = 1.12$, $p > 0.05$; Figure 1B). This indicates that WT and KO subjects had similar hearing, startle and pre-pulse inhibition. Finally, there was no effect of Sex or Sex*Genotype interaction for NST 8 kHz (Sex: $F[1, 37] = 0.010$; $p > 0.05$; Sex*Genotype $F[1, 37] = 0.299$; $p > 0.05$) or NST 40 kHz (Sex: $F[1, 37] = 0.175$; $p > 0.05$; Sex*Genotype $F[1, 37] = 0.144$; $p > 0.05$).

3.1.2 | Frequency-dependent auditory processing

We found no significant main effect of Genotype on Embedded Tone 100 at 10.5 kHz ($F[1, 39] = 0.01$, $p > 0.05$; Figure 2A), Embedded Tone 10 at 10.5 kHz ($F[1, 39] = 0.02$, $p > 0.05$; Figure 2B), nor Embedded Tone 100 at 40 kHz ($F[1, 39] = 0.17$, $p > 0.05$; Figure 2C). There was also no effect of Sex or Sex*Genotype interaction for EBT 100 at 10.5 kHz (Sex: $F[1, 37] = 0.000$; $p > 0.05$; Sex*Genotype $F[1, 37] = 3.100$; $p > 0.05$), EBT 10 at 10.5 kHz (Sex: $F[1, 37] = 0.410$; $p > 0.05$; Sex*Genotype $F[1, 37] = 0.300$; $p > 0.05$) or EBT 100 at 40 kHz (Sex: $F[1, 37] = 0.080$; $p > 0.05$; Sex*Genotype $F[1, 37] = 1.650$; $p > 0.05$). Additionally, we found no significant main

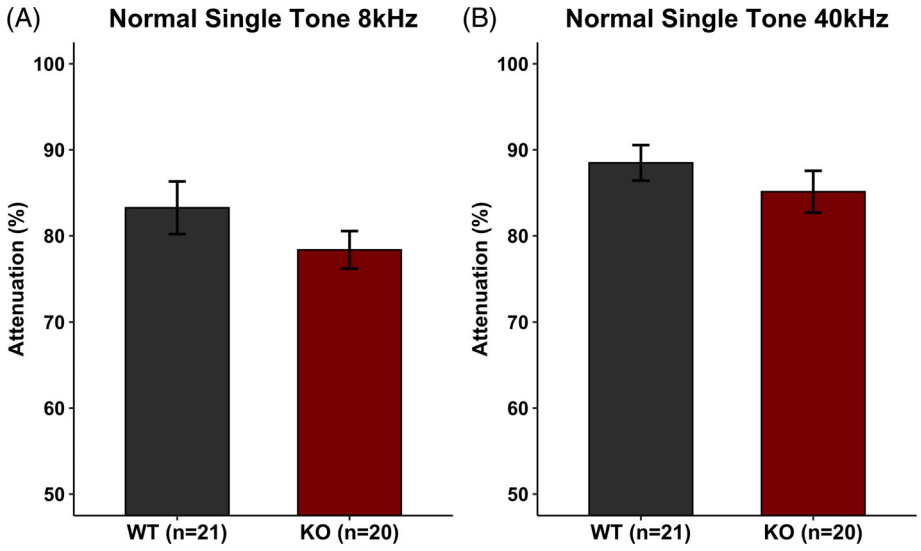

FIGURE 1 Normal Single Tone. *Kiaa0319* KO mice showed no baseline prepulse inhibition impairments or hearing abnormalities as compared with WT mice, using audible frequencies (8 kHz; A) and ultrasonic frequencies (40 kHz; B). *Lower attenuation scores indicate better performance*

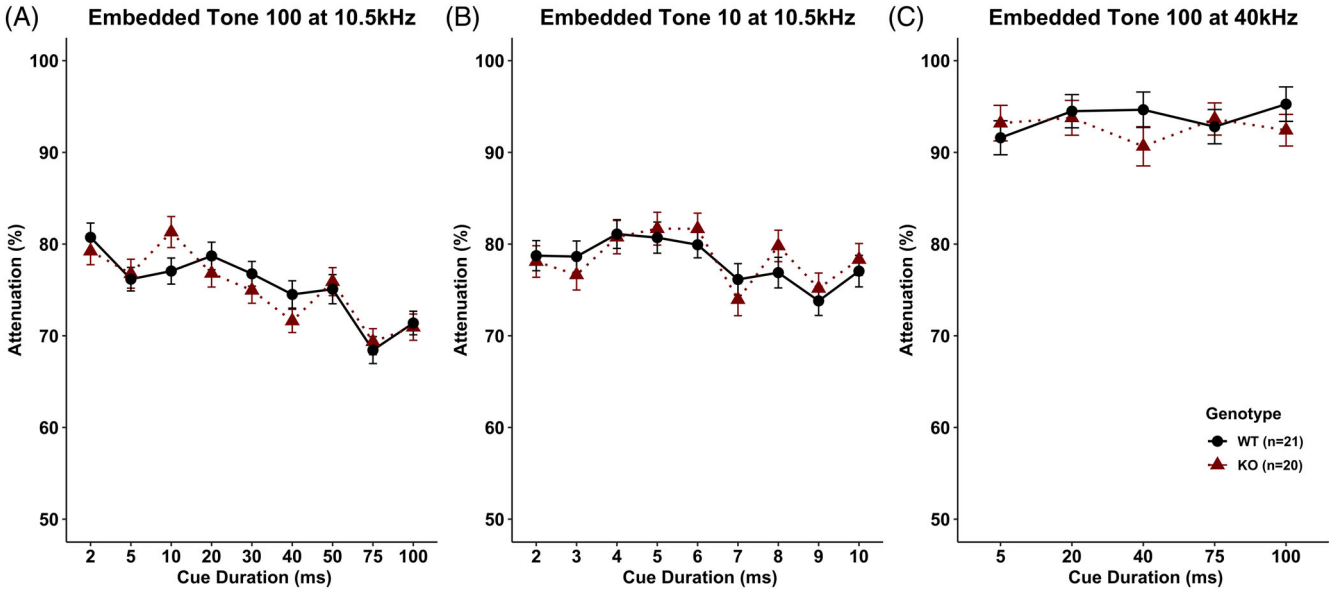

FIGURE 2 Embedded Tone. *Kiaa0319* KO mice performed comparably to WT mice on Embedded Tone 100 at 10.5 kHz (A), Embedded Tone 10 at 10.5 kHz (B), and Embedded Tone 100 at 40 kHz (C). *Lower attenuation scores indicate better performance*

effect of Genotype on PD at 10.5 kHz ($F[1, 39] = 0.38$, $p > 0.05$; Figure 3A), nor PD at 40 kHz ($F[1, 39] = 0.08$, $p > 0.05$; Figure 3B). There was also no effect of Sex or Sex*Genotype interaction for either PD at 10.5 kHz (Sex: $F[1, 37] = 0.030$; $p > 0.05$; Sex*Genotype $F[1, 37] = 0.000$; $p > 0.05$) or PD at 40 kHz (Sex: $F[1, 37] = 2.100$; $p > 0.05$; Sex*Genotype $F[1, 37] = 0.020$; $p > 0.05$). These results show that *Kiaa0319* KO mice were equally able to discriminate changes in frequency compared with WT.

3.1.3 | Temporal auditory processing

There was no main effect of Genotype on the longer version of silent gap (SG 300) ($F[1, 39] = 0.25$, $p > 0.05$; Figure 4A), indicating KO mice were processing longer duration cues similarly to controls. However, on the rapid version of silent gap (SG 100), we found that *Kiaa0319* KO mice were significantly impaired relative to WT controls ($F[1, 39] = 8.14$, $p < 0.01$; Figure 4B). Notably, an F value of 8.14 equates to a Cohen's d value of 1.28 (a robust effect size), calling for a minimum n of 12 per group at 80% power (15–16 per group at 90% power), and alpha = 0.05, to obtain significance. Therefore our n (20 KO, 21 WT) was adequate. Conversely, it is important to point out here that the n used in a prior study by Guidi et al.[50] was likely *not* adequate to detect impairments in rapid silent gap detection in the same line of *Kiaa0319* KOs ($n = 9$ KO, 18 WT) on similar silent gap detection tasks. Our novel findings confirm disruption in the processing of short acoustic gaps (less than 100 ms).

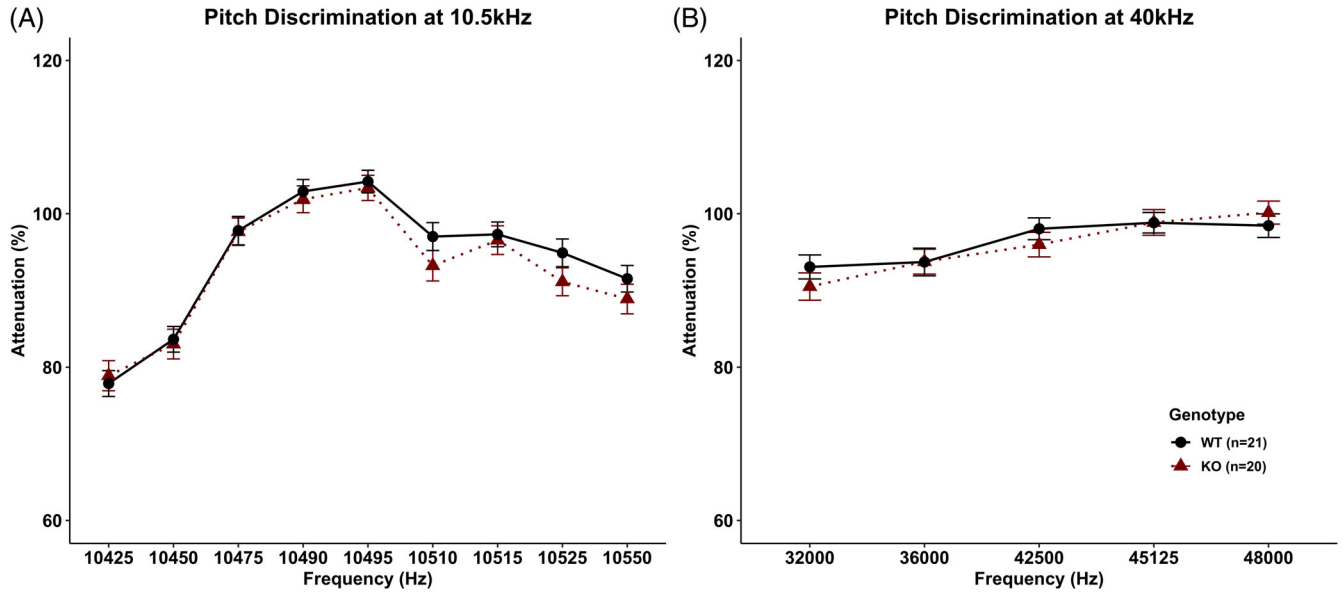

FIGURE 3 Pitch Discrimination. *Kiaa0319* KO mice performed comparably to WT mice on Pitch Discrimination at 10.5 kHz (A) and Pitch Discrimination at 40 kHz (B). *Lower attenuation scores indicate better performance*

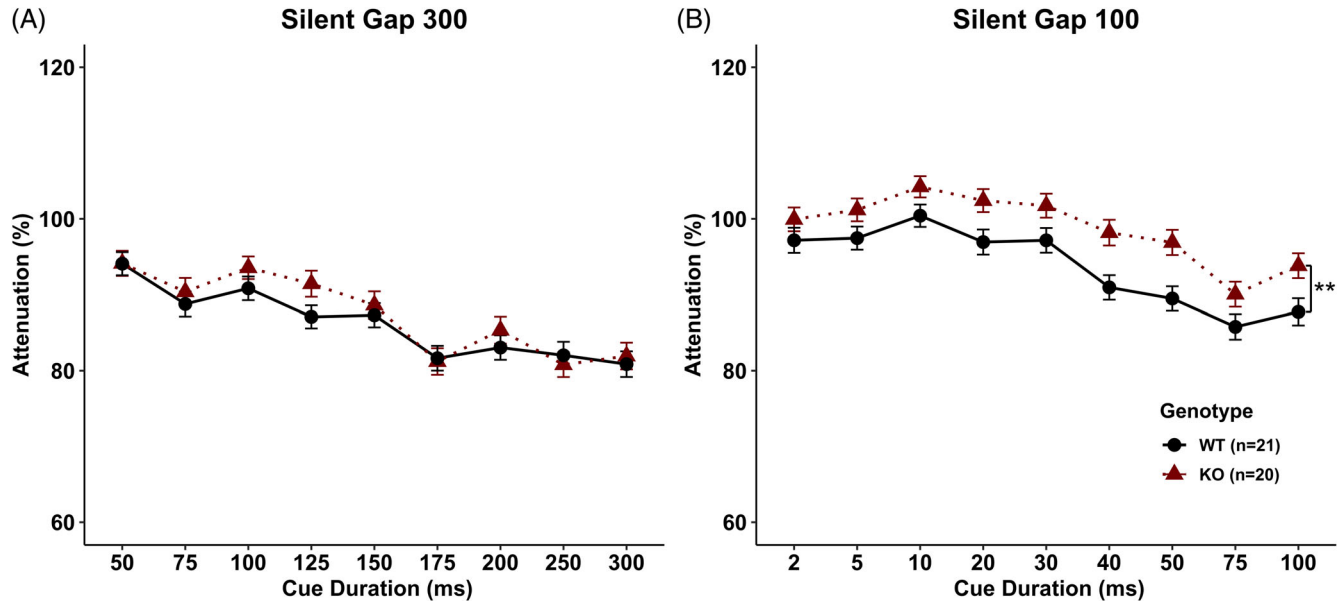

FIGURE 4 Silent Gap. *Kiaa0319* KO mice performed comparably to WT mice on Silent Gap 300 (A). However, *Kiaa0319* KO mice performed significantly worse on Silent Gap 100 as compared with WT mice (B). *Lower attenuation scores indicate better performance*. $^{**}p < 0.01$

With regards to Sex effects and Sex*Genotype interactions—for Silent Gap 300, there was a significant effect of Sex ($F[1, 37] = 12.92$; $p < 0.001$) with Males outperforming Females, however, there was no significant Sex*Genotype interaction ($F[1, 37] = 0.05$; $p > 0.05$). A similar pattern was seen on Silent Gap 100—there was a significant effect of Sex ($F[1, 37] = 7.28$; $p = 0.01$) with males outperforming females, however, there was no significant Sex*Genotype interaction ($F[1, 37] = 1.55$; $p > 0.05$). Because of lack of a significant Sex*Genotype interaction, Genotype remained the primary focus of these findings.

3.2 | Histological assessment of the MGN

No cortical laminar abnormalities were observed in *Kiaa0319* KO mice. Histological analysis of the MGN in *Kiaa0319* KO mice compared with controls showed no significant differences in volume of the Left MGN ($F[1, 37] = 3.68$, $p > 0.05$), a significant volumetric reduction in the Right MGN ($F[1, 37] = 4.51$, $p < 0.05$), and a significant reduction in overall MGN area ($F[1, 37] = 4.54$, $p < 0.05$) (Figure 5A). There were no significant Genotype differences when evaluating neuron population (Left MGN: $F[1, 37] = 1.66$, $p < 0.05$;

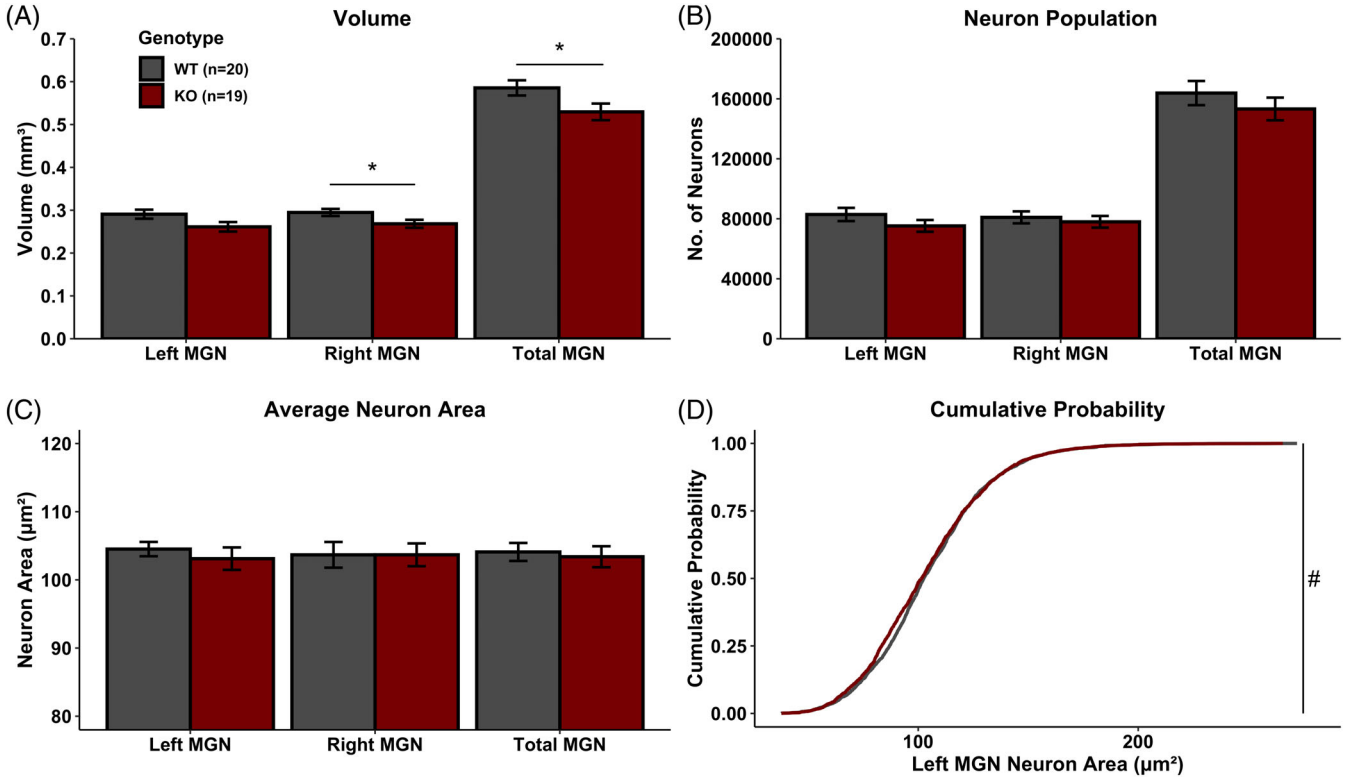

FIGURE 5 Histological assessment of the MGN. A volumetric reduction of the right and total MGN were observed in *Kiaa0319* KO mice as compared with WT subjects (A). There were no significant differences in number of neurons (B) and the average neurons size (C) between WT and KO mice. However, there was a shift toward smaller neurons in the Left MGN of *Kiaa0319* KO mice when compared with WT subjects (D). $^{\#}p < 0.10$; $^{*}p < 0.05$

Right MGN: $F[1, 37] = 0.28$, $p < 0.05$; Total MGN: $F[1, 37] = 0.92$, $p < 0.05$) (Figure 5B). We also saw no main effect of Genotype for a simple mean comparison of neuron area (Left MGN: $F[1, 37] = 0.53$, $p < 0.05$; Right MGN: $F[1, 37] = 0.00$, $p < 0.05$; Total MGN: $F[1, 37] = 0.12$, $p < 0.05$) (Figure 5C). However, a nonparametric K-S test on the comparability of continuous one-dimensional probability distributions showed a significant Genotype effect on cell-size distributions in the left and total MGN. Specifically, *Kiaa0319* knock-out mice showed a significant shift toward *fewer large and smaller neurons in the MGN* (Left MGN: $p < 0.01$; Total MGN: $p = 0.05$; Figure 5D). Notably, this test likely detected significance in MGN cell size distribution *despite* lack of significance in mean cell sizes (ANOVA) because the K-S test uses a Bayesian approach to directly assay nonparametric probability distributions, while standard ANOVA assumes normality of distributions.[62] No differences were seen in cell-size distribution in the Right MGN ($p > 0.05$).

4 | DISCUSSION

In the current study, we evaluated the behavioral and neuroanatomical consequences of *Kiaa0319* disruption using an engineered mouse model. Results showed that *Kiaa0319* KO mice do not have any obvious cortical lamination defects, but exhibit significant auditory processing impairments on a rapid acoustic processing task (detection of Silent Gaps under 100 ms). Importantly, they did not show impairments on an easier (longer-duration) version of the Silent Gap task, nor on frequency discrimination tasks, indicating otherwise normal auditory processing and PPI. We also found significant volumetric reductions of the right and total MGN, and a shift toward smaller and fewer larger neurons in the left and total MGN, in *Kiaa0319* KO mice. These results parallel prior evaluations of MGN in human post mortem brains of individuals with dyslexia, also showing shifts in MGN cell size toward more smaller and fewer large neurons.[63] Overall, the current results provide novel and important evidence of auditory processing impairments and thalamic neuroanatomical abnormalities in a *Kiaa0319* KO mouse model.

4.1 | Auditory processing impairments in Kiaa0319 KO mice

Our results show temporally-driven auditory processing impairments in *Kiaa0319* KO mice, as measured on a silent gap 100 ms. It is important to note that *Kiaa0319* KO mice showed auditory processing difficulties on the shorter version of silent gap but performed comparably to WT on the less temporally demanding version of the task (Silent Gap 300 ms), as well as on single-tone and frequency-based versions

of the task (EBT, PD). These disparate results indicate that mice had a *specific* difficulty with rapid (short duration) temporal processing in silent gap detection—a skill crucial to early processing and discrimination of phonemic speech sounds that form the foundation for subsequent language and reading.[64]

It is also important to note that a prior study failed to show significant deficits in *Kiaa0319* KO mice on a related silent gap 100 paradigm.[50] However, that study employed a smaller n (*Kiaa0319* KO = 9, WT = 18), and was under-powered based on current analyses. Therefore, few conclusions can be drawn regarding comparisons to current results. Guidi et al.[50] did report an auditory deficit in a behavioral gap-in-noise detection task in *Kiaa0319* and *Kiaa0319L* double knockout mouse, as well as abnormal recordings of click-evoked auditory brainstem responses (suprathreshold deficits in wave III amplitude) in *Kiaa0319L* (*AU040320*) mice, and more general deficits in dKOs. *AU040320* is the mouse homolog of *KIAA0319L*, which has also been suggested as a dyslexia-risk gene[65] and implicated in neuronal migration.[66]

Current findings are consistent with evaluations of auditory processing impacts from manipulations of other dyslexia candidate risk-genes. For example, Szalkowski et al.,[46] reported that *Kiaa0319* shRNA treated rats showed a marginal impairment on silent gap 0–10 (the most difficult version of Silent Gap used in their study) during the juvenile period, and a more robust deficit during adulthood when evaluated on a complex FM Sweep task. (Note that rats exhibit superior processing to mice, and require more demanding tasks to elicit acoustic processing deficits in genetic models; see Reference 55 for review). Although FM Sweep involves a cue which contains frequency *and* duration information, *Kiaa0319* shRNA rats showed impairments specifically when the cue presentation was shortest in duration, emphasizing the *temporal* demand as the critical differentiating factor in eliciting acoustic deficits. Similar findings have been reported for a wide variety of genetic and injury rodent models used to test core functional processes that (in humans) subserve language and commutation (e.g., induced cortical microgyria, autism risk-genes). In these varied models, deficits were consistently seen specifically on rapid versions of acoustic processing tasks (see Reference 52,67 for review).

In summary, cumulative findings indicate that rodent models of genes linked to language disability consistently show impairments on temporally-demanding or "rapid" (short-duration) versions of auditory processing tasks as characterized by brief silent gap detection.[25] This parallels data from human dyslexia research, where difficulties with temporal auditory processing *specifically with brief stimuli* are consistently and repeatedly reported.[15,16,68–72] These rapid acoustic processing deficits (e.g., as indexed via gap detection thresholds) are theorized to contribute to early disruptions of phonemic and phonologic language systems necessary to later reading acquisition.

4.2 | Neuroanatomical abnormalities in Kiaa0319 KO mice

A second aim of the current study was to evaluate how homozygous disruption of *Kiaa0319* affects brain regions crucial to auditory processing—specifically, the cortex and MGN of the thalamus. Our study did not examine cortical lamination defects in detail, but we visually confirmed a lack of major laminar abnormalities as previously reported in this model.[49,50] We also examined the MGN in the thalamus because a *postmortem* histological assessment of brain tissue from individuals with dyslexia, Galaburda et al.,[43] reported a shift toward smaller (and fewer large) MGN neurons. Those findings have since been validated in at least one imaging study.[73] Moreover, similar findings have been reported in rodent models for candidate genes of developmental dyslexia[47] and various other rodent models of cortical dysgenesis.[74–77] Here, we report that homozygous disruption of *Kiaa0319* results in a volumetric reduction in MGN size, as well as a shift toward smaller and fewer larger neurons. As an aside, we comment on the observation of reductions in volume on the right and overall MGN, versus shifts toward a smaller cell size on the left only. We do not view this as counter-intuitive. First, other variables besides cell size certainly contribute to structural volume (e.g., glia, neural density). Second, volume *was* reduced in the left MGN, just not significantly so. In short, anomalous impacts on the MGN appear on both sides, likely contributing together to behavioral difficulties. Nonetheless, future studies are needed to examine possible side-specific effects more closely, particularly given left language lateralization in humans.

Finally, the current MGN results may relate to functional differences observed in the lateral geniculate (visual) nucleus (LGN) of dyslexic human brains (where fewer large cells are also seen as compared with typical controls[78]). In the LGN, larger (magnocellular) cells are involved in rapid motion processing, whereas smaller parvocellular cells subserve color and detail processing. Thus, a selective loss of large cells in the dyslexic LGN could also relate to reading impairments. Although a similar functional dichotomy by cell size has not been shown in the MGN, it is nonetheless possible that the overall shift in cell size relates to impaired processing of acoustic *temporal* information.[24] Again, future studies will be necessary to identify and evaluate the implications of cell size shifts in the MGN.

4.3 | Controversies in the neurobiological basis of dyslexia

While the underlying neurobiological cause of developmental dyslexia remains poorly understood, anomalies in neuronal migration are thought to be a possible contributor to the pathology of the disorder. This hypothesis was founded based on the discovery of atypically migrated cortical neurons in individuals with dyslexia.[42] Since its inception, multiple rodent models have provided evidence for that hypothesis. Using in utero electroporation of small hairpin RNAs (shRNA) to downregulate *Kiaa0319* expression, Paracchini et al.,[35] Peschansky et al.,[45] Platt et al.,[66] Szalkowski et al.,[46] all reported atypical neuronal migration in *Kiaa0319* shRNA-electroporated rats. These anomalies were suggested to contribute to reported impairments in auditory processing, working memory and other anomalous behaviors in treated rats. As gene-editing technology has advanced,

mouse models of dyslexia-risk genes have showed more complex findings. Martinez-Garay et al.,[49] developed a *Kiaa0319* KO mouse using Cre-recombinase technology, and this KO model did *not* show atypical neuronal migration. Guidi et al.,[50] confirmed a lack of neuronal migration deficits in this *Kiaa0319* KO mouse model. The current study did not specifically examine cortical neuronal migration, but gross morphology of the cortex appeared normal on our Cresyl violet stained tissue, confirming previous findings.[49,50] These differences between *Kiaa0319* downregulation via RNA interference in rats, and *Kiaa0319* knockout in mice, challenge the validity of the neuronal migration hypothesis of dyslexia (see Reference 79 for a review of the evidence in favor and against the neuronal migration hypothesis of dyslexia across multiple risk genes). In the current study, we also used *Kiaa0319* KO mice derived from the Martinez-Garay et al.,[49] line. Despite visibly normal neuronal migration, we did find changes in temporally-driven auditory processing—as well as morphological alterations in the MGN. This is consistent with several gene mutations known to lead to clinical migration defects (e.g., doublecortin,[80,81] *LIS1*,[82] or *TUBA3*[83]), yet which show no apparent cortical migration phenotype in mouse KO models. Again, further research on the mechanistic relationship between *Kiaa0319* disruption and brain development is needed, as well exploration of impacts on behaviors such a rapid acoustic processing that may be relevant to human dyslexia.

4.4 | Limitations of current study

While the data and results presented here suggests that *Kiaa0319* disruption early in neurodevelopment results in temporally-driven auditory processing impairments and atypical MGN development, this study was not without limitations. For example, future studies should also assess other auditory-related brain structures in this model (e.g., cochlear nucleus, superior olivary complex, inferior colliculus and primary auditory cortex [A1]). Moreover, additional research is necessary to determine the specific molecular and cellular pathways underlying changes in MGN (and potentially additional structures) development. Further, both WT and KO subjects with *no* behavioral testing experience should be included in future histological assessments, to eliminate the potential confound of experience/exposure on brain development. Similarly, possible inter-active impacts of acoustic testing experience were not explicitly dissociated in the current study, and interpretations of findings would benefit from future inclusion of such additional controls.

A second important limitation of this study regards the potential impact of single housing on subject outcomes (all subjects were single-housed following weaning at P30). Although housing conditions were identical across Genotype groups, evidence shows that single-housed C57BL/6J mice were impaired in prepulse inhibition as compared with C57BL/6J mice that were group-housed.[84] Thus potential interactive effects of single-housing versus group-housing with Genotype-based deficits reported here should be examined in future studies.

5 | CONCLUSIONS

We report here that disruption of *Kiaa0319* results in relatively specific temporally-driven auditory processing difficulties, as well as atypical MGN development, despite seemingly typical cortical neuronal migration. Our findings provide supporting evidence of *KIAA0319*'s role in neurological processes that could be important in the pathology of developmental dyslexia, and prompt further investigation of alternative causal mechanisms outside disruption of the cortical neuronal migration process. Further research is needed to characterize in detail the behavioral and neuroanatomical consequences of alteration of gene *Kiaa0319* early in development, with the goal of better understanding neurodevelopmental processes contributing to dyslexia in humans. Work in this area is expected to lead to improved early genetic screening of infants, as well as the possibility of improved prognostication and tailored interventions based on specific dyslexia-risk mutations identified early in life.

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AUTHOR CONTRIBUTIONS

Peter A. Perrino was responsible for subject generation (i.e., animal husbandry & genotyping), conducting and overseeing behavioral and histological experiments/assessments, data analysis, and drafting the manuscript. Renee Y. Chasse was responsible for conducting behavioral experiments and histological assessments. Anthony P. Monaco, Zoltán Molnár, and Antonio Velayos-Baeza were primarily responsible for the original generation of the *Kiaa0319* KO mouse model, as well as provided valuable insight while drafting the manuscript. R. Holly Fitch was responsible for experimental design, manuscript drafting and providing the primary funding source. All authors have read and agreed to the published version of the manuscript. The authors have no conflicts of interest to declare.

ETHICS STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the University of Connecticut's Institutional Animal Care and Use Committee (IACUC) (Protocol No. A18-050; approval date February 27, 2020).

DATA AVAILABILITY STATEMENT

The data can be made available from the corresponding author upon reasonable request.

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