Apolipoprotein E genotypes and longevity across dementia disorders

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Abstract

INTRODUCTION: The ε4 allele of the apolipoprotein E (*APOE*) gene is a prominent risk factor for Alzheimer's disease (AD), but its implication in other dementias is less well studied.

METHODS: We used a dataset on 2858 subjects (1098 AD, 260 vascular dementia [VaD], 145 mixed AD and VaD [MIX], 90 other dementia diagnoses, 1265 controls) to examine the association of *APOE* polymorphisms with clinical dementia diagnoses, biomarker profiles and longevity.

RESULTS: The ε4 allele was associated with reduced longevity as ε4 vs ε3 homozygotes lived on average 2.6 years shorter (p=.006). In AD, ε4 carriers lived 1.0 years shorter than non-carriers (p=.028). The ε4 allele was more prevalent in AD, MIX and VaD patients compared to controls, but not in other dementia disorders.

DISCUSSION: The *APOE* ε4 allele is influential in AD, but might also be of importance in MIX and VaD; diseases in which concomitant AD pathology is common.

1. **Introduction**

Apolipoprotein E (apoE) is a protein involved in lipid metabolism and transport of cholesterol to neurons [1, 2]. There are three common alleles of the *APOE* gene (ε2, ε3 and ε4) that encode three isoforms of the protein (E2, E3 and E4) [3]. Being an ε4 allele carrier is the most prominent genetic risk factor for late onset Alzheimer's disease (AD), and has a reported prevalence of 24% in the Nordic population [4, 5]. Being a homozygous ε4 carrier increases the risk of developing sporadic AD by a factor 10-20 compared to being an ε3 homozygote [6-8]. *APOE* ε4 has also been implicated in other settings including atherosclerosis [9], depression [10], accelerated progression in multiple sclerosis [11] and brain atrophy in HIV patients [12]. The ε2 allele may be a protective trait, lowering the risk of developing AD [13], as compared to ε3, the most common allele. *APOE* has also been targeted for examination in longevity studies, where the ε4 allele has been found associated with early mortality [14].

The association of the *APOE* ε2/ε3/ε4 polymorphism with dementias other than AD is less clear. In vascular dementia (VaD), studies on the role of the *APOE* polymorphism has yielded conflicting results, possibly due to lack of consensus on the criteria required for diagnosis and the high overlap of VaD with other dementia disorders, primarily AD [15]. In meta-analysis, an increased VaD risk has been identified in *APOE* ε4 carriers [16], a result that has been confirmed by population-based data [17]. Dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD) patients share clinical and biochemical features; however, the impact of *APOE* genotype in these conditions might diverge. Evidence suggests an association of increased risk of DLB with a *APOE* ε4 carrier status [18, 19]. By contrast, the ε2 allele, but not ε3 or ε4, has been associated with increased risk of Parkinson's disease [20], although associations between ε4 and PDD has been reported [21]. Studies of *APOE* in frontotemporal dementia (FTD) have again produced conflicting results, but, in meta-analysis, carriage of the

ε4 allele was found to be associated with a slightly increased risk of disease [22]. In amyotrophic lateral sclerosis, another neurodegenerative disease which commonly overlaps with FTD, evidence suggests that ε4 carriage is associated with earlier onset and faster disease progression [23, 24].

In this study, we explored the prevalence of the *APOE* genotypes across a wide array of dementia disorders in a large dataset drawn from the Swedish dementia registry (Svedem) and the Swedish mortality registry. We hypothesized that (i) *APOE* ε4 would be most prevalent, and ε2 least prevalent in AD, (ii) the ε4 allele would be associated with AD-like CSF biomarker profiles in AD and in other neurodegenerative diseases, and (iii) the ε4 allele would be associated with shorter survival.

2. Methods

2.1 Subject cohort

Four sources of information were joined to prepare the dataset used in this study.

The first source was of a complete set of archived data on all clinical practice *APOE* genotype analyses and CSF Aβ42, T-tau and P-tau measurements made at the Sahlgrenska University Hospital, Sweden, from January 2002 to June 2012 extracted from the laboratory database.

The second source of information was the Swedish mortality registry, a national resource maintained by the Swedish National Board of Health and Welfare, keeping complete records on all deaths in Sweden, including causes of death as established by the physician issuing the death certificate. This database was queried for underlying cause (i.e. diagnosis codes) and date of death for the patients in our dataset, and information on 1166 records was retrieved.

The third data source was Svedem (the Swedish dementia registry) an initiative started in 2007, with an aim to improve the quality of diagnostics, treatment and care in dementia by collecting clinical information on dementia patients in Sweden [25, 26]. From this data source information on 557 patients was fetched including clinical diagnosis coded as one of nine preset options: early onset AD (EAD, < 65 years of age), late onset AD (LAD, > 65 years of age), FTD, dementia with Lewy bodies (DLB), Parkinson's with dementia (PDD), VaD, mixed AD and vascular dementia (MIX), dementia not otherwise specified (dementia NOS) and a group for the collected remainders of named dementia diagnoses called "other dementias", including corticobasal syndrome, alcohol-related dementias, and other rare diagnoses. Reporting clinicians are instructed to follow diagnostic guidelines as specified in ICD-10 to secure a unified basis for diagnosis [27]. For the purposes of this study, and since we lacked diagnosis date for the patients drawn from the mortality registry, the EAD and LAD groups were merged into a joint AD group, and the NOS and "other dementias" groups were stripped out of the dataset. The study cohort was further sub-classified into biochemically positive or negative profile according to the international working group (IWG-2) [28]. The following cutoffs for pathological biomarker concentrations were used: Aβ42 $\leq$ 550 pg/mL, T-tau $\geq$ 400 pg/mL, P-tau > 60 pg/mL for patients < 60 years, and P-tau > 80 for patients $\geq$ 60 years [29]. Pathological concentrations of Aβ42 and at least either T-tau or P-tau were required for a biochemical AD positive classification.

These data sources were cross-referenced using the Swedish personal identity number. Note that there was an overlap between the Svedem and the mortality registry records, where both sources were combined to retrieve data for a subset of the study cohort. Also note that date of death only was available for patients taken from the mortality registry.

The fourth source of information was a set of 1265 healthy control subjects from a previous study by Zetterberg et al. [30]. The controls were all western Swedish residents and the majority (n = 980) were selected from population-based cohort studies, while 285 were recruited by advertisements in newspapers and at senior citizen meetings.

2.2 Biochemical measurements

CSF T-tau and P-tau concentrations were measured using enzyme-linked immunosorbent assays (ELISAs) (INNOTEST hTau Ag and Phospho-tau [181P]; Fujirebio, Ghent, Belgium) as previously described [31, 32]. CSF Aβ42 concentration was measured using a sandwich ELISA (INNOTEST β-amyloid[1-42]), specifically constructed to measure Aβ containing both the 1st and 42nd amino acids, as previously described [33]. The between-assay coefficients of variation (CV) for the T-tau and P-tau tests were 10.35 % and 10.19 % respectively (as determined by internal control samples during the entire study period).

All CSF analyses were performed in clinical routine by board-certified laboratory technicians using procedures accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC). Longitudinal stability in the measurements was ascertained using an elaborate system of internal quality control samples and testing of incoming reagents, and further verified in the Alzheimer's Association CSF Quality Control Program [34].

2.3 APOE genotyping

APOE (gene map locus 19q13.2) genotyping was performed in blood by minisequencing or by TaqMan Single Nucleotide Polymorphism (SNP) Genotyping. Genotypes were obtained for the two SNPs, which are used to unambiguously define ε2, ε3, and ε4 alleles (rs7412 and rs429358).

2.4 Statistics

Age distributions in subject groups were tested with ANOVA, and sex distribution by χ2 statistics. Allele frequency and distributions in diagnosis groups and biochemical AD subgroups were analyzed by χ2-statistics and group differences by Kruskal-Wallis. Differences in life length between *APOE* genotype groups and between gene carriers and non-carriers were analyzed by ANOVA, as was analysis of differences in age of diagnosis in AD.

2.5 Ethics

The study was approved by the regional ethical committee at the University of Gothenburg.

3. Results

3.1 Dataset description

Demographics of study subjects are detailed in Table 1. The FTD subjects were significantly ($p < .001$) younger than the AD, MIX and VaD subjects. The VaD subjects were older ($p < .05$) than the AD, FTD, CJD and PDD subjects. Gender had no influence on any of the associations reported below.

3.2 APOE allele frequencies across diagnoses

Table 2 shows allele frequencies across diagnoses. The AD subjects had a higher ε4 allele frequency than the FTD ($p<.001$), VaD ($p<.001$) and healthy control ($p<.001$) groups. The MIX groups had a higher ε4 allele frequency than the FTD ($p<.002$), VaD ($p<.001$) and the healthy control groups ($p<.001$). The VaD group had a higher ε4 allele frequency than the healthy controls ($p<.001$).

The AD subjects had a lower ε3 frequency than the VaD (p<.001) and FTD (p=.012) subjects and the healthy controls (p<.001). The VaD subjects had a higher ε3 frequency than the MIX (p=.002) and the healthy controls (p=.003). The MIX subjects had a higher ε3 frequency than the FTD group (p=.031).

The ε2 allele frequency was lower in the AD and MIX groups than in the healthy controls (p<.001, p=.03).

3.3 APOE ε2/ε3/ε4 allele carriers across diagnoses

The AD group had a lower ε2 carrier proportion compared to the healthy controls (p<.05) (figure 1A). The ε3 carrier proportion was lower in the AD and MIX groups than in the healthy controls and VaD groups (p<.05) (figure 1B). The ε4 carrier proportion was higher in the AD and MIX groups than in the FTD, VaD and the healthy controls (p<.05), and also in VaD compared to the healthy controls (p<.05) (figure 1C). There were no other significant differences in ε2, ε3 and ε4 carrier proportions between subject groups.

3.4 APOE in the biochemically AD-like

Disregarding clinical diagnosis the ε4 allele frequency was higher (p<.001), and both the ε2 and ε3 allele frequency was lower (p<.001, p<.001) in the biochemical AD positive subjects (Figure 2A).

Figure 2 B shows the distribution of *APOE* alleles in the AD group, sub-classified into biochemical AD positive and negative subjects. The ε4 allele frequency was higher in the biochemical AD positive group than in the biochemical AD negative group (p<.001), and the ε3 allele frequency was lower (p<.001) but the ε2 allele frequency did not significantly differ (p=.262).

3.5 APOE and life length

In the full study cohort, excluding the healthy controls, the ε4 homozygous subjects had significantly shorter life length than the ε3 homozygous subjects (mean diff=2.1 years, p=.026). There were no other significant differences in life span between *APOE* genotype groups in the full study cohort.

When comparing life length within the individual diagnostic groups, the ε4 homozygous AD subjects had shorter life length those who were ε2/ε4 (mean diff=4.5 years, p=.048), ε3/ε3 (mean diff=2.6 years, p=.006) and ε3/ε4 (mean diff=2.3 years, p=.014) genotypes. There were no statistically significant differences in the other diagnostic groups.

The ε4 carriers in the AD group had significantly (mean diff=1.0 years, p=.028) shorter life length than the ε4 non-carriers. There were no other significant differences in mean life length between ε4 carriers and non-carriers in any of the diagnosis groups. In this analysis ε2 carriers were excluded, since ε2 might serve as a protective trait.

3.6 Age at diagnosis in AD

In the subgroup of patients with AD pathology (AD + MIX) where dates of diagnosis were available (n=434), patients who were ε4 homozygous were younger at the date of diagnosis than the ε3 homozygous patients (mean diff=3.5 years, p=.01), and the patients with an ε3ε4 configuration (mean diff=3.77 years, p=.001).

4. Discussion

We compared the *APOE* ε2/ε3/ε4 genotype frequencies across a wide array of dementia

disorders in a large cohort drawn from Swedish health registries. We found that the *APOE* ε4 allele was most common in patients with AD, and that the ε4 allele was associated with a shorter lifespan and a more distinguished AD-like biomarker pattern in AD.

Being an *APOE* ε4 carrier is a well-established and major genetic risk factor for AD[3, 8], and this was further corroborated in this study as the ε4 allele frequency was higher in the AD and MIX subjects than in any other group. The frequency of ε4 homozygous subjects and the proportion of ε4 carriers vs. non-carriers were also highest in AD with the MIX group a close second. Furthermore, the average lifespan of both ε4 carriers and homozygous ε4 subjects in AD was significantly shorter than non-carriers'. Previous studies have found evidence of the ε2 allele being associated with a longer lifespan [35], and that the ε4 allele is linked to a shorter life expectancy [36]; however, although the ε4 allele's link to poor survival in AD might be indirectly implied, it has not been thoroughly investigated [37]. The shorter lifespans of the ε4 carriers might, at least in part, be attributed to an earlier onset of disease, as we found ε4 homozygotes to be younger than ε3 homozygotes and patients with an ε3ε4 configuration at the date of diagnosis, corroborating previous studies [1, 38]. In fact, some studies have indicated that *APOE* might be a timing gene, rather than merely a risk gene, by showing that although disease onset is brought forward by being an ε4 carrier, the lifetime susceptibility of the disease might be unaffected [39]. We also found that the ε4 allele was associated with a biochemical AD-like profile, corroborating previous studies [40, 41].

In the other diagnostic groups, we found that the VaD subjects had a significantly higher ε4 allele frequency than the healthy controls, as previously shown in the literature [42]. However, these results should be interpreted with caution as AD and VaD are clinically hard to distinguish and concomitant AD pathology is common in VaD [43], as was further corroborated by the correlations between an biochemical AD positive biomarker profile and

the ε4 allele in this study [41]. The FTD subjects, also keeping with previous studies [44], closely resembled the healthy controls in *APOE* allele and genotype frequencies. The DLB and PDD groups were small, which might explain why, although the ε4 allele frequency was found to be higher in these groups compared to the healthy controls, this failed to reach statistical significance. These results were, however, in keeping with previous studies, as the ε4 allele has previously been found to be associated with a greater risk of developing DLB and PDD. The presence of ε4 has also been related to a greater amount of senile plaques and neurofibrillary tangles in these diseases, suggesting that this may be driven by concomitant AD, a promoting effect of α-synuclein on β-amyloid accumulation, or the other way around i.e. a promoting effect of β-amyloid on α-synuclein accumulation [21, 45].

We studied only a small number of patients with CJD; whilst these individuals had had a lower ε2 and higher ε4 allele frequency than the healthy controls, no significant differences between them and the other groups were found, likely reflecting the small sample size. These results are, however, keeping with previous studies. In meta-analysis an association between the ε4 allele and CJD has been shown [46], and synergistic effects between APOE ε4 and the PRNP gene, the most prominent risk factor of CJD, has been suggested in both AD and CJD [47].

The main strength of this study was the large study population size and the diversity of dementia disorders represented in it, but there are also a number of limitations to this study. The main one was that most *APOE* genotypes analyzed in this study were determined in clinical routine, introducing a risk of circular reasoning as the results of the genotyping might have influenced the physician's diagnostic formulation. Another limitation is that the majority of patients were diagnosed only clinically, which is known to introduce a high misdiagnosis rate, with low specificity figures (44-71%) for AD, and a large proportion (39%) of clinically

diagnosed FTD, LBD and VaD patients showing AD pathology at autopsy [48]. On the other hand, *APOE* genotype is not included in any of the diagnostic criteria for AD or any of the other dementia disorders and there are no guidelines on how to interpret *APOE* genotype results in clinical practice. Some of the diagnostic information in this study was taken from the death certificates of the included subjects, and some from Svedem. The diagnostic information taken from the death certificates might be more unreliable as the issuing physician is typically not an experienced neurologist or expert in dementia, in contrast to the physicians who report into Svedem, all of whom are specially trained in the evaluation of dementias. In the absence of autopsy confirmation, the clinical diagnosis may be less accurate in a proportion of cases. Finally, the lack of detailed genetic information on our study subjects preclude GWAS style analysis of other potential genes and SNPs that might influence the impact of the ApoE genotype in the diseases covered in this study.

5. Conclusions

The *APOE* ε4 allele frequency and ε4 carrier proportion was significantly higher in AD than in healthy controls and the ε4 allele was associated with a shorter lifespan and a more AD-like CSF biomarker pattern in AD. The *APOE* ε4 allele frequency was also higher in MIX and VaD as compared to the healthy controls. The results of this study highlight the significance *APOE* genotyping, and confirm the relationship between *APOE* ε4 and AD. Our finding that *APOE* ε4 genotype influences not only the CSF profile, but also life-span, may have clinical application not only in AD, but also in mixed dementia and VaD.

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Figure legends

Figure 1. Allele carrier proportions

Proportions of allele carriers vs. non-carriers in the study cohort groups. Diagnosis groups are represented on the x-axis, and percentages on the y-axis.

A. ε2. *Proportions of ε2 carriers.*

B. ε3. *Proportions of ε3 carriers.*

C. ε4. *Proportions of ε4 carriers.*

Figure 2. *APOE* genotypes

Proportions of APOE genotypes in biochemical AD positives vs. biochemical AD negatives. Genotypes are represented on the x-axis, and percentages on the y-axis.

A. Full study cohort. *Proportions in the full study cohort.*

B. AD group. *Proportions in the collected AD group (LAD+EAD+MIX).*

Table 1. Demographics of study cohort

Diagnosis		HC	AD	MIX	VaD	FTD	DLB	PDD	CJD
Sex	**F (n)**	792	654	79	107	23	3	6	5
	M (n)	473	444	66	153	26	10	15	2
Age at sampling	**n**	1265	1098	145	260	49	13	21	7
	Mean (SD)	73 (6)	72 (8)	75 (6)	76 (7)	64 (9)	70 (8)	69 (6)	66 (12)
	Mdn (IQR)		73 (67-78)	75 (71-79)	76 (71-81)	64 (58-72)	72 (64-76)	69 (67-74)	63 (61-78)
Aβ42 [ng/L]	**n**	160	1077	145	254	48	13	21	6
	Mean (SD)	712 (189)	404 (164)	436 (179)	521 (221)	624 (240)	589 (277)	587 (207)	402 (198)
	Mdn (IQR)		380 (290-485)	400 (330-490)	480 (360-620)	577 (500-750)	570 (430-800)	619 (410-687)	355 (260-573)
T-tau [ng/L]	**n**	160	1017	144	246	48	13	21	7
	Mean (SD)	351 (155)	744 (397)	639 (322)	475 (277)	423 (188)	345 (201)	370 (185)	2844 (1460)
	Mdn (IQR)		697 (485-900)	590 (410-820)	410 (280-596)	389 (290-568)	340 (220-390)	340 (250-394)	3810 (1280-4040)
P-tau [ng/L]	**n**	130	618	131	163	37	13	18	5
	Mean (SD)	62 (18)	95 (40)	83 (33)	59 (29)	55 (15)	51 (24)	53 (21)	49 (25)
	Mdn (IQR)		89 (67-114)	80 (57-104)	53 (42-66)	54 (43-66)	48 (35-60)	52 (37-60)	36 (34-68)

Table 2. Allele frequencies

Alleles	ε2	ε3	ε4	χ² p-value	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	χ² p-value
HC	188 (7.4%)	1956 (77.3%)	386 (15.3%)	n.a.	10 (0.8%)	140 (11.1%)	28 (2.2%)	760 (60.1%)	296 (23.4%)	31 (2.5%)	n.a.
AD	60 (2.7%)	1195 (54.4%)	941 (42.9%)	***	0 (0.0%)	30 (2.7%)	30 (2.7%)	335 (30.5%)	495 (45.1%)	208 (18.9%)	***
MIX	8 (2.8%)	158 (54.5%)	124 (42.8%)	***	0 (0.0%)	6 (4.1%)	2 (1.4%)	38 (26.2%)	76 (52.4%)	23 (15.9%)	***
VaD	78 (5.6%)	1173 (68.5%)	549 (26.0%)	***	3 (1.2%)	20 (7.7%)	3 (1.2%)	122 (46.9%)	92 (35.4%)	20 (7.7%)	***
FTD	7 (7.1%)	71 (72.4%)	20 (20.4%)		0 (0.0%)	4 (8.2%)	3 (6.1%)	26 (53.1%)	15 (30.6%)	1 (2.0%)	
DLB	2 (7.7%)	17 (65.4%)	7 (26.9%)		0 (0.0%)	1 (7.7%)	1 (7.7%)	6 (46.2%)	4 (30.8%)	1 (7.7%)	
PDD	3 (7.1%)	28 (66.7%)	11 (26.2%)		0 (0.0%)	3 (14.3%)	0 (0.0%)	8 (38.1%)	9 (42.9%)	1 (4.8%)	
CJD	0 (0.0%)	11 (78.6%)	3 (21.4%)		0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (57.1%)	3 (42.9%)	0 (0.0%)	

*** p < .001, ** p < .01, * p < .05

P-values were derived from the comparison of each patient group and controls.