



APOE and aging-related cognitive change in a longitudinal cohort of men



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ABSTRACT

We examined associations between APOE major isoforms, rs405509 promoter and rs440446 intron-1 polymorphisms, and nonpathologic cognitive aging. Men from the Helsinki Birth Cohort Study took the Finnish Defence Forces Basic Intellectual Ability Test twice, at age 20.1 ($n = 404$) and 67.6 years ($n = 247$). APOE major isoforms did not associate with cognitive ability. In the APOE major isoform-adjusted analyses, the number of rs405509 minor alleles was associated with a higher cognitive ability total and verbal, arithmetic, and visuospatial subtest scores at 67.6 years (p -values < 0.004). In the analyses of cognitive change, the visuospatial subtest score increased across time in rs440446 minor allele carriers but decreased in noncarriers ($p = 0.007$). Associations in the APOE major isoform-stratified analyses were significant in the APOE $\epsilon 3/\epsilon 3$ homozygotes only. The APOE locus harbors additional modifying alleles, independent of APOE major isoforms that are associated with better preserved general cognitive ability in nondemented elderly men and change in visuospatial ability across 5 decades. These results suggest that at least 2 distinct mechanisms link the APOE locus with cognitive ability.

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1. Introduction

Apolipoprotein E, coded for by the APOE gene, is involved in lipid transport and metabolism and in neural development, repair, and structure (Huang and Mahley, 2014). The $\epsilon 4$ major isoform of the gene has been associated with late-onset Alzheimer's disease (AD) (Corder et al., 1993), beta-amyloid plaque formation (Polvikoski et al., 2000), aging-related cognitive decline (Davies et al., 2014), and a range of neurocognitive functions in cognitively healthy adults (Wisdom et al., 2009). However, approximately half of AD

patients do not carry the $\epsilon 4$ major isoform (Farrer et al., 1997), which has led to the search for other variants that modify the risk. Among these variants is the functional APOE promoter polymorphism rs405509 (also known as -219 or Th1/E47cs), which affects the transcriptional activity of the gene (Artiga et al., 1998). Indeed, previous studies have suggested that carriers of the minor allele of this polymorphism, compared with major allele carriers, are at an increased risk for AD (Lambert et al., 1998a). However, it has not been clear whether the effects of the promoter polymorphism are truly independent or due to linkage disequilibrium with the $\epsilon 2$ or $\epsilon 4$ major isoforms (Jun et al., 2012; Roses et al., 2010).

A series of studies in Finland have examined the effect of this polymorphism in $\epsilon 3/\epsilon 3$ homozygotes, which enables unambiguous haplotyping in context of $\epsilon 3$ major isoform. In these studies, the minor allele of the rs405509 polymorphism has been associated

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with decreased AD risk (Myllykangas et al., 2002), with less brain amyloid plaque formation (Myllykangas et al., 2002), with lower dementia risk in an elderly population with cardiovascular diseases (Strandberg et al., 2005), and with less aortic atherosclerosis among smokers (Viiri et al., 2008). These results, obtained in 3 independent studies, demonstrate that other variation than the $\epsilon 3$ major isoform at the *APOE* locus influence neurologic and vascular traits.

It has been suggested that the *APOE* genetic effects on normal cognitive aging may be mediated by mechanisms that are at least partly independent of its effect on AD (Caselli et al., 2012; Deary et al., 2002). A recent study in a cohort of healthy elderly men found no association between rs405509 promoter polymorphism and performance in the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) but found that *APOE* $\epsilon 4$ major isoform and rs429358, a polymorphism of which the *APOE* major isoform is partially derived from, were associated with better performance in MMSE in the eldest quintile of the cohort (Prada et al., 2014).

We tested the effects of the *APOE* major isoforms and rs405509 promoter polymorphism among nondemented men on cognitive ability in young adulthood and old age, and on nonpathologic aging-related cognitive change across 5 decades. We tested *APOE* isoform dependent and independent associations, and extended our analyses by also examining the effects of rs440446 (also known as +113 or IE1), a functional polymorphism in the *APOE* gene intron-1.

2. Methods

2.1. Participants

The study cohort includes 4630 men born between 1934 and 1944 in Helsinki, Finland and are part of the Helsinki Birth Cohort Study (Barker et al., 2005; Eriksson et al., 2006). In 2001–2004, a random sample of 928 men participated in a clinical study. Data on *APOE* major isoforms and rs405509 were available for 737 men. Of them, cognitive ability data at military conscription at age 20.1 (standard deviation [SD] = 1.5, range = 17.1–26.6) years were available for 404 men, and in the invitation-based retest at age 67.6 (SD = 2.3, range = 64.5–74.5) years for 247 men; data on *APOE* major isoforms, rs440446, and cognitive ability were available for 374 and 223 men at 20.1 and 67.6 years, respectively. Of the 404 men with cognitive ability, data available at 20.1 years and who were genotyped but did not participate in the cognitive ability retest at 67.6 years, 28 had died before the retest, 27 had in a previous follow-up study declined participation in any further follow-up, and 7 lived abroad; the retest sample also excluded 14 men with cognitive ability data at 67.6 years, but who had received a stroke diagnosis (international classification of disease [ICD] codes 430–434 and 436–437 from ICD-8 and 9, 438 from ICD-9, and I60–I69 from ICD-10) according to hospital discharge register (HDR) data. There were no associations between *APOE* major isoforms or minor alleles in rs405509 or rs440446 and mortality of diagnosis of stroke (p -values > 0.46).

The analytic samples and the rest of the men in the genotyped sample differed from each other in 4 respects. Those in the analytic sample at age 67.6 years were more likely to have attained a higher maximum lifetime occupational status ($p < 0.001$), and a higher maximum level of education ($p < 0.001$) were less likely to have been diagnosed with coronary heart disease (codes 410–414 from ICD-8 and ICD-9 and I21–I25 from ICD-10) according to HDR ($p = 0.001$), and scored higher on general cognitive ability at age 67.6 years ($p < 0.01$). The Helsinki Birth Cohort Study has been approved by the Ethics Committee of the National Public Health Institute and the Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa. The Finnish Defence Command

has given permission for data linkage (AL18521). All participants have signed a written informed consent.

2.2. Cognitive ability

The Finnish Defence Forces Basic Intellectual Ability Test consists of time-limited verbal, arithmetic, and visuospatial [analogous to Raven's progressive matrices (Raven, 2000)] subtests as well as a total score. Each series consists of 40 multiple choice items with progressive difficulty. The test battery, with some modifications, has been in use since 1955, and its psychometric properties have been described in detail elsewhere (Raikkonen et al., 2009; Tiuhonen et al., 2005). Previous studies with the test have, for example, found test scores to be lower in 70-year-old men who have been exposed to early-life stress (Pesonen et al., 2013), and lower scores in the test have also been found to be predictive of higher cardiovascular disease and stroke risk (Kajantie et al., 2012). At age 20.1 years, the men took the test within the first 2 weeks of their compulsory military service. At age 67.6 years, the men were retested using the same test battery as previously described (Raikkonen et al., 2013). For all analyses, the cognitive ability test scores were square transformed to attain normality and standardized to a normal distribution with mean 100 and standard deviation 15. Two men had missing data in the total score and arithmetic subtest at 20.1 years. In the present study the intraclass correlations between the cognitive ability scores at the 2 testings were 0.78, 0.62, 0.74, and 0.68 for total score and for verbal, arithmetic, and visuospatial subtest scores, respectively, (all p -values < 0.001). Average mean-level changes were 0.22 (SD = 4.22, range = –16.33 to 13.33), 3.10 (SD = 6.15, range = –22.00 to 11.00), –0.85 (SD = 6.07, range = –19.00 to 30.00), and –1.62 (SD = 4.68, range = –11.00 to 16.00) raw score points in total score and in verbal, arithmetic, and visuospatial subtest scores, respectively.

2.3. Genotyping

DNA was extracted from peripheral blood leukocytes using standard methods. *APOE* $\epsilon 2/\epsilon 3/\epsilon 4$ genotyping was performed by poly amplification, HhaI restriction enzyme digestion, and agarose gel electrophoresis. Genotyping success rate was 99.8%. Hardy-Weinberg equilibrium was calculated for each of the 2 biallelic sites (Arg112/Cys112 and Arg158/Cys158) separately, and both sites were at Hardy-Weinberg equilibrium ($\chi^2 = 0.01$, 2 df, $p > 0.90$ and $\chi^2 = 2.27$, 2 df, $p > 0.30$, respectively).

Rs405509 was genotyped using modified Illumina 610 k array at Sanger Center, UK, with success rate of 99.9% in 2009. For rs440446, we used imputed data, which is a procedure of inferring unobserved genotypes based on known single nucleotide polymorphisms in linkage disequilibrium [for review see (Li et al., 2009)], with imputation success rate 99.1%. Minor allele frequencies were 45.0% for rs405509T and 28.6% for rs440446C, both markers were in Hardy-Weinberg equilibrium ($p > 0.28$). Linkage disequilibria between the markers were the following rs405509 and rs440446 ($D' = 0.98$, $R^2 = 0.474$, log of odds (LOD) 243.92), rs405509 and rs429358 ($D' = 0.984$, $R^2 = 0.092$, LOD 34.75), rs405509 and rs7412 ($D' 0.827$, $R^2 = 0.018$, LOD 6.45), rs429358 and rs7412 ($D' 1.0$, $R^2 = 0.016$, LOD 7.63).

2.4. Covariates and confounders

Covariates included the participant's age at 20.1 and at 67.6 years, father's occupational status in childhood derived from birth, child welfare, and school records, (laborer, junior clerical, and senior clerical), maximum attained lifetime occupational status

Table 1
Sample characteristics at 20.1 y according to rs405509 polymorphism

Characteristic	GG	GT	TT	<i>p</i> ^a
rs405509	125 (30.9%)	184 (45.5%)	95 (23.5%)	
Age	20.1 (1.3)	20.0 (1.6)	20.3 (1.8)	0.33
<i>APOE</i>				<0.001
ε3/3	78 (62.4%)	95 (51.6%)	40 (42.1%)	
ε2/3 ^b	31 (24.8%)	19 (10.3%)	0 (0.0%)	
ε4/2, ε4/3, ε4/4	16 (12.8%)	70 (38.0%)	55 (57.9%)	
rs440446 ^c				<0.001
GG	111 (98.2%)	63 (36.6%)	13 (14.6%)	
GC	2 (1.8%)	109 (63.4%)	39 (43.8%)	
CC	0 (0%)	0 (0.0%)	37 (41.6%)	
Cognitive ability score ^d				
Total	99.0 (15.4)	99.8 (14.7)	101.6 (14.8)	0.44
Verbal	99.2 (15.4)	99.1 (14.5)	102.8 (15.2)	0.12
Arithmetic	99.3 (15.9)	100.2 (14.8)	100.6 (14.2)	0.81
Visuospatial	99.2 (15.1)	100.2 (15.2)	100.5 (14.6)	0.79
Father's occupational status in childhood				<0.001
Laborer	79 (63.2%)	108 (58.7%)	44 (46.3%)	
Junior clerical	22 (17.6%)	35 (19.0%)	40 (42.1%)	
Senior clerical	24 (19.2%)	41 (22.3%)	11 (11.6%)	

Data are mean (SD) or n (%).

^a Comparison by analysis of variance for cognitive ability and age, χ^2 test for others.^b There were no *APOE* ε2/2 homozygotes in our study sample.^c Numbers do not sum up to total samples size because of missing genotypic data.^d Two men had missing data in the total and arithmetic subtest scores.

(laborer or self-employed, junior clerical, and senior clerical), maximum attained lifetime level of education (basic or less or unknown, upper secondary, lower tertiary, and upper tertiary) obtained from Statistics Finland providing data at 5-year intervals between 1970 and 2000, diagnoses of coronary heart disease (codes 410–414 from ICD-8 and ICD-9 and I21–I25 from ICD-10), and mood disorders (codes 296, 298.00, 3004, and 301.10 from ICD-8; 296, 3004A, and 3011D from ICD-9; and F30-F39 from ICD-10) derived from HDR, and Beck Depression Inventory (BDI) scores derived from 2001–2004 clinical examination for depressive symptoms (Beck et al., 1961). We also conducted multidimensional scaling analyses using whole genome data with Plink version 0.64 and derived the first three components explaining most variance in population substructure to control for population stratification.

2.5. Statistical methods

Linear regression analyses tested associations between *APOE* major isoform carrier status (ε3/3 as the referent and contrasted with ε2/3 and with ε4/2, ε4/3, and ε4/4; none were ε2/2 carriers), and the number of minor alleles in rs405509 and in rs440446 with cognitive ability at age 20.1 and 67.6 years. Mixed model regression tested associations with aging-related cognitive change. In the mixed model regression analyses, cognitive ability at age 20.1 and 67.6 years was the dependent outcome variable and *APOE* major isoform carrier status, and the number of minor alleles in rs405509 and rs440446 (all in separate models), time between the two cognitive testings and their interaction were the independent fixed effects. We first tested associations with the total cognitive ability score controlling for inflation of Type II error rate due to multiple testing with a false discovery rate procedure (Benjamini and Hochberg, 1995) setting the false discovery rate across 12 tests at 0.05. We then tested associations with the verbal, arithmetic, and visuospatial subtest scores (we did not apply the false detection rate procedure here as the subtests were highly correlated with the total score, r 's > 0.83, p -values < 0.001). When we tested if the effects of rs405509 and rs440446 were independent of *APOE* major isoforms we included *APOE* major isoform carrier status in the

regression equation, and when we tested if the effects were dependent on the *APOE* major isoforms, we tested the associations stratified according to the *APOE* major isoforms. All associations are presented as adjusted for the covariates and confounders as described in Section 3, table footnotes, and figure legends.

3. Results

Sample characteristics at 20.1 years and 67.6 years according to rs405509 genotype are presented in Tables 1 and 2. Sample characteristics at 20.1 years and 67.6 years according to *APOE* major isoforms are presented in Supplementary Tables 1 and 2.

3.1. Cognitive ability at age 20.1 and 67.6 years

Table 3 presents the linear regression analysis results predicting cognitive ability scores at 20.1 and at 67.6 years by *APOE* major isoform carrier status and by the number of minor alleles in rs405509 and rs440446. *APOE* ε2/3 and ε4/2, ε4/3 and ε4/4 carriers did not differ from ε3/3 carriers in the cognitive ability total or in any of the subtest scores at 20.1 or 67.6 years (p -values > 0.19). There were no significant associations between the number of minor alleles in rs405509 or rs440446 and the cognitive ability total or subtest scores at age 20.1 years (p -values > 0.23). However, the number of minor alleles in rs405509 and rs440446 was associated with a higher total cognitive ability score at age 67.6 years in models that adjusted for *APOE* major isoforms, age at cognitive testing, maximum lifetime occupational status, and population

Table 2
Sample characteristics at 67.6 y according to rs405509 polymorphism

Characteristic	GG	GT	TT	<i>p</i> ^a
rs405509	84 (34%)	107 (43.3%)	56 (22.7%)	
Age	67.8 (2.3)	67.7 (2.2)	67.1 (2.2)	0.14
<i>APOE</i>				<0.001
ε3/3	54 (64.3%)	56 (52.3%)	24 (42.9%)	
ε2/3 ^b	20 (23.8%)	11 (10.3%)	0 (0.0%)	
ε4/2, ε4/3, ε4/4	10 (11.9%)	40 (37.4%)	32 (57.1%)	
rs440446 ^c				<0.001
GG	75 (100.0%)	32 (33.0%)	6 (11.8%)	
GC	0 (0.0%)	65 (67.0%)	24 (47.1%)	
CC	0 (0.0%)	0 (0.0%)	21 (41.2%)	
Cognitive ability score				
Total	96.7 (14.6)	100.6 (14.8)	103.9 (15.2)	0.02
Verbal	97.3 (15.0)	100.8 (15.0)	102.7 (14.6)	0.09
Arithmetic	97.1 (14.7)	100.5 (14.9)	103.3 (15.1)	0.05
Visuospatial	97.1 (14.9)	100.1 (14.5)	104.2 (15.4)	0.02
Highest attained lifetime occupational status				0.14
Laborer or self-employed	16 (19.0%)	17 (15.9%)	10 (17.9%)	
Junior clerical	26 (30.1%)	19 (17.8%)	10 (17.9%)	
Senior clerical	42 (50.0%)	71 (66.4%)	36 (64.3%)	
Maximum attained lifetime education				0.04
Basic or less or unknown	30 (35.7%)	23 (21.5%)	8 (14.3%)	
Upper secondary	16 (19.0%)	26 (24.3%)	21 (37.5%)	
Lower tertiary	24 (28.6%)	33 (30.8%)	17 (30.4%)	
Upper tertiary	14 (16.7%)	25 (23.4%)	10 (17.9%)	
Beck depression inventory sumscore	4.0 (3.9)	4.7 (5.1)	4.6 (3.8)	0.36
Mood disorder diagnosis	2 (2.4%)	1 (0.9%)	0 (0%)	0.43
Coronary heart disease diagnosis	7 (8.3%)	11 (10.3%)	3 (5.4%)	0.56

Data are mean (SD) or n (%).

Key: BDI, Beck depression inventory.

^a Comparison by analysis of variance for cognitive ability, age, and BDI total score, χ^2 test for others.^b There were no *APOE* ε2/2 homozygotes in our study sample.^c Numbers do not sum up to total sample size because of missing genotypic data.

Table 3
Associations between *APOE* major isoform carrier status, and the number of minor alleles in rs405509 and rs440446 and cognitive ability total and subtest scores at 20.1 and 67.6 y

Genotype	Total score			Verbal subtest score			Arithmetic subtest score			Visuospatial subtest score		
	B	95% CI	<i>p</i>	B	95% CI	<i>p</i>	B	95% CI	<i>p</i>	B	95% CI	<i>p</i>
<i>APOE</i> ε3/3 vs. ε2/3 ^a												
20.1 y (n = 263)	-1.11	-5.44, 3.23	0.74	-0.40	-4.76, 3.98	0.86	-2.23	-6.64, 2.18	0.32	0.82	-3.65, 5.28	0.72
67.6 y (n = 165)	1.43	-3.54, 6.40	0.57	0.84	-4.56, 6.25	0.76	2.62	-2.48, 7.71	0.31	-0.94	-6.24, 4.37	0.73
<i>APOE</i> ε3/3 vs. ε4/2, ε4/3, ε4/4												
20.1 y (n = 354)	0.50	-2.51, 3.51	0.75	0.75	-2.28, 3.78	0.63	-0.34	-3.40, 2.72	0.83	1.13	-1.97, 4.22	0.47
67.6 y (n = 216)	-1.25	-4.75, 2.26	0.49	-0.86	-4.67, 2.96	0.66	-0.30	-3.90, 3.29	0.87	-2.53	-6.27, 1.22	0.19
rs405509 (GG/GT/TT)												
20.1 y (n = 404)	1.11	-1.00, 3.22	0.43	1.62	-0.51, 3.74	0.23	0.36	-1.75, 2.47	0.87	0.48	-1.65, 2.61	0.83
67.6 y (n = 247)	4.01	1.35, 6.67	0.003	3.06	0.33, 5.80	0.04	3.35	0.67, 6.04	0.02	3.96	1.29, 6.62	0.004
rs440446 (GG/GC/CC)												
20.1 y (n = 374)	0.71	-1.68, 3.09	0.70	1.16	-1.24, 3.55	0.49	0.16	-2.22, 2.55	0.97	-0.19	-2.59, 2.21	0.71
67.6 y (n = 223)	3.25	0.19, 6.32	0.03	2.11	-1.02, 5.24	0.21	2.38	-0.72, 5.49	0.14	3.96	0.91, 7.01	0.009

B refers to unstandardized regression coefficient. 95% CI refers to 95% confidence interval for B. The *p*-values are adjusted for *APOE* major isoforms, age at testing, occupational status, (father's occupational status in childhood for cognitive ability at 20.1 y, own highest attained occupational status for cognitive ability at 67.6 y), and the 3 first components from multidimensional scaling analysis for population stratification.
^a There were no *APOE* ε2/2 homozygotes in our study sample.

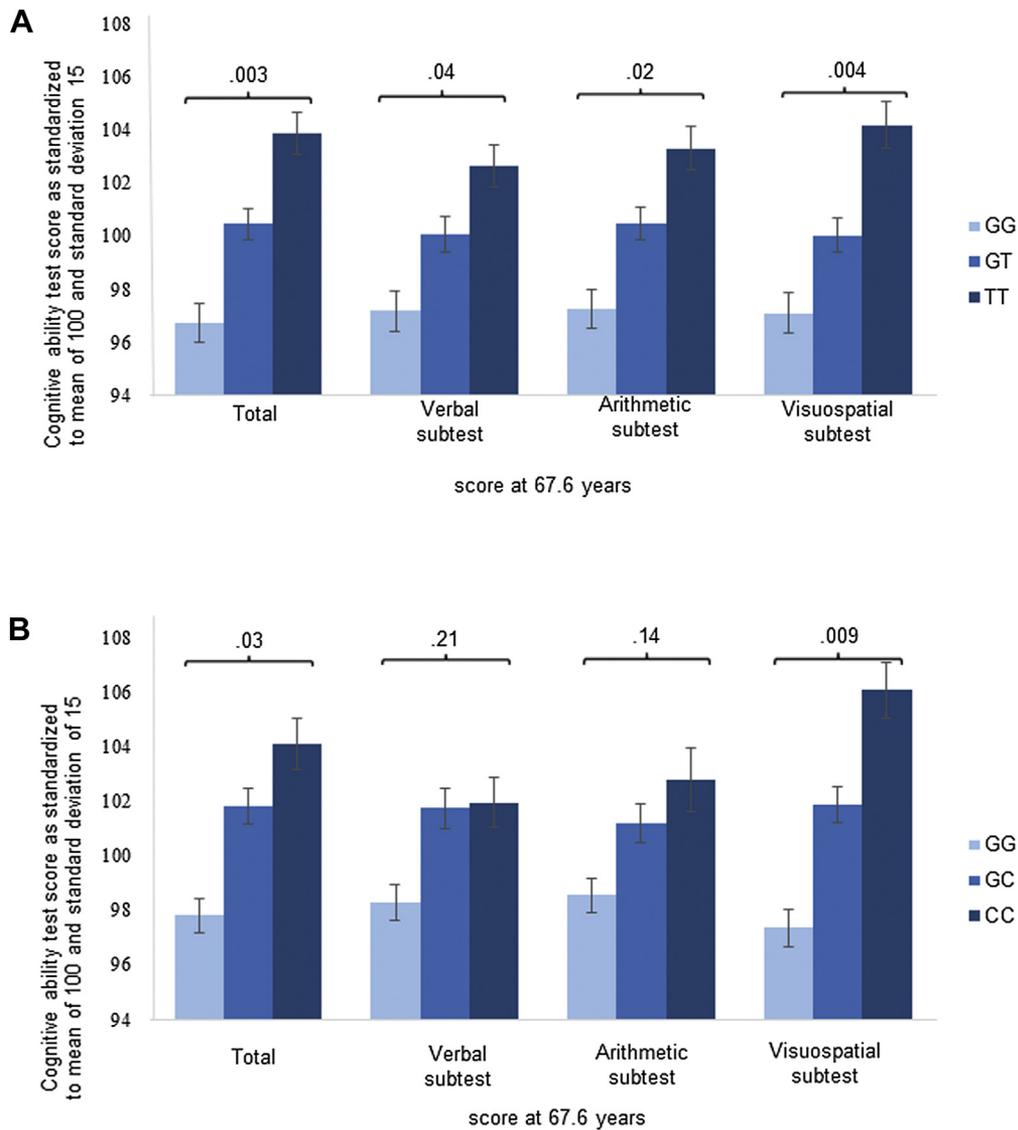


Fig. 1. Estimated marginal means for cognitive ability total and subtest scores at 67.6 years according to the number of minor alleles in rs405509 (A) and rs440446 (B) polymorphisms. Error bars represent standard errors. The *p*-values are adjusted for *APOE* major isoforms, age at cognitive testing, maximum attained lifetime occupational status, and population stratification. Cognitive ability scores are standardized to mean = 100, standard deviation = 15.

stratification ($p = 0.003$ and $p = 0.03$, respectively). However, the association between rs440446 and total cognitive ability score did not survive the correction for multiple testing. The number of minor alleles in rs405509 was also significantly associated with verbal, ($p = 0.04$); arithmetic, ($p = 0.02$); and visuospatial ($p = 0.004$) subtest scores at 67.6 years, and the number of minor alleles in rs440446 was associated with higher visuospatial subtest score at 67.6 years ($p = 0.009$) in models adjusting for *APOE* major isoforms, age at cognitive testing, maximum lifetime occupational status and population stratification. These findings are depicted in illustrative form in Fig. 1 and demonstrate the *APOE* independent effects of minor alleles in rs405509 and rs440446 on cognitive ability at 67.6 years.

All these associations remained significant after additionally adjusting for coronary heart disease diagnoses (p -values < 0.04), mood disorder diagnoses (p -values < 0.04), and BDI score (p -values < 0.05). The association between the number of minor alleles in rs405509 and rs440446 and the cognitive ability total ($p = 0.03$ and 0.05 , respectively) and visuospatial subtest scores ($p = 0.03$ and 0.01 , respectively) also remained significant when we replaced maximum attained lifetime occupational status with the maximum lifetime achieved level of education. The associations between the number of minor alleles in rs405509 and verbal ($p = 0.10$) and arithmetic ($p = 0.10$) subtest scores were rendered nonsignificant when maximum occupation was replaced by maximum education.

In analyses stratifying for *APOE* major isoforms and adjusting for the covariates and confounders, the number of minor alleles in rs405509 was associated with higher visuospatial subtest score at age 67.6 years in the *APOE* $\epsilon 3/3$ homozygotes ($p = 0.03$; Table 4). This association remained significant when additionally adjusting for coronary heart disease diagnoses ($p = 0.03$), mood disorder diagnoses ($p = 0.04$), and BDI score ($p = 0.02$) and when replacing maximum occupation by maximum education ($p = 0.04$). Rs405509 was not significantly associated with cognitive ability in the other *APOE* major isoform subgroups (Table 4), indicating that the main effect is driven by the *APOE* $\epsilon 3/3$ homozygotes. Rs440446 was not

significantly associated with the total cognitive ability or subtest scores in any of the *APOE* isoform groups (Table 4).

3.2. Cognitive change

APOE $\epsilon 2/3$ and $\epsilon 4/2$, $\epsilon 4/3$ and $\epsilon 4/4$ carriers did not differ from $\epsilon 3/3$ carriers in the change in cognitive ability total or subtest scores between 20.1 and 67.6 years (p -values > 0.10 ; Supplementary Fig. 1). The number of minor alleles in rs405509 and rs446446 was not significantly associated with the change in cognitive ability total, verbal or arithmetic subtest scores between 20.1 years and 67.6 years in models adjusting for *APOE* major isoforms and covariates and confounders (p -values > 0.15 ; Fig. 2, Panels A and B). However, the visuospatial subtest score increased from age 20.1 to age 67.6 years in carriers of 2 minor alleles and decreased in carriers of 2 major alleles of the rs440446 in models adjusting for *APOE* major isoforms and covariates and confounders ($p = 0.007$; Fig. 2, Panel B). Visuospatial subtest score also increased from age 20.1 to age 67.6 years in carriers of 2 minor alleles and decreased in carriers of 2 major alleles in rs405509 and rs440446 in the *APOE* $\epsilon 3/3$ homozygotes ($p = 0.01$ and $p = 0.01$, respectively; Fig. 2 Panels C and D).

These associations remained significant after additionally adjusting for coronary heart disease (p -values < 0.01), mood disorder diagnoses (p -values < 0.01), and BDI score (p -values < 0.01) and when maximum occupation was replaced by maximum education (p -values < 0.01). These results suggest that the effects of rs405509 and rs440446 on the change in cognitive ability are driven by *APOE* $\epsilon 3/3$ homozygotes and that the association with rs440446 is slightly stronger.

4. Discussion

We found associations between the number of minor alleles in rs405509 and rs440446 and better cognitive ability at 67.6 years though the association with rs440446 did not survive the correction for multiple testing. We also found that visuospatial subtest score increased across 5 decades between the ages of 20.1 and

Table 4

Associations between the number of minor alleles in rs405509 and rs440446 and cognitive ability total and subtest scores at 20.1 and 67.6 y stratified by *APOE* major isoforms

Genotype	Total score			Verbal subtest score			Arithmetic subtest score			Visuospatial subtest score		
	B	95% CI	<i>p</i>	B	95% CI	<i>p</i>	B	95% CI	<i>p</i>	B	95% CI	<i>p</i>
rs405509 (GG/GT/TT)												
20.1 y												
<i>APOE</i>												
$\epsilon 2/3^a$ (n = 50)	2.97	-5.77, 11.70	0.57	-0.90	-9.78, 7.97	0.76	4.65	-4.05, 13.34	0.36	3.24	-5.58, 12.07	0.45
$\epsilon 3/3$ (n = 213)	0.29	-0.24, 3.03	0.93	1.34	-1.43, 4.09	0.39	-0.05	-2.80, 2.69	0.88	-1.19	-3.91, 1.53	0.32
$\epsilon 4/2, \epsilon 4/3, \epsilon 4/4$ (n = 141)	2.38	-1.28, 6.04	0.26	2.59	-1.09, 6.27	0.34	0.42	-3.26, 4.09	0.71	3.23	-0.53, 6.99	0.13
67.6 y												
<i>APOE</i>												
$\epsilon 2/3^a$ (n = 31)	9.68	-1.65, 21.00	0.96	9.30	-2.17, 20.78	0.87	9.94	-1.26, 21.13	0.76	3.44	-0.79, 14.77	0.71
$\epsilon 3/3$ (n = 134)	3.69	0.34, 7.04	0.06	2.23	-1.23, 5.68	0.38	3.38	-0.04, 6.79	0.12	3.91	0.61, 7.20	0.03
$\epsilon 4/2, \epsilon 4/3, \epsilon 4/4$ (n = 82)	2.99	-1.95, 7.93	0.06	2.74	-2.22, 7.69	0.16	1.85	-3.09, 6.79	0.14	3.64	-1.32, 8.60	0.06
rs440446 (GG/GC/CC)												
20.1 y												
<i>APOE</i>												
$\epsilon 2/3^a$ (n = 43)	4.81	-4.68, 14.31	0.38	-1.55	-11.27, 8.17	0.66	7.13	-2.24, 16.50	0.22	5.49	-3.92, 14.90	0.18
$\epsilon 3/3$ (n = 200)	-0.46	-3.32, 2.41	0.63	0.65	-2.24, 3.54	0.62	-0.79	-3.66, 2.08	0.65	-1.90	-4.73, 0.94	0.19
$\epsilon 4/2, \epsilon 4/3, \epsilon 4/4$ (n = 131)	3.33	-1.66, 8.32	0.34	3.54	-1.46, 8.55	0.33	1.34	-3.69, 6.36	0.82	3.59	-1.58, 8.75	0.27
67.6 y												
<i>APOE</i>												
$\epsilon 2/3^a$ (n = 26)	11.07	-1.28, 23.41	0.30	11.72	-0.02, 23.62	0.28	9.65	-2.56, 21.86	0.73	4.41	-8.04, 16.87	0.42
$\epsilon 3/3$ (n = 125)	2.80	-0.73, 6.32	0.19	1.37	-2.25, 4.98	0.70	2.37	-1.25, 5.99	0.30	3.42	-0.03, 6.87	0.08
$\epsilon 4/2, \epsilon 4/3, \epsilon 4/4$ (n = 72)	1.39	-5.90, 8.68	0.38	0.08	-7.15, 7.31	0.74	-2.00	-7.50, 7.10	0.66	4.76	-2.55, 12.07	0.12

B refers to unstandardized regression coefficient. 95% CI refers to 95% confidence interval for B.

The p -values are adjusted for age at testing, occupational status (father's occupational status in childhood for cognitive ability at 20.1 y and own highest attained occupational status for cognitive ability at 67.6 y), and the 3 first components from multidimensional scaling analyses for population stratification.

^a There were no *APOE* $\epsilon 2/2$ homozygotes in our study sample.

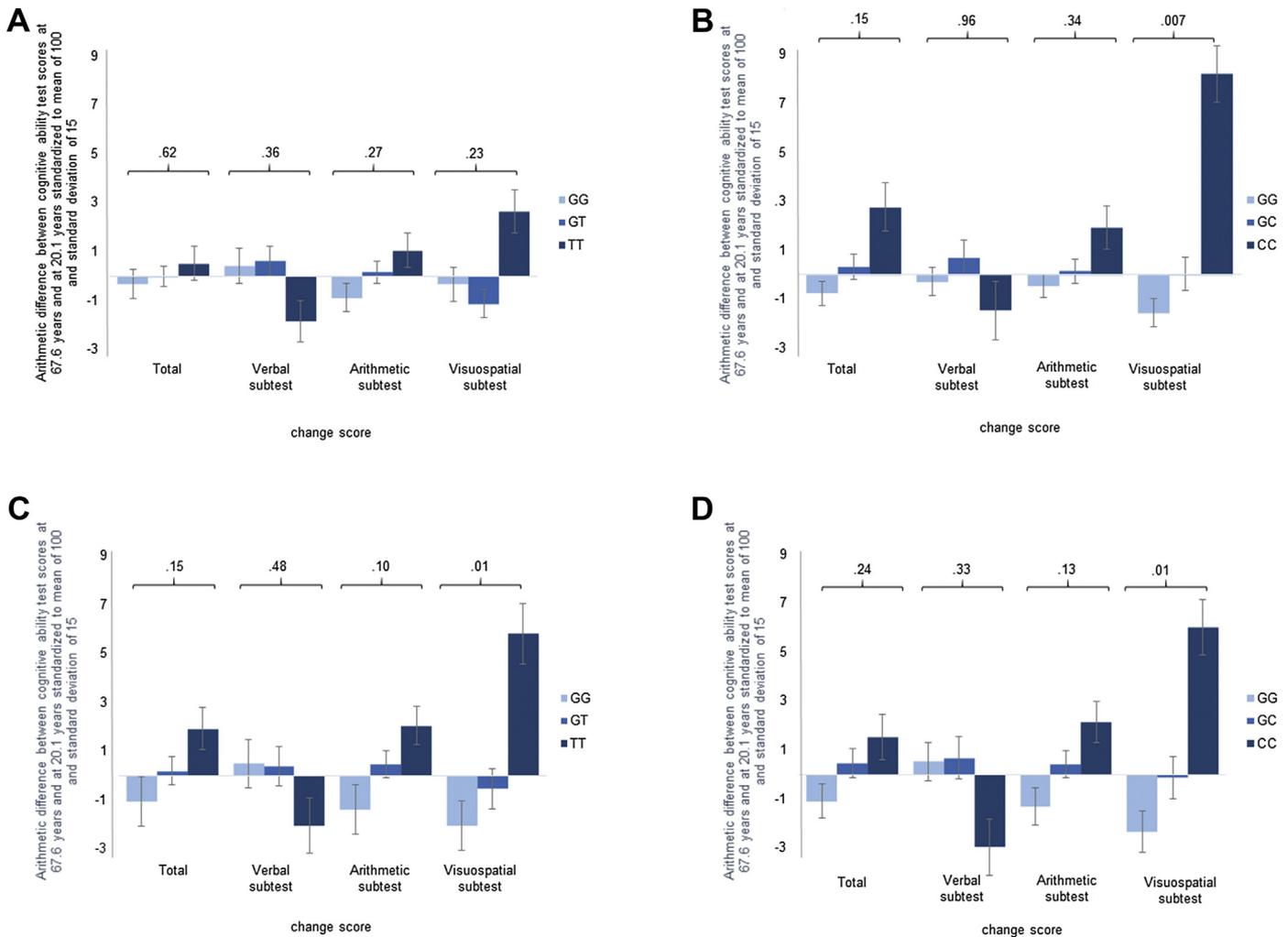


Fig. 2. Change in cognitive ability total and subtest scores between 20.1 and 67.6 years according to the number of minor alleles in rs405509 and rs440446 polymorphisms. Bars represent change scores, error bars represent standard errors. The *p*-values are adjusted for *APOE* major isoforms, age at cognitive testing, maximum attained lifetime occupational status, and population stratification (A and C), and in *APOE* $\epsilon 3/\epsilon 3$ homozygotes for age at cognitive testing, maximum attained lifetime occupational status, and population stratification (B and D).

67.6 years in carriers of 2 minor but decreased in carriers of 2 major alleles in rs440446. Notably, *APOE* major isoforms were not associated with these measures. The associations between the minor alleles in rs405509 and rs440446 and cognitive ability were independent of the *APOE* major isoforms. In the *APOE* major isoform-stratified analyses, which showed that the visuospatial subtest scores increased across 5 decades in carriers of 2 minor and decreased in carriers of 2 major alleles in rs405509 and rs440446, the associations were significant in *APOE* $\epsilon 3/\epsilon 3$ homozygotes only. These associations did not substantially change when we made adjustments for age at cognitive testing, occupational status of the participant's father in childhood, own highest attained occupational status and/or attained level of education across the lifespan, diagnoses of coronary heart disease or mood disorders, depressive symptoms, and population stratification. As no associations were found with cognitive ability at 20.1 years, our findings do not reflect individual differences in cognitive ability across the lifespan but rather point to changes in cognitive abilities across 5 decades. Yet, our findings, and particularly those suggesting that the associations are driven by the *APOE* $\epsilon 3/\epsilon 3$ homozygotes, ought to be considered as preliminary in light of our relatively small sample size. Hence, future studies on *APOE* and cognitive ability and change across time

are needed in samples that are larger than the present one to either confirm or refute our study findings.

Our result showing that the number of minor alleles in the rs405509 polymorphism was associated with higher cognitive ability in the old age, and with an increase in the visuospatial subtest score across 5 decades, especially among the *APOE* $\epsilon 3/\epsilon 3$ homozygotes concurs with previous studies in the Finnish *APOE* $\epsilon 3/\epsilon 3$ homozygous individuals (Myllykangas et al., 2002; Strandberg et al., 2005). These previous studies have demonstrated that minor alleles in this promoter polymorphism are associated with lower AD risk and lower brain beta amyloid deposition (Myllykangas et al., 2002) as well as smaller aging-related change in cognitive function in presence of risk factors such as herpes virus or low attained education (Strandberg et al., 2005).

Regarding rs440446, previous studies which have found associations with AD, independent of *APOE* major isoforms, have most often linked the minor allele with lower risk regardless of the population studied (Bullido et al., 2000; Lambert et al., 1998b; Myllykangas et al., 2002). Our results are also compatible with this pattern.

Our results, however, differ from a recent study that found no association between rs405509 and rs440446 with performance in

the MMSE in a sample of healthy elderly men from the U.S. (Prada et al., 2014). Although this difference may be attributable to population differences, another explanation may underpin the differences in the cognitive outcome measures: MMSE, which was used in the US study (Prada et al., 2014), is a screening tool for cognitive deficits related to dementia, although our measure tested for verbal, arithmetic, and visuospatial ability and was at the age of 20.1 years used for selecting conscripts for leadership training during their military service (Tiihonen et al., 2005) and in the retest at the age of 67.6 years for testing stability and cognitive change in a nondemented population (Pesonen et al., 2013; Raikkonen et al., 2009). Yet, the U.S. study did not test for *APOE* independent and isoform-specific effects of the rs405509 and rs440446 on MMSE (Prada et al., 2014) precluding direct comparisons.

We did not find *APOE* major isoforms to be associated with either cognitive ability at 20.1 or 67.6 years or with cognitive change. A meta-analysis suggests that although particularly the $\epsilon 4$ isoform has in many studies been found to be associated with lower cognitive function in old age, it is only associated with some domains of cognition, such as executive function or episodic memory but not with others, such as verbal ability or visuospatial ability (Small et al., 2004). It is possible that the lack of significant associations with *APOE* $\epsilon 4$ isoform in our study reflects this domain selectivity because we did not specifically test executive function and episodic memory. However, we cannot rule out that the null associations may reflect the small number of participants carrying the $\epsilon 2$ and $\epsilon 4$ isoforms.

It is of note that the visuospatial subtest used in this study is analogous to Raven's progressive matrices (Raven, 2000), which is an established indicator of the 'g' factor or general intelligence (Gray and Thompson, 2004). The visuospatial test is also an independent predictor of another neurologic disorder, stroke, whereas the 2 other subtests are not (Kajantie et al., 2012).

Strengths of the study include measurement of cognitive ability at age 20.1 years, decades before aging-related change in cognitive ability is shown to begin, and in a retest at age 67.6 years with the same test battery in a well-characterized cohort of men. The main limitations are the relatively small sample size, limited number of *APOE* $\epsilon 2$ and $\epsilon 4$ major isoform carriers, and studying men only. Also, possible sources of bias because of sample attrition over 5 decades may attenuate significant associations. Therefore, the findings await replication in other samples and including women, as well as samples that differ from ours in ethnicity. In addition, the testing conditions at 20.1 years may have been more stressful than that at the retest at 67.6 years. However, as the intraclass correlations between the abilities across 5 decades were high, and because there is no reason to presume that any potential stress-effects on cognition vary according to the *APOE* major isoform carrier status or rs405509 and rs440446 polymorphisms, it is unlikely that this would have introduced a bias into our results.

In sum, we found that the number of minor alleles in rs405509 and rs440446 polymorphisms was associated with higher cognitive ability at 67.6 years independent on the *APOE* major isoforms, and with an increase in the visuospatial ability across 5 decades in *APOE* $\epsilon 3/3$ homozygous men. Our findings concur with earlier studies in Finnish women and men showing a lower AD and cognitive impairment risk in minor allele carriers of these polymorphisms (Mylykangas et al., 2002; Strandberg et al., 2005). These results suggest that at least 2 distinct mechanisms may link the *APOE* locus with aging-related cognitive change: the *APOE* isoform independent mechanism, which is shared between AD and nonpathological cognitive change in our cohort, and the *APOE* isoform dependent mechanism which is not. The *APOE* isoform independent mechanism is probably mediated at the level of gene regulation of *APOE*

and nearby genes. Given the major role of the *APOE* locus in cognitive change, elucidation of the causal variant(s) and their mechanism of action will have major public health impact.

Disclosure statement

There are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2016.04.024>.

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