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ARTICLE

Genetic association of the presenilin-1 regulatory region with early-onset Alzheimer's disease in a population-based sample

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Genetic association has been reported between a di-allelic polymorphism in intron 8 of presenilin-1 (*PSEN1*) and Alzheimer's disease (AD) in some studies but not in others. In a population-based series of 102 patients with early onset AD and 118 community controls we examined whether polymorphisms in linkage disequilibrium with intron 8 of *PSEN1* may explain the association. In addition to the intron 8 polymorphism ($P = 0.05$), a promoter polymorphism ($P = 0.03$) and the simple tandem repeat (STR) polymorphism D14S1028 located upstream of *PSEN1* ($P = 0.04$) were found to be marginally significantly associated to AD. When excluding PSEN1 mutation cases ($n = 6$), the intron 8 association was explained by linkage disequilibrium to the dominant *PSEN1* mutations. In the non-mutation cases, the weak associations between the polymorphisms in the regulatory region remained. Our study suggests that a polymorphism/mutation in the promoter or regulatory region of *PSEN1* rather than the polymorphism in intron 8 of *PSEN1* is associated with early onset AD.

Keywords: Alzheimer's disease; early-onset; presenilin-1; genetic association

Introduction

PSEN1 is a gene involved in autosomal dominant forms of early onset Alzheimer's disease (AD).[1] In addition to the dominant mutations, genetic association was reported between a di-allelic polymorphism in intron 8 of *PSEN1* and AD, in which a twofold increase of the 11 genotype was observed.[2] The association was confirmed in a number of case series,[3–7] but not in others.[8–14] No evidence was found for an influence of the *PSEN1* intron 8 polymorphism on the amount or molecular form of β amyloid deposition, one of the main pathological characteristics of AD, suggesting the absence of a functional relation to AD.[15] Until now, there has been little evidence for genetic variability in *PSEN1* that could account for the association with the *PSEN1* intron 8 polymorphism.[16] We conducted a study of polymorphisms flanking *PSEN1* in a population-based series of 102 patients with early onset AD and 118 community controls.

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Table 1 Allele and genotype frequencies of polymorphisms in *PSEN1* in early-onset AD cases and controls

			Cases		*Controls*		
Polymorphism			*n*	%	*n*	%	*P value*
Promoter	Allele	1	194	95	209	89	0.03
		2	10	5	25	11	
	Genotype	11	93	91	94	80	0.04
		12	8	8	21	18	
		22	1	1	2	2	
5′UTR	Allele	1	168	82	187	83	0.75
		2	38	18	39	17	
	Genotype	11	3	3	3	3	0.95
		12	32	31	33	29	
		22	67	66	77	68	
Intron 8	Allele	1	90	45	127	54	0.05
		2	110	55	107	46	
	Genotype	11	26	26	34	29	0.03
		12	38	38	59	50	
		22	35	36	24	21	
3′UTR	Allele	1	172	86	195	84	0.57
		2	28	14	37	16	
	Genotype	11	74	74	83	72	0.79
		12	24	24	29	25	
		22	2	2	4	3	

Subjects and Methods

Patients ($n = 102$) were derived from a population-based epidemiological study of early-onset AD.[17] Within two areas of the Netherlands, the study aimed at a complete ascertainment of all AD patients in whom the disease onset was at or before the age of 65 years. For this study, the clinical diagnosis of AD was independently confirmed by two neurologists using a standardised protocol according to the NINCDS-ADRDA criteria for AD. Family history of dementia addressed all first, second and third degree relatives of the patients. Patients who were known to be related were excluded from the association studies presented here. The mean age at onset of the patients was 56.7 ± 5.4 years and the mean attained age at the time of the study was 63 ± 4.4 years. Patients were compared to an age-matched control series ($n = 118$; mean age 63 ± 4.4 years) that was drawn randomly from the Rotterdam Study.[17,18] According to the family histories (up to 2 degrees), these subjects were not related. None of the control subjects showed symptoms of dementia and none had cognitive test scores suspect for dementia.[17,18]

All patients were previously subjected to mutation analysis of exons 16 and 17 of the amyloid precursor protein gene (APP)[19] and all exons of *PSEN1* and presenilin-2 (*PSEN2*).[16] No APP mutations were found, whereas six patients carried a *PSEN1* mutation and one a *PSEN2* mutation.[16] For *PSEN2*, three polymorphisms in exons 3 and 4 and intron 11[16] and STR marker D1S479 located near PSEN2[20] were analysed to exclude the possibility of genetic association. Allele and genotype distributions of *PSEN2* polymorphisms were similar in cases and controls. The frequency of the ε4 allele of the apolipoprotein E gene (*APOE*), the most important susceptibility gene for AD in the general population,[21] was 2.3 times increased in patients compared to controls.[17]

In this study, four di-allelic *PSEN1* polymorphisms in the promoter region, the 5' untranslated region (UTR),[16] intron 8[2] and the 3'UTR, respectively, (this study) were analysed. The 3'UTR polymorphism was detected using PCR primers 3UTR7 (5'-AAACAGTACAGCTATTTCTCATCA-3') and 3UTR8 (5'-GCTTCAACAGCCATTTTACTC-3') to amplify an A to G polymorphism at nucleotide 952 in the 3'UTR of *PSEN1*. The amplification products were digested with 5 U *Nla*III resulting in a constant fragment of 37 bp and 2 alleles of 308 bp (G-allele) and 162 + 146 bp (A-allele), respectively. For each PCR-RFLP, the longer restriction fragment was assigned allele number 1, whilst the shorter one was assigned allele number 2. Further, linkage disequilibrium with STR polymorphisms flanking *PSEN1* was examined (D14S1028, D14S77, D14S1004, D14S43 and D14S61). Alleles for the STR polymorphisms were numbered as in the CEPH Genotype Database (http://www.cephb.fr./cephdb).

The association between early-onset AD and the polymorphisms studied was assessed using the likelihood ratio test or the Fisher exact test when appropriate. For di-allelic markers, genotype and allele frequencies were compared between cases and controls. For the STR markers, comparisons were restricted to allele frequencies because of the larger number of (rare) genotypes. Linkage disequilibrium was tested using the EH program as described by Terwilliger and Ott.[22] Since the markers tested were in strong linkage disequilibrium, adjustment for multiple testing is not straightforward, as statistical tests are not independent. In the tables we therefore report exact *P* values. When the overall likelihood ratio test had a *P* value of 0.05 or less, the strength of

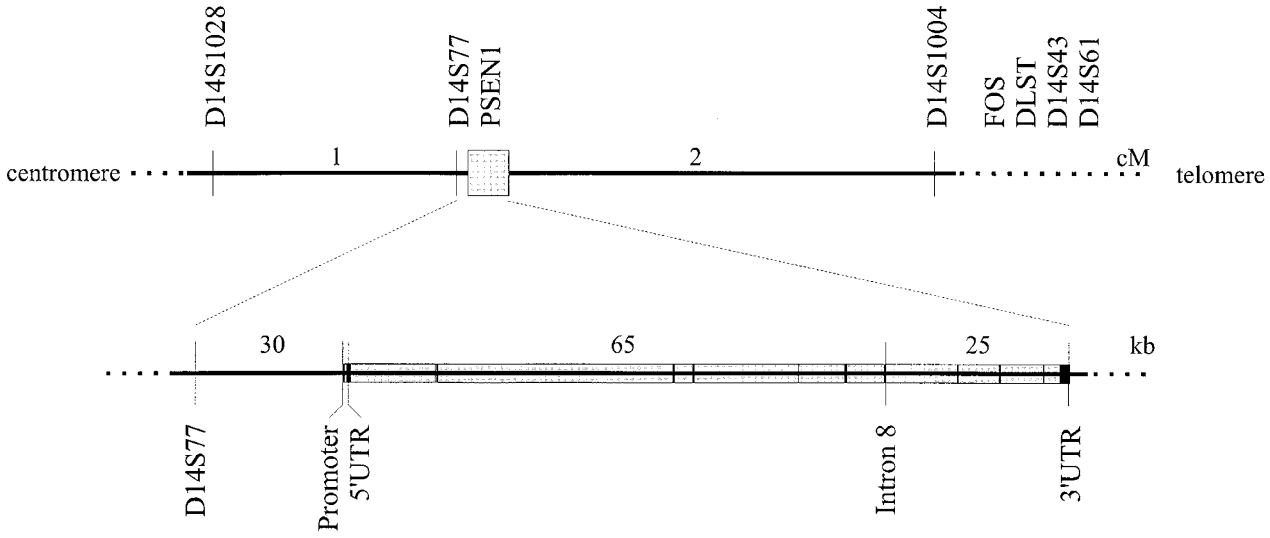

Figure 1 *Map of the chromosome 14q24.3 region containing* PSEN1. *The position of the STR polymorphisms and the 5'→3' orientation of* PSEN1 *are as published by Cruts* et al.[26] *Distances are in centimorgan (cM) according to the Généthon sex averaged genetic map of chromosome 14,*[27] *or in kilobases (kb) defined by restriction mapping of* PSEN1[28] *and YAC fragmentation*[29]

association between the alleles or genotypes and early-onset AD was evaluated with the odds ratio (OR) presented with 95% confidence intervals (CI). Genotype and allele frequencies were in Hardy-Weinberg equilibrium for all markers. Stratified analyses were conducted based on the presence of known *PSEN1* mutations[16] or an *APOE* ε4 allele.[19]

Results

Within the *PSEN1* gene, four di-allelic polymorphisms were examined (Table 1). In our population, the allele ($P = 0.05$) and genotype ($P = 0.03$) distributions of the intron 8 polymorphism in patients differed marginally from that in controls. Also, the promoter polymorphism was weakly associated with AD when comparing allele ($P = 0.03$) and genotype ($P = 0.05$) distributions between patients and controls. No differences were observed for the 5'UTR and 3'UTR polymorphisms. When analysing STR markers upstream of *PSEN1* (Figure 1), the allele distribution of D14S1028 was different between AD cases and controls ($P = 0.04$). No association was detected with D14S77 located 30 kb upstream of *PSEN1* (Figure 1) (data not shown). Downstream of *PSEN1*, no association was found with D14S1004 or the more distantly located STRs, D14S43 and D14S61, or the di-allelic polymorphisms in FOS and DLST (Figure 1) (data not shown).

The differences in genotype distributions between cases and controls are explained by increased homozygosity. In some cases an increased frequency was found of the 22 genotype for the intron 8 polymorphism, the 11 genotype for the promoter polymorphism and the 44 genotype for D14S1028. Genotype frequencies were stratified for the presence of mutations in *PSEN1* (Table 2). Five of the six patients with a *PSEN1* mutation carried the promoter genotype 11 (83% compared with 80% in controls), whilst four carried the intron 8 genotype 22 (67% compared with 21% in controls; $P = 0.02$) and D14S1028 genotype 44 (67% compared with 6% in controls; $P = 0.0002$). In patients without *PSEN1* mutations, the association remained for the promoter genotype 11 (OR indicated a 2.6 fold increase in frequency; 95% CI 1.1–6.1; $P = 0.01$) and the D14S1028 genotype 44 (OR indicated a 2.9 fold increase in frequency; 95% CI 1.1–7.9; $P = 0.005$), but not for the intron 8 polymorphisms. Allele 4 (231 bp) of D14S1028 is in linkage disequilibrium with allele 1 of the promoter polymorphism; all carriers of the 44 genotype were homozygous for allele 1 at the promoter polymorphism. Similar genotype frequencies were found for the promoter and D14S1028 in *APOE* ε4 carriers and non-carriers (data not shown).

Discussion

This is the first study to examine several polymorphic markers spanning the whole *PSEN1* gene as well as flanking regions. Our study shows association to multiple markers in the region upstream of *PSEN1*. The finding of an early-onset AD associated haplotype suggests that various patients received the PSEN1

Table 2 Genotype frequencies in early onset AD cases stratified for the presence of *PSEN1* mutations

			PSEN1 mutations		*No PSEN1 mutations*		
Polymorphism			*n*	%	*n*	%	*P value*
D14S1028	Genotype	44	4	67	13	14	0.05
		Other	2	33	77	86	
PSEN1 promoter	Allele	1	11	92	181	95	0.04
		2	1	8	9	5	
	Genotype	11	5	83	87	92	0.04
		12	1	17	7	7	
		22	0	67	1	1	
PSEN1 intron 8	Allele	1	4	33	84	46	0.10
		2	8	66	100	54	
	Genotype	11	2	33	23	25	0.10
		12	0	0	38	41	
		22	4	67	31	34	

region from a common ancestor, suggesting the presence of a disease related mutation/polymorphism.

In contrast to the study by Wragg *et al*,[2] who reported an increased homozygosity of the genotype 11 of the intron 8 polymorphism in late-onset AD patients, we found an increased frequency of genotype 22. The association we detected was for a large part explained by the finding that three of the four patients with the same *PSEN1* Ala79Val mutation shared the intron 8 genotype 22 as well as STR polymorphisms flanking *PSEN1*. This indicates that these patients most likely inherited the mutation from a common ancestor.[16] Also Sorbi *et al*[11] found evidence for association with the *PSEN1* intron 8 polymorphism in patients of families segregating *PSEN1* mutations, but not in patients with sporadic forms of AD.

In contrast to the intron 8 association, the associations with the *PSEN1* promoter and D14S1028 polymorphisms remained in our study when *PSEN1* mutation carriers were excluded. In patients without *PSEN1* mutations, a 2.6 fold increase of the 11 genotype for the promoter genotype 11 (95% CI 1.1–6.1; $P = 0.03$) and a 2.9 increased of the 44 genotype of D14S1028 (95% CI 1.1.–7.9; $P = 0.005$) were found. It is important to realise that given the marginal level of significance in the overall findings, the findings can be interpreted as possibly false positives. However, findings from molecular biological studies of AD are supportive of a true relationship.

Exons 1A and 1B are alternatively used exons and sequence analysis of *PSEN1* clones from a hippocampal cDNA library suggested that only exon 1A containing transcripts are present in brain.[23] In accordance with this finding, in our study, genetic association was absent with the polymorphism located in exon 1B.[16]

We do not know whether the promoter polymorphism has any influence on the expression level of *PSEN1*. Differential expression of *APOE* in brain of AD patients with specific *APOE* promoter polymorphisms has been associated with an increased risk for late-onset AD independent of the *APOE* ε4 genotype.[24,25] Another possibility is that other, not yet identified polymorphisms in the PSEN1 promoter region are responsible for the observed association with *PSEN1*. In this respect the finding with D14S1028 is important. According to our physical mapping data using yeast artificial chromosomes (YACs), D14S1028 is located upstream of the *PSEN1* gene (Figure 1).[26] With D14S1028 we found the strongest evidence for association in terms of statistical significance: a 2.9 times increased frequency of the genotype 44 ($P = 0.005$). Allele 4 is in linkage disequilibrium with allele 1 of the promoter polymorphism; all carriers of the 44 genotype were homozygous for allele 1 at the promoter polymorphism.

Our data suggest that the association of early-onset AD with *PSEN1* may result from polymorphism(s) in the promoter region or regulatory regions upstream of *PSEN1*. Given the marginal significance, the association remains to be confirmed in an independent sample of patients with early-onset AD. Because of the rarity of the phenotype, such sample was not available to us. Only partial information has been published on the

PSEN1 promoter sequence[23] and no studies are available that demonstrate how *PSEN1* expression is regulated in different tissues including brain. Our study indicates that such studies may be useful for the understanding of the AD pathogenesis.

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