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Alzheimer risk loci and associated neuropathology in a population-based study (Vantaa 85+)

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Abstract

Objective
To test the association of distinct neuropathologic features of Alzheimer disease (AD) with risk loci identified in genome-wide association studies.

Methods
Vantaa 85+ is a population-based study that includes 601 participants aged ≥85 years, of which 256 were neuropathologically examined. We analyzed 29 AD risk loci in addition to *APOE* ε4, which was studied separately and used as a covariate. Genotyping was performed using a single nucleotide polymorphism (SNP) array (341 variants) and imputation (6,038 variants). Participants with Consortium to Establish a Registry for Alzheimer Disease (CERAD) (neuritic Aβ plaques) scores 0 (n = 65) vs score M + F (n = 171) and Braak (neurofibrillary tangle pathology) stages 0–II (n = 74) vs stages IV–VI (n = 119), and with capillary Aβ (CapAβ, n = 77) vs without (n = 179) were compared. Cerebral amyloid angiopathy (CAA) percentage was analyzed as a continuous variable.

Results
Altogether, 24 of the 29 loci were associated (at $p < 0.05$) with one or more AD-related neuropathologic features in either SNP array or imputation data. Fifteen loci associated with CERAD score, smallest $p = 0.0002122$, odds ratio (OR) 2.67 (1.58–4.49) at *MEF2C* locus. Fifteen loci associated with Braak stage, smallest $p = 0.004372$, OR 0.31 (0.14–0.69) at *GAB2* locus. Twenty loci associated with CAA, smallest $p = 7.17E-07$, β 14.4 (8.88–20) at *CR1* locus. Fifteen loci associated with CapAβ smallest $p = 0.002594$, OR 0.54 (0.37–0.81) at *HLA-DRB1* locus. Certain loci associated with specific neuropathologic features. *CASS4*, *CLU*, and *ZCWPW1* associated only with CAA, while *TREM2* and *HLA-DRB5* associated only with CapAβ.

Conclusions
AD risk loci differ in their association with neuropathologic features, and we show for the first time distinct risk loci for CAA and CapAβ.

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Glossary

Aβ = amyloid-β; **AD** = Alzheimer disease; ***APOE*** = apolipoprotein E; **CAA** = cerebral amyloid angiopathy; **CapAβ** = capillary Aβ; **CERAD** = Consortium to Establish a Registry for Alzheimer Disease; **Chr9 region** = chromosome 9 region; **CI** = confidence interval; **GWAS** = genome-wide association study; **IHC** = immunohistochemistry; **LOAD** = late-onset Alzheimer disease; **OR** = odds ratio; **SNP** = single nucleotide polymorphism.

Late-onset Alzheimer disease (LOAD) is neuropathologically characterized by cerebral accumulation of amyloid-β (Aβ) peptide containing neuritic plaques and hyperphosphorylated tau-protein, and by cerebral amyloid angiopathy (CAA) and capillary Aβ (CapAβ) deposition. LOAD is known to have a fairly strong hereditary risk,[1] the apolipoprotein E (*APOE*) ε4 being the strongest genetic risk factor.[2,3] Recently, genome-wide association studies (GWASs) have identified approximately 30 Alzheimer disease (AD)-associated risk loci,[4–10] which are known to encode proteins involved in immune system and inflammation (*CLU*, *CR1*, *ABCA7*, *MS4A*, *CD33*, *EPHA1*, *MEF2C*, *HLA-DRB1/DRB5*, *TRIP4*, and *TREM2*), cholesterol metabolism (*APP*, *CLU*, *ABCA7*, and *SORL1*), synaptic and membrane function (*PICALM*, *BIN1*, *CD33*, *CD2AP*, *EPHA1*, *INPP5D*, *PTK2B*, *SORL1*, and *SLC2A4*), tau pathology (*BIN1*), and Aβ metabolism (*APP*, *CLU*, *CR1*, *ABCA7*, *INPP5D*, and *SORL1*).[6,8,11–15] Most of the previous GWASs have been based on large clinically diagnosed hospital-based samples,[5–10,14] but recently, a few GWASs have been published based on neuropathologically verified data sets.[16] In those studies, *ABCA7* and *CD2AP* as well as a variant near *APP* (rs2829887) and *ABCG1*, *GALNT7* and an intergenic region on chr 9 (9: 129,280,000–129,380,000) loci have been found to be associated with neuritic plaque pathology.[4,16] The Consortium to Establish a Registry for Alzheimer disease (CERAD) score and Braak stage have also been associated with *ABCA7*, *BIN1*, *CASS4*, *MEF2C*, and *PICALM*, and Braak stage with *CLU*, *SORL1*, *ZCWPW1*, and CERAD score with *MS4A6A*, and *CD33*.[4]

Here, we analyzed possible associations of AD risk loci with each neuropathologic feature (neuritic plaque, neurofibrillary tangle and CAA, and CapAβ) in a population-based sample of very elderly Finns (Vantaa 85+ Study).

Methods

Study population

The Vantaa 85+ Study includes 601 individuals, aged at least 85 years, who were living in the city of Vantaa on April 1, 1991. Autopsy and neuropathologic examination were performed on 300 (mean age 92.4 ± SD 3.7 years, range 85–105). The clinical characteristics of the whole genotyped subpopulation (N = 512) and the whole genotyped neuropathologically examined subpopulation (N = 300) are shown in table e-1 (http://links.lww.com/NXG/A16).

Neuropathologic examination

Evaluation of Braak stages of Alzheimer-type neurofibrillary pathology[17] and CERAD scores of neuritic plaques[18] have been described previously.[19] The percentage of CAA-affected noncapillary blood vessels was estimated using histologic Congo red as described previously and confirmed by immunohistochemistry (IHC).[20] The presence of CapAβ was analyzed as described before[20,21] using IHC.[21]

Evaluation of the *APOE* genotype, SNP array, and candidate gene approach

APOE genotyping was performed as described previously.[22] A GWAS was conducted as previously described[23] by Infinium Human370 BeadChips (Illumina, San Diego, CA) for 327,521 variants in blood samples from 512 participants. Data quality control was done using the standard PLINK v1.9[24,25] protocol.[26] In summary, related individuals (identity by descent >0.185), individuals with discordant sex information, divergent ancestry, elevated missing data (>3%) rate, or outlying heterozygosity rate (±2 SD) were excluded. Variants with missing per person rate >10%, minor allele frequency <1% and missing data rate >5%, or significantly different genotype call rates between cases and controls ($p < 0.00001$) were excluded. Variants not in the Hardy-Weinberg equilibrium ($p < 0.00001$) were also discarded.

A PubMed search was performed to identify all the loci that have been reported in previous GWAS analyses in samples from participants with clinically or neuropathologically diagnosed AD. Besides *APOE*, we found reports on 44 variants at 29 loci. Variants at genes near these candidate loci were extracted from the quality-controlled genome-wide single nucleotide polymorphism (SNP) array. To cover nearby variants of possible interest, variants within 1 kb of each candidate gene were also included in the study (table e-2, http://links.lww.com/NXG/A16).

Imputation

Imputation was performed using IMPUTE2.[27] 1000 Genomes phase3 data (October 2014 release) supplied by IMPUTE2 were used as the reference panel. Imputation was performed on the same candidate genes as in the SNP array–based analyses and on the 44 previously reported index variants. The whole available Vantaa 85+ data set (n = 512) was imputed. We have whole-genome sequences of a subset of the Vantaa 85+ study (n = 286), and we

compared the imputed genotypes to the whole-genome sequencing-derived genotypes. The median discordance between genotypes was 0.7%, which indicates successful imputation. We performed the same quality control steps and association analyses as we did to the SNP array data, but the genotyping rate threshold was not defined for the 44 index variants.

Statistical analyses

In the analyses, participants with moderate or frequent CERAD scores were compared with participants with no neuritic plaques (CERAD 0). Similarly, participants with Braak stages 0–II were compared with the high-stage group (Braak stages IV–VI). All participants without CapAβ were regarded as controls in analyses related to that pathology. The associations between *APOE ε4* allele and neuropathologic features were performed using logistic or linear regression analysis with age and sex as covariates on SPSS (version 23) (table 1). Other statistical analyses were performed using PLINK. Case-control association tests were calculated using logistic regression. Quantitative trait associations were calculated using linear regression. Each regression analysis was performed twice with either age and sex or age, sex, and *APOE* ε4 status as covariates. In this candidate gene analysis of GWAS known AD loci, $p < 0.05$ was considered statistically significant.

Standard protocol approvals, registrations, and patient consents

The Vantaa 85+ study was approved by the Ethics Committee of the Health Centre of the City of Vantaa in 1991 and by the Coordinating Ethics Committee of the Helsinki University Central Hospital in 2014. The Finnish Health and Social Ministry has approved the use of the health and social work records and death certificates. Blood samples were collected only after the participants or their relatives provided written informed consent. The National Authority for Medicolegal Affairs (VALVIRA) has approved the collection of the tissue samples at autopsy as well as their use for research. Written informed consent for autopsy was obtained from the nearest relatives.

Table 1 Results of association analyses between the *APOE* ε4 allele and neuropathologic features (CERAD, Braak, CAA, and CapAβ)

Variable	*p* Value	OR/β	95% CI
CERAD	9.25E-7	14.91	5.07–43.85
Braak	2.01E-7	8.25	3.72–18.27
CAA	1.50E-8	6.491	4.311–8.67
CapAβ	2.13E-10	7.54	4.04–14.06

Abbreviations: *APOE* = apolipoprotein E; CAA = cerebral amyloid angiopathy; CapAβ = capillary Aβ; CI = confidence interval; OR = odds ratio.
Univariate logistic (CERAD, Braak, and CapAβ) and linear (CAA%) regression analysis with values adjusted for age at death and sex.
APOE genotyping (rs429358, rs7412).
CERAD/neuritic plaques = moderate or frequent neuritic plaques according to the Consortium to Establish a Registry for Alzheimer Disease vs no neuritic plaques. Braak = Braak stage IV–VI vs Braak stage 0–II. CAA = mean percentage of subarachnoid and cortical noncapillary blood vessels with CAA in 6 samples. CapAβ = capillary Aβ present vs not present.

Results

SNP array and imputation

After quality control, 341 variants at 26 candidate loci remained in the SNP array data (table e-2, http://links.lww.com/NXG/A16). There were no variants in *EXOC3L2*, *HLA-DRB1*, and *TREM2* loci. In the imputed data set, 6,038 variants remained in 28 loci after quality control. Imputation was not successful in *INPP5D*, but it was covered with 26 variants in the SNP array data. Thus, all 29 candidate loci were covered in either the original SNP array or imputed data sets. Imputation of the index variants in *ABCG1*, *APP*, and chromosome 9 region (Chr9 region) was not successful because of too small minor allele frequency or low genotyping quality. Associations between the candidate loci and neuropathologic features are summarized in table 2.

Of the 512 samples, 487 passed the quality control criteria. Samples from 3 individuals were excluded because of difference in reported and estimated sex, 4 because of relatedness of participants, and 18 because of excessive missing data rate or heterozygosity.

Neuropathologic findings

The characteristics of the whole Vantaa 85+ sample (n = 512) and the neuropathologically and genetically examined subpopulations (n = 256) are shown in table e-1 (http://links.lww.com/NXG/A16). Neuropathologic analysis and data details have been previously reported.[19–21] No statistically significant differences were found in age at death or sex between the whole study population and the neuropathologically examined subpopulation, but there were slightly more females in the neuropathologically examined subpopulation.

APOE

As expected and already previously published using other types of analyses,[28,29] the *APOE* ε4 allele was strongly associated with all AD-related neuropathologic features (table 1). Further analyses were performed with and without *APOE* ε4 adjustment.

Association of the 29 risk loci with distinct neuropathologic features

Overall distribution of associations between the 26 candidate loci covered by the SNP array and neuropathologic features are shown in table e-3 (http://links.lww.com/NXG/A16). *EXOC3L2*, *HLA-DRB1*, and *TREM2* could not be analyzed since there were no variants at these loci in the SNP array. Variant details and *p* values are shown in table e-4. *APOE* was treated as a covariate and not included in the list of tested loci. Nine of the 26 SNP array loci were not associated with any histopathologic variables in SNP array data.

Table 2 Associations of candidate loci with neuropathologic features

	CapAβ[a]	CapAβ[b]	CAA[a]	CAA[b]	CERAD[a]	CERAD[b]	Braak[a]	Braak[b]	Variants at locus (n)
ABCA7			+	+	+		+	+	65
ABCG1			+	+	+	+	+	+	279
APP	+	+	+	+		+	+	+	733
BIN1	+	+	+	+	+	+			204
CASS4			+	+					52
CD2AP			+				+	+	363
CD33									10
CELF1									81
CLU			+						16
CR1		+	+	+			+	+	227
EPHA1									21
EXOC3L2									12
FERMT2	+	+	+	+	+	+			177
GAB2				+	+	+	+	+	237
GALNT7	+	+	+				+	+	199
HLADRB1	+	+	+	+					66
HLADRB5	+								10
INPP5D									26
MEF2C	+				+	+	+		295
MS4A cluster	+	+	+		+	+	+	+	714
NME8	+	+	+	+	+	+	+	+	270
PICALM	+		+	+	+	+			400
PTK2B	+	+			+	+	+	+	459
SLC24A4		+	+	+	+	+	+	+	533
SORL1	+		+	+	+	+	+	+	242
Region in chr 9	+	+	+	+	+	+	+	+	287
TREM2		+							2
TRIP4			+	+			+	+	49
ZCWPW1			+	+					32

Abbreviations: *APOE* = apolipoprotein E; CAA = cerebral amyloid angiopathy; CapAβ = capillary Aβ; CERAD = Consortium to Establish a Registry for Alzheimer Disease; M = moderate neuritic plaques; F = frequent neuritic plaques (moderate or frequent) logistic regression analysis vs no neuritic plaques.
Loci with associated variants ($p < 0.05$) marked with "+."
CapAβ (CAA type1) logistic regression analysis vs all other participants with[a] and without[b] APOE ε4 carrier status; CAA linear regression analysis; Braak stage (IV–VI) logistic regression analysis vs Braak stage (0–II).

After imputation of all 29 loci, associations were found with 24 loci. The 5 loci that did not show association with any neuropathologic feature were *CD33*, *CELF1*, *EPHA1*, *EXOC3L2*, and *INPP5D*. We found an association at $p < 0.05$ with a neuropathologic feature for 7 of the previously reported 44 index variants, while 9 other variants showed a trend at $0.05 < p < 0.10$ (table e-5). However, the genotyping rate of index variants was <95% for 14 variants.

CERAD score of neuritic plaques

In the SNP array data, we identified 8 loci that were associated with the CERAD score when adjusted for age at death and sex

Table 3 Associations in SNP array data between the CERAD score (CERAD score 0 vs M + F) and previously known AD risk loci (341 variants)

CERAD	Rs	*p* Value[a]	OR (95% CI)	*p* Value[b]	OR (95% CI)
***ABCG1* chr 21**	rs225443			0.03456	1.67 (1.04–2.67)
	rs183436	0.01556	1.78 (1.12–2.84)		
	rs225385	0.01347	0.56 (0.35–0.89)	0.02533	0.62 (0.41–0.94)
	rs2234718	0.01432	0.53 (0.31–0.88)	0.01775	0.56 (0.35–0.90)
***FERMT2* chr 14**	rs1112777	0.01733	0.56 (0.35–0.90)		
***MEF2C* chr 5**	rs187270	0.003969	1.99 (1.25–3.18)	0.006607	1.82 (1.18–2.81)
	rs190438	0.003805	2.20 (1.29–3.78)	0.003842	2.14 (1.28–3.58)
	rs34318	0.004267	1.98 (1.24–3.16)	0.009478	1.76 (1.15–2.71)
	rs412458	0.0005646	3.04 (1.62–5.71)	0.0004791	3.01 (1.62–5.59)
	rs661311	0.0005646	3.04 (1.62–5.71)	0.0004791	3.01 (1.62–5.59)
	rs700591	0.02687	1.69 (1.06–2.68)	0.01942	1.66 (1.09–2.54)
	rs700588	0.0002122	2.67 (1.59–4.49)	0.0003895	2.40 (1.48–3.88)
	rs160044	0.0002684	2.62 (1.56–4.38)	0.0004573	2.37 (1.46–3.83)
	rs10044342	0.0008274	3.65 (1.71–7.79)	0.001522	3.27 (1.57–6.8)
	rs3850653	0.0304	0.54 (0.31–0.94)		
	rs770463	0.02303	0.59 (0.37–0.93)	0.017	0.60 (0.40–0.91)
***MS4A* chr 11**	rs4939387			0.03929	0.44 (0.21–0.96)
	rs6591595	0.03037	0.47 (0.23–0.93)	0.003674	0.38 (0.20–0.73)
	rs2847212	0.04648	0.51 (0.27–0.99)	0.008148	0.44 (0.24–0.81)
	rs4939416	0.04648	0.51 (0.27–0.99)	0.008148	0.44 (0.24–0.81)
	rs474347			0.007831	0.44 (0.24–0.81)
***PICALM* chr11**	rs2077815	0.04248	0.55 (0.31–0.98)		
	rs10501604	0.0108	2.41 (1.23–4.74)	0.04079	1.98 (1.03–3.80)
	rs713346	0.02167	1.98 (1.11–3.55)		
	rs475639	0.01235	1.77 (1.13–2.76)		
	rs680119	0.013	0.58 (0.38–0.89)		
	rs642949	0.04878	1.63 (1.00–2.66)		
	rs10501608	0.01042	2.42 (1.23–4.76)	0.03225	2.05 (1.06–3.94)
***PTK2* chr 8**	rs2322606	0.01452	2.34 (1.18–4.61)	0.0109	2.30 (1.21–4.37)
	rs10097861	0.01611	2.20 (1.16–4.20)	0.01598	2.12 (1.15–3.91)
	rs1879188	0.007295	2.43 (1.27–4.65)	0.02249	2.01 (1.10–3.65)
	rs1879189	0.007295	2.43 (1.27–4.65)	0.02249	2.01 (1.10–3.65)
	rs3735759	0.01496	2.43 (1.19–4.95)	0.02571	2.17 (1.10–4.28)
	rs4733058	0.0406	0.55 (0.31–0.98)	0.02569	0.55 (0.33–0.93)

Continued

Table 3 Associations in SNP array data between the CERAD score (CERAD score 0 vs M + F) and previously known AD risk loci (341 variants) *(continued)*

CERAD	Rs	*p* Value[a]	OR (95% CI)	*p* Value[b]	OR (95% CI)
***SLC24A4* chr 14**	rs11623019	0.04134	0.58 (0.35–0.98)	0.03965	0.61 (0.37–0.97)
***SORL1* chr 11**	rs2298525	0.01498	0.37 (0.17–0.82)	0.02715	0.45 (0.22–0.91)

Abbreviations: *APOE* = apolipoprotein E; CERAD = Consortium to Establish a Registry for Alzheimer Disease; CI = confidence interval; M = moderate neuritic plaques; F = frequent neuritic plaques (moderate or frequent, n = 171) vs CERAD (no neuritic plaques, n = 65); OR = odds ratio; SNP = single nucleotide polymorphism.
Binary logistic regression analysis with and without adjustment for *APOE* ε4.
Binary logistic regression analysis adjusted for age at death and sex and with[a] and without[b] carrier status of the *APOE* ε4 allele.
All variants with $p < 0.05$ are shown.

but not for *APOE* ε4 (table 3). When *APOE* ε4 was included as a covariate, all these associations remained significant, and an additional association was detected with *FERMT2* (rs1112777, $p = 0.01733$, odds ratio [OR] 0.5587, 95% confidence interval [CI] 0.35–0.90). The strongest association was found between the CERAD score and the *MEF2C*—locus (rs700588) (when adjusted with age, sex, and *APOE* ε4, $p = 0.0002122$, OR 2.67, 95% CI 1.59–4.49 and without *APOE* ε4 $p = 0.0003895$, OR 2.40, 95% CI 1.48–3.88). *MEF2C* did not associate with any other histopathologic variables (Braak, CAA, and CapAβ).

In the imputed data, we identified 14 loci that were associated with the CERAD score (table 2, tables e-6 and e-7, http://links.lww.com/NXG/A16). The strongest association found was the same as in the SNP array data: rs700588 at *MEF2C*.

Braak stage

In the SNP array data, 6 loci were associated with a high Braak stage (IV–VI vs 0–II) (*APP*, *GALNT7*, *PTK2B*, *SLC24A*, *SORL1*, and *TRIP4*), and when adjusted for *APOE* ε4, associations were also found with *ABCG1* (table 4). Overall, the associations with the Braak stage were weaker than those with the CERAD score. The strongest association was found with *ABCG1* (rs532345, $p = 0.02671$, OR 0.5571, 95% CI 0.33–0.93 with *APOE* ε4 adjustment).

In the imputed data, we identified 15 loci that showed association with the Braak stage (*ABCA7*, *ABCG1*, *APP*, *CD2AP*,

Table 4 Associations in SNP array data between the Braak stage and previously known AD risk loci (341 variants) comparing participants with Braak stage IV–VI (n = 119) vs Braak stage 0–II (n = 74)

Braak	Rs	*p* Value[a]	OR (95% CI)	*p* Value[b]	OR (95% CI)
***ABCG1* chr 21**	Rs225443	0.0267	0.57 (0.34–0.94)		
	Rs532345	0.02671	0.56 (0.33–0.93)		
	Rs691687	0.04217	0.59 (0.35–0.98)		
***APP* chr 21**	Rs400603			0.04806	0.42 (0.18–0.99)
	Rs2830104	0.04174	1.93 (1.03–3.62)		
***GALNT7* chr 4**	Rs7658148			0.03616	0.63 (0.41–0.97)
***PTK2b* chr 8**	Rs2322606	0.04005	1.96 (1.03–3.73)	0.02477	1.96 (1.09–3.52)
	Rs10097861	0.04979	1.84 (1.00–3.40)		
	Rs1879188	0.033	1.94 (1.06–3.57)		
	Rs1879189	0.033	1.94 (1.06–3.57)		
***SLC24A4* chr14**	Rs2402130	0.02	2.24 (1.12–4.46)	0.03353	1.99 (1.06–3.76)
***SORL1* chr 11**	Rs1532763	0.048	0.59 (0.35–1.00)	0.0324	0.60 (0.37–0.96)
***TRIP4* chr15**	Rs936689			0.03045	2.24 (1.08–4.66)

Abbreviations: AD = Alzheimer disease; *APOE* = apolipoprotein E; CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism.
Binary logistic regression analysis with and without adjustment for *APOE* ε4.
Binary logistic regression analysis adjusted for age at death and sex and with[a] and without[b] carrier status of the *APOE ε4* allele for 341 variants.
All variants with $p < 0.05$ are shown.

Chr9 region, *CR1*, *GAB2*, *GALNT7*, *MEF2C*, *MS4A*, *NME8*, *PTK2B*, *SLC24A4*, *SORL1*, and *TRIP4*) (table 2, tables e-8 and e-9, http://links.lww.com/NXG/A16). All except for *MEF2C* showed association regardless of *APOE* ε4 adjustment. Rs2512518 at *GAB2* locus had the strongest association (p = 0.004372, OR 0.31, 95% CI 0.14–0.69) when adjusted for age and sex.

CAA

In the SNP array data, 7 loci were associated with CAA (*ABCA7*, *CR1*, *FERMT2*, *NME8*, *SLC24A4*, *SORL1*, and *ZCWPW1*), and when adjusted for *APOE* ε4, an additional locus (*GALNT7*) was found (table 5). The strongest association was with *CR1* (rs65087, p = 0.004934, β 2.52, 95% CI 0.78–4.26 without *APOE* ε4 adjustment); *CR1* and *ABCA7* were not associated with any other histopathologic variable than CAA.

In the imputed data, we identified 20 loci that were associated with CAA (table 2, tables e-10 and e-11, http://links.lww.com/NXG/A16). Fifteen loci were associated regardless of *APOE* ε4 adjustment (*ABCA7*, *ABCG1*, *APP*, *BIN1*, *CASS4*, Chr9 region, *CR1*, *FERMT2*, *HLA-DRB1*, *NME8*, *PICALM*, *SLC24A4*, *SORL1*, *TRIP4*, and *ZCWPW1*), 4 loci when adjusted for age, sex, and *APOE* ε4 (*CD2AP*, *CLU*, *GALNT7*, and *MS4A* locus) and 1 locus (*GAB2*) when adjusted for age and sex. The strongest association was found for rs185310342 at *CR1* locus (p = 7.17E-07, β 14.4, 95% CI 8.88–20) when adjusted for age and sex.

CapAβ

In SNP array data, 4 loci were associated with CapAβ (*APP*, *BIN1*, *MS4A*, and *PTK2B*), and when adjusted for *APOE* ε4, age, and sex, 3 additional loci were associated with CapAβ (*GALNT7*, *NME8*, and *FERMT2*, table 6). The strongest

Table 5 Associations in SNP array data between CAA and previously known AD risk loci (341 variants) adjusted for age at death and sex and with and without carrier status of the *APOE ε4* allele

CAA	Rs	*p* Value[a]	β (95% CI)	*p* Value[b]	β (95% CI)
***ABCA7* chr 19**	rs3752240	0.007962	−1.97 (−3.41 to −0.53)	0.01813	−1.86 (−3.38 to −0.33)
	rs2279796	0.04255	1.44 (0.06 to 2.82)		
***CR1* chr 1**	rs11117959	0.03422	1.83 (0.15 to 3.52)	0.004963	2.53 (0.78 to 4.27)
	rs650877	0.03592	1.81 (0.13 to 3.49)	0.004934	2.52 (0.78 to 4.26)
	rs11118131	0.03592	1.81 (0.13 to 3.49)	0.004934	2.52 (0.78 to 4.26)
	rs677066			0.01169	2.09 (0.48 to 3.70)
	rs6691117			0.01169	2.09 (0.48 to 3.70)
	rs12734030			0.03786	1.95 (0.12 to 3.78)
***FERMT2* chr14**	rs8007536	0.008585	3.29 (0.86 to 5.72)	0.04077	2.701 (0.13 to 5.28)
	rs4901318	0.009914	2.79 (0.69 to 4.89)	0.04571	2.28 (0.05 to 4.50)
***GALNT7* chr 4**	rs10001613	0.04873	−1.46 (−2.90 to −0.02)		
***NME8* chr 7**	rs2722301	0.01244	2.13 (0.47 to 3.79)	0.01564	2.18 (0.42 to 3.93)
***SLC24A4* chr 14**	rs4904896	0.03997	−1.51 (−2.95 to −0.08)	0.01082	
	rs4904903			0.03422	−1.90 (−3.64 to −0.15)
	rs10498633	0.04763	1.78 (0.03 to 3.53)	0.04017	1.946 (0.10 to 3.80)
***SORL1* chr 11**	rs661057	0.02727	−1.74 (−3.27 to −0.20)		
	rs676759	0.04524	−1.54 (−3.04 to −0.04)		
	rs666004	0.03078	−1.62 (−3.08 to −0.16)	0.01868	−1.86 (−3.40 to −0.32)
	rs3781827	0.01306	1.87 (0.40 to 3.33)	0.008359	2.09 (0.55 to 3.64)
***ZCWPW1* chr 7**	rs5015756	0.009438	−1.89 (−3.31 to −0.47)	0.02247	−1.76 (−3.26 to −0.26)

Abbreviations: AD = Alzheimer disease; *APOE* = apolipoprotein E; CAA = cerebral amyloid angiopathy; CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism.
The severity of CAA is expressed using the percentage of blood vessels with CAA of all blood vessels visible in the tissue slide.
Linear regression analysis adjusted for age at death and sex and with[a] and without[b] carrier status of the *APOE* ε4 allele for 341 variants (N = 256).
All variants with $p < 0.05$ are shown.

Table 6 Associations in SNP array data between CapAβ and previously known AD risk loci (341 variants)

CapAβ	Rs	*p* Value[a]	OR (95% CI)	*p* Value[b]	OR (95% CI)
***APP* chr 21**	Rs1783016	0.005933	2.01 (1.22–3.30)	0.02242	1.67 (1.08–2.61)
	Rs214488	0.0259	0.52 (0.29–0.92)		
	Rs383700	0.03333	0.34 (0.13–0.92)	0.0479	0.40 (0.16–0.99)
	Rs2014146	0.03392	0.61 (0.38–0.96)		
	Rs2830000	0.02793	0.53 (0.30–0.93)		
	Rs466609			0.03013	0.40 (0.18–0.92)
***BIN1* chr 2**	Rs873270	0.01358	0.43 (0.22–0.84)	0.01855	0.54 (0.31–0.97)
***FERMT2* chr 14**	Rs1112777	0.04187	0.62 (0.39–0.98)		
	Rs7494379	0.01501	1.78 (1.12–2.83)		
***GALNT7* chr 4**	Rs2332655	0.03211	0.62 (0.39–0.96)		
	Rs10001613	0.01362	0.57 (0.36–0.89)		
	Rs12644699	0.04208	0.56 (0.32–0.98)		
***MS4A* chr 11**	Rs2847212			0.04211	0.47 (0.23–0.97)
	Rs4939416			0.04211	0.47 (0.23–0.97)
***NME8* chr 7**	Rs10488617	0.04465	0.58 (0.34–0.99)		
***PTK2b* chr 8**	Rs6986075	0.02349	1.67 (1.07–2.59)	0.027	1.60 (1.09–2.34)

Abbreviations: AD = Alzheimer disease; *APOE* = apolipoprotein E; CapAβ = capillary Aβ; CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism.
Comparison between participants with CapAβ (n = 77) and all other participants (n = 179) using binary logistic regression analysis.
Binary logistic regression analysis adjusted for age at death and sex and with[a] and without[b] carrier status of the *APOE* ε4 allele for 341 variants.
All variants with $p < 0.05$ are shown.

association was found with *APP* (rs1783016, $p = 0.005933$, OR 2.01, 95% CI 1.22–3.30 with *APOE* ε4 adjustment).

In imputed data, we found association for 15 loci (table 2, tables e-12 and e-13, http://links.lww.com/NXG/A16). Of these, 9 were associated regardless of *APOE* ε4 adjustment (*APP*, *BIN1*, *FERMT2*, *GALNT7*, *HLA-DRB1*, *MS4A*, *NME8*, *PTK2B*, and Chr9 region), 3 loci (*MEF2C*, *PICALM*, and *SORL1*) when adjusted for age, sex, and *APOE* ε4, and 3 loci (*CR1*, *SLC24A4*, and *TREM2*) when adjusted for age and sex. The strongest association was found for rs66962766 at *HLA-DRB1* locus ($p = 0.002594$, OR 0.54, 95% CI 0.37–0.81).

Discussion

In this study, we focused on previously reported genetic AD risk loci identified in GWAS analyses on clinically or neuropathologically verified patients with AD and controls. We confirmed the association of 24 of the 29 previously known AD risk loci with one or more AD-related neuropathologic features (CERAD, Braak, CAA, and CapAβ) (table 2).

In this study, we found strong associations between *APOE* ε4 and all AD-related neuropathologic features (CERAD, Braak, CAA, and CapAβ, table 1). This is in line with previous studies.[4,21] To take into account the strong effect of *APOE* on the other loci,[30] we performed analyses in 2 ways, testing each neuropathologic feature with and without the adjustment for the *APOE* ε4 carrier status. Previously, certain loci have been reported to be more likely influenced by *APOE ε4* than others; e.g., *PICALM* and *EXOC3L2* have been found to show stronger associations with neuropathologically confirmed AD without *APOE* ε4 adjustment, whereas adjustment with *APOE* was reported to have no effect on the association between *CR1*, *CLU*, or *BIN1* and neuropathologic AD.[30] We found that the *APOE* adjustment did not remarkably alter the associations between neuropathologic features in most loci (table 2).

In addition to *APOE*, *APP*, Chr9 region, *NME8*, *PICALM*, and *SLC24A4* were associated with all neuropathologic variables (CERAD, Braak, CAA, and CapAβ) (table 2).

On the other hand, certain loci were associated only with specific neuropathologic features. *CASS4*, *CLU*, and *ZCWPW1* were associated only with CAA. *TREM2* and *HLA-DRB5* were associated with only CapAβ, whereas *HLA-DRB1* was associated with both CAA and CapAβ but not with other pathologies (table 2). These are interesting findings suggesting that the risk loci (and mechanisms) for CAA and CapAβ may be partially distinct. It is of note that there has

been only 1 previous GWAS that investigated the genetic background of CAA, in which the only significant association was found between CAA and the *APOE* locus.[4] However, in a previous candidate gene analysis, *CR1* was associated with CAA pathology burden.[31] The association between CAA and *CR1* was confirmed in our study. No previous GWAS has been performed using CapAβ as the phenotype. Here, we reported significant associations between CapAβ and 15 loci (*APP*, *BIN1*, Chr9 region, *CR1*, *FERMT2*, *GALNT7*, *HLA-DRB1*, *MEF2C*, *MS4A*, *NME8*, *PICALM*, *PTK2B*, *SLC24A4*, *SORL1*, and *TREM2*). Our results provide information on the partly shared and partly distinct genetic backgrounds of AD-related neuropathologic features.

Author contributions

Mira Mäkelä and Karri Kaivola: acquisition of data, analysis and interpretation of data, and drafting the manuscript. Miko Valori: acquisition of data and analysis and interpretation of data. Anders Paetau and Tuomo Polvikoski: acquisition of data and critical revision of the manuscript for intellectual content. Andrew B. Singleton: acquisition of data and analysis and interpretation of data. Bryan J. Traynor: acquisition of data and critical revision of the manuscript for intellectual content. David J. Stone and Terhi Peuralinna: acquisition of data and analysis and interpretation of data. Pentti J. Tienari: analysis and interpretation of data, study concept, and design and critical revision of the manuscript for intellectual content. Tanskanen Maarit: acquisition of data and critical revision of the manuscript for intellectual content. Liisa Myllykangas: analysis and interpretation of data, supervision, study concept and design, and critical revision of the manuscript for intellectual content.

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