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Gray matter maturation and cognition in children with different APOE ε genotypes

ABSTRACT

Objective: The aims of the current study were to determine whether children with the 6 different APOE ε genotypes show differences in gray matter maturation, particularly for those with ε4 and ε2 alleles, which are associated with poorer outcomes in many neurologic disorders.

Methods: A total of 1,187 healthy children (aged 3–20 years, 52.1% boys, 47.9% girls) with acceptable data from the cross-sectional Pediatric Imaging Neurocognition and Genetics Study were evaluated for the effects of 6 APOE ε genotypes on macroscopic and microscopic cortical and subcortical gray matter structures (measured with 3-tesla MRI and FreeSurfer for automated morphometry) and on cognition (NIH Toolbox).

Results: Among APOE ε4 carriers, age-related changes in brain structures and cognition varied depending on genotype, with the smallest hippocampi in ε2ε4 children, the lowest hippocampal fractional anisotropy in younger ε4ε4 children, the largest medial orbitofrontal cortical areas in ε3ε4 children, and age-dependent thinning of the entorhinal cortex in ε4ε4 children. Younger ε4ε4 children had the lowest scores on executive function and working memory, while younger ε2ε4 children performed worse on attention tasks. Larger parietal gyri in the younger ε2ε4 children, and thinner temporal and cingulate isthmus cortices or smaller hippocampi in the younger ε4ε4 children, predicted poorer performance on attention or working memory.

Conclusions: Our findings validated and extended prior smaller studies that showed altered brain development in APOE ε4-carrier children. The ε4ε4 and ε2ε4 genotypes may negatively influence brain development and brain aging at the extremes of age. Studying APOE ε polymorphisms in young children may provide the earliest indicators for individuals who might benefit from early interventions or preventive measures for future brain injuries and dementia. Neurology® 2016;87:585-594

GLOSSARY

AD = Alzheimer disease; FA = fractional anisotropy; GAF = genetic ancestry factor; GAM = general additive model; PING = Pediatric Imaging, Neurocognition, and Genetics; ROI = region of interest; SES = socioeconomic status; WM = working memory.

APOE ε4 is a well-known risk allele for Alzheimer disease (AD), especially late-onset AD, and may lead to poorer outcome in neurologic disorders.1–4 In addition, APOE ε4 may influence brain development.5–7 However, the APOE ε4 allele demonstrates antagonistic pleiotropy, with deleterious effects on cognition, brain morphometry, and activation primarily after 55 years of age, but no negative8 or even beneficial effects in adults younger than 50 years9,10 and children aged 6 to 15 years.11–13 Compared to non–ε4 carriers, healthy children carrying ε4 (8–20 years) tended to have thinner entorhinal cortex,14 while healthy infants carrying ε4 showed altered brain measures in regions affected by AD.6,7 Whether these structural differences influence cognitive performance in children with ε4 remains controversial.5,12–14

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**RESULTS**

Participant characteristics. The 1,187 children were aged 12.1 ± 5.0 years; 569 were girls (table e-1 on the Neurology® Web site at Neurology.org).

The 5 APOE ε allele groups were similar in sex proportion. The ε2ε2 children were the youngest and ε2ε3 children were the oldest; ε3ε3 was most common (61.78%), followed by ε3ε4 (21.8%), ε2ε3...
(11.9%), \(\varepsilon 2\varepsilon 4\) (2.6%), and \(\varepsilon 4\varepsilon 4\) (1.75%), with \(\varepsilon 2\varepsilon 2\) the rarest (0.17%). All genotype groups showed significant European GAF (60%–70%), except for \(\varepsilon 4\varepsilon 4\) (35% European, but higher African ancestry than other groups). Parents/guardians of \(\varepsilon 4\varepsilon 4\) children had the lowest household income, education, and occupation levels.

**Subcortical volume differences across genotypes.** The hippocampi differed across genotype groups (figure 1, A and B; table e-2). Independent of age, \(\varepsilon 3\varepsilon 4\) children had the largest, while \(\varepsilon 2\varepsilon 4\) had the smallest hippocampi across groups (figure 1B). Hippocampal volumes increased linearly with age but differed by APOE \(\varepsilon\) genotype, with an inverted U shape in \(\varepsilon 3\varepsilon 3\) children (peaking at 13.2 years). The 2 children with \(\varepsilon 2\varepsilon 2\) had relatively large hippocampi.

**Genotype-by-age on subcortical FA.** Age-dependent FA changes were evaluated in all subcortical structures (table e-2; figure 1, B–F). In the right hippocampus, FAs in \(\varepsilon 4\varepsilon 4\) children were lower at younger ages (younger than 7 years) but normalized thereafter, with no changes with age (figure 1B). As a group, \(\varepsilon 2\varepsilon 4\) children had the highest FA, while the younger \(\varepsilon 2\varepsilon 2\) child showed the lowest hippocampal FA. Conversely, in the left amygdala, younger children (<7 years) with \(\varepsilon 4\varepsilon 4\), \(\varepsilon 2\varepsilon 4\), and \(\varepsilon 3\varepsilon 4\) had lower FA than \(\varepsilon 3\varepsilon 3\) and \(\varepsilon 3\varepsilon 4\) groups, whose FA was constant with age (figure 1D). The left thalamus FA increased with age in all children (figure 1E), but children younger than 7 years with \(\varepsilon 2\varepsilon 4\) and \(\varepsilon 4\varepsilon 4\) had lower FA, while children older than 12 years with \(\varepsilon 4\varepsilon 4\) showed higher FA (figure 1E).

**APOE \(\varepsilon\) genotype on cortical measures.** Several cortical areas differed by APOE \(\varepsilon\) genotype (figure 2A), with parallel age-related trajectories (validated in the ROI model) (figure 2, A–D; table e-3). For these regions,
e2ε4 children showed the smallest areas, while e4ε4 or ε3ε4 children had the largest areas (figure 2, B–D). The 2 children with ε2ε2 had exceptionally large areas. The selected cortical volumes decreased with age for all genotypes. Computed tomography analysis showed that, relative to the reference ε3ε3 group (brown), the ε2ε4 group (blue) had significantly smaller right medial orbitofrontal cortical areas (p = 0.006) and smaller right cuneus areas (p = 0.002), while the ε3ε4 group (purple) had larger lateral occipital (p = 0.016) and medial orbitofrontal cortical areas (p = 0.006). The ε4ε4 group (red) also had the largest areas among all groups in the right cuneus (ε4ε4 > ε2ε4, p = 0.07) and right lateral occipital area (ε4ε4 > ε2ε4, p = 0.04). The children with ε2ε2 (black) had relatively large cortical areas in the cuneus and right medial orbitofrontal regions, largest in the younger ε2ε2 child. See also table e-3 for additional results. *Data for the ε2ε2 children are not included in the group analyses.

For cortical thickness, the right isthmus cingulate showed age-dependent thinning, except for ε4ε4 children who showed no change with age (figure 3C). Conversely, temporal pole thickness was constant with age, except for ε4ε4 children who showed age-related increases, with thinner cortices in younger and thicker cortices in older individuals (figure 3D). Furthermore, since prior reports compared ε4 carriers (ε4ε4, ε4ε3) with non-ε4 carriers (ε3ε3, ε2ε3),5 we performed vertex-based, whole-brain analyses using the same grouping. Compared to non-ε4 carriers, ε4 carriers had nonsignificantly thicker left entorhinal cortex (figure 4A, yellow region), verified on ROI analyses (figure 4B), and nonsignificantly thinner dorsal postcentral and lateral temporal cortices (figure 4A, lighter blue). However, cortical thickness showed a trend for group differences on age-dependent measures in parahippocampal regions and the left postcentral and entorhinal cortices (figure 4C, yellow regions). ROI analyses verified group differences in age-dependent thinning in the left entorhinal cortex. Specifically,
e4/e4 children showed the steepest slope ($r = -0.66$, $p = 0.02$), with thicker cortices in younger children but thinner cortices in older children (>11 years; figure 4D), as reported for adolescents.5

### APOE ε genotypes and age on cognitive performance.

Age-by-genotype interactions were found on executive function, attention, and WM (figure 5, A–C) but not episodic memory. Compared to other genotype groups, younger e4/e4 children had lower scores on executive function and WM, but similar or better performance after age 8 years (figure 5, A and B). On the attention task, younger e2/e4 children performed worse than other genotype groups, but their scores normalized after age 10 years (figure 5C).

#### Relationships between brain morphometry and cognition.

After adjustments for sex, SES, and GAF, brain regions with age-related genotype variations also showed differential correlations with attention or WM. In the right superior parietal gyrus, e2/e4 children differed from other groups; those with larger superior parietal gyral volumes had the lowest attention ($r = -0.58$, $p = 0.001$; figure 5D) or WM scores ($r = -0.59$, $p = 0.005$; figure 5E). Similarly, across genotypes, e2/e4 children with thicker cortices had the poorest attention (right isthmus cingulate: $r = -0.62$, $p = 0.0002$, figure 5F; temporal pole: $r = 0.43$, $p = 0.01$, figure 5G), while e4/e4 children with thinner temporal poles had poorer attention ($r = +0.5$, $p = 0.02$; figure 5G). All children with smaller hippocampal volumes had poorer WM performance, especially e4/e4 children ($r = +0.48$, $p = 0.03$; figure 5H).

### DISCUSSION

The APOE ε gene is polymorphic with 3 alleles, with ε3 being the most common (approximately 78%), followed by ε4 (14%) and ε2 (8%).15 Evaluating all 6 APOE ε genotypes in a large cohort of typically developing children clarified the
associations of heterozygous vs homozygous e2 and e4 on brain development. The major findings are as follows: (1) compared to other genotype groups, e4e4, e2e2, and e2e4 children had altered age-related slopes in brain regions often affected in AD; (2) smaller hippocampal volumes in younger e2e4 children and lower hippocampal FA in younger e4e4 children mirror the smaller volumes and steeper age-dependent atrophy of the hippocampi in elderly e4 and/or e2 carriers; and (3) the younger e2e4 and e4e4 children with altered age-related changes in brain measures also showed poorer performance on attention or WM tasks.

Children with the most common e3e3 genotype served as our reference group. These children showed typical age-related increases in hippocampal volumes and the medial orbitofrontal and occipital cortical areas until early adolescence. They also showed the typical age-dependent increase in thalamic FA, reflecting ongoing myelination, but not age-related increases in hippocampal and amygdala FA, as reported in healthy children without genotype groupings. Age-dependent decreases in parietal cortical volume and thinning of the isthmus likely reflect pruning of neuronal synapses and cell shrinkage. Furthermore, our e3e3 children with thinner isthmus had similarly better performance in WM and attention.

The children with APOE e2e3 genotype had comparable brain morphometry and cognitive performance as e3e3 children. However, younger (<7 years) e2e3 children, like those with e2e2 or e4e4, had relatively lower FA in left amygdala, suggesting lesser microstructural integrity, such as lower cellular density or lesser myelination. Since the amygdala is involved in emotional processing, these children may have greater vulnerability to emotional problems. Lower amygdala FA was also found in infants whose mothers had greater prenatal depressive symptoms; hence, future studies should include maternal depressive symptoms as a covariate to determine whether these symptoms might account for the lower amygdala FA in younger e2e3 children.

Relative to e3e3 children, those with one e4 allele, specifically APOE e3e4, had larger hippocampi, occipital and frontal cortical areas, and thicker...
temporal poles, but similar cognitive functioning. These larger brain measures are consistent with an antagonistic pleiotropic effect of $e4$, similar to findings in middle-aged (51–59 years) heterozygous $e4$ carriers (primarily $e3e4$).3,10 Normal or thicker cortices in our $e3e4$ children contrast with prior findings of thinner left entorhinal and orbitofrontal cortices (92% $e3e4$ compared to $e3e3$ children).3 However, larger cortical areas in our $e3e4$ children resemble larger parietal volumes in $e3e4$ infants (ages 8.5–14 months)7 relative to $e3e3$ participants. Moreover, consistent with our results, prior studies found similar IQ or cognitive performance between $e3e4$ and $e3e3$ children.3,13

Despite the relatively normal hippocampal volumes, the younger $e4e4$ participants had the lowest FA in hippocampus, amygdala, and thalamus, suggesting slower development initially with lesser myelination or lower cellularity in these regions. These children also did not show typical age-dependent cortical thinning in posterior cingulate (isthmus) cortex, which suggests aberrant brain maturation, possibly due to reduced synaptic pruning. Such possible aberrant brain maturation with lesser cortical thinning was found in children with prenatal alcohol exposure and fetal alcohol syndrome,36 although such children were excluded in the current study. Furthermore, $e4e4$ children showed age-dependent thickening of the temporal pole, which along with the isthmus, is often affected in AD30,37 and in cognitively healthy $e4$ carriers,30 who showed age-dependent increases in amyloid deposition.18

Figure 5  Cognitive performance in relation to age, brain volumes, or cortical thickness

APOE $\times$ genotype-by-age interactions on executive function (A), attention (B), and WM (C). Children with larger right superior parietal gyral volumes had poorer attention scores (D) or WM scores (E) across all genotype groups, especially those with APOE $\varepsilon2\varepsilon4$. Similarly, those with thicker cortices in the isthmus cingulate cortical thickness (F) and temporal poles (G) had poorer attention and WM scores, especially children with APOE $\varepsilon2\varepsilon4$. In contrast, children with APOE $\varepsilon4\varepsilon4$ with thicker cortices had better attention and WM scores (F and G). All children with larger hippocampal volumes had higher WM scores (H), especially children homozygous for APOE $\varepsilon4$ (H). *Data for the $e2e2$ children (black dots) are not included in the group analyses. ROI = region of interest; WM = working memory.

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Younger ε4ε4 children showed the poorest executive function and WM among groups, even after SES adjustment, similar to school-age ε4 carriers with AD family history. The normal cognition of our older ε4ε4 children is consistent with prior findings in older ε4ε4 children and in young and middle-aged adults. The ε4ε4 children with smaller hippocampal volumes and thinner temporal pole had poorer WM and attention; these findings resemble the thinnest frontal cortices in middle-aged ε4ε4 individuals without dementia, who showed the most rapid decline on mental arithmetic tasks requiring WM. Hence, ε4 homozygosity might slow maturation of the hippocampus and cortical thickness, which in turn might negatively affect WM and attention. Mirroring these findings, older individuals with ε4 homozygosity had the highest prevalence for AD (50%–91%) and the greatest hippocampal and temporal lobe atrophy among genotypes.

Unlike ε3ε4 children with the largest hippocampi, ε2ε4 children had the smallest hippocampi and orbitofrontal and occipital surface areas among groups and across ages. Therefore, ε4 allele effects differ greatly when combined with ε2 vs ε3. Although the younger ε2ε4 children had larger parietal cortices and thicker posterior cingulate cortices, they had poorer attention and WM. These findings also mirror those in the 3 ε2ε4 oldest old (>90 years) among 89 participants, since all 3 met criteria for dementia but not neuropathology for AD. While ε2 carriers showed reduced cognitive decline, ε2ε4 is a risk genotype for AD across ethnicity. Unfortunately, ε2ε4 participants are often excluded from studies because of potentially opposite effects of ε4 and ε2.

In the ε2ε2 children, the relatively large hippocampi are similar to the case reports of ε2ε2 adults, and the lower hippocampal FA in the younger child suggests less coherent fibers in the large hippocampi. They also had relatively higher thalamic FA, similar to findings in ε2 heterozygous adults. Our ε2ε2 children also had poorer attention and executive function, while ε2ε2 children in a prior study showed above-average IQ. Furthermore, a 92-year-old ε2ε2 woman showed no cognitive deficits until her stroke, despite the postmortem finding of prominent AD neuropathology. The relative preservation of cognition in aging ε2ε2 individuals may be due to ApoE ε2’s antioxidant, anti-inflammatory, and antiproteolytic effects. However, these processes may not be relevant in the developing brain.

For comparison with the literature, we also evaluated all ε4 carriers. Hippocampal volumes in ε4 carriers ranged from smaller (ε2ε4) to no difference (ε4ε4) or slightly larger (ε3ε4) relative to ε3ε3 children. Similarly, cortical surface areas were smallest in ε2ε4 but largest in ε3ε4 or ε4ε4. Hence, combining all ε4 carriers might have attenuated or abolished group differences compared to non–ε4 carriers, and the larger parietal volumes in the youngest ε2ε4 children or the thinnest isthmus gyrus and temporal poles in the youngest ε4ε4 children might have been missed. In fact, our ε4 carriers collectively had nonsignificantly thicker temporal lobes, contrasting with thinner entorhinal cortices in a prior study of healthy ε4 children. However, further analysis showed that ε4ε4 children had steeper age-dependent thinning in this region, leading to thinner entorhinal cortices in adolescents, similar to prior findings. Moreover, our youngest ε4 carriers performed poorer on WM than the non–ε4 carriers, which resemble WM deficits in older healthy ε4 carriers.

The age-dependent brain measures of ε4ε4 and ε2ε4 children often deviated from those in the other genotype groups. The youngest children with one of these genotypes had less mature brain structures and poorer cognitive function, but tended to normalize or exceed the other genotype groups during late adolescence. Prior studies of ε4ε4 and ε2ε4 young adults showed larger specific brain structures and better cognitive performance, whereas older adults showed poorer cognitive performance or less efficient neural networks relative to other genotype groups. Incidentally, ε2ε4 participants have a low odds ratio of AD until age 50 years, but the highest odds at age 70 years, while ε4 homozygosity leads to earliest AD onset (approximately 68 years).

This study has several limitations. Despite this relatively large sample, the age-related brain measures may be biased by the cohort effect, driven by participants with these rare genotypes at the extremes of age range, in this cross-sectional study. Longitudinal follow-ups are needed to confirm the true developmental trajectories in the less prevalent ε4 or ε2 carriers. In addition, some younger children (<5 years) could not perform all NIH Toolbox tasks; future studies with more young children using age-appropriate assessments are needed. Lastly, although we covaried for GAF, ethnicity may influence the effects of ε4 and ε2 on brain and cognitive measures. However, repeating all the analyses only in children with >50% European ancestry yielded similar results (table e-4, figures e-1 to e-3). Future studies should include a larger sample of children with other ancestry and evaluate them separately.

This large sample of children validated and extended prior smaller studies that showed altered brain development in ε4 carriers. The ε4ε4 and ε2ε4 carriers appear to show the strongest antagonistic pleiotropic effects, with negative influences on brain structures and cognition at younger age, mirroring those in elderly participants and patients with
AD. Future studies of APOE ε should evaluate each genotype separately, since brain development, and possibly brain aging and recovery, may vary substantially across specific ε4 or ε2 genotypes. Finally, studying APOE ε polymorphism in young children may provide early indications of risk of future brain injuries and dementia. Given the urgent need to determine how early patients with AD should receive interventions or preventive treatments, a thorough understanding of how AD risk genes, such as APOE ε4, might independently or interactively influence the brain across the ages, is needed.

AUTHOR CONTRIBUTIONS
Linda Chang, Vanessa Douet, and Thomas Ernst developed the concept, designed the study, performed the statistical analyses, interpreted the data, drafted and revised the manuscript. Linda Chang, Thomas Ernst, Alexandra Pritchett, Kristin Lee, Terry Jernigan, Natacha Akshoomoff, Sarah Murray, Cinnamon Bloss, David Kennedy, Jean Fraser, David Amaral, Jeffrey Gruen, Walter Kaufmann, B.J. Casey, and Elisabeth Sowell were all involved in the data acquisition. Sarah Murray was responsible for all genotyping and genetic imputations. The USCD team was responsible for all image processing. All authors were involved in the critical revision and approval of the manuscript.

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DISCLOSURE
The authors report no disclosures relevant to the manuscript. Go to DISCLOSURE (G12-MD007601).

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