LETTER TO JMG

Insulin-degrading enzyme is genetically associated with Alzheimer's disease in the Finnish population

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The gene for insulin-degrading enzyme (*IDE*), which is located at chromosome 10q24, has been previously proposed as a candidate gene for late-onset Alzheimer's disease (AD) based on its ability to degrade amyloid β-protein. Genotyping of single nucleotide polymorphisms (SNPs) in the *IDE* gene in Finnish patients with AD and controls revealed SNPs rs4646953 and rs4646955 to be associated with AD, conferring an approximately two-fold increased risk. Single locus findings were corroborated by the results obtained from haplotype analyses. This suggests that genetic alterations in or near the *IDE* gene may increase the risk for developing AD.

Alzheimer's disease (AD) is characterised by the progressive and severe accumulation of amyloid β-protein (Aβ) in the brain, and insulin-degrading enzyme (product of the *IDE* gene) is a possible candidate enzyme responsible for the degradation and clearance of Aβ.[1 2] *IDE* is located at chromosome 10q24, close to a region for which linkage and association with late-onset AD has been previously described.[3] In addition, genetic linkage was simultaneously reported on chromosome 10 in a region roughly 30 Mb from *IDE*.[4 5] Numerous subsequent case–control studies have reported genetic association between *IDE* and AD, but others have failed to observe significant effects (http://www.alzgene.org).

Because *IDE* is an excellent candidate on both positional and functional grounds, we investigated whether single nucleotide polymorphisms (SNPs) in the *IDE* gene or in nearby regions were associated with AD in the eastern Finnish population. Two of the six SNPs tested showed significantly different allele and genotype distributions between AD and control cohorts. These findings were corroborated by the results of haplotype analyses.

METHODS

All patients fulfilled the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) clinical criteria for probable AD without significant vascular contribution for dementia.[6] The study group consisted of 370 patients with AD (mean (SD) age at onset 72 (7) years; range 43 to 90 years; 69% women) and 454 healthy control subjects (70 (5) years; range 60 to 87 years; 61% women). In the AD cohort, 12% and 25% of patients had history of diabetes and hypertension, respectively. In total, 46% of patients with AD had a positive family history of AD, but did not show conclusive evidence of autosomal dominant transmission.[7] Controls had no signs of dementia as determined by interview and neuropsychological testing. There were 61 patients with AD (16%) and 116 controls (26%) with an age of onset or age at examination of $\leqslant$65 years.

Five non-coding SNPs in IDE and one SNP in the gene encoding haematopoetically expressed homeobox protein (*HHEX*) were selected for genotyping, based on previous studies.[8 9] Genotyping of SNPs were performed using either a mini-sequencing technique (SNaPshot Multiplex Ready Reaction Mix; Applied Biosystems, Foster City, California, USA) or by high-efficiency fluorescence polarisation detected single-base extension (rs1544210 and rs2251101) (Criterion Analyst AD High-Throughput Fluorescence Detection System; Molecular Devices, Sunnyvale, California, USA). Apolipoprotein E (*APOE*) genotyping was performed as previously.[7]

Statistical analysis

Single locus allele, genotype and logistic regression analyses were carried out using SPSS V.11.0 (SPSS Inc., Chicago, IL, USA). Pairwise linkage disequilibrium (LD), Hardy-Weinberg equilibrium, haplotype estimation and haplotype block structure analyses were performed with Haploview V. 3.2 (http://www.broad.mit.edu/mpg/haploview/). Statistical significance was set at $p<0.05$.

RESULTS

The *APOE* e4 allele was significantly over-represented among the 370 patients with AD compared with the 454 control subjects ($p<0.001$; OR 4.8; 95% CI 3.8 to 6.1) (table 1). The minor allele frequency of the *IDE* intron 20 SNP (12973709) was $\leqslant$0.01 in both the AD and control cohorts, which led us to exclude this SNP from further analysis.

SNPs rs3758505, rs4646953 and rs4646955, located within the same 40 kb region in the 5′ half of *IDE*, were in strong LD (D′ values $\geqslant$0.994), and originated from the same haplotype block. Comparison of the genotype and allele distribution of SNPs between the AD and control cohorts showed that rs4646953 and rs4646955 showed nominal evidence of association with AD (table 1). When the sample was stratified based on onset age using 65 years as the cut-off point, nominal genotype and allele association of rs4646953 and rs4646955 were observed for patients with late-onset, but not those with early-onset AD (data not shown). SNPs rs4646953 and rs4646955 conferred an approximately two-fold increased risk for AD in logistic regression analyses in both whole and late-onset cohorts (table 2). Stratification according to gender and *APOE* status did not reveal any major differences between subgroups for either of these SNPs.

Using SNPs rs3758505, rs4646953 and rs4646955 (5′ haplotype block), four haplotypes were identified (table 3). Global association test with TTT, TCC, and pooled (frequencies $\leqslant$0.05) haplotypes revealed a borderline association with AD ($p = 0.06$). Assessment of individual haplotype distributions showed that the TTT may be significantly over-represented in the AD cohort (nominal $p = 0.03$). Conversely, haplotype TCC was under-represented among patients with AD (nominal

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; IDE, insulin-degrading enzyme; LD, linkage disequilibrium; SNP, single nucleotide polymorphism

Table 1 Allele and genotype frequencies of *IDE* and *HHEX* SNPs

NCBI rs number and location (kb)*	Allele	Allele frequency Control (n = 908)	AD (n = 740)	p Value for allele‡	Genotype	Genotype frequency Control (n = 454)	AD (n = 370)	p Value for genotype‡
rs1544210† *HHEX* (0)	A	0.566	0.535	0.21	AA	0.322	0.300	0.32
	G	0.434	0.465		AG	0.476	0.446	
					GG	0.192	0.232	
rs3758505 *IDE* 5′-UTR (153)	G	0.052	0.053	0.93	GG	0.004	—	0.48
	T	0.948	0.947		GT	0.095	0.105	
					TT	0.901	0.895	
rs4646953 *IDE* 5′-UTR (154)	C	0.338	0.284	0.02 (0.10)	CC	0.126	0.076	0.04 (0.20)
	T	0.662	0.716		CT	0.425	0.416	
					TT	0.449	0.508	
rs4646955 *IDE* Intron 3 (194)	C	0.380	0.327	0.03 (0.15)	CC	0.152	0.095	0.04 (0.20)
	T	0.620	0.673		CT	0.456	0.465	
					TT	0.392	0.441	
rs2251101 *IDE* 3′UTR (276)	C	0.177	0.174	0.87	CC	0.030	0.027	0.87
	T	0.823	0.826		CT	0.289	0.295	
					TT	0.678	0.678	

*Distance from *HHEX* SNP rs1544210. All SNPs were in Hardy-Weinberg equilibrium in both cases and controls ($p>0.1$).
†Patients with AD (n = 362), controls (n = 449).
‡Allele and genotype frequencies were compared using two-sided Pearson's χ^2 and Fisher's exact tests, respectively. p Values were correct for multiple testing using Bonferroni correction (in parentheses).
AD, Alzheimer's disease; SNP, single nucleotide polymorphism.

Table 2 Age, gender and *APOE* status adjusted odds ratios obtained from univariant and multivariant logistic regression for *IDE* and *HHEX* SNPs

SNP	OR (95% CI) univariate Whole population	>65 years	OR (95% CI) adjusted for age, gender and *APOE* Whole population	>65 years
rs1544210 AA+AG vs GG	1.09 (0.81 to 1.47)	1.25 (0.89 to 1.74)	1.02 (0.72 to 1.44)	1.19 (0.81 to 1.76)
rs3758505 GG+GT vs TT	0.93 (0.59 to 1.47)	0.88 (0.53 to 1.45)	1.01 (0.60 to 1.70)	0.96 (0.53 to 1.75)
rs4646953 CT+TT vs CC	1.75 (1.09 to 2.82)*	2.21 (1.29 to 3.80)**	2.08 (1.20 to 3.60)**	3.00 (1.58 to 5.69)*
rs4646955 CT+TT vs CC	1.72 (1.11 to 2.64)*	2.08 (1.29 to 3.36)**	1.95 (1.18 to 3.23)**	2.58 (1.45 to 4.57)**
rs2251101 CT+CC vs TT	1.00 (0.75 to 1.34)	1.00 (0.72 to 1.39)	1.10 (0.78 to 1.54)	1.11 (0.75 to 1.63)

SNP, single nucleotide polymorphism.
*$p<0.05$; **$p<0.01$.

p = 0.02). The frequencies of the TCC and TTT haplotypes were similar in the patients with early-onset and those with late-onset AD compared with the whole cohort, but the distribution of these haplotypes was significantly different from patients with late-onset AD compared with age-matched controls (TCC nominal p = 0.03 and TTT nominal p = 0.03).

DISCUSSION

Our data from both single locus and haplotype association analyses lend further support to *IDE* being a susceptibility gene for AD. The approximately two-fold risk increase observed for rs4646953 and rs4646955 in an eastern Finnish population appeared to be independent of gender, *APOE* status and age. Several case-control studies have reported that genetic variants in *IDE* increase the risk for AD (http://www.alzgene.org). To date, there appear to be more negative than positive findings, and at the time of writing (20 April 2007) none of the continuously updated meta-analyses conducted as part of the AlzGene database show significant risk effects for any of the *IDE* SNPs. This situation is not uncommon in genetically heterogeneous and complex diseases, for which study populations often stem from different genetic backgrounds and ethnic groups. The control allele and genotype frequencies of all the SNPs studied here differed from those previously observed in Caucasians, probably owing to the isolated genetic background of the Finnish population. Interestingly though, our study is in line with analyses from a Chinese case–control sample, which also found significant association between rs4646953 and AD risk, albeit with the opposite allele.[10] Moreover, we observed the *IDE* risk effect among the unstratified cohort and not in the *APOE* e4 carriers, as observed in the Chinese study. Taken together, our findings and those of previous reports suggest the presence of an AD risk-modifying variant in the *IDE* gene. More studies are warranted to investigate these associations and their functional implications further, and to evaluate the potential of using IDE as a therapeutic tool in the prevention and treatment of AD.

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Table 3 Haplotype frequencies of IDE SNPs rs3758505, rs4646953 and rs4646955 in patients with AD and controls

IDE haplotypes for SNPs rs3758505, rs4646953 and rs4646955	Control (n = 908 alleles)	AD (n = 740 alleles)	p Value
TCC	0.34	0.28	0.02
TTT	0.57	0.62	0.03
GTT	0.05	0.05	0.93
TTC	0.04	0.04	0.94
Overall			0.06

AD, Alzheimer's disease; SNP, single nucleotide polymorphism.

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