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Association of genetic risk factors with cognitive decline: the PATH through life project

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ABSTRACT

We examined the association of 28 single nucleotide polymorphisms (SNPs), previously associated with dementia or cognitive performance, with tests assessing episodic memory, working memory, vocabulary, and perceptual speed in 1689 nondemented older Australians of European ancestry. In addition to testing each variant individually, we assessed the collective association of the 12-risk SNPs for late-onset Alzheimer's disease using weighted and unweighted genetic risk scores. Significant associations with cognitive performance were observed for *APOE* ε4 allele, *ABCA7*-rs3764650, *CR1*-rs3818361, *MS4A4E*-rs6109332, *BDNF*-rs6265, *COMT*-rs4680, *CTNNBL*-rs6125962, *FRMD4A*-rs17314229, *FRMD4A*-rs17314229, intergenic SNP chrX-rs12007229, *PDE7A*-rs10808746, *SORL1*-rs668387, and *ZNF224*-rs3746319. In addition, the weighted genetic risk score was associated with worse performance on episodic memory. The identification of genetic risk factors, that act individually or collectively, may help in screening for people with elevated risk of cognitive decline and for understanding the biological pathways that underlie cognitive decline.

1. Introduction

Cognitive differences in the elderly consist of differences in stable, life-long cognitive traits, and differences in age-associated cognitive change. For both of these there is significant inter-individual variability in the population (Wilson et al., 2002). Loss of cognitive function due to age-associated cognitive decline is associated with increased difficulties in performing tasks involving memory or rapid information processing and can have a major impact on an individual's quality of life, even in the absence of dementia (Boyle et al., 2012; Kobayashi et al., 2014; Tucker-Drob, 2011; Wilson et al., 2013; Yam and Marsiske, 2013; Zahodne et al., 2013). Identifying factors that predispose individuals to a faster rate of cognitive decline is an important step for developing intervention and treatment strategies aimed at maintaining cognitive health.

Genetic factors likely contribute to the interindividual variability observed in cognitive decline, with common genetic variants estimated to account for between 40% and 50% of the variability associated with general cognitive functioning in later life and 24% of the variability in lifetime cognitive change (Davies et al., 2011; Deary et al., 2012). To date, most of the genetic research on cognitive decline has focused on candidate genes that have been previously associated with age-related disease, traits or mechanisms (Harris and Deary, 2011; Payton, 2009), and particularly with genes related to neurotransmitters, neurotrophins, cognitive function, and neurodegenerative disease. Two of the most widely studied such genes are *COMT*, which encodes the neurotransmiter catechol-O-methyl transferase, and *BDNF*, which encodes the neurotrophin brain-derived neurotrophic factor. Functional variants in these genes have been primarily associated with decline in executive functioning and memory, respectively, although results are inconsistent (Payton, 2009). Late-onset Alzheimer's disease (LOAD) susceptibility genes are also good candidates for association with cognitive decline as the pathologic features of LOAD progress to varying degrees in individuals without dementia or cognitive impairment and are associated with nonclinical cognitive decline (Boyle et al., 2013b; Savva et al., 2009). This cross-over effect is exemplified by the *APOE* *ε4 allele, which confers the largest known genetic risk for LOAD, approximately 2–3 times and 10–12 times for heterozygotes and homozygotes, respectively (Farrer et al., 1997). The *APOE* genotypes are also associated with specific effects on the cognitive domains of episodic memory, executive functioning, perceptual speed, and global cognitive ability (Wisdom et al., 2011).

Despite the publication of numerous genetic associations with cognitive decline, the variants identified typically explain a very small fraction of the phenotypic variability and many remain to be

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replicated. Furthermore, failure to replicate an initial positive result is common because of differences in participant characteristics (e.g., baseline education, mean age, gender, and ethnicity) and methodologies (e.g., sample size, duration of the study, number of follow-ups, population stratification, variation in classification, and cognitive measures; Payton, 2009).

Here, we investigate the association between selected genetic risk factors with cognitive decline in a longitudinally followed community-based cohort of 1689 older adults without dementia who have undergone comprehensive cognitive testing. First, we investigate whether 12 single nucleotide polymorphisms (SNPs) from the top replicated LOAD–associated genes (Morgan and Carrasquillo, 2013; Supplementary Tables 1 and 2); are individually, or collectively as a genetic risk score (GRS), associated with cognitive decline. Second, we investigate whether 16 SNPs, previously associated with either dementia or cognition (Supplementary Tables 1 and 2) are also associated with cognitive decline.

2. Methods

2.1. Participants

Participants were recruited randomly from the electoral rolls (registration is a legal requirement for Australian citizens) of Canberra and Queanbeyan into the Personality and Total Health (PATH) Through Life Project. PATH consists of 3 cohorts; 20–24 (20+), 40–44 (40+), and 60–64 (60+) years at baseline, who have participated in a large longitudinal community survey of health and well-being in adults, the background, and procedures for which have been described in detail elsewhere (Anstey et al., 2012). Written informed consent was obtained from all participants and approval for the study was obtained from the Human Research Ethics Committee of the Australian National University.

The 60+ cohort is the focus of this study. Individuals were assessed at 4-year intervals for a period of 8 years with interviews conducted in 2001–2002 (n = 2551), 2005–2006 (n = 2222), and 2009–2010 (n = 1973). Individuals were excluded from further analysis based on the following criteria: attendance at only 1 interview (n = 309), no genomic DNA available for genotyping (n = 185), *APOE* *ε2/*ε4 genotype (n = 60; to avoid the conflation of *ε2 protective and *ε4-risk effect), non-European ancestry (n = 110), probable dementia at any wave (mini-mental state examination [MMSE] score <24; Folstein et al., 1975), self-reported medical history of epilepsy, stroke, transient ischemic attack, brain tumor, or brain infection (n = 327). Missing values, which can reduce power and result in biased estimates, were imputed for the covariate "Education" (total years of education) using random forests via the "missForest" package available in R (Stekhoven and Bühlmann, 2012; n = 139). This left a final sample of 1689 individuals. At baseline, the individuals retained in the final sample had on average of 0.69 more years of education and scored 0.74 points higher on the MMSE than those excluded (Table 1).

Table 1
Sample demographics

Variable	Excluded ($n = 861$)	Included ($n = 1689$)	Degrees of freedom	t/χ^2	p
Age[a]	62.46 ± 1.49	62.54 ± 1.51	1753	−1.22	0.21
Education[a]	3.31 ± 3.09	14 ± 2.59	1488	−5.62	<0.001
MMSE[a]	28.6 ± 2.13	29.35 ± 0.92	1010	−9.77	<0.001
Male *n* (%)[b]	443 (51.4)	873 (51.7)	1	0.005	0.94
APOE Genotypes *n* (%)					
*ε2/*ε2	6 (0.70)	13 (0.77)			
*ε3/*ε3	395 (45.82)	1048 (62)			
*ε4/*ε4	20 (2.32)	29 (1.71)			
*ε2/*ε3	70 (8.12)	204 (12.07)			
*ε2/*ε4	60 (6.96)	0 (0)			
*ε3/*ε4	137 (15.89)	395 (23.37)			

Key: MMSE, mini-mental state examination.
[a] Unpaired 2-tailed *t*-test.
[b] Pearson's χ^2 2-tailed test.

2.2. Cognitive assessment

All participants were assessed at baseline and at each subsequent interview for the following 5 cognitive abilities: perceptual speed was assessed using the Symbol Digit Modalities Test, which asks the participant to substitute as many digits for symbols as possible in 90s (Smith, 2002); episodic memory was assessed using the immediate recall and delayed recall of the first trial of the California Verbal Learning Test, which involves recalling a list of 16 nouns (Delis et al., 1987); working memory was assessed using the digit span backward from the Wechsler Memory Scale, which presents participants with series of digits increasing in length at the rate of 1 digit per second and asks them to repeat the digits backward (Wechsler, 1945); and vocabulary was assessed with the Spot-the-Word test, which asks participants to choose the real words from 60 pairs of words and nonsense words (Baddeley et al., 1993; Supplementary Tables 5 and 6).

2.3. Genotyping

Sixty-four SNPs were selected for genotyping based on previous associations with dementia, cognition, neuroanatomical differences, and blood pressure (Supplementary Table 2). Genomic DNA was extracted from cheek swabs (n = 4597) using Qiagen DNA blood kits or from peripheral blood leukocytes (n = 64) using QIAamp DNA 96 DNA blood kits.

Preamplification of the targeted loci was performed using the TaqMan PreAmp Master Mix Kit (Life Technologies). Each reaction included 2.5-μL TaqMan PreAmp Master Mix (2x), 1.25-μL Preamplification Assay Pool, 0.5-μL H_2O, and 1.2-μL genomic DNA. These reactions were incubated in a Biorad thermocycler for 10 minutes at 95 °C, followed by 12 cycles of 95 °C for 15 seconds and 60 °C for 4 minutes, and then incubated at 99.9 °C for 10 minutes. The preamplified products were then held at 4 °C until they were diluted 1:20 in 1× TE buffer and then stored at −20 °C until use; 2.5-μL diluted preamplified products were mixed with 2.5-μL TaqMan OpenArray Master Mix. The resulting samples were dispensed using the OpenArray AccuFill System onto OpenArray plates with each plate containing 48 samples and 64 SNP assays per sample. The QuantStudio 12K Flex instrument (Applied Biosystems, Carlsbad, CA, USA) was used to perform the real-time polymerase chain reactions on the loaded OpenArray plates. The fluorescence emission results were read using the OpenArray SNP Genotyping Analysis software, v1 (Applied Biosystems), and the genotyping analysis was performed using TaqMan Genotyper, v1.3, using the autocalling feature. Participant-specific quality controls included filters for genotype success rate (>90%), genotype-derived gender concordant with reported gender, and sample provenance error assessed via pairwise comparisons of genotype calls between all samples to identify samples with >90% similarity. Samples that were flagged in the initial quality control checks were repeated, those that still failed quality control were excluded. SNP-specific filters included genotype call rate (>90%) and Hardy-Weinberg equilibrium ($p > 0.001$) assessed using an exact test with the PLINK toolkit (Purcell et al., 2007).

For this study, data for 28 of the 64 genotyped SNPs were extracted based on a priori hypotheses (Supplementary Table 1). These SNPs have being previously identified as being associated with dementia or cognition through genome wide association