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ORIGINAL COMMUNICATION

Genetic variation in apolipoprotein D and Alzheimer's disease

Seppo Helisalmi
Mikko Hiltunen
Saila Vepsäläinen
Susan Iivonen
Elizabeth H. Corder
Maarit Lehtovirta
Arto Mannermaa
Anne Maria Koivisto
Hilkka Soininen

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S. Helisalmi · M. Hiltunen · S. Vepsäläinen · S. Iivonen · M. Lehtovirta · A. M. Koivisto · H. Soininen
Dept. of Neuroscience & Neurology
University Hospital
and University of Kuopio
Kuopio, Finland

S. Helisalmi · M. Hiltunen · S. Vepsäläinen · S. Iivonen · A. M. Koivisto · H. Soininen
Brain Research Unit
Clinical Research Centre/Mediteknia
University of Kuopio
Kuopio, Finland

S. Helisalmi, PhD (✉)
Dept. of Neuroscience & Neurology
Brain Research Unit
Clinical Research Centre/Mediteknia
Kuopio University
Harjulantie 1
70211 Kuopio, Finland
Tel.: +358-17/162259
Fax: +358-17/163539
E-Mail: seppo.helisalmi@uku.fi

E. H. Corder
Center for Demographic Studies
Duke University
Durham (NC), USA

M. Lehtovirta
Dept. of Neurology
Jorvi Hospital
Espoo, Finland

A. Mannermaa
Dept. of Pathology & Forensic Medicine
University Hospital & University of Kuopio
Kuopio, Finland

Abstract Apolipoprotein D (apoD) is a lipoprotein-associated glycoprotein, structurally unrelated to apoE, that transports small hydrophobic ligands including cholesterol and sterols. Levels are increased in the hippocampus and CSF of Alzheimer's disease (AD) patients. We tested whether variation in the APOD gene affects AD risk. Four single nucleotide polymorphisms (SNPs) were investigated (in map order): exon 2, 15T→C encodes an amino acid substitution Phe→Ser at codon 15; intron 2, –352G→A; intron 3, +45C→T; intron 4, +718C→T, determined by SNaPshot assay. SNP frequencies for 394 eastern Finnish AD patients were compared with those found for 470 control subjects, dividing subjects also into early-onset AD (EOAD; ≤ 65 years) and late-onset AD (LOAD; > 65 years) groups. The –352G allele was associated with a significant 3-fold increase in the risk of EOAD (OR: 2.7; 95 % CI: 1.1–6.5). The –352G containing haplotypes were more common for EOAD cases (TGCC: 0.48 vs 0.41; TGCT: 0.08 vs 0.01 ($p = 0.002$). In the Grade-of-membership analysis, APOD genotype frequencies at each SNP site and disease status were used to construct two latent groups: the affected group carried –352 as GG or GA and +45 CC, was often women and enriched in APOE ε4. Each method suggested that the –352G allele frequency is higher for EOAD in the eastern Finnish population.

Key words allelic association · Alzheimer's disease · apolipoprotein D · grade-of-membership analysis · linkage disequilibrium

Introduction

The etiology of Alzheimer's disease (AD) remains unclear. Multiple susceptibility genes such as APOE and environmental risk factors are implicated. This study investigates apolipoprotein D (APOD, gene; apoD, protein; MIM 107740) as a risk factor for AD for the eastern Finnish population. APOD is localized to the p26.2-qter region on chromosome 3, encodes a single polypeptide chain (169-amino acids) of ~30 kDa [5], and is composed of five exons spanning 20 kbp with a coding transcript ~850 bp long [7, 17].

ApoD is a component of high-density plasma lipoproteins structurally unrelated to other apolipoproteins, e. g. apoE. It exhibits sequence homology with the

lipocalins, a superfamily of proteins involved in the transport of small hydrophobic ligands e. g. cholesterol, bilirubin, pregnenolone, progesterone and arachidonic acid implying multiple tissue-specific functions [7, 9]. Little APOD is expressed in the liver and intestine, major sites of synthesis of other lipoproteins, whereas high expression is found in many other tissues, e. g. brain, spleen, testes, kidney, placenta and pancreas.

The high level of apoD synthesis in brain, as well as in regenerating and remyelinating peripheral nerves, suggests a role in lipid transport and neuronal remodeling in the CNS. Increased expression in stressed cortical neurons of sporadic late-onset AD patients further implicates apoD in AD pathogenesis [1]. A more general role in neuropsychiatric disorders is consistent with protein accumulation at sites of regenerating peripheral nerves and in the cerebrospinal fluid of patients with stroke, meningoencephalitis, motor neuron disease [21], Niemann-Pick [20] and schizophrenia [22].

APOD polymorphisms have been associated with 1) obesity and hyperinsulinemia [25], 2) cardiovascular risk for African blacks, specifically, women [5], making an indirect association with AD via diabetes (a risk factor for AD) [16] and demonstrating a gender-specific outcome. More relevant to this study, variation in intron 1 was associated with an increased AD risk among APOE ε4 carriers [6]. Moreover, genome-wide linkage-disequilibrium (LD) mapping with samples from eastern Finland [10] localized an AD susceptibility locus to the APOD region.

To evaluate the hypothesis that allelic variations in APOD modify risk for AD, we genotyped four single nucleotide polymorphisms (SNPs) within the gene and compared frequencies found for AD patients and unaffected control subjects. We also used Grade-of-Membership (GoM) analysis to identify two groups differing in SNP frequencies and AD status.

Materials and methods

Case-control samples

The study subjects were ascertained as part of a project on risk genes for AD in the eastern Finnish population. They were examined in the Department of Neurology, Kuopio University Hospital [10, 12]. The study was approved by the hospital ethical committee and the use of tissue by the Office of Legal Health Care Affairs. The 394 cases were divided into two groups according to age of onset: early-onset AD (EOAD; onset age ≤ 65) and late-onset AD (LOAD; onset age > 65). AD groups were compared with control subjects dividing at age 65 (Table 1).

Thirty-six percent of AD cases had a positive familial history of AD, but insufficient evidence for autosomal dominant transmission and were unrelated to each other. The disease was considered to be familial if at least two first-degree relatives with dementia in two generations were documented [12]. All case subjects underwent a comprehensive clinical evaluation during which the clinical diagnosis of probable AD was made according to NINCDS-ADRDA [14]. Nine percent (n = 34) of cases were confirmed as definite and one percent (n = 4) as probable AD at autopsy according to the neuropathological CERAD criteria [15]. This study included 63 EOAD cases (16 % of all AD cases) and they were screened not to carry known mutations in APP, PSEN-1 or PSEN-2 genes. However, it is still possible that some of these patients may carry variants in these genes. Control subjects had no signs of dementia by interview and neuropsychological testing. Each was screened for cognitive decline (Mini Mental State Examination score ≥ 25).

DNA isolation and SNP detection

Genomic DNA was extracted from white blood cells [24]. The genomic sequence of APOD was obtained by alignment of the cDNA sequence gi:4502162; (NM_001647) to the genomic sequence from clone gi:22042603 (NT_033016) using the BLAST program (http://www.ncbi.nlm.nih.gov/entrez). Several published SNPs in APOD were randomly selected for screening from the clone gi:22042603; the exact locations in the genomic sequence were validated through use of a direct sequencing (Applied Biosystems).

SNPs having a minor allele frequency < 10 % were not included, with the exception of a SNP encoding an amino acid. Fig. 1 shows the location and orientation of four selected SNPs (the NCBI SNP cluster ID, rs-number): exon 2, 15T→C (rs5952) encoding an amino acid sub-

Table 1 The study subjects

	All subjects		≤ 65 years		> 65 years	
Attribute	AD (n = 394)	Controls (n = 470)	EOAD (n = 63)	Controls (n = 112)	LOAD (n = 331)	Controls (n = 358)
Age in years, mean ± SD	70 ± 5	71 ± 7	60 ± 5	63 ± 1	74 ± 5	72 ± 4
% female (n)	70 (276)	60 (284)	75 (47)	50 (55)	69 (229)	64 (229)
% APOE ε2; ε3; ε4	2; 52; 46	4; 80; 16	2; 38; 60	3; 79; 18	2; 54; 44	5; 80; 15

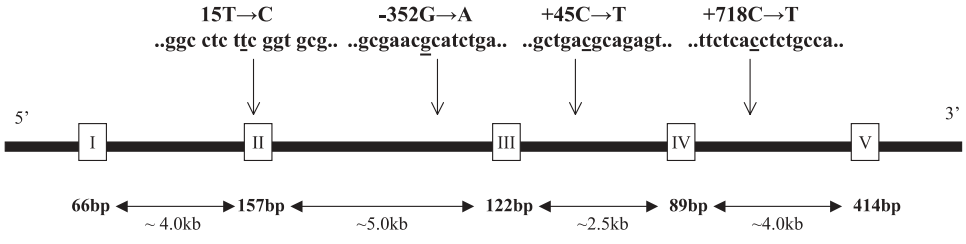

Fig. 1 Schematic for the APOD gene showing intron-exon structure and SNP positions. Below each SNP is a part of gene sequence; underlined bases are polymorphic. The boxes with numbers indicate exons; sizes for exons and introns are indicated beneath

stitution (Phe→Ser at codon 15); intron 2, –352G→A (rs1568565); intron 3, +45C→T (rs1568566) and intron 4, +718C→T (rs1467282).

Genotyping

Assays for the selected SNPs were performed in two multiplex PCR, subsequently mixed to perform a single SNaPshot reaction. Amplification assays were designed for each SNP with the following forward and reverse primers: 15T→C, 5'-CTCCAGGTCCCTTCTCCAG-3', 5'-GGCACTTCCCAAGATGAAAT-3'; –352G→A, 5'-ATCCCTCCTCAGGGTCAGAT-3', 5'-TTCATCTGAAACAGTGCACAA-3'; +45C→T, 5'-CATCCAGGCCAACTACTCACT-3', 5'-CTGCTAGGAGCAGGCAGTC-3'; +718C→T, 5'-CCCAGAACTGTGAGGCTTTC-3', 5'-TAGGCACTTGACTGGTGTGG-3'.

A product of each PCR-amplification was used as a template in an ABI PRISM® SNaPshot™ Multiplex assay (Applied Biosystems). The following specific primers were used: 15T→C, 5'-C_{24}GCACTGGCTGGCCTCT-3'; –352G→A, 5'-T_{25}CTAGCTCCCCACA GCGAAC-3'; +45C→T, 5'T_{49}CAGGCCTGCGGAGTGCTGA-3' and +718C→T, 5'-T_{32}CCTGCACTTCCT ACTTCTCA-3'. Finally, samples were analysed and allele peak determination was done by use of ABI 3100 Genetic Analyzer and Genotyper 3.7 program (Applied Biosystems).

Apolipoprotein E (APOE) genotyping was determined by using a standard method [23].

Statistical methods

Allele and genotype frequencies were compared for case and control subjects (SPSS version 10.0). The level of statistical significance was set at $p = 0.05$. Haplotype frequencies were estimated from genotype data using the expectation-maximization algorithm assuming Hardy-Weinberg equilibrium (HWE) [8]. When tested, genotype frequencies did not violate this assumption. Haplotype frequencies were compared for case and control subjects using the RxC-program employing the metropolis algorithm to obtain unbiased estimates for exact p-values and corresponding standard errors [2, 18].

Grade-of-membership analysis (GoM) was developed for studies of complex biological systems [13, 27]. Information for individuals is condensed into a limited number of latent groups each defined by frequencies for the variables. Individuals resemble one or more groups either entirely or in part, i. e. the groups are fuzzy rather than crisp. Typically, the subscript i is used to index the I subjects, j indexes the J variables, and k indexes the K latent groups. Here, $I = 155$ (persons under the age of 65 years, 56 EOAD cases and 99 unaffected controls), $J = 5$ variables (the four SNPs plus disease status), $K = 2$, i. e. two latent groups were requested. Each variable has a specific number of possible responses L, indexed by l. Using this notation, each of the $K = 2$ latent groups is described by response frequencies for the variables, i. e. profiles of probabilities (λ_{jlk}).

This approach resembles the clinical setting where the patient is examined and given a diagnosis, possibly with some uncertainty or with ambiguous presentation. Multiple comparison problems that arise when comparing genotypic frequencies for case and control subjects for each of many SNPs are avoided. Haplotyping cannot identify all combinations of genotypes related to AD risk such as gene dose effects, important only on certain high-risk ancestral haplotypes or for subgroups, e. g. based on gender, at typical sample sizes.

In this study, each individual contributed information on the genotype found at each SNP site and disease status information to identify two latent groups (*Grade of Membership Software*, Center for Demographic Studies, Duke University, Durham, NC (1987)). Information on gender and APOE genotype was not used to define the two groups. Instead, the model was asked to generate gender and APOE frequencies for each of the $K = 2$ GoM groups.

Results

APOE distributions

The distributions of APOE ε2/ε3/ε4 alleles expectedly differed for the 394 AD cases compared with the 470 control subjects ($p < 0.001$, Table 1): The cases were nearly 5-fold more likely to carry one or two ε4 alleles (OR = 4.7; 95 % CI 3.8 to 5.9). Age at onset was approximately three years earlier for ε4+ vs. ε4– cases (71 vs. 74 years, $p < 0.001$).

APOD comparisons

We evaluated the possibility that the coding 15T→C substitution of Phe→Ser at codon 15 was a risk factor for AD. Frequencies were 3 % for cases (n = 11) and controls (n = 14) providing no support for this hypothesis (Table 2).

Next, associations with AD were evaluated for the three non-coding intronic SNPs: There was no statistically significant evidence that they were related to AD, or to LOAD when older subjects were separately investigated (Table 2).

EOAD and intronic SNPs

EOAD was associated with the –352G→A SNP (Table 2). The 63 EOAD cases and 112 controls had differing genotypic distributions for G/G, G/A, and A/A: 29 %, 56 %, 16 % and 22 %, 43 %, 35 %, respectively ($p = 0.02$). Thus cases were more likely to carry G/G or G/A (85 % vs 65 %) and less likely to carry A/A (16 % vs 35 %), an almost 3-fold elevation in risk associated with the G allele. Age, sex and APOE adjusted OR for the –352G allele carriers versus non-carriers was 2.7 (95 % CI: 1.1–6.5). Risk for EOAD women when separately evaluated was modestly higher (OR = 3.6; 95 % CI 1.2 to 10.9; $p = 0.03$). Younger and older control subjects had essentially the same distributions of –352 genotypes.

Haplotype analysis

The finding is that a particular combination of SNPs near –352 helps to define the ancestral haplotype associated with disease-related variability in APOD. Four common combinations of alleles, i. e. haplotypes, were identified for EOAD cases and their control subjects (Map order: +15, –352, +45, +718): TGCC, TACC, TATC and TGCT in descending order of frequency (Table 3). All four carried +15T; none of the common haplotypes had the rare +15C variant. Thus +15 was not informative. Both haplotypes containing –352G (TGCC and

Table 2 SNP genotypic frequencies for AD cases and controls

SNP	Genotype	Controls[a]	AD	Controls[a]	AD	Controls[a]	AD
		All (n = 470)	All (n = 394)	> 65 years (n = 358)	> 65 years (n = 331)	≤ 65 years (n = 112)	≤ 65 years (n = 63)
+15T→C	T/T	0.97	0.97	0.97	0.97	0.97	0.98
	T/C	0.03	0.03	0.03	0.03	0.03	0.02
	C/C	0	0	0	0	0	0
–352G→A[b]	GG	0.23	0.20	0.23	0.19	*0.22*	*0.29*
	GA	0.45	0.49	0.46	0.48	*0.43*	*0.56*
	AA	0.32	0.31	0.31	0.33	*0.35*	*0.16*
+45C→T	CC	0.63	0.60	0.63	0.59	0.61	0.70
	CT	0.32	0.35	0.31	0.36	0.35	0.27
	TT	0.05	0.05	0.06	0.05	0.04	0.03
+718C→T	CC	0.82	0.81	0.80	0.81	0.86	0.79
	CT	0.17	0.18	0.18	0.18	0.13	0.19
	TT	0.01	0.01	0.01	0.01	0.01	0.02

[a] Genotype frequencies were not statistically different between the three control groups

[b] Cases ≤ 65 years and their control subjects (in italics) had different genotypic distributions –352 (Fisher's Exact test, Monte Carlo Sig., 2-sided; $\chi^2 = 7.418$, $p = 0.025$). Using the RxC program (50,000 total replicates to estimate a p-value and its SE), they were also significantly different: $p = 0.021 \pm 0.002$. Allelic ratios (G:A) were 0.56:0.44 for cases and 44:56 for controls. No significant differences in genotype frequencies were observed in any study groups for SNPs +15, +45 and +718

Table 3 APOD four-locus haplotype frequencies for EOAD cases and control subjects

Haplotype[a]	Haplotype frequency[b]	
	Controls	EOAD
TGCC	0.41	0.48
TACC	0.33	0.26
TATC	0.18	0.14
TGCT	0.01	0.08
Other	0.07	0.04

[a] Frequencies for the four major APOD haplotypes are shown. Underlined bases differ from the most frequent TGCC haplotype. Map order for the SNPs is +15; –352; +45; +718 (Fig. 1).

[b] Haplotype frequencies for cases (n = 126 alleles) vs controls (n = 224 alleles) were compared using the RxC program (50,000 total replicates to estimate a p-value and its SE). They were significantly different ($p = 0.006 \pm 0.002$). The TGCT genotype was significantly more common for cases ($p = 0.002 \pm 0.001$)

TGCT) had +45C and differed for +718, either C (common) or T (infrequent). Thus –352G was essentially always found with +45C. However, +45C was not always found with –352G, i. e. TACC.

The haplotype frequencies differed for EOAD case and control subjects (Table 3). Both of the –352G containing haplotypes were more common for case compared to control subjects (TGCC: 0.48 vs 0.41; TGCT: 0.08 vs 0.01). The 8-fold overrepresentation of TGCT for cases was significantly over-represented among cases compared with the other three haplotypes.

No significant difference in haplotype frequencies was found between all AD cases and controls or LOAD cases separately and their controls (data not shown).

GoM approach

To further define the association of SNP –352G with EOAD, information on SNP genotype and disease status was used to construct two GoM groups described in Table 4 that are labeled I and II. The latent groups differed in that Group I had a 100 % probability of being an 'EOAD' case, i. e. subjects matching the latent group if all respects were affected, while Group II was the 'at-risk' unaffected. The genetic profiles for affected latent Group I and unaffected latent Group II demonstrated major differences in genotypic frequencies for SNPs –352 and +45: Affected Group I carried one (42 %) or two (58 %) copies of –352G, i. e. there was a 58 % chance for 'EOAD' cases to carry the G/G genotype and otherwise carried G/A. Unaffected Group II did not carry G/G, although G/A was consistent with being unaffected at age 65 (controls ranged up to age 65). Thus the GoM analysis associated EOAD with –352G. The GoM approach more definitively indicated that genotype –352 G/G conferred risk.

One feature identified in the GoM analysis not revealed by the haplotype analysis is that affected subjects whether –352 G/G or G/A carried +45 C/C (not C/T or T/T): The affected Group I carried four-locus genotype was T/*, G/*, C/C, */* while at-risk Group II carried T/T, */A, */*, C/*. Note that the Group II four-locus genotype was consistent with the high-risk Group I profile: This may imply that not all persons with the high-risk set of genotypes become affected by age 65. The GoM approach placed the infrequent +15T/C genotype with group I, not group II. Both latent groups had rather similar genotypic distributions for +718, although C/T and T/T were more consistent with case status.

Table 4 The two GoM groups defined by SNP and EOAD status frequencies

SNP frequencies and EOAD status

			SNP in the APOD gene											
			+15T→C			−352G→A			+45C→T			+718C→T		
Group	Case	Control	TT	TC	CC	GG	GA	AA	CC	CT	TT	CC	CT	TT
I: 'EOAD'	100	0	93	7	0	58	42	0	100	0	0	73	23	3
II: 'At-risk'	0	100	100	0	0	0	52	49	43	52	5	87	13	0

Gender and APOE genotype

	Gender		APOE genotype				
Group	Women	Men	ε2/3	ε3/3	ε2/4	ε3/4	ε4/4
I: 'EOAD'	74	26	2	18	2	44	34
II: 'At-risk'	51	49	4	62	2	27	5

Note: GoM group I was labelled 'EOAD' as persons matching the group had 100 % probability of being affected. Conversely, group II was labelled 'At-risk' and had 100 % probability of being unaffected. Group I carried –352 GG (58 %) or GA (42 %) and + 45 CC (100 %), three-fourths women, and enriched in APOE ε4. The GoM run concerned 155 persons under the age 65 years, 56 EOAD cases and 99 unaffected subjects. The groups are by their nature extremes presenting a strong contrast. A total of 34 EOAD cases matched the 'EOAD' latent group exactly and 73 controls matched the 'Control' group exactly. Others presented imperfect matches

Information on gender and APOE genotype was not used to identify the latent groups. The unaffected group II profile of genotypes was equally likely to be found for men and women, as might be expected. If this result had not been found it would have been necessary to assume that EOAD is extremely common or, alternatively, that a common rapidly fatal disorder was related to the low-risk for EOAD profile (II). A gender-specific role for –352G/* in combination with +45 C/C in EOAD is indicated by the 3-fold overrepresentation of women in affected latent group I. In short, the GoM approach strengthens suspicion that the high-risk genotype is more important for women.

The ε4 allele for APOE is a risk-factor for AD and, as might be expected common for affected latent group I (80 % probability for one or two copies).

Discussion

Since the APOE polymorphism is an established risk factor for AD we determined allele distributions of APOE [4]: the ε4 allele was expectedly more common for cases. APOE ε4 allele frequency was higher for early-onset cases than for the LOAD subjects. We did not specifically investigate interaction in this study because the sample size was too low, but it was clear that EOAD cases were enriched in both genetic risk-factors, APOE ε4 and a specific APOD haplotype. At advanced ages, frequencies for both risk-factors were less common among cases (i. e. the relative risk decreased with age). This heterogeneity may result from the presence of other unevaluated modifying factors speeding or retarding the AD process providing a spectrum of susceptibility and onset age, or simply the depletion of vulnerable subjects.

There were 331 LOAD and 63 EOAD cases, analysed separately for APOD SNPs using age 65 as the cut off age. There was little evidence that APOD was associated with LOAD (or AD in the whole sample, largely composed of LOAD cases). There was substantial evidence that the –352G allele was related to the risk of EOAD despite the limited sample size, especially in double dose, when found with the +45 C/C genotype, and for women.

While we acknowledge the possibility of a false-positive observation mainly caused by stratification with age or/and sex, three approaches were taken to demonstrate that the –352G allele located in intron two was associated with increased risk in EOAD. First, the G/G and G/A genotypes were over-represented among EOAD cases compared with control subjects age ≤ 65: 29 % vs 22 % for G/G, 56 % vs 43 % for G/A, and 16 % vs 35 % for A/A. The odds of being a case were increased 3-fold for –352G carriers controlling for gender and the presence or absence of ε4 with the 95 % CI not including the referent value of one.

Second, both 4-locus haplotypes that contained –352G were more common for EOAD cases. These haplotypes were TGCC (48 % found vs 41 % expected) and TGCT (8 % found vs 1 % expected). The latter with +718T was on its own significantly over-represented among cases ($p = 0.002$). Both haplotypes had +45C (Table 3).

Third, the GoM approach defined two latent groups based on disease status and genotypes found for the four SNPs. Group I was affected (100 %), 58 % –352 G/G and 42 % –352 G/A, carried +45 C/C, enriched in APOE ε4 and frequently women. The 4-locus 'EOAD' genotype (I) was T/*, G/*, C/C and */*. This genotype was consistent with the 'at-risk' unaffected genotype (II): T/T, */A, */*, C/*, suggesting that not all 'at-risk' subjects become affected by age 65. Other GoM analyses for older ages demonstrate continued risk to age 75. The APOE and gender distributions for the 'at-risk' group are similar to those for the eastern Finnish population [11, 12]. As sug-

gested in logistic analyses, women were at higher risk when carrying the high-risk four-locus genotype: 74% of the affected group (I) was female.

Since the –352G→A cannot be related directly to change in apoD protein function or expression, the assumption is that it may be in linkage disequilibrium (LD) with biologically relevant variability elsewhere in the APOD gene that has an impact on function or expression. It cannot be excluded that variations in another adjacent gene in LD with the –352G→A were responsible for the observed association, as well. According to the genetic location database (http://cedar.genetics.soton.ac.uk), APOD is flanked at its 5' side by the gene encoding the human transferrin receptor (TFRC [MIM 190010]), which plays a major role in the uptake of transferrin-bound iron [19]. Changes in iron homeostasis have been described to be a contributory factor to neurodegeneration in AD [3, 26].

In summary, our data representing the eastern Finnish population indicate that the –352G allele for APOD is associated with an increased risk of EOAD, especially in double dose, when found with the nearby +45 C/C APOD genotype, and for women. Independent replication studies are needed to verify the finding and extend it to other ethnic groups.

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References

1. Belloir B, Kövari E, Surini-Demiri M, Savioz A (2001) Altered apolipoprotein D expression in the brain of patients with Alzheimer disease. J Neurosci Res 64:61–69
2. Boldt A, Petzl-Erler M (2002) A new strategy for mannose-binding lectin gene haplotyping. Hum Mutat 19: 296–306
3. Connor JR, Milward EA, Moalem S, Sampietro M, Boyer P, Percy ME, Vergani C, Scott RJ, Chorney M (2001) Is hemochromatosis a risk factor for Alzheimer's disease? J Alzheimer's Dis 3:471–477
4. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261:921–923
5. Desai PP, Bunker CH, Ukoli FAM, Kamboh MI (2002) Genetic variation in the apolipoprotein D gene among African blacks and its significance in lipid metabolism. Atherosclerosis 163: 329–338
6. Desai PP, Hendrie HC, Evans RM, Murrell JR, DeKosky ST, Kamboh MI (2003) Genetic variation in apolipoprotein D affects the risk of Alzheimer disease in African-Americans. Am J Med Genet 116:98–101
7. Drayna DT, McLean JW, Wion KL, Trent JM, Drabkin HA, Lawn RM (1987) Human apolipoprotein D gene: gene sequence, chromosomal localization, and homology to the $\alpha_{2\mu}$-globulin superfamily. DNA 6:199–204
8. Excoffier L, Slatkin M (1995) Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 12:921–927
9. Flower DR (1994) The lipocalin protein family: a role in cell regulation. FEBS Lett 354:7–11
10. Hiltunen M, Mannermaa A, Thompson D, Easton D, Pirskanen M, Helisalmi S, Koivisto AM, Lehtovirta M, Ryynänen M, Soininen H (2001) Genome-wide linkage disequilibrium mapping of late-onset Alzheimer's disease in Finland. Neurology 57:1663–1668
11. Kuusisto J, Koivisto K, Kervinen K, Mykkänen L, Helkala E-L, Vanhanen M, Hänninen T, Pyörälä K, Kesäniemi A, Riekkinen P, Laakso M (1994) Association of apolipoprotein E phenotype with late-onset Alzheimer's disease: a population-based study. BMJ 309: 636–638
12. Lehtovirta M, Soininen H, Helisalmi S, Mannermaa A, Helkala E-L, Hartikainen P, Hänninen T, Ryynänen M, Riekkinen P Sr (1996) Clinical and neuropsychological characteristics in familial and sporadic Alzheimer's disease: relation to apolipoprotein E polymorphism. Neurology 46:413–419
13. Manton KG, Woodbury MA, Tolley HD (1994) Statistical Applications Using Fuzzy Sets. John Wiley & Sons, New York
14. McKhann G, Drachman D, Folstein M, Katzman R, Prica D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34:939–944
15. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L, CERAD neuropathologists (1991) The Consortium to Establish a Registry for Alzheimer's disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 41:479–486
16. Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM (1999) Diabetes mellitus and the risk of dementia: the Rotterdam study. Neurology 53:1937–1942
17. Rassart E, Bedirian A, Do Carmo S, Guinard O, Sirois J, Terrisse L, Milne R (2000) Apolipoprotein D Biochim Biophys Acta 1482:185–198
18. Raymond ML, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283
19. Richardson DR, Ponka P (1997) The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. Biochim Biophys Acta 1331:1–40
20. Suresh S, Yan Z, Patel RC, Patel YC, Patel SC (1998) Cellular cholesterol storage in the Niemann-Pick disease type C mouse is associated with increased expression and defective processing of apolipoprotein DJ Neurochem 70: 242–251
21. Terrisse L, Poirier J, Bertrand P, Merched A, Visvikis S, Siest G, Milne R, Rassart E (1998) Increased levels of apolipoprotein D in cerebrospinal fluid and hippocampus of Alzheimer's patients. J Neurochem 71:1643–1650

22. Thomas EA, Dean B, Pavey G, Sutcliffe JG (2001) Increased CNS levels of apolipoprotein D in schizophrenic and bipolar subjects: implications for the pathophysiology of psychiatric disorders. Proc Natl Acad Sci USA 98: 4066–4071
23. Tsukamoto K, Watanabe T, Matsushima T, Kinoshita M, Kato H, Hashimoto Y, Kurokawa K, Teramoto T (1993) Determination by PCR-RFLP of apoE genotype in a Japanese population. J Lab Clin Med 121:598–602
24. Vandenplas S, Grobler-Rabie A, Brebner K, Rickets M, Wallis G, Bester A, Boyd C, Mathew C (1984) Blot hybridisation of genomic DNA. J Med Genet 21:164–172
25. Vijayaraghavan S, Hitman GA, Kopelman PG (1994) Apolipoprotein-D polymorphism – a genetic marker for obesity and hyperinsulinemia. J Clin Endocrinol Metab 79:568–570
26. Ward RJ, Zhang Y, Crichton RR (2001) Aluminium toxicity and iron homeostasis. J Inorg Biochem 87:9–14
27. Woodbury MA, Clive J, Garson A Jr (1978) Mathematical typology: a grade of membership technique for obtaining disease definition. Comput Biomed Res 11:277–298