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A combined effect of two Alzheimer's risk genes on medial temporal activity during executive attention in young adults

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ABSTRACT

A recent history of failed clinical trials suggests that waiting until even the early stages of onset of Alzheimer's disease may be too late for effective treatment, pointing to the importance of early intervention in young people. Early intervention will require markers of Alzheimer's risk that track with genotype but are capable of responding to treatment. Here, we sought to identify a functional MRI signature of combined Alzheimer's risk imparted by two genetic risk factors. We used a task of executive attention during fMRI in participants genotyped for two Alzheimer's risk alleles: *APOE*-ε4 and *CLU*-C. Executive attention is a sensitive indicator of the progression of Alzheimer's even in the early stages of mild cognitive impairment, but has not yet been investigated as a marker of Alzheimer's risk in young adults. Functional MRI revealed that *APOE*-ε4 and *CLU*-C had an additive effect on brain activity such that increased combined genetic risk was associated with decreased brain activity during executive attention, including in the medial temporal lobe, a brain area affected early in Alzheimer's pathogenesis.

1. Introduction

Developing the empirical groundwork for preventive interventions against Alzheimer's disease (AD) is a priority because the recent history of failed clinical trials suggests that waiting until even the early stages of frank disease onset may be too late for effective treatment (Zahs & Ashe, 2010). Identifying neurocognitive markers of genetic risk for AD in young people is an important component of this groundwork (Goldberg & Weinberger, 2004; Green, Fugelsang, Kraemer, & Dunbar, 2008; Meyer-Lindenberg & Weinberger, 2006; Tan, Callicott, & Weinberger, 2008). Genetic association studies of AD have repeatedly confirmed that the ε4 allele of the apolipoprotein E (APOE) gene is by far the strongest common genetic risk factor for late onset AD (e.g., Bertram, McQueen, Mullin, Blacker, & Tanzi, 2007; Harold et al., 2009; Lambert et al., 2009). Inheritance of one copy of APOE-ε4 markedly increases the risk of AD and decreases the average age of onset (Farrer et al., 1997). The mechanism by which APOE affects AD risk is still unclear, although mouse studies show that in normal brain, APOE-ε4 is associated with alterations in synaptic components (Dumanis, DiBattista, Miessau, Moussa, & Rebeck, 2013; Dumanis et al., 2009) and activity (Hunter et al., 2012). These effects may lead to earlier amyloid deposition observed in mouse models of AD (Kim, Basak, & Holtzman, 2009). Studies in humans suggest that, prior to clinical symptoms, APOE genotype affects medial temporal lobe (MTL) activity (Bookheimer & Burggren, 2009; Bookheimer et al., 2000) and distributed network connectivity (Pena-Gomez et al., 2012), and that APOE-ε4 increases the risk of converting from mild cognitive impairment (MCI) to AD (Fei & Jianhua, 2012). These data suggest that APOE-ε4 is associated with an increased susceptibility of the hippocampus and surrounding medial temporal regions to damage that occurs early in the development of AD (Liu, Kanekiyo, Xu, & Bu, 2013).

While alleles of APOE have the strongest known effects on genetic risk for AD, genome-wide association studies have identified polymorphisms in other genes that have small but significant effects on the risk of AD (Harold, et al., 2009; Lambert et al., 2009). Together, these genes identify several potential pathways that could affect the risk of AD, including neuroinflammation, cholesterol homeostasis, and endocytic regulation (Bertram et al., 2007). Individual genes may affect common pathways to AD pathogenesis, or entirely independent pathways. One of these genetic risk factors is CLU, the gene for clusterin (or apolipoprotein J). Extant evidence indicates that the CLU-C polymorphism is associated with a slightly higher risk of Alzheimer's disease. Large-scale meta analyses have replicated that the CLU-C allele is associated with

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elevated AD risk (Odds ratio=1.22; $p=8.6\times10^{-5}$) (Carrasquillo et al., 2010; Harold et al., 2009).

To the authors' knowledge, there are no reports demonstrating an interaction effect between APOE and CLU to increase Alzheimer's disease risk. However, the apoE and apoJ proteins share a number of important characteristics: they are among very few proteins associated with brain lipoproteins (Elliott, Weickert, & Garner, 2010; Koch et al., 2001); they interact with a shared set of cell surface receptors (Kounnas et al., 1995); they promote neurite outgrowth (Kang et al., 2005; Nathan et al., 1994); and elimination of apoE or apoJ in an AD mouse model caused similar effects on accumulation of Aβ, a component of amyloid plaques associated with AD neuropathology (DeMattos et al., 2004). Due to these biological connections, APOE and CLU polymorphisms may affect similar pathways leading to the development of AD (Wu, Yu, Li, & Tan, 2012).

The neural effects of CLU genotype in young humans have not yet been well characterized. Extant studies indicate that the risk-associated CLU-C allele alters structural and functional connectivity as well as memory-related neuronal activity (Braskie et al., 2011; Erk et al., 2011; Lancaster et al., 2011) in ways that may ultimately contribute to disordered blood flow (Thambisetty et al., 2013) and atrophy (Thambisetty et al., 2012). To our knowledge, no prior research has investigated combined neural effects of CLU and APOE in young people.

The most conspicuous neurocognitive deficit associated with AD is memory impairment, but the disease also has dramatic effects on a set of complex thinking skills referred to as executive function (Kane & Engle, 2003; Silveri, Reali, Jenner, & Puopolo, 2007). One of these skills, executive attention, or the ability to maintain appropriate focus despite the presence of salient but irrelevant stimuli, appears to be a sensitive indicator of the progression of AD even in the early stages of mild cognitive impairment, yielding effects on both behavioral (Saunders & Summers, 2011; Wylie, Ridderinkhof, Eckerle, & Manning, 2007) and brain-based (Neufang et al., 2013; Schroeter et al., 2012) measures. Executive attention relies most strongly on prefrontal and cingulate regions associated with top-down response inhibition and selection (Kane & Engle, 2003), though MTL has also been implicated to a lesser extent (Banich et al., 2009; Casey, Thomas, Davidson, Kunz, & Franzen, 2002; Epstein, Harris, Stanley, & Kanwisher, 1999; Preston & Gabrieli, 2008; Ryan, Lin, Ketcham, & Nadel, 2010). The few brain-imaging studies carried out thus far on the effects of the APOE-ε4 allele in young healthy individuals have focused on memory tasks and have not yet examined executive attention (Borghesani et al., 2008; Bunce, Anstey, Burns, Christensen, & Easteal, 2011; Dennis et al., 2010; Filippini et al., 2009; Mondadori et al., 2007; Scarmeas et al., 2005).

Here, we investigated a task of executive attention as a brain-imaging marker for AD risk in a cohort of healthy young adults, testing for combined effects of APOE and CLU genotype. Based on their likely involvement in shared molecular biological pathways, we hypothesized that possession of the CLU-C risk allele would exacerbate neural effects of the APOE-ε4 allele. Because of the early involvement of MTL in AD pathogenesis, we focused on combined genetic risk effects in this region in our young cohort.

2. Methods

2.1. Participants

Participants were selected from a superset of 160 healthy, right-handed native English speakers (131 male, mean age=23.7 years) who were undergraduate students and community members with no history of mental illness, brain injury, or psychoactive medication, providing informed consent for fMRI. Several exclusions were made to maximally match groups on genetic variables (except for those we sought to directly contrast), and to make group sizes proximate. Table 1 displays demographic data for the individuals included in our analyses. Carriers of the APOE-ε2 allele were excluded due to the potential confound of protective effects conferred by the ε2 allele (Farrer et al., 1997; Bertram et al., 2007). APOE-ε4ε4 ($N=4$) were excluded because the impact of putative exacerbating effect of ε4 homozygosity (Bertram et al., 2007; Farrer et al., 1997) could not be meaningfully assessed given the small group size. Because there were far more APOE-ε3ε3/CLU-C individuals ($N=83$) than APOE-ε3ε3/CLU-NonC individuals ($N=16$) or APOE-ε4ε3/CLU-C individuals ($N=23$), sixteen APOE-ε3ε3 were randomly selected for analysis by taking the first sixteen in a randomly assigned order using the "RAND" function in Microsoft Excel (2011). This enabled proximate group sizes for contrasts between combined APOE and CLU genotype groups and between all of the included ε4-positive ($N=26$) vs. ε4-negative ($N=32$) individuals. The randomly selected group of sixteen APOE-ε3ε3/CLU-C individuals did not differ from the full group of eighty-three APOE-ε3ε3/CLU-C individuals with respect to IQ, MSIT performance, or MSIT executive attention-related activity within an a priori region of interest in bilateral medial temporal lobe (all $p>.1$). In the two groups of CLU-C-positive individuals (i.e., APOE-ε3ε3/CLU-C and APOE-ε4ε3/CLU-C), the proportion of individuals who were heterozygous (CC) vs. homozygous (CT) did not differ, $c^2(1, N=43)=0.26$, $p=.61$). The four genotype groups selected for analysis did not differ on age ($F(3, 54)=1.14$, $p=.34$; all between-group $p>.1$) or IQ ($F(3, 54)=.90$. $p=.45$; all between-group $p>.1$). Additionally, selected participants did not differ by Age or IQ when grouped as APOE-ε4-positive vs. APOE-ε4-negative or as CLU-C-positive vs. CLU-C-negative (all $p>.25$). Our study was composed predominantly of men. The effects of APOE genotype on AD risk appear to be similar for men and women in the broader population (Farrer et al., 1997; Ghebremedhin et al., 2001). However, reports of sex differences in the effects of APOE on brain biomarkers (Damoiseaux et al., 2012; Lehmann et al., 2006) motivated a test of the effect of sex within our sample, which we report below. Our study was predominantly Caucasian. Only 4 of the 55 participants in the selected genotype groups were not Caucasian, with both the ε4-positive and ε4-negative groups including one participant identifying as Black and one identifying as Asian. Thus, it is highly unlikely that our data are substantially affected by potential confounds related to ethnic stratification.

Table 1
Demographic information.

Genotype	Sex	Age	IQ
APOE-E3E3/CLU-NonC	M: 15; F: 1	26 ± 6	127.56 ± 8.21
APOE-E3E3/CLU-C (CC: 4; CT: 12)	M: 15; F: 1	25.25 ± 5.87	121.47 ± 13.97
APOE-E3E3/CLU-NonC	M: 3; F: 0	20 ± 1.73	119.5 ± 11.32
APOE-E4E3/CLU-C (CC: 5; CT: 18)	M: 19; F: 4	24 ± 5.49	123.22 ± 12.30

Values for age and IQ represent the mean ± standard deviation.

2.2. Genotyping

Human APOE genotypes were determined using TaqMan® SNP Genotyping Assays per manufacturer's protocol (Applied Biosystems, Inc.). Briefly, extracted DNA samples were amplified using the standard Allelic Discrimination Protocol on an ABI 7900HT system and SDS software using either the rs429358 (codon 112) or rs7412 (codon 158) primer/probe sets for APOE and rs11136000 primer/probe set for CLU. For APOE genotyping, human DNA of known APOE genotypes (ε2ε2, ε2ε3, ε2ε4, ε3ε3, ε4ε3, ε4ε4) obtained from the National Cell Repository for Alzheimer's Disease (NCRAD, Indiana University) were run on reaction plates as standards. Both APOE and CLU genotype runs included negative controls lacking DNA template. We obtained 100% correct calls using the APOE standards for this gene and for both genes we obtained ≥95% quality value on all calls and 100% recall on ~20% of samples that were rerun for quality control purposes.

2.3. Experimental procedure

All experimental procedures occurred within a single scanning session. Participants performed the Multi-Source Interference Task (MSIT; Bush & Shin 2006), illustrated in Fig. 1, during event-related fMRI. On each MSIT trial, participants pressed one of three buttons to indicate which of three concurrently presented digits differed numerically from the other two. There were two trial types, "Incongruent" and "Congruent." Incongruent trials included distractor number choices that were distinctive due to size but not numerically different from each other and therefore not the correct choice. These numbers were salient because they were potentially valid choices (1–3), and because their distinct sizes drew attention. Thus, Incongruent trials elicited attentional conflict, requiring the use of executive attention to overcome distractions in order to focus on the correct answer. Congruent trials did not involve attentional conflict. Specifically, all numbers other than the correct choice were the same size and were always 0 s, designed to be minimally salient. However, all characteristics of the task