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*APOE*  $\epsilon$ 4, rs405509 and rs440446 promoter and intron-1 polymorphisms and dementia risk in a cohort of elderly Finns – Helsinki Birth Cohort Study

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**ABSTRACT (168/170)**

We tested if the  $\epsilon 4$  major isoform of the *APOE* gene and rs405509 and rs440446 promoter and intron-1 polymorphisms predicted risk of any dementia or Alzheimer's disease with diagnoses derived from the Hospital Discharge and Causes of Death Registers in 1453 participants of the Helsinki Birth Cohort Study. We used Cox proportional hazard models adjusted for sex, year of birth, maximum lifetime occupational status and education, and diagnoses of stroke, coronary heart disease, mood disorders and depressive symptoms. *APOE*  $\epsilon 4$  predicted higher risk of any dementia (hazard ratios [HR]'s > 3.68; 95% confidence interval [CI] 1.76, 7.70) across all statistical models, and when adjusted for rs405509 and rs440446. The number of minor alleles in rs405509 or rs440446 was not associated with dementia risk (HR's < 1.43; 95% CI 0.87, 2.36). As rs405509 or rs440446 have been associated with non-pathological cognitive aging in this and other cohorts independent of the *APOE* major isoforms, these findings lend credence that *APOE* locus may be linked with dementia risk and non-pathological cognitive aging via separate mechanisms.

**Highlights**

- \**APOE*  $\epsilon 4$  major isoform predicted dementia risk independent of rs405509 and rs440446.
- \*rs405509 and rs440446 have been previously linked with cognitive aging in this cohort.
- \*rs405509 and rs440446 did not predict dementia risk.
- \*Separate mechanisms may link the *APOE* locus with cognitive ability and dementia.

## 1 Introduction

The  $\epsilon 4$  major isoform of the *APOE* gene is a well-documented risk factor for Alzheimer's disease [AD] (Corder et al, 1993), dementia with Lewy bodies (Bras et al, 2014) as well as vascular dementia (Sun et al, 2015), and has also been found to be associated with cognitive functioning without dementia (Wisdom et al, 2009). In addition to the  $\epsilon 4$  major isoform, the functional polymorphism rs405509 in the *APOE* gene promoter region has been found to be associated with AD risk (Lambert et al, 1998) as well as non-pathological cognitive aging (Rantalainen et al, 2016). However, research on this polymorphism has not been unanimous. Some studies have found higher AD risk associated with the major allele (Myllykangas et al, 2002; Beyer et al, 2005) and others with the minor allele (Lambert et al, 1998; Nicodemus et al, 2004). Furthermore, some studies have reported risks differing with ethnicity (Lambert et al, 2002) or age of dementia onset (Beyer et al, 2005). It has also been unclear whether the promoter polymorphism's effects are truly independent of or due to linkage disequilibrium with *APOE* major isoforms (Jun et al, 2012; Roses et al, 2010). It has been suggested that the *APOE* locus is associated with non-pathological cognitive aging and dementia risk via separate mechanisms (Deary et al, 2002). However, there have thus far been no studies to report associations of *APOE* major isoforms and rs405509 promoter polymorphisms with both outcomes in a single study population. We recently reported that while the *APOE* major isoforms were not associated with cognitive ability in elderly men from the Helsinki Birth Cohort Study (HBCS), the number of minor alleles in rs405509 and in another functional polymorphism, rs440446 in the *APOE* gene intron-1, predicted better preserved cognitive ability independent of the *APOE* major isoforms (Rantalainen et al, 2016). Here we extend these analyses and study whether these genetic variants predict dementia risk in participants derived from this same study population.

## 2 Methods

### 2.1 Participants

The HBCS includes a random sample of 2003 participants. Of them, data on *APOE* major isoforms, rs405509, rs440446 and dementia diagnoses were available for 1453 participants (72.5%).

Compared to the rest of the cohort, the participants in the analytic sample were more likely to have attained maximum lifetime upper clerical occupation ( $p < .001$ ) and tertiary education ( $p < .001$ ).

## 2.2 Ethics statement

The HBCS 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.

## 2.3 Genotyping

Genotyping and linkage disequilibria between markers have been described (Rantalainen et al, 2016). In this sample the biallelic sites that are used to determine *APOE* major isoforms (Arg112/Cys and Arg158/Cys) and rs405509 and rs440446 were at Hardy-Weinberg equilibrium (not shown).

## 2.5 Dementia diagnoses

Diagnoses of any organic dementias, given in inpatient (1969-2013) and outpatient (1998-2013) specialty care (codes 290.00-290.10 from International Classification of Diseases (ICD)-8, 290, 2912A, 2928C, 2941A, 3310A and 3311A from ICD-9, and F00, F01, F03, F051 and G30 from ICD-10) and AD (codes 331.0 and 290.1 from ICD-9, and G30 and F00 from ICD-10) until the end of 2013 were derived from the Finnish Hospital Discharge (HDR) and Causes of Death (CDR) Registers.

## 2.4 Covariates and confounders

These included the participant's sex, year of birth extracted from hospital records, highest attained occupational status (manual worker, junior clerical, senior clerical) and highest attained level of education in adulthood (lower secondary or less, upper secondary, lower tertiary, upper tertiary)

recorded at five-year intervals between 1970-2000 derived from Statistics Finland, and diagnoses of stroke (codes 430–434 and 436–437 from ICD-8 and -9, 438 from ICD-9, and I60–I69 from ICD-10) (Barker et al, 2015), coronary heart disease (codes 410–414 from ICD-8 and ICD-9 and I21–I25 from ICD-10) (Osmond et al, 2007) 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) until the end of 2013 derived from the HDR, depressive symptoms reported using the Beck Depression Inventory (Beck et al, 1961) at the time of drawing blood for DNA extraction, and the first three multidimensional scaling components derived from genome-wide data to account for population stratification.

## 2.6 Statistical methods

Cox Proportional Hazards models examined the associations between *APOE*  $\epsilon 4$  ( $\epsilon 4$  carriers vs. noncarriers as referent), and the number of minor alleles in rs405509 and in rs440446 and risk of any dementia and AD (no dementia as referent). The participants were followed up until their first hospitalization for any dementia, death, migration, or until December 31, 2013. We first examined the associations of *APOE*  $\epsilon 4$ , rs405509 and rs440446 with dementia risk in separate models, and then entered rs405509 and rs440446 in the models with *APOE*  $\epsilon 4$ . We also tested the associations of rs405509 and rs440446 with dementia risk separately in  $\epsilon 4$  carriers and  $\epsilon 3/3$  homozygotes, which enables unambiguous haplotyping (see Myllykangas et al, 2002; Rantalainen et al, 2016; Strandberg et al, 2005; Viiri et al, 2008) as rs405509 and rs440446 are in linkage disequilibrium with the  $\epsilon 2$  or  $\epsilon 4$  major isoforms (Jun et al, 2012; Rantalainen et al, 2016; Roses et al, 2010). The analyses were adjusted first for sex, year of birth and population stratification, and then for the other covariates.

## 3 Results

Sample characteristics according to *APOE* major isoforms are presented in Supplementary Table 1.

*APOE*  $\epsilon 4$  was associated with a higher risk of any dementia in all statistical models (hazard ratios [HR]'s > 3.68; 95% confidence interval [CI] 1.76, 7.70) (Table 1, Supplementary Figure 1). The

number of minor alleles in rs405509 or rs440446 were not significantly associated with any dementia risk in any of the models (all HR's < 1.43; 95% CI 0.87, 2.36), and were not either associated with any dementia risk in *APOE*  $\epsilon$ 3/3 homozygotes (all HR's < 0.81; 95% CI 0.33, 1.99) or *APOE*  $\epsilon$ 4 carriers (all HR's < 1.09; 95% CI 0.39, 3.06) (for any dementia, n=11 in *APOE*  $\epsilon$ 3/3 homozygotes and n=20 in *APOE*  $\epsilon$ 4 carriers). When we entered *APOE*  $\epsilon$ 4 simultaneously with rs405509 and rs440446 in respective models, *APOE*  $\epsilon$ 4 remained a significant predictor of dementia risk (Table 1). In analyses with risk of AD, the results were similar (Supplementary Table 2; data of stratified analyses in *APOE*  $\epsilon$ 3/3 homozygotes and *APOE*  $\epsilon$ 4 carriers not shown, n=7 and n=16 with AD, respectively).

#### 4 Discussion

Our result is in accordance with the suggestion that the *APOE* gene may be associated with dementia risk and non-pathological cognitive aging via separate mechanisms (Deary et al, 2002). Together with the finding that rs405509 and rs440446, but not  $\epsilon$ 4, were associated with non-pathological cognitive aging (Rantalainen et al, 2016), the result suggests that the *APOE* locus may be linked with dementia risk via an isoform-dependent mechanism and with non-pathological cognitive aging via an isoform-independent mechanism in this cohort. However, further studies are needed to determine if a similar pattern of associations is found in other samples derived from one study population.

Our results replicate the expected association between *APOE*  $\epsilon$ 4 and a higher risk of any dementia and AD (Corder et al, 1993, Sun et al, 2015). These associations were independent of participant's sex, year of birth, highest attained lifetime occupation and level of education, diagnoses of stroke, cardiovascular disease or any mood disorder, and depressive symptoms, as well as the number of minor alleles in rs405509 or rs440446. However, the number of minor alleles in rs405509 or rs440446 was not associated with risk of dementia. This latter finding is in disagreement with some (Lambert et al, 1998; Myllykangas et al, 2002; Beyer et al, 2005) but not all (Jun et al, 2012)

previous studies. Our study is unable to confirm or refute the possibility that these discrepancies may be due to ethnicity (Lambert et al, 2002) or age of dementia onset (Beyer et al, 2005).

Strengths of this study include availability of data on dementia diagnoses from national registers in a well-characterized lifespan longitudinal cohort followed-up to 69-79-years-of-age. Our study has the major limitation of a small sample size and a small number of dementia or AD cases. Our findings should therefore be considered preliminary and await replication in larger samples with lifespan data on non-pathological cognitive aging and dementia. However, dementia prevalence (2.1%) in our sample is similar to Finnish nation-level estimates (2.4%) (Prince et al, 2015), and the relative contributions of *APOE*  $\epsilon$ 4, rs405509 and rs440446 to dementia risk are less likely to change as our cohort ages. As the effect of *APOE*  $\epsilon$ 4 on dementia risk diminishes after age 70 (Farrer et al, 1997), and as rs405509 and rs440446 are in linkage disequilibrium with  $\epsilon$ 4 (Jun et al, 2012; Rantalainen et al, 2016; Roses et al, 2010), the proportion of the rs405509 T and rs440446 G alleles, that are more common in  $\epsilon$ 4 carriers with dementia, will likely diminish according to age as well, and an increasing proportion of carriers of these alleles among participants who receive a dementia diagnosis will likely be  $\epsilon$ 4 non-carriers. As the cohort was born in 1930s and 1940s, generalizations to more recent cohorts cannot be made. This however remains an unavoidable limitation of studying aging-related diseases in today's elderly. Finally, residual confounding cannot be entirely excluded.

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**Table 1. Risk of any dementia according to *APOE*  $\epsilon$ 4 and rs405509 and rs440446 polymorphisms**

	Model 1 <sup>a</sup>		Model 2 <sup>b</sup>		Model 3 <sup>c</sup>		Model 4 <sup>d</sup>	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
<b><i>APOE</i> <math>\epsilon</math>4 and number of rs405509 minor T alleles and rs440446 minor C alleles in separate models</b>								
<i>APOE</i> $\epsilon$ 4	3.70 (1.75, 7.64)	.001	3.86 (1.84, 8.10)	<.001	3.68 (1.76, 7.70)	.001	3.83 (1.82, 8.04)	<.001
rs405509 T	1.40 (0.86, 2.29)	.182	1.41 (0.86, 2.31)	.172	1.41 (0.86, 2.32)	.176	1.43 (0.87, 2.36)	.158
rs440446 C	0.76 (0.41, 1.42)	.394	0.75 (0.40, 1.40)	.363	0.76 (0.41, 1.43)	.401	0.76 (0.40, 1.43)	.387
<b><i>APOE</i> <math>\epsilon</math>4 and number of rs405509 minor T alleles in the same model</b>								
<i>APOE</i> $\epsilon$ 4	3.77 (1.67, 8.52)	.001	4.06 (1.77, 9.30)	.001	3.83 (1.69, 8.71)	.001	3.96 (1.74, 9.05)	.001
rs405509 T	0.95 (0.54, 1.68)	.864	0.93 (0.53, 1.63)	.789	0.94 (0.53, 1.67)	.829	0.95 (0.54, 1.67)	.850
<b><i>APOE</i> <math>\epsilon</math>4 and number of rs440446 minor C alleles in the same model</b>								
<i>APOE</i> $\epsilon$ 4	3.14 (1.41, 6.96)	.005	3.29 (1.48, 7.31)	.003	3.14 (1.41, 6.97)	.005	3.30 (1.48, 7.36)	.004
rs440446 C	1.00 (0.51, 1.94)	.988	0.96 (0.49, 1.88)	.909	1.00 (0.51, 1.96)	1.000	0.99 (0.50, 1.96)	.980

Abbreviations: HR = hazard ratio; 95% CI=95% confidence interval.

a Model 1 adjusting for sex, year of birth and population stratification.

b Model 2 adjusting for sex, year of birth, maximum attained lifetime occupational status and population stratification.

c Model 3 adjusting for sex, year of birth, maximum attained lifetime educational status and population stratification.

d Model 4 adjusting for sex, year of birth, maximum attained lifetime occupational status, population stratification, diagnoses of stroke, coronary heart disease or any mood disorder, and BDI score.