

Evidence against association of the FE65 gene (APBB1) intron 13 polymorphism in Alzheimer's patients

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Received 15 September 2000; received in revised form 12 October 2000; accepted 20 October 2000

Abstract

A genetic polymorphism in intron 13 of the FE65 gene (APBB1) was reported to be associated with Alzheimer's disease (AD). Our analyses of this polymorphism, both in a family-based or a case-control sample, fail to support the association between the FE65 intron 13 polymorphism and AD. We performed the sibship disequilibrium test (SDT, $P = 0.77$) and the sib transmission/disequilibrium test (Sib-TDT, $P = 0.56$) in a family-based study which included 526 subjects from 158 sibships. In addition, we compared the genotype and allele frequencies of this biallelic polymorphism in 311 AD patients to those of a control group consisting of 260 subjects and found no significant difference (χ^2 , $P = 0.847$ and $P = 0.586$, respectively). Furthermore, our two-point linkage analysis in a family-based sample was in agreement with a genome wide scan for linkage to AD and showed no evidence for linkage to the short arm of chromosome 11 where the FE65 gene is located. We conclude that the association of the FE65 intron 13 polymorphism with AD, if any, is smaller than previously reported. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Alzheimer's disease; FE65; APBB1; Polymorphism; Family-based association; Case control sample

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by age-dependent cognitive decline. The post-mortem identification of both amyloid plaques and neurofibrillary tangles in the hippocampus and cortex are confirmation of clinical diagnosis of AD dementia. Genetic factors play a significant role in both early-onset (<65 years) and late-onset AD (= 65 years). Mutations in three different genes, PS1, PS2 and amyloid precursor protein (APP), account for ~40% of early-onset AD while genetic risk factors and/or modifiers such as apolipoprotein (ApoE) are associated with late-onset AD.

Increased β -amyloid peptide (A β) production is associated with mutations in the presenilin and APP genes [18]. Therefore, candidate genes for AD include those genes coding for proteins that have the potential to alter either production or clearance of the amyloid peptide (A β). Studies examining the consequence of cellular over-

expression of members of the FE65 family have shown that these proteins can alter APP processing [9,17]. Notably, overexpression of FE65 in Madin–Darby Canine Kidney (MDCK) cells increases the amount of A β recovered in conditioned media [17]. Members of the FE65 protein bind the cytoplasmic domain of APP at the clathrin-coated pit internalization sequence [6,9,10,20]. This motif is involved in APP sorting into the endocytic pathway where a significant proportion of A β is produced [16].

In a recent study that screened for mutations/polymorphisms in the FE65 gene (APBB1, [5]), a biallelic polymorphism was identified in intron 13 which interrupts the FE65 exons coding for the APP-binding domain phosphotyrosine interaction domain (PID2) [12]. The minor allele of this biallelic polymorphism usually occurs as a haplotype, containing both a trinucleotide deletion (CTA) occurring at positions 6–8 from the donor site and a single basepair substitution in the branch point sequence of intron 13. The CTA deletion was reported to be associated with a decreased risk for AD in a case-control sample [12] and is the polymorphism tested in this study. In view of these

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findings, we tested for a genetic association of the FE65 intron 13 CTA deletion and AD in a family-based association study using the National Institute of Mental Health (NIMH) Genetics Initiative AD sample [2]. We also extended our study to include a case-control sample because the initial report for the association of this polymorphism with AD was performed in a case-control study.

The collection of AD families under the NIMH Genetics Initiative was previously described [3]. The total NIMH sample used for linkage and genotype frequency estimations in this study included 795 subjects, 576 affected and 219 unaffected, from 291 families. The NIMH sample used for association tests was a subset of the total sample containing 526 subjects from 158 families consisting of discordant sibships. The average age of onset of affected individuals was 69.9, SD 8.3. Autopsy confirmation of clinical diagnosis in the NIMH sample is 94% AD. The case-control sample collected by the Massachusetts Alzheimer's Disease Research Center (ADRC) included DNA collected from blood or brain tissue from 311 AD patients (95 were neuropathologically confirmed for AD) and a control group comprising 260 subjects [7]. The mean age of onset for the clinically diagnosed cases was 70.4, SD 8.8 ($n = 251$) while controls had a mean age of 68.6, SD 12.2 (84% of sample). Within the control group there are 193 subjects with no neurological symptoms or pathologies and 67 non-AD neurological disease controls. Genotypes were in Hardy–Weinberg equilibrium for both cases and controls.

Genotyping of the FE65 intron 13 polymorphism was performed either by digestion of the 178 bp polymerase chain reaction (PCR) product obtained using the following primers, 5'-CACATGTTCTGGTGCGAGC-3' and 5'-AACGGGCATCCAGACTTC-3', with the *SpeI* restriction enzyme as previously described [12] or by separation of the ³²P-labeled PCR products on polyacrylamide gels. The minor allele (FE65 2-allele following the nomenclature of Hu et al. [12]) can be seen as a 178 bp PCR product because the CTA deletion in intron 13 destroys the *SpeI* restriction enzyme site.

Two family-based association tests were used to test for the association of the intron 13 polymorphism with AD in the NIMH families: the sibship disequilibrium test (SDT) [11] and the sib transmission/disequilibrium test (sib-TDT) [19]. In the SDT, the average number of candidate alleles between affected and unaffected siblings is compared within each family, while the Sib-TDT compares the allele distribution in discordant sibpairs. In addition, we performed conditional logistic regression analyses using the FE65 2-allele and the Apoε4-allele carrier status within families to examine the independent and combined contribution of these genes. We also performed parametric two-point linkage analyses (using FASTLINK) using two autosomal disease models as previously described [4]. The ADRC case-control sample was analyzed by comparing allele and genotype frequencies using χ^2 -tests comparing cases and controls.

In our study using the NIMH discordant sibship sample, we found no evidence of conferred risk due to the presence of the FE65 1-allele (Table 1). Both the sibship disequilibrium test (SDT) and the sibling transmission disequilibrium test (S-TDT) failed to reveal an association between the FE65 intron 13 polymorphism and late-onset AD ($Z = 0.09$, $P = 0.77$ and $Z = 0.33$, $P = 0.56$, respectively). In the same sample the SDT results showed a highly significant association between ApoE and AD ($Z = 28.4$, $P = 0.000001$) consistent with previous reports for the NIMH sample [3]. The Maentel–Haenzel odds ratio for the effect of carrying the FE65 2-allele on risk for AD was 1.251 (95% confidence interval (CI) = (0.691, 2.263)). Conditional logistic regression analyses adjusted for the effect of the Apo-ε4 did not significantly alter the risk ratio (1.267, 95% CI = (0.690, 2.324)). In addition, we repeated the SDT analyses restricting the sample to siblings with age of onset (in affected) or age (in unaffected) greater than 70 or 75. These two age-restricted analyses were no different than the analysis on the full sample, and gave no evidence of association between the FE65 intron 13 polymorphism and AD.

Furthermore, we find no evidence for linkage to the region of the short arm of chromosome 11 using parametric two-point linkage analyses of the total NIMH sample (798 subjects from 291 families).

In contrast to the published case-control study of Hu et al. [12] in which 267 control individuals and 190 AD patients were examined, we determined the FE65 intron 13 genotypes of 260 control individuals and 311 AD patients (Table 2). The frequencies of the FE65 intron 13 alleles were similar in the control populations from these two studies (Table 2). However, our results did not show any significant difference in the allele frequency between cases and controls (Table 2). We also examined age stratified FE65 2-allele frequencies and found no significant differences between cases and controls.

In this study, we have used both population- and family-based samples to examine the previously reported association of the FE65 intron 13 polymorphism with AD. Our data do not support an association of the FE65 intron 13 polymorphism with AD.

The discrepancy between our case-control study results which failed to show an association of the FE65 intron 13 polymorphism with AD and the previously published case-

Table 1
Genotype frequencies of the APBB1 (FE65) polymorphism in the NIMH Genetics Initiative AD samples

Genotype	Affected ^a $n = 576$ (%)	Unaffected ^a $n = 219$ (%)
1,1	426 (73.9)	164 (74.9)
1,2	136 (23.6)	48 (21.9)
2,2	14 (2.4)	7 (3.2)

^a Genotype and allele frequencies are not independent in family-based samples.

Table 2
Allele and genotype frequencies of the APBB1 intron 13 polymorphism in a case-control sample^a

	Cases		Controls	
	This study (<i>n</i> = 311) (%)	Ref. [12] (<i>n</i> = 190) (%)	This study (<i>n</i> = 260) (%)	Ref. [12] (<i>n</i> = 267) (%)
<i>Genotype</i>				
1,1	212 (68.2)	157 (82.6)	182 (70.0)	188 (70.4)
1,2	90 (28.9)	29 (15.3)	72 (27.7)	72 (27.0)
2,2	9 (2.9)	4 (2.1)	6 (2.3)	7 (2.6)
<i>Allele frequency</i>				
1	514 (82.6)	343 (90.3)	436 (83.8)	448 (83.9)
2	108 (17.4)	37 (9.7)	84 (16.2)	86 (16.1)

^a Alleles: $\chi^2 = 0.296$, $P = 0.586$, genotypes: $\chi^2 = 0.332$, $P = 0.847$ in cases vs. controls.

control result [12] might be due to population admixture and/or Type I error. Given that we also failed to observe an association of the FE65 intron 13 polymorphism with AD in our family-based association study, which is not susceptible to population admixture, it is likely that the contribution of this polymorphism to the aetiology of AD is smaller than previously reported. Our negative results for linkage to the short arm of chromosome 11, using parametric two-point linkage analyses of the NIMH sample, are consistent with our findings. Furthermore, no evidence for linkage to the short arm of chromosome 11 was found in a recent whole genome scan of affected sib-pairs in the NIMH sample [13].

Interestingly, because of its proximity to the cathepsin D gene (*CTSD*) on the short arm of chromosome 11, it has been suggested that the FE65 gene might account for the association of the *CTSD* exon 2 polymorphism with AD [15]. However, our data do not confirm either the association of the FE65 intron 13 polymorphism or the *CTSD* exon 2 polymorphism with AD [1]. Furthermore, the genetic distance between the two genes is estimated to be 10.03 cM, which is roughly equivalent to 10 Mb (The genetic location database, <http://www.cedar.genetics.soton.ac.uk>). In an association study examining *APOE* as a susceptibility locus for AD in which single nucleotide polymorphisms around the *APOE* gene were examined, the maximum distance from the *APOE* gene for which an association was detectable was 440 kb [14]. In one extreme case, background linkage disequilibrium between pairs of marker loci in a genetically homogeneous population was reported at a genetic distance of 5.51 cM (approximately 5.5 Mb) [8]. Taken together, these findings suggest that linkage disequilibrium between the FE65 and the *CTSD* gene is unlikely.

Despite our negative findings, it remains possible that there are other polymorphisms within or near the FE65 gene that are associated with AD but are not in linkage disequilibrium with the one reported here. In support of this notion, a recent study testing ten single nucleotide polymorphisms (SNPs) in the region surrounding the ApoE locus for allelic association with AD showed that only the APOC1 SNP, which is in linkage disequilibrium with ApoE, gave highly significant evidence of association with AD in

both case-control and family-based samples [14]. Finally, the discrepancy between the results of our study and the published report [12] may be due to the degree to which this polymorphism is in linkage disequilibrium with a possible underlying 'causative' DNA variation in the two populations studied.

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