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Genetic variations in the CLU and PICALM genes are associated with cognitive function in the oldest old

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Abstract

Recently, two large, and independent genome wide association studies of late-onset Alzheimer's disease (AD) established association with the same rs11136000 variation in the clusterin (CLU) gene. In addition, one variation, rs3851179, in the phosphatidylinositol binding clathrin assembly protein (PICALM) gene and one variation, rs6656401, in the complement component (3b/4b) receptor 1 (CR) gene were associated with AD. Here, we replicate these associations with cognitive functioning in 1380 individuals from the Danish (1905) birth cohort study of the oldest old (92–93 years at intake) using measures of Mini Mental State Examination (MMSE) and a cognitive composite score. We found a significant association between the highly frequent CLU rs11136000 T allele (38%) and better performance on the cognitive composite score ($p = 0.016$) explaining 0.5% of the mean variation in cognitive composite score, and for men a significant association between the highly frequent PICALM rs3851179 A allele (38%). Better performance was found ($p = 0.024$), explaining 1.4% of the mean variation in cognitive composite score in men. These alleles correspond to the minor alleles initially found more frequent in controls than in cases of AD.

Keywords

Aging; Dementia; Genes

1. Introduction

Sustaining cognitive abilities is a fundamental element for successful aging and a major component of quality of life. Neurodegenerative Alzheimer's disease (AD) is a key epidemiological factor affecting the heath of elderly. Early onset AD has been linked to rare mutations in genes encoding key proteins or peptides in the synthesis of β-amyloid (Aβ), these are amyloid beta (A4) precursor protein (APP), presenilin 1 (PS1), and presenlin 2

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The authors disclose no actual or potential conflicts of interest.
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(PS2) (Campion et al., 1999). The much more abundant form, late onset AD, is partly caused by complex genetics of which the apolipoprotein E (APOE) allele ε4 is an important risk factor (Bertram et al., 2007).

Recently, two large independent genome wide association studies (GWAS) of late onset AD established an association with the same rs11136000 variation in the clusterin (CLU) gene as well as to the well described APOE gene (Harold et al., 2009; Lambert et al., 2009). In addition, Lambert and co-workers found a significant association with a single nucleotide polymorphism (SNP), rs6656401, in the complement component (3b/4b) receptor 1 (CR1) gene (Lambert et al., 2009), while the GWAS by Harold and coworkers found a significant association with rs3851179 in the phosphatidylinositol binding clathrin assembly protein (PICALM) gene, and also suggested several genetic variations with $p < 1 \times 10^{-5}$ which did not pass the statistical criteria for correction for multiple testing but did include variations in the CR1 gene identified in the other GWAS (Harold et al., 2009).

In the present study we aim to assess the relevance of these three variations further by investigating the possible association between these variations and cognitive functioning in elderly individuals, measured both by the well established Mini Mental State Examination (MMSE) (McGue and Christensen, 2001) and a cognitive composite score (McGue and Christensen, 2002). While AD is clearly distinct from normal cognitive aging, there are several reasons why genetic factors that influence risk for AD might also be associated with cognitive functioning in a sample of older individuals without dementia. First, a sample of older individuals is likely to include some individuals with incipient but undiagnosed dementia who are experiencing early cognitive loss (Bondi et al., 1994). Second, genetic risk for AD reflects the cumulative impact of multiple genetic factors, the impact of any one of which is statistical rather than determinative (Bookheimer and Burggren, 2009). In the absence of an overall configuration of genetic risk, the impact of any single genetic risk factor may be to diminish cognitive functioning within the normal range. For example, the APOE polymorphism that is a risk factor for late onset AD is also a risk factor for mild cognitive impairments in the absence of AD (Small et al., 2004).

2. Methods

2.1. Subjects

The subjects included in this study were participants in The Danish 1905 Cohort Study (Nybo et al., 2003). The Danish 1905 cohort study is a prospective investigation of an entire national birth cohort. The survey was initiated in 1998, when the participants were 92–93 years and followed by three follow-up studies of the participating survivors in 2000, 2003, and 2005. Of the 3600 individuals still alive at intake, 2262 participated, and 1651 provided either a blood spot sample or a cheek swap at their first assessment in 1998. Data from participants who provided biological material were included in the present study. Each survey of the 1905 Cohort study comprises multidimensional face-to-face interviews focusing on health and lifestyle issues, as well as objective assessment of cognitive and physical abilities. Written informed consent was obtained from all participants and the study was approved by the Danish Scientific-Ethical Committees.

2.2. Cognitive functions

Cognitive functioning was assessed using the MMSE. The MMSE ranges from 0 to 30 and can be graded as severely impaired for scores between 0 and 17, mildly impaired for scores between 18 and 23, and nonimpaired for scores between 24 and 30. The cognitive composite score was generated by combining the scores of five cognitive measures: fluency (the number of animals the participant was able to name within 1 minute), forward and backward

digit span, and immediate and delayed recall of a modified 12-word learning test. Each of the five cognitive measures were standardized to mean = 0, standard deviation = 1. The cognitive composite score was computed by taking the sum of the five standardized measures (McGue and Christensen, 2002). Table 1 shows that both the mean cognitive composite and MMSE score is slightly higher for the 1905 genetic subsample than for the entire 1905 cohort sample.

2.3. Genetic analysis

DNA was isolated from cheek swabs or blood spots, with the use of QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Genotyping of rs11136000, rs6656401, and rs3851179 was performed by allelic discrimination using predesigned TaqMan® SNP genotyping assays (Applied Biosystems, Pleasonton, USA). Reactions were conducted in a 10 μL volume as recommended by the manufacturer. Polymerase chain reaction (PCR) was performed in the Step One Plus™ Real-Time PCR system and genotypes called using the Step One™ Software version 2.1 (Applied Biosystems).

2.4. Statistical analysis

Statistical analysis was performed using STATA 10.1 (StataCorp, College Station, TX, USA). Linear regression analysis was performed on genotypes recoded 0, 1, and 2, where 0 are homozygotes for the major allele, 1 are heterozygotes, and 2 are homozygotes of the minor allele. Both the cognitive composite score and the MMSE were analyzed as continuous variables and means are reported. All analyses were either adjusted for sex or stratified by sex. Corrections for multiple testing were not applied to these analyses of allele effects because an a priori hypothesis of association was conducted for each of the selected SNP alleles. Neither was there adjustment for the sex stratified populations, because previous research has suggested that genetic effects on cognitive functioning and dementia may vary by gender. Models of interactions between sex and genotypes were tested for significant deviation from noninteraction using a likelihood ratio test. Posthoc analysis of genotype association was performed using categorical genotypes in regression analysis of codominant, recessive, and dominant models (Mario, 2005). Because these genotype-based association analyses were not based on a priori hypotheses, we corrected for testing the three models. A Bonferroni correction was applied and the significance level was equivalent to the *p*-value 0.017.

3. Results

Genotype distribution of the three genetic variations, rs11136000 in the CLU gene, rs3851179 in the PICALM gene, and rs6656401 in the CR1 gene in the 1905 birth cohort are presented in Table 2. All three genetic variations were in Hardy-Weinberg equilibrium in the 1905 birth cohort.

The three genetic variations previously found to be associated with the neurodegenerative disease Alzheimer's were here compared with cognitive measures in the oldest old (Table 3). Significant association was found between better performance in the cognitive composite score and the CLU rs11136000 minor T allele (regression coefficient [reg. coef.] 0.33, 95% confidence interval [CI], 0.06–0.61, $p = 0.016$, $R^2 = 0.005$). Interestingly, stratification by sex showed that this association was stronger in women (reg. coef. 0.46, 95% CI, 0.14–0.79, $p = 0.005$, $R^2 = 0.009$). However, there were no significant interaction between sex and rs11136000 (likelihood ratio [LR] test: $\chi^2 = 1.92$, $p = 0.16$). The influence of sex on the association with cognitive function was however evident for the PICALM rs3851179 variation. In men the rs3851179 minor allele A (reg. coef. 0.60, 95% CI, 0.08 –1.12, $p = 0.024$, $R^2 = 0.014$) was significantly associated with better cognitive composite scores.

There was also a significant interaction between sex and rs3851179 (LR test: $\chi^2 = 5.44$, $p = 0.02$).

Also in males indications of an association between the CR1 rs6656401 minor allele A and poorer performance on the cognitive composite score (reg. coef −0.58%, 95% CI, −1.21 to 0.05, $p = 0.07$) was found, but the association did not reach significance at the 0.05 level.

In addition, we studied mean cognitive composite scores and mean MMSE by genotype groups (Table 4). Both the rs1113000 and the rs3851179 variations showed indications of a dominant genetic effect, thus we tested these variations in codominant, dominant, and recessive models. For the rs1113000 variation the dominant effect (AA vs. AT and TT) was significant in the combined sex group ($F = 6.96$, $p = 0.008$) and in women ($F = 8.92$, $p = 0.003$), and for the rs3851179 variation the dominant effect (GG vs. AG and AA) was significant in men ($F = 7.48$, $p = 0.007$). Also the rs6656401 variation was significantly associated with recessive effects in men (GG and AG vs. AA) ($F = 4.85$, $p = 0.03$), but did not reach significance when applying the Bonferroni correction for using three models.

4. Discussion

Recently, two large genome wide association studies of Alzheimer's disease (AD) were published, including more than 14,600 and 16,100 participants, respectively. These studies are interesting because of their strong power to detect novel associations due to their large sample sizes, but particularly interesting because both studies established convincing evidence of an association between AD and the exact same locus, rs11136000, in the CLU gene as well as to the well described APOE gene (Harold et al., 2009; Lambert et al., 2009). In addition to finding the association between CLU and AD, one variation, rs3851179, in the PICALM gene (Harold et al., 2009) and one variation, rs6656401, in the CR1 gene (Lambert et al., 2009) were suggested as contributors to AD.

In the present study, we aimed to replicate these findings by testing for association between the three loci and cognition in 1380 elderly individuals from the nationwide Danish 1905 Cohort Study.

Overall, our results confirmed an association between the CLU rs11136000 variation and cognitive functioning, measured both by MMSE and the cognitive composite score. Importantly, this association was in the direction expected based on the GWAS of AD. The association between the highly frequent CLU rs11136000 T allele (38%) and significantly better performance on the cognitive composite score in our sample (Table 3) is in complete agreement with the minor rs11136000 T allele being more prevalent among controls than cases of AD as was found by both Lambert et al. (2009) and Harold et al. (2009) who estimated identical odds ratios (0.86). The T allele was estimated to account for 0.5% of the variance in the cognitive composite score (Table 3).

Our second significant association was also consistent with the earlier GWAS of AD. That is, for the highly frequent PICALM rs3851179 A allele (38%) we found for men a significant better performance on the cognitive composite score (Table 3). This result is in agreement with Harold and co-workers who in populations with both sexes found the minor A allele to be more prevalent among controls than cases (odds ratio [OR] = 0.86) (Harold et al., 2009). Although the overall effect of the PICALM rs3851179 A allele on the cognitive composite score (0.5%) was less than that of the CLU rs11136000 T allele the effect was stronger in males (1.4%) than the effect found for the CLU rs11136000 T allele in women (0.9%).

The association between CR1 rs6656401 and the cognitive composite score just barely reached significant association with poorer cognitive performance in men carrying the minor allele A. This is similar to what Lambert and coworkers estimated with the rs6656401 minor allele A being more prevalent among cases than controls (OR = 1.21). Posthoc analysis of the genotypes did however show significant association between poorer cognitive function and the recessive rs6656401 AA genotype ($p = 0.03$), but this result may be a chance finding as it was not significant when corrected for multiple testing.

In the present study of the oldest old, we found reliable evidence that the CLU rs11136000 T allele and the PICALM rs3851179 A allele in men, was associated with better cognitive functioning. These genetic variations were associated with lower risk for AD in contrast to the well known APOE ε4. Nonetheless, whether these associations arise because the CLU rs11136000 and PICALM rs3851179 variations are functional or are in linkage disequilibrium with other functional polymorphisms in the region is not yet known. Our analysis of genotypes did indicate a dominant effect for both genotypes, which may suggest that the CLU rs11136000 CC and PICALM rs3851179 GG genotypes are the actual risk factors for poorer cognitive performance/AD or that these genotypes are linked to other functional variations. The rs11136000 variation is located in an intron of the CLU gene, which encodes the multifunctional molecule clusterin. Clusterin has functionalities similar to APOE which regulates the toxicity of Aβ and its conversion into an insoluble form (Harold et al., 2009). Harold and coworkers identified several genetic variations within the PICALM gene as being associated with AD, some were even functional, but none had the strength of the rs3851179 variation located 88.5 kb 5′ to the PICALM gene. This gene encodes a protein involved in clathrin-mediated endocytosis, which is essential for intracellular trafficking of a range of substrates, e.g., neurotransmitters, proteins, and lipids. Thus PICALM may traffic the Aβ precursor protein itself or traffic proteins from the pathway of Aβ synthesis or processing, e.g., vesicle-associated membrane protein 2 (VAMP2) (Harold et al., 2009). Future functional studies may reveal the underlying nature of these associations and thereby bring us one step further to understand the genetic contribution to normal cognitive abilities and Alzheimer's disease.

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Table 1

Descriptives in the 1905 birth cohort

Cohort	1905	
	All replied	Genetic subsample
Age	92–93 years	92–93 years
Number of individuals	1798	1380
% Female	73	69
Mean composite score	0.007	0.33
SD composite score	3.57	3.45
Mean MMSE	21.40	21.96
SD MMSE	6.04	5.63
Median MMSE	23	23
% Nonimpaired	45.77	48.64
% Mildly impaired	31.98	32.06
% Severely impaired	22.25	19.30

Key: MMSE, Mini Mental State Examination.

Table 2

Genotypes and frequencies in the 1905 birth cohort

SNP	Gene	Minor allele (frequency)	Genotypes	Observed genotype, *n* individuals	Observed genotype frequency
rs11136000	CLU	T (0.38)	CC	541	0.39
			CT	625	0.45
			TT	214	0.16
rs3851179	PICALM	A (0.38)	GG	510	0.38
			AG	652	0.48
			AA	188	0.14
rs6656401	CR1	A (0.18)	GG	915	0.67
			AG	405	0.30
			AA	49	0.04

Total genotypes rs11136000 (*n* = 1380), rs3851179 (*n* = 1369), and rs6656401 (*n* = 1350).

Key: SNP, single nucleotide polymorphism.

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Table 3

Regression analysis of cognitive composite scores and MMSE by rs11136000, rs3851179, and rs6656401 additive coded genotypes

SNP (gene)	Mean cognitive composite score reg. coef. (95% CI), R^2	Mean MMSE reg. coef. (95% CI), R^2	Mean cognitive composite score reg. coef. (95% CI), R^2		Mean MMSE reg. coef. (95% CI), R^2	
			Females	Males	Females	Males
rs11136000 (CLU)	0.33[a] (0.06 to 0.61), 0.005	0.31 (−0.13 to 0.76), 0.005	0.46[b] (0.14 to 0.79), 0.009	0.04 (−0.45 to 0.54), 0.0001	0.45 (−0.07 to 1.00), 0.003	0.006 (−0.84 to 0.83), < 0.0001
rs3851179 (PICALM)	0.09 (−0.20 to 0.37), 0.001	0.07 (−0.40 to 0.53), 0.002	−0.14 (−0.48 to 0.20), 0.0007	0.60[a] (0.08 to 1.12), 0.014	−0.28 (−0.83 to 0.27), 0.001	0.85[c] (−0.02 to 1.72), 0.010
rs6656401 (CR1)	−0.12 (−0.47 to 0.23), 0.001	−0.43 (−0.99 to 0.15), 0.004	−0.09 (−0.33 to 0.52), 0.0002	−0.58[c] (−1.21 to 0.05), 0.009	−0.27 (−0.95 to 0.42), 0.0007	−0.77 (−1.82 to 0.29), 0.005

Reference groups are rs11136000 CC, rs3851179 GG, or rs6656401 GG, respectively.

Key: CI, confidence interval; MMSE, Mini Mental State Examination, reg. coef., regression coefficient.

[a] Significant at < 0.05 level.

[b] Significant at < 0.01 level.

[c] Significant at < 0.1 level.

Table 4

Mean cognitive composite scores and MMSE by rs11136000, rs3851179, and rs6656401 genotypes

SNP (gene)	Genotypes	Mean cognitive composite score (SD)	Mean MMSE (SD)	Mean cognitive composite score (SD)		Mean MMSE (SD)	
				Females	Males	Females	Males
rs11136000 (CLU)	CC	0.02 (0.16)	21.64 (0.26)	−0.15 (0.19)	0.42 (0.28)	21.32 (0.31)	22.39 (0.47)
	CT	0.53 (0.14)	22.17 (0.23)	0.55 (0.17)	0.54 (0.26)	22.03 (0.27)	22.48 (0.44)
	TT	0.60 (0.25)	22.16 (0.42)	0.65 (0.30)	0.47 (0.47)	22.09 (0.49)	22.33 (0.83)
rs3851179 (PICALM)	GG	0.15 (0.16)	21.71 (0.26)	0.28 (0.19)	−0.14 (0.29)	21.86 (0.29)	21.37 (0.54)
	AG	0.49 (0.14)	22.13 (0.23)	0.31 (0.16)	0.90 (0.27)	21.71 (0.28)	23.12 (0.41)
	AA	0.17 (0.27)	21.62 (0.44)	−0.09 (0.34)	0.78 (0.45)	21.24 (0.56)	22.51 (0.70)
rs6656401 (CR1)	GG	0.40 (0.12)	21.71 (0.26)	0.30 (0.14)	0.65 (0.22)	21.88 (0.23)	22.62 (0.35)
	AG	0.15 (0.18)	22.13 (0.23)	0.04 (0.22)	0.38 (0.32)	21.52 (0.35)	22.30 (0.58)
	AA	0.66 (0.58)	21.62 (0.44)	1.82 (0.63)	−1.51 (0.94)	21.24 (0.56)	19.64 (1.73)

Key: MMSE, Mini Mental State Examination; SNP, single nucleotide polymorphism.