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RESEARCH ARTICLE

ABCA7 risk variant in healthy older African Americans is associated with a functionally isolated entorhinal cortex mediating deficient generalization of prior discrimination training

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Abstract

Using high-resolution resting state functional magnetic resonance imaging (fMRI), the present study tested the hypothesis that ABCA7 genetic risk differentially affects intra-medial temporal lobe (MTL) functional connectivity between MTL subfields, versus internetwork connectivity of the MTL with the medial prefrontal cortex (mPFC), in nondemented older African Americans. Although the association of ABCA7 risk variants with Alzheimer's disease (AD) has been confirmed worldwide, its effect size on the relative odds of being diagnosed with AD is significantly higher in African Americans. However, little is known about the neural correlates of cognitive function in older African Americans and how they relate to AD risk conferred by ABCA7. In a case–control fMRI study of 36 healthy African Americans, we observed ABCA7 related impairments in behavioral generalization that was mediated by dissociation in entorhinal cortex (EC) resting state functional connectivity. Specifically, ABCA7 risk variant was associated with EC-hippocampus hyper-synchronization and EC-mPFC hypo-synchronization. Carriers of the risk genotype also had a significantly smaller anterolateral EC, despite our finding no group differences on standardized neuropsychological tests. Our findings suggest a model where impaired cortical connectivity leads to a more functionally isolated EC at rest, which translates into aberrant EC-hippocampus hyper-synchronization resulting in generalization deficits. While we cannot identify the exact mechanism underlying the observed alterations in EC structure and network function, considering the relevance of Aβ in ABCA7 related AD pathogenesis, the results of our study may reflect the synergistic reinforcement between amyloid and tau pathology in the EC, which significantly increases tau-induced neuronal loss and accelerates synaptic alterations. Finally, our results add to a growing literature suggesting that generalization of learning may be a useful tool for assessing the mild cognitive deficits seen in the earliest phases of prodromal AD, even before the more commonly reported deficits in episodic memory arise.

KEYWORDS

ABCA7, African American, Alzheimer's disease, entorhinal cortex, high-resolution fMRI functional connectivity

1 | INTRODUCTION

Multiple studies have shown qualitative and quantitative differences in Alzheimer's disease (AD) between African Americans and Caucasians. At the population level, the prevalence of AD dementia is nearly doubled in African Americans compared with Caucasians (Alzheimer's Association, 2018; Tang et al., 2001). Cognitively, African Americans with AD are more likely than Caucasians to have a slower decline (Barnes et al., 2005). The causes of this health disparity in AD are not sufficiently understood. A recent study examining racial differences in

cerebrospinal fluid (CSF) and structural magnetic resonance imaging (MRI) biomarkers of AD in an elderly cohort, found that despite comparable CSF Aβ42 levels, white matter hyperintensity (WMH) volume, and hippocampal volume, the same degree of WMH had a greater impact on cognition in African Americans as compared to Caucasians (Howell et al., 2017). Since WMH is a marker of cerebrovascular disease, it may be a mediating mechanism in the association between cognitive deficits and the risk factors that cause vascular dysfunction.

One such factor known to contribute to vascular dysfunction and potentially to cognition as well, is ABCA7 gene expression, which has been associated with AD via the dysregulation of lipid metabolism (Aikawa, Holm, & Kanekiyo, 2018; Zhao, Yu, Tan, & Tan, 2015). ABCA7 is a member of the highly conserved superfamily of ATP-binding cassette (ABC) transporters which function to regulate the homeostasis of phospholipids and cholesterol in the central nervous system and peripheral tissues. It is expressed in a variety of tissues/organs, including the brain, as well as, blood cells, and, data from several genome-wide association studies indicate that ABCA7 is a genetic risk factor for late-onset AD (Beecham et al., 2014; Hollingworth et al., 2011; Kamboh et al., 2012; Liu et al., 2014; Reitz et al., 2013). Studies to date have mainly implicated two possible mechanisms whereby ABCA7 loss-of-function contributes to AD pathology. First, ABCA7 facilitates amyloid precursor protein (APP) processing and increases amyloid-β (Aβ) deposition in mouse models (Sakae et al., 2016; Satoh, Abe-Dohmae, Yokoyama, St George-Hyslop, & Fraser, 2015). Second, ABCA7 critically regulates phagocytic function in macrophages, contributing to immune responses along with the host defense system (Tanaka, Abe-Dohmae, Iwamoto, & Yokoyama, 2011), and since microglia are the resident macrophages of the central nervous system (Brown & Neher, 2014), ABCA7 has also been found to mediate microglial phagocytosis of Aβ (Fu, Hsiao, Paxinos, Halliday, & Kim, 2016). Hence, Aikawa et al. (2018) posit that ABCA7 dysregulation may influence the properties of brain cell types, in particular neurons and microglia, by disturbing brain lipid homeostasis, and these alterations likely facilitate APP processing and suppression of cellular Aβ clearance, contributing to AD development.

Three common ABCA7 loci have been confirmed to increase the risk of AD, but the associations vary among different races. ABCA7 single nucleotide polymorphism (SNP) rs115550680 is linked to the development of late-onset AD in African Americans with an effect size (OR = 1.79; 70%–80% increase in risk) that is comparable to the effect size of APOE ε4 (Reitz et al., 2013). In contrast, the two ABCA7 variants (rs3752246; OR = 1.15 and rs3764650; OR = 1.23, ~10%–20% increase in risk) identified as susceptibility loci for AD in Caucasians have significantly lower effect sizes (Hollingworth et al., 2011; Naj et al., 2011). Hence, increasing evidence indicates that the ABCA7 gene confers greater AD risk in African Americans. While the ABCA7 variants implicated in Caucasians (rs3752246 and rs3764650) are associated with changes in amyloid deposition (Bamji-Mirza et al., 2016; Shulman et al., 2013) and lipid metabolism (Giri, Zhang, & Lü, 2016; Li et al., 2017), emerging research on the functional consequences of rs115550680 (significant in African Americans) is relatively sparse. However, rs115550680 in an African American cohort was found to be in linkage disequilibrium (LD) with rs3752246 and rs3764650 in a Caucasian sample (Reitz et al., 2013), implying a common underlying mechanism. Furthermore, there are differences in minor allele frequency (MAF) and LD patterns between ethnic groups; rs115550680 has MAF of 7% in African Americans, whereas it is monomorphic on the nonrisk minor "A" allele in Caucasians (Machiela & Chanock, 2015; Reitz et al., 2013). Additionally, within the African American population the three ABCA7 variants are not in LD with one another (Machiela & Chanock, 2015), indicating that they are not linked and occur independently of each other. This further supports the notion that while there may be a common underlying mechanism, distinct alleles confer AD risk in different ethnicities with variable impact on protein structure or function. Consistent with this, rs115550680 was found to be in LD with a 44-base pair deletion (rs142076058) in ABCA7 that is associated with AD in individuals of African ancestry (Cukier et al., 2016). This deletion is rare in Caucasians but relatively common in African Americans and confers AD risk by interfering with the transportation of lipids and increased Aβ brain levels (Cukier et al., 2016). Therefore, ABCA7 rs115550680 may be an ethnicity-specific risk variant that contributes to AD in African Americans through APP processing and suppression of Aβ clearance. As such, in African Americans, this ABCA7 risk variant (rs115550680) may have consequences for neural network function along pathways affected by amyloid deposition.

ABCA7 risk variants have been associated with well-established AD biomarkers, namely cortical and hippocampal atrophy in cognitively normal and mild cognitive impairment (MCI) individuals (Ramirez et al., 2016), as well as, with memory decline in MCI and late-onset AD patients (Carrasquillo et al., 2015). Hence, while ABCA7 is possibly responsible for both the development and progression of AD, the precise neural systems or networks through which it may exert its AD related effects remain unknown. However, considering the relevance of Aβ in the pathogenesis of AD through ABCA7 expression, it seems likely that ABCA7 related neuronal dysfunction manifests in early Aβ accumulating brain regions and propagates through synaptically connected neural networks. A recent study on ADNI and BioFINDER cohorts showed that Aβ accumulation preferentially starts in several of the core regions of the default mode network (DMN), including the orbitofrontal cortex (OFC), an area within the medial prefrontal cortex (mPFC) (Palmqvist et al., 2017). Furthermore, the earliest Aβ accumulation was associated with hypoconnectivity within the DMN (Palmqvist et al., 2017). Consistent with this, several studies have reported reduced functional connectivity among major DMN nodes in aging (Andrews-Hanna et al., 2007; Damoiseaux et al., 2007), AD (Greicius, Srivastava, Reiss, & Menon, 2004; Sperling et al., 2010; Wang et al., 2006) and in asymptomatic APOE ε4 carriers at increased risk of developing AD (Fleisher et al., 2009).

Another core DMN node, the medial temporal lobe (MTL) is one of the earliest brain regions where tau tangles aggregate, and its subregions show a selective topography of pathological involvement during early AD (Braak & Braak, 1991). The formation of tau neurofibrillary tangles is first seen in the transentorhinal and entorhinal cortex (EC) (Braak & Braak, 1991, 1997) followed by the dentate gyrus (DG) and CA3 subfield in the hippocampus (Leal & Yassa, 2018). Although their pathological relationship remains unclear (Mann & Hardy, 2013), MTL tau is considerably increased in normal subjects

with cortical Aβ (Braak, Thal, Ghebremedhin, & Del Tredici, 2011; Sperling, Mormino, & Johnson, 2014). Furthermore, Song et al. (2015) showed that in cognitively normal elderly subjects cortical Aβ load was related to disrupted resting state functional connectivity within the MTL. Hence, Aβ plaques may play a key role in facilitating tauopathy in the MTL, and therefore lead to disrupted functional connectivity in the MTL circuitry. As such, the function of ABCA7 in AD may be explained more comprehensively as Aβ facilitated tauopathy (Hardy & Selkoe, 2002), such that, as Aβ deposition increases in cortical DMN nodes, it facilitates concurrent accumulation of tau tangles in MTL, via reciprocal connections through the EC (Pooler et al., 2015).

Hence, the cortico-MTL circuit within the DMN may be the neural network underlying ABCA7 related AD pathology. Measures of functional connectivity obtained within specific sub-regions of MTL, and between MTL subfields and cortical DMN regions may potentially be more sensitive to early disease stages. In MCI patients, studies found increased connectivity within the MTL, between EC and subregions of the MTL (Das et al., 2013), and intra-hippocampal connectivity (Pasquini et al., 2015), which was associated with impaired memory. These studies also reported decreased connectivity of the MTL from other cortical nodes of the DMN (Das et al., 2013; Pasquini et al., 2015). In nondemented elderly, Salami, Pudas, and Nyberg (2014) found age-related elevation of connectivity between left and right hippocampus along with attenuated hippocampal–prefrontal cortex connectivity, which was associated with lower cross-sectional episodic memory performance and declining longitudinal memory performance over 20 years.

In the present study, we tested the hypothesis that in older nondemented African Americans, ABCA7 rs115550680 risk variant differentially affects intra-MTL functional connectivity between MTL subfields, versus inter-node connectivity between MTL and the mPFC, a major cortical DMN region. Consistent with previously noted results, we expected to observe increased connectivity within the MTL. We further predicted that this increase would be accompanied by decreases in inter-network connectivity of the MTL with mPFC. To constrain interpretations of this dissociation in functional connectivity, we related it to cognitive performance on a concurrent discrimination and generalization task (Myers et al., 2002), previously known to depend on MTL function (Johnson, Schmitz, Asthana, Gluck, & Myers, 2008; Myers et al., 2002; Myers, Kluger, Golomb, Gluck, & Ferris, 2008). It is a two-phase task in which participants initially learn a series of visual discriminations and are then tested on their ability to generalize when stimulus information changes. Performance on the generalization phase of the task distinguished between hippocampal-atrophied and nonatrophied individuals, even though standard tests, such as delayed paragraph recall, did not show this distinction (Myers et al., 2002, 2008). Conversely, in healthy aging, there were no deficits in those aged 40–75, but performance was impaired for those older than 75 (Krishna, Moustafa, Eby, Skeen, & Myers, 2012), likely reflecting preserved MTL function until advanced old age (Shing et al., 2011). Hence, as this task selectively engages the MTL, its correlation with cortico-MTL connectivity would elucidate the functional consequences of genetic risk related neuronal network alterations.

2 | MATERIALS AND METHODS

2.1 | Participants

Participants in this study were recruited through the *African-American Brain Health Initiative: A University-Community Partnership* (AABHI) at Rutgers University-Newark. Created in 2006, the AABHI partners with community-based organizations to promote brain health literacy, Alzheimer's awareness, brain-healthy lifestyle choices, and participation in brain research among older African Americans in and around Newark, New Jersey. The research participants recruited to our study came primarily from long-standing partnerships with local churches; senior centers; city, county, and state offices for health and aging; as well as from outreach to public house and other federally subsidized low-income housing sites. Our outreach and research efforts are informed by community input from the *AABHI Community Advisory Board* comprised of over a dozen community leaders from local community organizations (see *Acknowledgments* at end for a list of members and their affiliations). For additional details on our community engagement, outreach, and recruitment strategies, see www.brainhealth.rutgers.edu as well as Gluck, Shaw, & Hill (2018).

From a larger parent study of 60 individuals, participants in the present study were selected for analysis in a case–control matched design. Of the parent sample, 18 individuals were carriers of ABCA7 rs115550680 "G" allele, which is the strongest AD genetic risk factor for African Americans (outside of the APOE ε4 allele) with an odds ratio of 1.8 (Reitz et al., 2013). We then matched these 18 ABCA7 "G" risk allele individuals with 18 individuals who were homozygous for the nonrisk "A" allele, based on age and years of education. Hence, individuals with the ABCA7 rs115550680 AG genotype constituted the risk group (no participant homozygous for the "G" allele was present in the sample), while individuals with the AA genotype were the nonrisk group. Overall, the current study included 36 healthy adults, 3 male (1 in the AA group and 2 in the AG group) and 33 female, aged 63 to 90 years, with an average age of 72 years (Table 1).

Participants exhibiting any signs of dementia as revealed in the standardized neuropsychological assessments (detailed below), and those who took medications that could affect cognition were excluded from the study. Other exclusion criterion included history of excessive alcohol intake and/or drug use, psychiatric disorders (including Bipolar Disorder and Schizophrenia), Epilepsy or related seizure disorders, and significant cardiovascular and cerebrovascular diseases. Participants were also required to be fluent English speakers. All participants completed written informed consent prior to participation in the study.

2.2 | Standardized neuropsychological assessments and self-reported measures

To exclude anyone showing evidence of cognitive impairment consistent with early dementia or another age-related disorder, we gave participants a short battery of neuropsychological tests. Prior to MRI scanning, the neuropsychological battery consisting of the Mini Mental State Exam (MMSE) (broad assay of cognitive impairment), Rey Auditory Verbal Learning Test (RAVLT) Immediate and Delayed Recall

(sensitive to verbal memory), North American Adult Reading Test (NAART35) (sensitive to verbal intellectual ability), Wechsler Adult Intelligence Scale (WAIS-IV) Digit Span (sensitive to working memory), and Beck Depression Inventory (severity of depressive symptoms) was administered to characterize cognition (Table 1).

2.3 | Behavioral paradigm: Concurrent discrimination and generalization task

The testing took place in a quiet room, with the participant seated in front of a laptop computer with a color screen. The keyboard was masked except for two keys, labeled "Left" and "Right" which the participant used to enter responses.

This task has been previously described in Myers et al. (2002). In brief, it is a two-phase task in which participants learn a series of visual discriminations and are then challenged to generalize when stimulus information changes. Phase 1 (acquisition) was an eight-pair concurrent discrimination. On each trial, two colored shapes appeared, ~1-in. high on the screen and set about 3 in. apart (~1.5° of visual angle, at normal viewing distance). The participants were instructed to press the left or right key to choose one object. The chosen object rose onscreen and, if the choice was correct, a smiley face was revealed underneath (Figure 1). There was no limit on response time, and there was an interval of approximately 1 s between participant response and start of the next trial, allowing the participant to view the discrimination pair and feedback (presence or absence of the desired smiley face icon).

This was an incrementally acquired, feedback-based learning task in which participants were to learn which object was correct. They were given no information about the correct object ahead of time. Within each object pair, the same object was always rewarded. For four of the discrimination pairs, objects differed in shape but not color (example, blue checkerboard versus blue funnel); for the other four pairs, objects differed in color but not shape (example, orange spider versus blue spider). Thus, within each pair, one dimension (shape or color) was relevant to predicting the location of the smiley face, and one dimension was irrelevant. Trials were organized into blocks, each containing 16 trials: 1 presentation of each discrimination pair in each possible left–right ordering. Trials in a block occurred in a pseudo-random but fixed order. Phase 1 continued until the participant reached a criterion of 16 consecutive correct responses, or for a maximum of 96 trials (6 blocks).

TABLE 1 Demographics and neuropsychological assessments

Measurement	AA genotype	AG (risk) genotype	Difference (*t* test)
Sample size	18 (1 M)	18 (2 M)	
Age	72 (5.74)	72.56 (8.23)	$p = .52$
Education (years)	14.33 (2.03)	13.86 (1.98)	$p = .48$
MMSE	28.0 (1.5)	26.89 (2.68)	$p = .13$
DigitSpan	22.94 (4.86)	23.06 (4.54)	$p = .94$
NAART	35.5 (11.83)	37.11 (9.02)	$p = .65$
RAVLT - immediate	11.28 (2.22)	12.28 (2.52)	$p = .21$
RAVLT - delayed	10.61 (2.52)	10.44 (2.71)	$p = .85$

Data are presented as mean (standard deviation). Group differences were assessed via simple pairwise *t* tests. No statistically significant differences ($p < .05$) were found.

As soon as the acquisition phase ended, Phase 2 (generalization) began without any warning to the participant. The screen events were identical to concurrent discrimination phase except that the discrimination pairs were altered so that the relevant features remained constant but the irrelevant features were altered. For example, the blue checkerboard versus blue funnel might change to lavender checkerboard versus lavender funnel as shown in the first row, second column (Figure 1a); the shapes remain the same but the irrelevant color changes from blue to lavender. In the second discrimination learned, the orange versus blue spider discrimination might change to an orange versus blue circle; shape remains irrelevant but color continues to be predictive.

Individuals who had solved the concurrent discrimination by basing associations on the relevant features (funnel beats checkerboard and blue beats orange) could perform perfectly in the generalization phase, since the relevant features were still predictive. By contrast, individuals who had approached concurrent discrimination phase by learning to respond to whole objects (blue funnel beats blue checkerboard), treating all features equally are effectively confronted with novel objects (lavender funnel and lavender checkerboard) in the generalization phase, and might perform near chance.

The generalization phase was organized into blocks of 16 trials, 1 trial with each discrimination pair in each possible left–right ordering, in a pseudo-random but fixed order. It continued until the participant reached a criterion of 16 consecutive correct responses, or a maximum of 96 trials (6 blocks). The entire procedure took about 15–20 min to complete.

2.4 | MRI data acquisition

MRI data were acquired on a 3 T Siemens Allegra, using a 32-channel Multiband parallel encoding coil, at the Rutgers University Brain Imaging Center at Rutgers University-Newark. If required, MRI-compatible glasses were used on the day of scanning. A high-resolution 3D magnetization-prepared rapid gradient echo (MP-RAGE) structural scan was acquired in the sagittal plane for each participant: repetition time (TR) = 1,900 ms, echo time (TE) = 2.52 ms, 9° flip angle, 176 slices (no gap), voxel size 1.0 × 1.0 × 1.2 mm, field of view (FOV) = 270 × 254 × 212, with a total acquisition time of 9 min. High-resolution Multiband echo-planar images were collected using a FOV of 208 × 208 × 125, a TR of 664 ms, an TE of 30 ms, a flip angle of 30°, an isotropic resolution of 1.8 mm with no gap, and, a Multiband acceleration factor of 5. Forty-five axial slices were acquired covering the entire brain. Multiband parallel imaging enabled the acquisition of high-resolution functional images, with large sampling rates for full-brain coverage, through the acquisition of multiple slices simultaneously. This resulted in significantly reduced acquisition time, which also limited distortion resulting from magnetic susceptibility. Furthermore, the high temporal efficiency has been shown to provide greater statistical power (Feinberg et al., 2010).

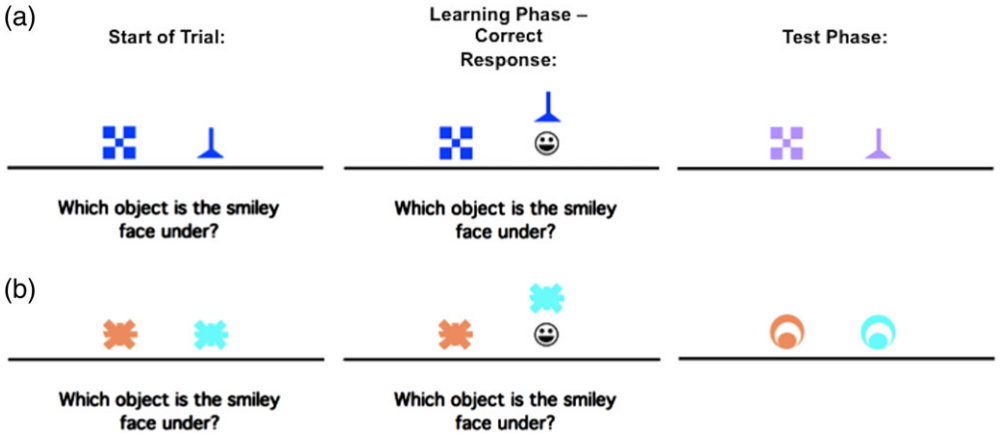

FIGURE 1 An example of the concurrent discrimination and generalization task. On each trial of Phase 1 (acquisition), the discrimination pair is presented and if the participant responds correctly, the chosen object is raised to reveal a smiley face icon underneath. During Phase 2 (generalization) events are similar to Phase 1, but the objects are changed so that the relevant dimension is the same, whereas the irrelevant dimension is novel [Color figure can be viewed at wileyonlinelibrary.com]

2.5 | Functional Magnetic Resonance Imaging (fMRI) data analysis

2.5.1 | Preprocessing

All neuroimaging data were preprocessed and analyzed using Analysis of Functional NeuroImages Linux and Mac OSX platforms. Analyses largely took place in accordance with the standardized afni_proc.py pipeline. Data were despiked (3dDespike), slice timing corrected (3dtshift), coregistered with T1-weighed anatomical images (align_epi_anat.py), motion corrected (3dvolreg), smoothed to 2 mm isotropic (3dmerge) with a Gaussian FWHM kernel, and automasked to exclude voxels outside the brain (3dautomask). Trials with motion in excess of 0.3 mm were excluded from the time series using a custom script. Critically, based on processing steps suggested by Power et al. (2012), we also regressed signal in white matter and ventricles to account for noise related to motion and scanner artifact. This was accomplished using ANATICOR (Jo et al., 2010), which uses local white matter and ventricular signal estimates applied to nearby gray matter voxels. Functional scans were aligned to each subject's skull-stripped MP-RAGE (align_epi_anat.py). Final voxel time courses were estimated using univariate regression (3dDeconvolve), which included nuisance variables for six motion parameters (pitch, roll, and yaw; *x*, *y*, and *z* frame displacement) and linear scanner drift.

We used Advanced Normalization Tools (Avants et al., 2011) to warp each individual participant's structural scan into an in-house high-resolution 0.65 mm isotropic template using a diffeomorphic nonlinear registration algorithm (SyN) (Klein et al., 2009). The transformation parameters were then applied to the coplanar functional data output from the regression described above in order to align them to the custom template, for individual and group level analyses.

2.5.2 | Region of interest (ROI) analyses

Based on our a priori hypothesis, the differences in functional connectivity were examined in MTL cortical regions (perirhinal cortex, parahippocampal cortex, and EC), hippocampal subfields (subiculum, CA1, and DG/CA3), and the mPFC; ROIs are shown in Figure 2. We note that our mPFC ROI largely corresponds to the ventromedial aspect of the prefrontal cortex, though our delineation of the region was fairly liberal given ambiguous anatomical delineations used in the literature. We did, however, intentionally exclude OFC and dorsal anterior cingulate cortex given the specialized affective and evaluative roles ascribed to those regions. For subsequent analyses, a structural ROI approach was used, selecting all the voxels within an anatomical ROI, which was based on manual delineations of the subfields and regions of interest on the custom template. ROIs in the MTL were segmented based on published protocols (Reagh et al., 2018). Anterolateral and posteromedial EC were segmented based on results from Maass, Berron, Libby, Ranganath, and Düzel (2015), as also applied by Reagh et al. (2018). Resulting voxel *z* statistics were averaged and all subsequent statistical analyses were conducted on these averages. For each subject, ROIs in the MTL, including hippocampal subfields, were used as seed regions for functional connectivity analysis. Each seed ROI was used to define the reference time course (3dmaskave), after which, a correlation was computed between each reference time course and the signal time series in each voxel within the acquired whole-brain image set (3dfim++), and then standardized to generate *z*-score functional connectivity maps, thresholded at a false discovery rate of $q = 0.05$. Hence, a whole-brain *z*-score connectivity map was generated for each seed region (3dcalc), displaying all those voxels whose signal time series were correlated with it. From these seed to whole-brain connectivity maps, correlation values were extracted from each ROI (3dmaskave) and were evaluated for significance across groups.

2.6 | Genetic data collection and processing

Saliva samples were collected using Oragene kits during the neuropsychological testing visit before MRI scanning. DNA extraction and genotyping were conducted at the Rutgers University Human Genetics Institute. ABCA7 SNP rs115550680 genotyping was carried out by quantitative polymerase chain reaction (PCR) on an Eppendorf Mastercycler thermal cycler, using a TaqMan Custom Genotyping assay.

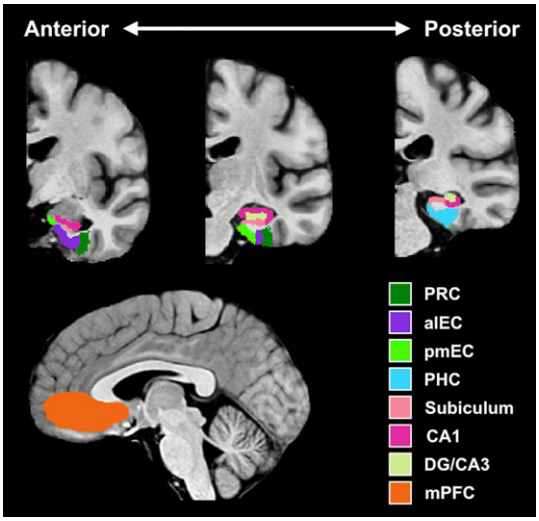

FIGURE 2 Regions of interest (ROIs). PRC = perirhinal cortex; aLEC = anterolateral entorhinal cortex; pMEC = posteromedial entorhinal cortex; PHC = parahippocampal cortex; DG = dentate gyrus; mPFC = medial prefrontal cortex [Color figure can be viewed at wileyonlinelibrary.com]

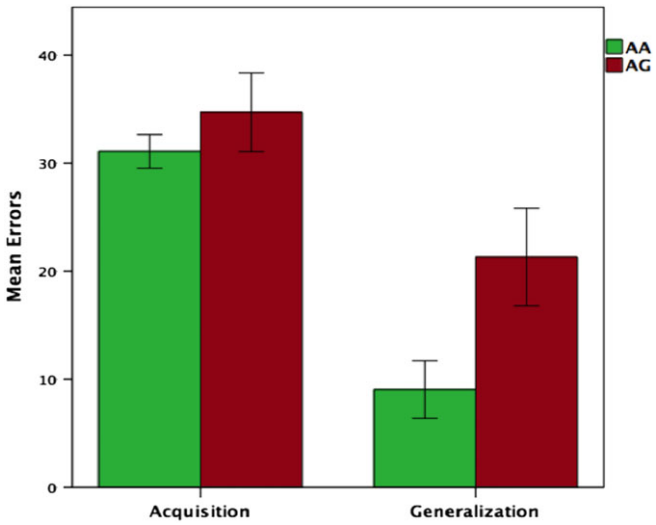

FIGURE 3 Performance (total errors) on the concurrent discrimination and generalization task based on ABCA7 genotype. While there were no group differences on the initial learning (acquisition phase), carriers of the AG risk genotype made significantly more errors during generalization [Color figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS

3.1 | Behavioral results

All participants underwent a battery of standardized neuropsychological assessments, and were included in our analyses only if they were within the age and education adjusted norms (Table 1). No differences were observed on these standardized measures of cognitive functioning (MMSE, Digit Span, NAART, and RAVLT).

On the concurrent discrimination task, all participants reached the criterion of 16 consecutive correct responses on the acquisition phase, indicating that they successfully learned the task. Figure 3 shows the mean errors for the acquisition and generalization phases of the task. There was no effect of group, based on ABCA7 genotype, on acquisition; $t(34) = 0.916$, $p = .366$. An ANOVA revealed a significant effect of group ($F(1,33) = 6.24$, $p = .018$), as well as, a significant effect of acquisition errors on generalization performance ($F(1,33) = 13.017$, $p = .001$). Post hoc comparison of generalization performance, adjusted for acquisition errors, revealed that the AA group made significantly fewer errors ($M = 10.91$, $SD = 2.4$), compared to the AG risk group ($M = 19.57$, $SE = 2.4$).

3.2 | Functional neuroimaging results

As described in Section 2, whole brain, *z*-score functional connectivity maps were generated with MTL subfields as seed regions. Based on a priori hypotheses, we conducted ROI analysis on these seed to whole-brain connectivity maps to examine group differences in functional connectivity within the MTL and between the MTL regions and mPFC. Corrections were made for multiple comparisons using the Holm–Bonferroni method (Holm, 1979). The AG risk group showed increased functional connectivity between EC and other MTL regions, including the hippocampal subfields (subiculum: $t(34) = 3.22$, $p = .003$, p-corrected = .015; CA1: $t(34) = 4.24$, $p = .001$, p-corrected = .006; DG/CA3: $t(34) = 3.42$, $p = .003$, p-corrected = .015; perirhinal cortex: $t(34) = 2.46$, $p = .019$, p-corrected = .038; parahippocampal cortex: $t(34) = 1.24$, $p = .224$; p-corrected = .224); significant between-group differences are shown in Figure 4a. Conversely, the AG risk group showed decreased functional connectivity between EC and mPFC: $t(34) = 2.86$, $p = .007$, p-corrected = 0.021 (Figure 4b).

To investigate the potential link between the observed functional connectivity and the behavioral ability to generalize, we performed partial correlations between connectivity *z*-scores and generalization total errors, controlling for the effect of acquisition errors. The analysis was conducted on functional connectivity scores that showed significant group differences; as such EC-subiculum, EC-CA1, EC-DG/CA3, EC-perirhinal cortex, and EC-mPFC *z*-scores were considered. Correlations were assessed at the level of AG carriers, AA carriers, and collapsed across the entire sample (Figure 5). For the correlations assessed across groups, corrections were made for multiple comparisons using the Holm–Bonferroni method (Holm, 1979). Generalization errors across groups were positively correlated with increased connectivity between EC and hippocampal subfields (subiculum: $r(33) = .418$, $p = .013$, p-corrected = .039; CA1: $r(33) = 0.502$, $p = .002$, p-corrected = .01; DG/CA3: $r(33) = .359$, $p = .034$, p-corrected = .063). A positive correlation was also observed between increased EC-perirhinal cortex connectivity and generalization errors ($r(33) = .244$, $p = .157$, p-corrected = .157), but this relationship did not reach significance. Within-group correlations were trending or significant for the EC-CA1 ($r(15) = .474$, $p = .054$) and EC-subiculum ($r(15) = 0.495$, $p = .043$) connectivity in the AA group but not in the AG group (EC-CA1: $r(15) = .27$, $p = .29$; EC-subiculum: $r(15) = .17$, $p = .514$). The other nonsignificant correlations within each group also trended in the same direction (positive correlations between generalization errors and increased connectivity). Thus, in general, greater synchronization of EC and hippocampal sub-regions was associated with poorer ability to generalize based on previously learned information. Conversely, increased connectivity between EC and

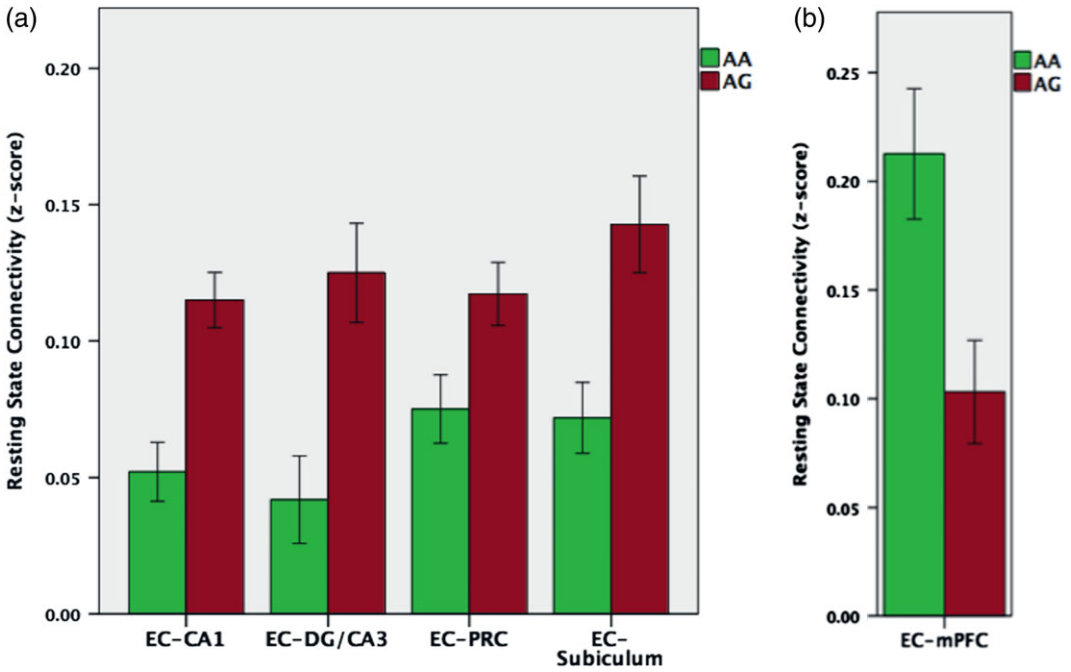

FIGURE 4 Resting state functional connectivity based on ABCA7 genotype. The AG risk group showed increased functional connectivity between entorhinal cortex and other MTL regions, including hippocampal subfields and perirhinal cortex (a), but decreased functional connectivity between entorhinal cortex and mPFC (b) [Color figure can be viewed at wileyonlinelibrary.com]

mPFC was negatively associated with generalization errors across groups ($r(33) = -.494$, $p = .003$, p-corrected = .012). Within group correlations were significant for the AA group ($r(15) = -0.628$, $p = .007$), but not for the AG group ($r(15) = -0.173$, $p = .51$). Thus, opposite to the relationship observed in EC-hippocampal connectivity, greater synchronization between EC and mPFC was associated with better generalization performance.

3.3 | Mediation analysis

We examined whether the effect of AG risk genotype on generalization is mediated by EC functional connectivity. To minimize multicollinearity, principal components analysis (PCA) was used to examine the variance shared by the z-scores on the functional connectivity between EC and hippocampal subfields (CA1, DG/CA3, and Subiculum). The Eigenvalues >1 criterion suggested a single factor representing EC-hippocampus connectivity, accounting for 90.15% of the variance. Hence, a multiple mediator model (Preacher & Hayes, 2008) was tested with two mediator variables: EC-hippocampus and EC-mPFC functional connectivity; acquisition error was entered in the model as a covariate. The mediation model is shown in Figure 6 and the indirect effect estimates and 95% CIs are in Table 2. Overall EC functional connectivity significantly mediated the relationship between AG risk genotype and generalization scores (total indirect effect = 8.13, CI = 3.69–15.07). An examination of the specific indirect effects indicated that both EC-hippocampus (indirect effect = 4.32, CI = 0.5–10.94) and EC-mPFC (indirect effect = 3.81, CI = 0.64–9.51) connectivity were significant mediators. Furthermore, the direction of the paths was consistent with the interpretation that the AG risk genotype was associated with increased EC-hippocampus connectivity ($\beta = 0.99$, $p = .007$) and decreased EC-mPFC connectivity ($\beta = -0.10$, $p = .012$), which was subsequently associated with higher generalization errors ($\beta = 4.38$, $p = .037$; $\beta = -37.75$, $p = .011$). Consistent with the correlational findings, the mediation analysis suggests that the ABCA7 risk variant is associated with EC-hippocampus hyper-synchronization and EC-mPFC hypo-synchronization, which, in turn, result in generalization deficits.

3.4 | Structural MRI results

Volumetric analyses across MTL regions and hippocampal subfields showed significant group differences in the EC volume ($t(34) = 2.02$, $p = .005$, p-corrected = .03) and surface area ($t(34) = 2.84$, $p = .008$, p-corrected = .048). Further examination following an anterolateral versus posteromedial functional division (alEC; pmEC) revealed that the difference in EC structure between the two groups was driven by the anterolateral entorhinal cortex (alEC). As shown in Figure 7, in AG risk genotype carriers, the alEC was significantly smaller in surface area ($t(34) = 3.41$, $p = .002$) and volume ($t(34) = 2.45$, $p = .02$). There was however no difference between the two groups in pmEC surface area ($t(34) = 1.31$, $p = .2$) or volume ($t(34) = 0.69$, $p = .49$). No other region of the MTL showed a significant group difference.

4 | DISCUSSION

In this study, we observed ABCA7 risk genotype related impairments in generalization, mediated by hyper-synchronization between EC and hippocampus, and, hypo-synchronization between EC and mPFC. Moreover, impaired generalization on the behavioral paradigm was positively correlated with increased connectivity between EC and

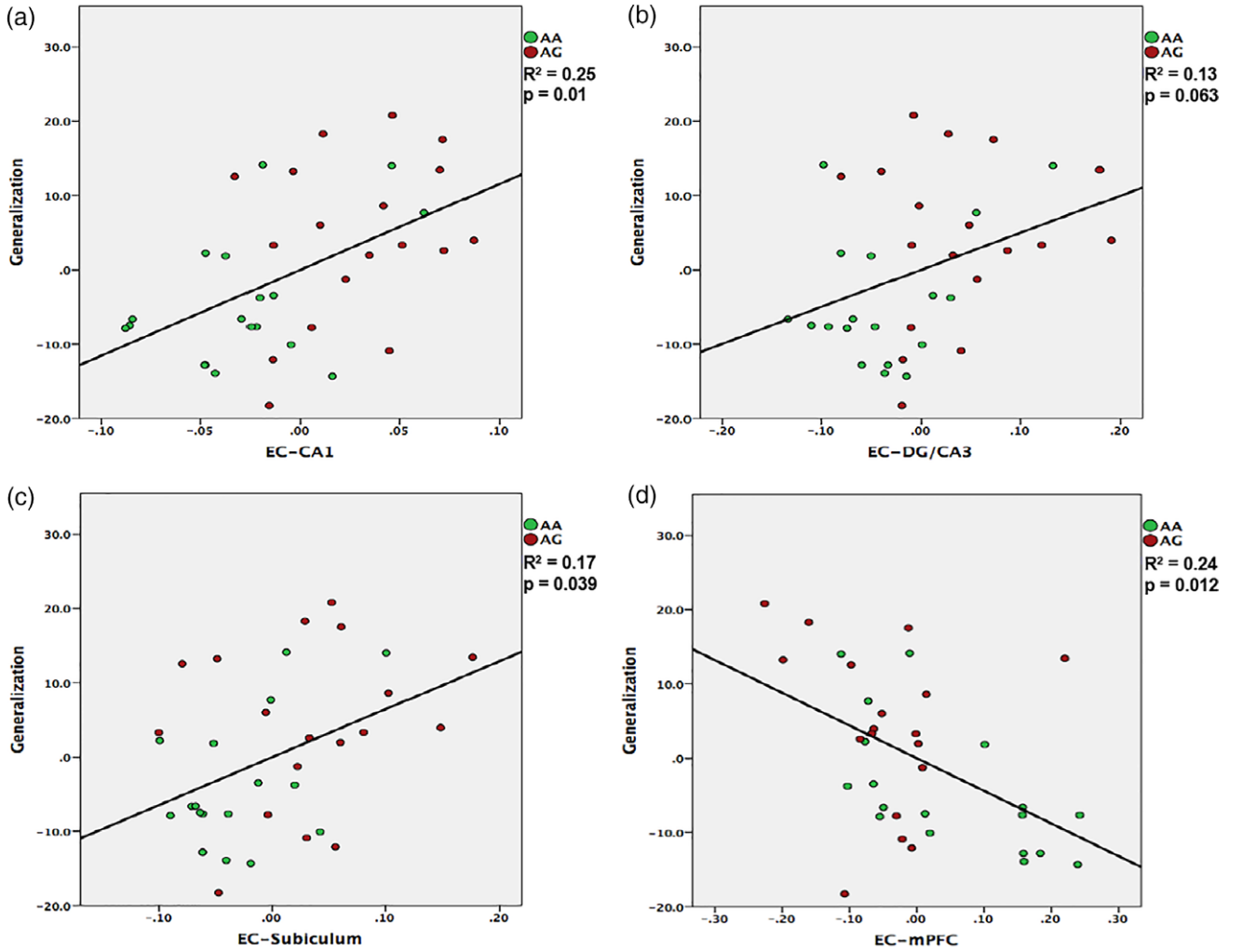

FIGURE 5 Partial correlations between functional connectivity *z*-scores and generalization total errors, controlling for the effect of initial learning (acquisition) errors. Across groups, generalization deficits were associated with increased connectivity between entorhinal cortex and hippocampal subfields (a–c). conversely, greater connectivity between entorhinal cortex and mPFC was associated with better generalization performance (d) [Color figure can be viewed at wileyonlinelibrary.com]

hippocampal subfields, but negatively correlated with increased connectivity between EC and mPFC. Carriers of the AG risk genotype also had a significantly smaller alEC, in both volume and surface area. Importantly, there were also no group differences on standardized neuropsychological tests, and, across groups participants were asymptomatic in terms of dementia or major indices of cognitive decline.

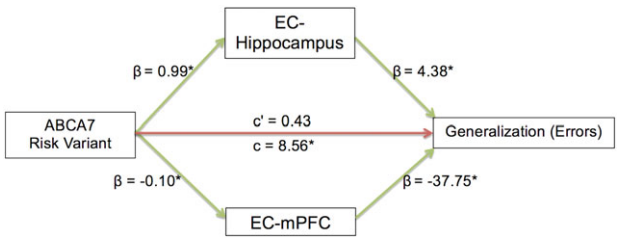

FIGURE 6 An illustration of the mediation model. β values indicate the coefficient of the arrow's partial effect. Green arrows indicate significant effects and asterisk (*) is appended to significant coefficients. c' (*c*-prime) represents the direct effect and *c* represents the total effect of ABCA7 risk variant on generalization errors. Indirect effect coefficients indicate that ABCA7 risk variant was associated with EC-hippocampus hyper-synchronization and EC-mPFC hypo-synchronization, in turn resulting in cognitive deficits [Color figure can be viewed at wileyonlinelibrary.com]

On the behavioral paradigm, both groups learned to solve the concurrent discrimination equally well. However, generalization of this initial learning to a novel context or task demand was found to be significantly decreased in AG group compared to the AA group, indicating that there is a specific generalization deficit associated with the ABCA7 risk genotype. Notably, no group differences were observed on common standardized measures of declarative memory (delayed recall of semantic or episodic memories), suggesting that the AG risk genotype is specific to impairing generalization of learning in older adults who are otherwise cognitively healthy on measures of declarative memory. In other studies with nondemented elderly (Andrews, Das, Anstey, & Easteal, 2017; Andrews, Das, Cherbuin, Anstey, & Easteal, 2016; Engelman et al., 2013), as well as, AD patients (Chung et al., 2014; Nettiksimmons, Tranah, Evans, Yokoyama, & Yaffe, 2016), ABCA7 risk variants were related to variations in episodic memory using standardized assessments. Our results therefore suggest that the concurrent discrimination and generalization behavioral paradigm may be a useful tool for assessing the mild cognitive deficits

TABLE 2 Mediation of the effect of ABCA7 risk variant on generalization (errors) through entorhinal-hippocampal and entorhinal-mPFC functional connectivity

	Product of coefficients			Bootstrapping 95% CI		Coefficients	
	Point estimate	*SE*	*Z*	Lower	Upper	ABCA7 -> mediator	Mediator -> generalization errors
EC-hippocampus	**4.32***	2.55	1.84	0.50	10.94	0.99	4.38
EC-mPFC	**3.81***	2.30	1.83	0.64	9.51	−0.10	−37.75
TOTAL	**8.13***	2.83		3.69	15.07		

Any statistically significant indirect effects (mediators) are bolded.

seen in the earliest phases of prodromal AD before the more severe and more commonly reported deficits in episodic memory arise later in the course of the disease.

Commensurate with behavioral impairments, on resting state fMRI, the AG risk group showed significantly increased functional connectivity between EC and hippocampal sub-regions, which was positively correlated with impairments in generalization, suggesting that the increased synchronization is maladaptive and is a marker for neuronal dysfunction. In particular, this EC-hippocampus hyper-synchronization may reflect low network flexibility (Bassett et al., 2011, 2013) implying that the neural pathways are so heavily connected that they cannot accommodate any new information (Bassett, Yang, Wymbs, & Grafton, 2015; Rosenberg-Lee et al., 2015). Conversely, the AG risk group showed significantly decreased functional connectivity between EC and mPFC, which was negatively correlated with generalization errors. Hence, a deficit in generalization of learning was associated with elevated EC-hippocampus synchronization, but reduced functional integration of EC with the prefrontal cortex. Taken together, our results imply that cognitive decline may be associated with a relative disconnect of EC from the prefrontal cortex, particularly mPFC, but hyper-connectivity between EC and hippocampus. This is consistent with recent studies of functional connectivity in MCI and AD patients reporting a similar disconnection of the MTL from other nodes of the DMN, particularly mPFC, but increased connectivity locally within the MTL, specifically between EC and sub-regions of the MTL (Das et al., 2013), which was also associated with impaired memory (Pasquini et al., 2015). However, we build significantly on these lines of research by providing, for the first time to our knowledge, evidence that in cognitively healthy older adults, the pathological dissociation in EC functional connectivity (as observed in MCI and AD patients) is associated with ABCA7 risk genotype.

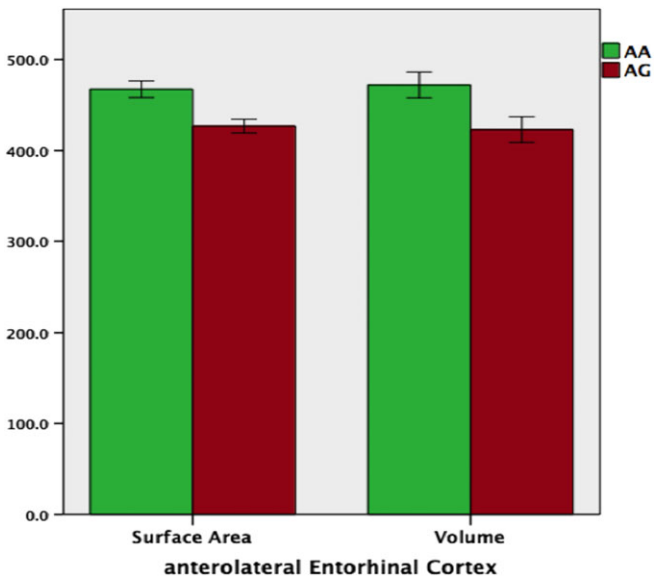

FIGURE 7 Volumetric analyses of volume (a) and surface area (b) showed that in the AG risk genotype carriers, the anterolateral entorhinal cortex (alEC) was smaller in both volume and surface area [Color figure can be viewed at wileyonlinelibrary.com]

Additionally, our mediation analysis suggests that the dissociation in EC functional connectivity plays a role in mediating the effect of ABCA7 risk on the generalization of learning. In particular, the ABCA7 risk variant was associated with EC-hippocampus hyper-synchronization and EC-mPFC hypo-synchronization, which, in turn, provide a putative mechanism for generalization deficits. These results indicate that, ABCA7 risk variant related neuronal dysfunction is propagated through the cortico-hippocampal network, with the EC as an important hub region of early vulnerability. Furthermore, we also found that compared to the nonrisk group, carriers of the AG risk genotype showed significantly reduced volume and surface area in the alEC, suggesting that structural neurodegeneration may underlie the observed dysfunctional dissociation in the mPFC-EC-hippocampus circuit. Hence, overall our findings imply that ABCA7 loss of function induces neurodegeneration in EC neurons, which in turn causes trans-synaptic deficits initiating the cortical-hippocampal network dysfunction. While we cannot identify the exact mechanism underlying the observed alterations in EC structure and network function, considering the relevance of Aβ in ABCA7 related AD pathogenesis, the results of our study may reflect the synergistic reinforcement between amyloid and tau pathology in the EC, which significantly increases tau-induced neuronal loss and accelerates synaptic alterations (Pooler et al., 2015). Consistent with this, in an AD mouse model, selective overexpression of APP/Aβ in EC neurons caused an excitatory EC-hippocampal network dysfunction leading to behavioral abnormalities (Harris et al., 2010), as we have seen in our ABCA7 risk variant group.

Although the association of ABCA7 risk variants with AD has been confirmed worldwide (Beecham et al., 2014; Hollingworth et al., 2011; Kamboh et al., 2012; Liu et al., 2014; Reitz et al., 2013), the effect size of the ABCA7 locus on the relative odds of being diagnosed with AD is significantly higher in African Americans (Reitz et al., 2013). In particular, the ABCA7 SNP (rs115550680) being examined in our study has an odds ratio of 1.79 for African Americans, an effect size comparable with that of APOE ε4 (Reitz et al., 2013). Furthermore, this SNP is monomorphic in Caucasians. It therefore remains a significant question whether the results observed in the present study

can be replicated across ethnicities with other causative variants that may have a similar underlying mechanism as rs115550680.

There are several remaining study limitations and specific future directions that should be acknowledged. First, we did not have any participants who were homozygous in the ABCA7 "G" risk allele, and therefore could not examine differences between carriers of one (AG) versus two (GG) risk alleles. Furthermore, due to the relatively small sample size, we were unable to adjust for the effects of APOE or investigate the additive effect in participants who have the risk variant for both APOE and ABCA7. Hence, additional studies with a larger sample size are required to explore differences in ABCA7 risk allele heterozygotes versus homozygotes, as well as, the effects of APOE ε4 and potential gene–gene interactions.

Lastly, there was a gender imbalance in our study, with just three male participants, which limits the generalizability of our findings. The biological influences of sex differences are an important factor to consider in cognitive aging and AD research. While men are at greater risk of developing MCI (Petersen et al., 2010), women are more likely to develop AD (Alzheimer's Association, 2018), possibly due to sex-related differences in the rate of progression from MCI to probable AD. In patients with MCI and AD, brain volumes, particularly hippocampal, have been found to decline faster in women than men (Ardekani, Convit, & Bachman, 2016; Skup et al., 2011), supporting the evidence of faster progression of women from MCI to AD. Furthermore, genes implicated in dementias have been found to increase risk and progression of AD in women; the effect of the APOE ε4 genotype is more pronounced in women (Altmann et al., 2014), while the MET66 allele of BDNF (brain-derived neurotrophic factor) is selectively associated with increased risk of AD in women but not in men (Fukumoto et al., 2010). Particularly notable in the context of this study is *219K* allele of the *ABCA1* gene, which has a 1.75-fold increased risk of developing AD in women, but was found to be protective in men (Sundar, Feingold, Minster, DeKosky, & Kamboh, 2007). Since ABCA7 also belongs to the A subfamily of ABC transporters and shares 54% sequence identity with ABCA1, it may similarly confer a greater AD risk in women, and therefore driving the current results. Stratification by sex in future work is needed to elucidate whether ABCA7-related mechanisms differentially affect AD pathology and neural network dysfunction in men and women.

In conclusion, the results of the study show that in cognitively healthy elderly, ABCA7 risk variant contributes to a maladaptive dissociation in EC resting state functional connectivity, resulting in elevated EC-hippocampus synchronization, but reduced functional integration of EC with mPFC. Our findings suggest a model where impaired cortical connectivity leads to a more functionally isolated EC at rest, which translates into aberrant EC-hippocampus hypersynchronization resulting in generalization deficits.

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CONFLICT OF INTEREST

The authors confirm that there are no known conflicts of interest associated with the publication of this manuscript.

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