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APOE and A-betaPP Gene Variation in Cortical and Cerebrovascular Amyloid-beta Pathology and Alzheimer's Disease: A Population-Based Analysis

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Abstract

Cortical and cerebrovascular amyloid-beta (A-beta) deposition is a hallmark of Alzheimer's disease (AD), but also occurs in elderly people not affected by dementia. The *apolipoprotein E* (*APOE*) *epsilon4* is a major genetic modulator of A-beta deposition and AD risk. Variants of the *amyloid-beta protein precursor* (*A-betaPP*) gene have been reported to contribute to AD and cerebral amyloid angiopathy (CAA). We analyzed the role of *APOE* and *A-beta PP* variants in cortical and cerebrovascular A-beta deposition, and neuropathologically verified AD (based on modified NIA-RI criteria) in a population-based autopsy sample of Finns aged 85 years (Vantaa85 + Study; $n = 282$). Our updated analysis of *APOE* showed strong associations of the *epsilon4* allele with cortical ($p = 4.91 \times 10^{-17}$) and cerebrovascular ($p = 9.87 \times 10^{-11}$) A-beta deposition as well as with NIA-RI AD ($p = 1.62 \times 10^{-8}$). We also analyzed 60 single nucleotide polymorphisms (SNPs) at the *A-betaPP* locus. In single SNP or haplotype analyses there were no statistically significant *A-betaPP* locus associations with cortical or cerebrovascular A-beta deposition or with NIA-RI AD. We sequenced the promoter of the *A-betaPP* gene in 40 subjects with very high A-beta deposition, but none of these subjects had any of the previously reported or novel AD-associated mutations. These results suggest that cortical and cerebrovascular A-beta depositions are useful quantitative traits for genetic studies, as highlighted by the strong associations with the *APOE epsilon4* variant. Promoter mutations or common allelic variation in the *A-betaPP* gene do not have a major contribution to cortical or cerebrovascular A-beta deposition, or very late-onset AD in this Finnish population based study.

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INTRODUCTION

Cortical and cerebrovascular amyloid-beta (A-beta) deposition is a neuropathological hallmark of Alzheimer's disease (AD), but these depositions also occur in elderly people not affected by dementia. The prevalence of A-beta deposition and AD increases by age and is partially genetically determined. The *epsilon4* variant of the *apolipoprotein E* (*APOE*) gene is an unequivocally accepted major genetic risk factor modulating both A-beta deposition, late-onset AD risk and cerebrovascular A-beta deposition [1–3]. A handful of other weaker risk loci for late-onset AD have been found and replicated in recent genome-wide association (GWA) studies [4, 5]. The role of other suggested genetic risk variants for late-onset AD, mostly identified on candidate gene analyses, has remained unclear. One such candidate gene is *amyloid-beta protein precursor* (*A-betaPP*).

The role of missense mutations in the *A-betaPP* gene in early-onset familial AD has been extensively studied. About a dozen missense mutations have been described thus far [6–8]. The common denominator of these mutations is that they result in increased generation of A-beta species that form A-beta oligomers. These oligomers are believed to play a crucial role in mediating the neurodegenerative effects of A-beta. Copy number variation of the *A-betaPP* gene on chromosome 21q, as in Down's syndrome [10, 11] or in *A-betaPP* locus duplication, leads to prominent A-beta deposition and presenile dementia and, in some cases, marked cerebral amyloid angiopathy (CAA) [12–14]. In addition, six *A-betaPP* mutations (A692 G, E693Q, E693 G, E693K, N694D, and L705V) have been identified leading to prominent CAA. Most of these mutations are associated with cerebral hemorrhages and/or ischemic stroke, and some, such as the Arctic (E693 G) and Iowa (N694D) variants, result in abundant neurofibrillary and amyloid plaque pathology [15, 16].

Although the above mentioned genetic mutations are very rare and virtually absent in late-onset AD cases, genetic variation of *A-betaPP* has been suggested to play a role in AD in several linkage and association studies. Particularly, a possible role of allelic variation at the 5' region of the *A-betaPP* gene (variants –3830 T/C, –3102 G/C, –2919A/G –1023 C/T, +37 G/C), has been suggested in several studies [20–23], mostly based on early-onset AD cases. However, not all studies have detected association with the *A-betaPP* gene, and in recent GWA studies of late-onset AD, designed to detect common risk alleles, the *A-betaPP* locus has not yielded any *p*-values approaching Bonferroni threshold of statistical significance [4, 5].

In the present study, we have performed a detailed analysis on whether common or rare variations in the *A-betaPP* gene, encoding the precursor molecule of A-beta, contribute to cortical or cerebrovascular A-beta deposition or neuropathologically verified AD risk. Thus far, such neuropathology-based analysis of the *A-betaPP* gene has not been reported. In parallel with the analyses of the *A-betaPP* gene we report updated results on the contribution of *APOE* in cortical and cerebrovascular A-beta deposition and neuropathologically verified AD in the whole neuropathologically examined Vantaa 85+ population (*n* = 282). Our previous reports on *APOE* have been based on smaller subsets of this population [2, 25, 26].

MATERIALS AND METHODS

Subjects

The demographic characteristics of the study material (Vantaa 85 + population) are shown in Table 1. The Vantaa 85 + study includes all persons aged 85 years or older who were living in the city of Vantaa (Southern Finland), on April 1st, 1991. Out of the eligible 601 subjects, DNA extraction and *APOE* genotyping was possible in 534 subjects. Autopsies were carried out in 306 subjects, and DNA was available in 282 of this neuropathologically examined

subpopulation. The study was approved by the Ethical Review Committee of the City of Vantaa. The use of the health and social work records and death certificates was approved by the Finnish Ministry of Social Affairs and Health. The collection of the tissue samples at autopsy, and their use for research, was approved by the National Authority for Medicolegal Affairs. Consent for participation in the study and autopsy was obtained from the subjects and/or their nearest relatives.

Neuropathological analyses

Cortical A-beta deposition was defined as the percentage (A-beta %) of neocortex covered by methenamine silver-positive plaques, as reported earlier. This staining was selected because it is as sensitive or almost as sensitive as A-beta immunohistochemistry in detecting senile plaques, including diffuse plaques. This technique is quick and easy to do and gives excellent contrast between the cortex and white matter, which was necessary for the morphometry [2, 27, 28]. Deposition of A-beta -derived amyloid in the cerebral cortical and leptomeningeal small and midsized blood vessels (cerebral amyloid angiopathy; CAA) was diagnosed using histological Congo red staining. All samples positive in Congo red were further stained using IHC against A-beta peptide (clone 4 G8, residues 17–24, Senetec/Biodesign, in dilution of 1: 1000/1: 3000, applied on four μm -thick paraffin sections). The severity of cerebrovascular A-beta deposition was assessed by counting the percentage of the A-beta immunoreactive blood vessels out of all vessels in four neocortical areas (frontal, temporal, parietal, occipital), hippocampus, and cerebellum to yield the individual percentage of the A-beta immunoreactive blood vessels (cerebrovascular A-beta deposition), throughout the whole section. The diagnosis of neuropathological AD was established according to the modified NIA-RI criteria. Briefly, diagnosis of AD according to the modified NIA-RI criteria required plaque scores “moderate” or “frequent” according to the CERAD protocol and Braak stages IV–VI of neurofibrillary pathology ($n = 113$). The individuals belonging to the neuropathologically examined subpopulation who either lacked neocortical neuritic plaques or had the CERAD neuritic plaque score “sparse”, and whose Braak stage for neurofibrillary pathology was less than III, were defined as neuropathological controls ($n = 48$) [2, 32]. Subjects of the neuropathologically examined subpopulation that did not fulfill the criteria for either of these categories were excluded.

Genetic studies

The method for *APOE* genotyping has been described earlier. We analyzed 60 single nucleotide polymorphisms (SNPs) in the *A-betaPP* gene and its flanking regions using Illumina HumanCNV370 Beadchips (52 SNPs), restriction endonuclease methods (6 SNPs), and sequencing (2 SNPs). We also re-sequenced a 971 bp region (-767 ± 204) of the *A-betaPP* promoter in 40 subjects with very high A-beta % (7%) in order to unravel previously reported rare mutations [23,33] or novel variants. The region -90 ± 204 was sequenced from whole study population in order to genotype two rare SNPs (rs45476095/-9, rs459543/+37) and the novel variant at position -62 found in this study. Sequencing was performed using BigDye (Applied Biosystems, Foster City, CA, USA). Primers are available on request. Sequence analysis was carried out with the Sequencer 4.5 Software (Gene Codes Corporation, Ann Arbor, MI, USA).

Statistical analyses

The associations between the *APOE epsilon4* and *A-betaPP* alleles and cortical and cerebrovascular A-beta depositions were analyzed with PLINK, a toolset for genome association analysis (<http://pngu.mgh.harvard.edu/purcell/plink/>), using asymptotic Wald test. The association between age at death and cortical A-beta deposition were analyzed by linear regression analysis using the SPSS for Windows v. 15.0. Association between *APOE epsilon4* allele and NIA-RI criteria based AD were also done with PLINK using Cochran-

Armitage trend test. *A*-beta PP haplotype associations and LD structure were calculated with the Haploview software. Haplotype blocks were defined using criteria by Gabriel et al.. All other statistical analyses were performed using SPSS for Windows (v15.0).

RESULTS

Neuropathological findings

In this elderly population-based sample, 83% of the subjects had detectable A-beta in the cortical regions examined. The mean and median percentages of the cortical A-beta were 3.41% and 2.13% (range 0–17.3%). 70% of the subjects had detectable cerebrovascular A-beta deposition (range 0–77.2%, mean 4.6%, median 1.2%). There was no association between the age at death and the extent of cortical A-beta (p -value = 0.52) or cerebrovascular A-beta deposition (p -value = 0.66). There were 113 subjects in this cohort who fulfilled the criteria for NIA-RI AD, and 87.6% of those also showed cerebrovascular A-beta pathology.

Association of APOE with cortical and cerebrovascular A-beta, tau pathology, and AD

A robust association between cortical A-beta % and *APOE* epsilon4 was found ($p = 4.91 \times 10^{-17}$, Fig. 1A), updating our previously reported results based on smaller number of neuropathologically verified subjects [2, 32]. Presence of cerebrovascular A-beta deposition was also very strongly associated with *APOE* epsilon4 ($p = 9.87 \times 10^{-11}$, Fig. 1B), in accordance with our previous focused report on the >95 year-old subpopulation of the Vantaa 85+ Study. Distribution of *APOE* epsilon4 positive and negative subjects in Braak stages 0–6 is shown in Fig. 1C. When Braak stage 0–II versus IV–VI were compared (according to our previous study), a robust association between *APOE* epsilon4 and Braak stage was found ($p = 3.45 \times 10^{-7}$, Figure 1C). *APOE* epsilon4 was also associated with AD verified by the modified NIA-RI criteria ($p = 1.62 \times 10^{-8}$, odds ratio = 12.8, 95% confidence interval (CI) from 3.9 to 42).

A-beta PP gene variation

To investigate the effect of *A*-beta PP variation in cortical and cerebrovascular A-beta deposition, and neuropathologically verified NIA-RI AD, we genotyped a total of 60 single nucleotide polymorphism (SNP) markers in the *A*-beta PP gene region, including markers in the promoter area (–3830T/C (rs11911934), –3102G/C (rs463946), –2919A/G (rs438031), –1023C/T (rs466448), and +37 G/C (rs459543)), which have been previously implicated in early-onset or late-onset AD [18, 20, 21, 23]. Figure 2 illustrates the position of the markers, their association with cortical and cerebrovascular A-beta deposition and the haplotype structure of the *A*-beta PP locus. None of the SNPs were associated with cortical or cerebrovascular A-beta (Fig. 2), after taking into account Bonferroni correction for multiple testing, nor did they show association with neuropathologically verified NIA-RI AD (Table 2). One of the previously suggested markers (rs11911934) was so rare (1.7% in NIA-RI AD versus 0% in controls) that the power of the present study does not allow us to make conclusions on its role in AD. The linkage disequilibrium (LD) structure of the *A*-beta PP locus in this elderly Finnish population was similar to the Caucasian population in the Hapmap project (<http://hapmap.ncbi.nlm.nih.gov>). Haplotype association analyses with the 12 haplotype blocks shown in Fig. 2 did not show association with cortical or cerebrovascular A-beta or NIA-RI AD (data not shown). Conditioning on the presence or absence of *APOE* epsilon4 did not show any associations in single marker or haplotype analyses (data not shown).

Sequencing of A-betaPP promoter

Since rare mutations in the promoter region of the *A-betaPP* gene have been reported to increase susceptibility to early-onset AD and increase the production of A-beta, a part of the promoter region was sequenced in 40 subjects with the highest cortical A-beta deposition (7% of the cortex covered by A-beta). A novel mutation was discovered at the promoter region at position -62 (gagcgG/Aggtgg) in one subject with cortical A-beta % = 7. This mutation was tested in all 282 neuropathologically examined subjects, but no one else carried this mutation. Any of the previously reported rare mutations (positions -118, -369, -479 and -534), which have been described in early-onset AD patients and reportedly increase transcriptional activity of *A-betaPP* [23–33], were not found.

DISCUSSION

Although the search to find genetic risk factors for late-onset AD has been going on for over 20 years, only a small number of unequivocal genetic risk factors for late-onset AD have been identified. Problems in many genetic studies have included small and heterogeneous study populations, selection bias involved in the study design, and lack of neuropathologically verified diagnosis of cases and controls. The Vantaa 85+ Study material provides an alternative opportunity to identify genetic risk factors for late-onset AD: it is possible to study genetics of AD-related histologically determined quantitative traits, such as A-beta deposition, in a population-based setting. Furthermore, studying subjects derived from the genetically homogeneous Finnish population increases the chances for detecting associated variants. The power of this approach is highlighted by the very strong association of the *APOE* epsilon4 variant with cortical and cerebrovascular A-beta deposition. Although the number of subjects in our study was relatively low, our sample is one of the largest among population-based studies in which neuropathological quantitative data is available. To the best of our knowledge, the associations that we report here are among the strongest that have been reported for epsilon4, highlighting the statistical power of quantitative trait analysis even in this relatively small material. In addition, these results reinforce the major role of *APOE* in both A-beta deposition and AD risk. The robust effect of *APOE* in amyloid, tangle, and CAA pathologies has also been shown in other population-based neuropathological studies.

The role of gene variation in the *A-betaPP* locus in modulating the risk of late-onset AD has remained unclear despite active research in a variety of populations. However, the same problems as above apply to these previous studies, e.g., none have focused on very elderly populations (>85 y), and no neuropathology-based analysis has been carried out. In our study we performed a detailed analysis of the significance of the *A-betaPP* locus variation for both A-beta load and risk for AD. Furthermore, given the link between CAA and overexpression of A-beta PP in the duplication mutation previously reported, it was of interest to extend the present analysis to the CAA phenotype for the first time. Our analysis of the *A-betaPP* locus did not provide any evidence for the role of the previously suggested *A-betaPP* variants in A-beta deposition or NIA-RI AD risk with the exception of rs11911934 (-3829 C/T), which was too rare for meaningful analysis. The high-density SNP and haplotype analyses did not provide any support for a role of common variation within the *A-betaPP* locus in modulating A-beta deposition or risk of NIA-RI-based AD risk. Furthermore, by re-sequencing we did not find any of the previously reported rare mutations in the proximal promoter region in the 40 subjects with the highest cortical A-beta % (7–18%). Thus, it appears that genetic variation in the *A-betaPP* locus does not play a major role in the development of cortical or cerebrovascular A-beta deposition or AD risk in the Finnish elderly population. Our results agree with those of Nowotny et al., who showed this lack of association in a large case – control study containing 730 cases and 650 controls.

It is noteworthy that the age of onset of AD has been relatively early in those studies reporting associations with variants at the 5' region of the *A*-betaPP locus. In the study of Theuns et al. reporting the rare promoter mutations (−118, −369 and −534), the age of onset was <70 y in all cases. In the study by Brouwers and colleagues, the ages of onset were 61, 74, and 78 y for the −369C→G mutation carriers, 77 y for the −534G→mutation carrier, and 62, 71, 74, and 79 y for the −479C→T mutation carriers. In the study of Guyant-Merchal et al. the mean age-of-onset of patients with the −3102 variant was <70 y. Our study population of subjects aged 85 is clearly older than in any of the previous studies. It is possible that the variation of the *A*-betaPP locus affects the risk of AD at an earlier age and that this effect could not be detectable in this very old age group. This hypothesis would be consistent with the view that increased A-beta generation (via mutations or overexpression) might contribute to earlier onset AD, whereas in the very elderly individuals A-beta clearance (rather than its generation) might be impaired. This might be partly influenced by the increased prevalence of cerebrovascular disease, a known inducer of A-beta PP and A-beta expression, and possible problems in perivascular drainage along the walls of capillaries and arteries in the aged. Senescence of microglial cells might also contribute to the failure in A-beta clearance in the elderly. Furthermore, enzymatic mechanisms of degrading A-beta and LRP mediated transport are known to be related to oxidative stress, a common phenomenon in the aging brain. In accordance with this view, genes encoding at least two proteins, APOE and A2 M, involved in A-beta clearance, have been shown to contribute to cortical A-beta deposition and AD pathology in the Vantaa 85 + material [2, 25].

In conclusion, our results suggest that both the cortical and the cerebrovascular A-beta depositions can be used as powerful quantitative traits for genetic studies, as highlighted by the strong associations with the *APOE* epsilon4 variant. This first neuropathologically examined population-based study on the effect of *A*-betaPP on late-onset AD demonstrates that promoter mutations or common allelic variation of *A*-betaPP do not appear to provide a major contribution to cortical or cerebrovascular A-beta deposition in very elderly Finns.

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Fig. 1.

(A) Cortical A-beta deposition and APOE epsilon4. There was a clear association between the APOE epsilon4 allele and cortical A-beta deposition ($p=4.91 \times 10^{-17}$). Mean cortical A-beta deposition in subjects with APOE epsilon4=5.85 (SD=3.93, range 0%–15.66) and without APOE epsilon4=2.29 (SD=3.08, range 0%–17–31%). (B) Cerebrovascular A-beta deposition and APOE epsilon4. There was a clear association between the ApoE epsilon4 allele and cerebrovascular A-beta deposition ($p=9.87 \times 10^{-11}$). Mean cerebrovascular A-beta deposition in subjects with APOE epsilon4=8.76 (SD=12.39, range 0%–72.67%) and without APOE epsilon4=2.00 (SD=4.82, range 0%–38.83%). (C) Braak stages from 0 to 6 and APOE epsilon4.

Fig. 2. Associations of the A-betaPP variations with cortical and cerebrovascular A-beta and A-betaPP LD structure. None of the SNPs was associated with either cortical or cerebrovascular A-beta% after Bonferroni correction.

Table 1

Demographic characteristics of the whole Vantaa 85+population and the neuropathologically examined subpopulation

Characteristics	Whole Vantaa 85+ study population	Neuropathologically examined subpopulation
n	534	282
Women (%)	79.4	83
Age in 1991 (mean y $\pm$ SD)	88.59 $\pm$ 2.9	88.87 $\pm$ 3.1
Age at death (y $\pm$ SD)	92.38 $\pm$ 3.7	92.55 $\pm$ 3.6
Cortical A% (mean $\pm$ SD)	-	3.41 $\pm$ 3.8
CAA% (mean $\pm$ SD)	-	4.13 $\pm$ 8.6
Braak Stage (1–2/4–6)	-	81/129
CERAD (0/2–3)	-	68/185
NIARI AD (control/cases)	-	48/113

Table 2
Association of A-betaPP locus markers with neuropathologically verified AD (based on modified NIA-RI criteria)

	SNP	Chr. 21 position	Position to gene	MAF*AD/control s	P-value AD/control s
1	rs1571660	26121367	3' side	0.450/0.446	0.948
2	rs2829929	26124180	3' side	0.243/0.239	0.942
3	rs1394839	26136162	3' side	0.147/0.217	0.125
4	rs2829946	26138228	3' side	0.157/0.228	0.155
5	rs2829949	26144693	3' side	0.073/0.033	0.181
6	rs7282634	26147048	3' side	0.477/0.424	0.391
7	rs2040273	26154069	3' side	0.431/0.402	0.624
8	rs8126493	26158485	3' side	0.486/0.413	0.232
9	rs41175	26160169	3' side	0.425/0.422	0.969
10	rs2829961	26163866	3' side	0.477/0.413	0.292
11	rs3787620	26176988	intron 17	0.073/0.098	0.47
12	rs373521	26179531	intron 17	0.395/0.359	0.532
13	rs11145	26181885	intron 17	0.113/0.130	0.668
14	rs2829973	26182327	intron 17	0.384/0.446	0.334
15	rs1783025	26188634	intron 16	0.179/0.156	0.617
16	rs380417	26194030	intron 15	0.248/0.283	0.539
17	rs1787438	26195960	intron 15	0.101/0.130	0.464
18	rs1783016	26201909	intron 14	0.275/0.272	0.947
19	rs214488	26209795	intron 13	0.202/0.250	0.371
20	rs383700	26220640	intron 13	0.064/0.109	0.201
21	rs2829984	26226123	intron 13	0.128/0.152	0.589
22	rs2234983	26238745	intron 13	0.142/0.163	0.658
23	rs216779	26245575	intron 13	0.055/0.109	0.112
24	rs400603	26247159	intron 13	0.046/0.076	0.301
25	rs440666	26250046	intron 11	0.202/0.239	0.472
26	rs2014146	26256893	intron 11	0.353/0.359	0.923
27	rs216762	26258039	intron 11	0.412/0.413	0.986

	SNP	Chr. 21 position	Position to gene	MAF*AD/control s	P-value AD/control s
28	rs2070657	26276888	intron 8	0.165/0.18	0.678
29	rs2830000	26283639	intron 8	0.202/0.283	0.141
30	rs7278838	26296475	intron 6	0.257/0.250	0.904
31	rs2830008	26313330	intron 6	0.257/0.228	0.615
32	rs2830012	26319478	intron 5	0.366/0.391	0.682
33	rs768039	26329891	intron 5	0.229/0.304	0.194
34	rs2830028	26349119	intron 3	0.222/0.211	0.838
35	rs3991	26350127	intron 3	0.275/0.207	0.227
36	rs2830033	26352796	intron 3	0.317/0.283	0.584
37	rs2830036	26357396	intron 3	0.225/0.283	0.277
38	rs2830038	26364467	intron 3	0.381/0.413	0.619
39	rs7283136	26365981	intron 3	0.060/0.054	0.86
40	rs2830040	26368303	intron 3	0.023/0.022	0.948
41	rs2830044	26376866	intron 3	0.312/0.304	0.898
42	rs2830051	26387226	intron 2	0.225/0.294	0.212
43	rs2830052	26387468	intron 2	0.262/0.228	0.571
44	rs11702267	26390611	intron 2	0.092/0.065	0.49
45	rs2830054	26397975	intron 2	0.357/0.424	0.29
46	rs3827166	26403188	intron 2	0.165/0.130	0.444
47	rs2830058	26406839	intron 1	0.165/0.133	0.487
48	rs2830068	26418538	intron 1	0.298/0.272	0.667
49	rs466609	26428188	intron 1	0.106/0.065	0.259
50	rs2830088	26436611	intron 1	0.495/0.478	0.782
51	rs2186302	26442585	intron 1	0.280/0.283	0.963
52	rs2830104	26459455	intron 1	0.197/0.174	0.628
53	rs459543#	26464920	5' UTR	0.034/0.091	0.037
54	rs45476095	26464965	5' UTR	0.010/0.045	0.045
55	rs466448#	26465979	promoter	0.523/0.554	0.613
56	rs438031#	26467875	promoter	0.229/0.196	0.524

	SNP	Chr. 21 position	Position to gene	MAF*AD/control s	P-value AD/control s
57	rs463946#	26468058	promoter	0.033/0.061	0.386
58	rs11911934#	26468785	promoter	0.017/0.000	0.246
59	rs2156079	26470201	5' side	0.220/0.222	0.971
60	rs463164	2650073	5' side	0.257/0.196	0.258