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ARTICLE

Genetic variability in the regulatory region of presenilin 1 associated with risk for Alzheimer's disease and variable expression

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Mutations in the presenilin 1 (*PSEN1*) gene have been implicated in 18–50% of autosomal dominant cases with early-onset Alzheimer's disease (EOAD). Also, *PSEN1* has been suggested as a potential risk gene in late-onset AD cases. We recently showed genetic association in a population-based study of EOAD, pointing to the 5′ regulatory region of *PSEN1*. In this study we systematically screened 3.5 kb of the *PSEN1* upstream region and found four novel polymorphisms. Genetic analysis confirmed association of two polymorphisms with increased risk for EOAD. In addition, we detected two different mutations in EOAD cases at –280 and –2818 relative to the transcription initiation site in exon 1A of *PSEN1*. Analysis of the mutant and wild-type –280 variants using luciferase reporter gene expression in transiently transfected neuroblastoma cells showed a 30% decrease in transcriptional activity for the mutant –280G *PSEN1* promoter variant compared with the wild-type variant –280C. Our data suggest that the increased risk for EOAD associated with *PSEN1* may result from genetic variations in the regulatory region leading to altered expression levels of the *PSEN1* protein.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of senile dementia and the fourth leading cause of death in western societies. Although AD occurs most frequently in the elderly population [late-onset or senile AD (LOAD)], in 1–2% of the cases the first symptoms become apparent before the age of 65 years [early-onset or presenile AD (EOAD)] (1). In 40–60% of AD cases a positive family history for dementia has been documented (2). In 10% of familial cases, AD is inherited as an autosomal dominant trait. Eighteen to fifty per cent of familial autosomal dominant EOAD cases can be explained by mutations in the presenilin 1 gene (*PSEN1*) (3–5). Other genes that are mutated in autosomal dominant EOAD cases are the amyloid precursor protein (*APP*) and presenilin 2 (*PSEN2*) genes (6,7). Also, the E4 allele of the apolipoprotein E gene (*APOE4*) was shown to increase risk for developing LOAD and EOAD (8,9).

No *PSEN1* mutations were found in LOAD patients; however, a genetic association was reported between a di-allelic polymorphism in intron 8 of *PSEN1* and LOAD (10). Association was present in a Caucasian North-American but not in an African-American population, suggesting that the intron 8 polymorphism may be in linkage disequilibrium with functionally more relevant sequence variations elsewhere in the *PSEN1* gene (10). However, sequence analysis failed to detect sequence variations in the coding region. We recently analysed several polymorphisms within and near the *PSEN1* gene for genetic association with EOAD in a population-based case–control sample (11). We found significant association with the intron 8 polymorphism ($P = 0.05$) as well as with a promoter polymorphism located 48 bp upstream of the transcription initiation site of *PSEN1* (–48C→T; $P = 0.03$) (3,11) and a simple tandem repeat (STR) polymorphism *D14S1028* located upstream of *PSEN1* (11). However, the intron 8 association was fully explained by linkage disequilibrium with the dominant *PSEN1* mutations. The association with the polymorphisms in the regulatory region remained after excluding *PSEN1* mutation carriers from the analysis. Of the promoter polymorphism –48C→T (3), the most frequent allele C was associated with an increased risk for EOAD [odds ratio (OR) = 2.6] due to over-representation of the CC genotype in the cases, independent of the *APOE4* allele (11). These data suggested that genetic variants within the *PSEN1* regulatory region might be implicated in AD pathogenesis. The

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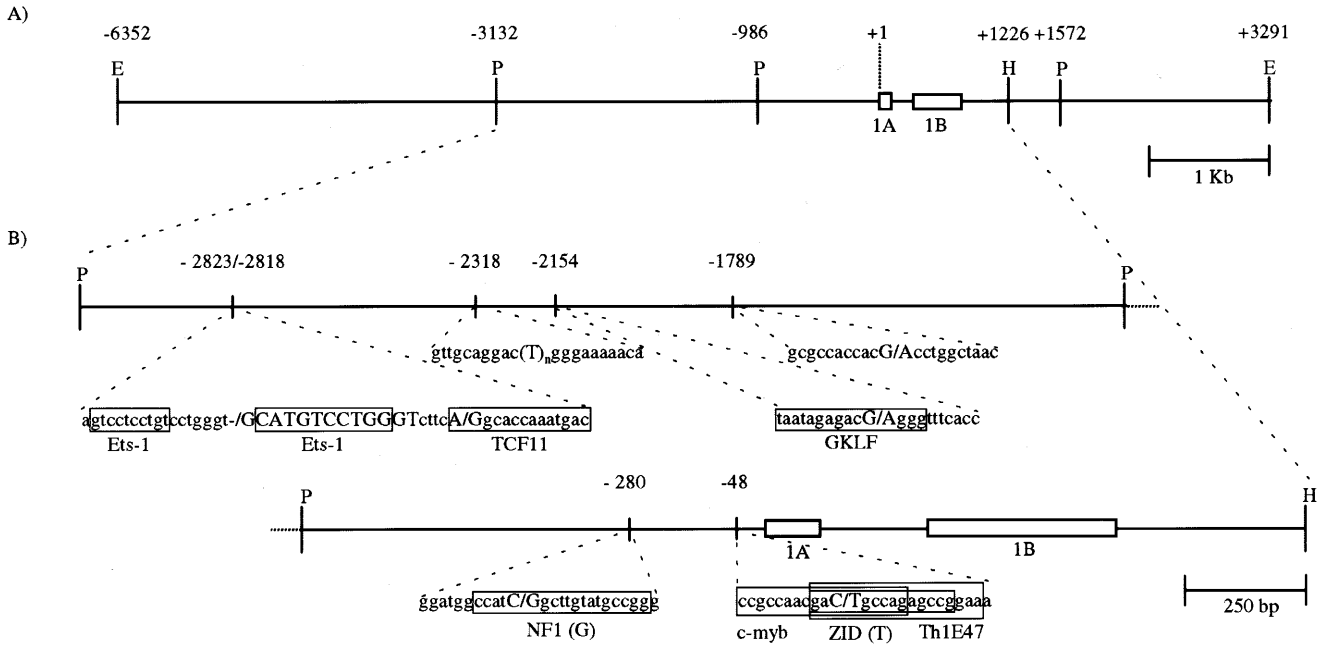

Figure 1. Sequence variations in the 5′ upstream region of *PSEN1*. Positions are numbered relative to the transcription start site of exon 1A (13). Open bars represent exons. (**A**) Plasmid B22 showing the restriction sites used for the subcloning: E, *Eco*RI; P, *Pst*I; H, *Hin*dIII. (**B**) Region of B22 subjected to systematic polymorphism/mutation analyses. Sequence variations are represented by upper case (allele present in plasmid B22/alternative allele). The sequences of potential cis elements detected by MatInspector V 2.2 (35) are within rectangles. In the case of allele-specific presence of the binding site the particular allele is indicated in parentheses.

Table 1. Sequence variations detected in the *PSEN1* 5′ upstream region

Position	Variation	Restriction site change	Primers
–48	C→T	–*Hga*I	5′-AGCAGCCTCAGAACCCCGACAA-3′
			5′-ACTCCCCATCACGCACATACG-3′
–1789	G→A	–*Hga*I (mismatch)	5′-CAGGCATGCGCCACGAC-3′
			5′-TTGAACAAATACAGGCTAAAACCCATCTA-3′
–2154	G→A	–*Bsm*BI	5′-AAGGCTGGTTATTCAATGTTAG-3′
			5′-GTCCACCCACCTCACAGAAT-3′
–2319	T-stretch (T_n)		5′-AAGGCTGGTTATTCAATGTTAG-3′
			5′-GTCCACCCACCTCACAGAAT-3′
–2823	Deletion/insertion of 13 bp: 5′-GCATGTCCTGGGT-3′	+*Nla*III	5′-TTAAAGGGTAGTGAGAAGGCTGGAGAAGAG-3′ 5′-AACTGCCCCACCCCCATTTC-3′
–280	C→G	+*Nco*I	5′-GCGATTTTAACAGCATTCTCTTGATTG-3′
			5′-ATTTCCGGCTCTGGCGTCGTT-3′
–2818	A→G	+*Ban*I	5′-TTAAAGGGTAGTGAGAAGGCTGGAGAAGAG-3′
			5′-AACTGCCCCACCCCCATTTC-3′

Nucleotide positions are relative to the transcription initiation site of exon 1A (13).

PSEN1 gene consists of 13 exons, three non-coding exons (exons 1A, 1B and 2) and 10 coding exons (exons 3–12) (12). *PSEN1* is ubiquitously expressed; however, exons 1A and 1B are alternatively used with the exon 1B transcript found only once in a colon adenocarcinoma cDNA library (13). We determined the genomic organization of *PSEN1* by fibre-FISH analysis and restriction mapping of a clone contig spanning *PSEN1* (14). In addition to the coding region the clone contig contains ~28 kb of sequences upstream of exon 1B. In this study we sequenced 6.7 kb of upstream sequences and systematically analysed 3.5 kb for genetic variations associated with EOAD.

RESULTS

PSEN1 promoter polymorphisms

We subcloned four restriction fragments of plasmid B22 (14), representing a 6.7 kb sequence upstream of exon 1B in the pOCUS-2 vector for transposon-based genomic sequencing: the 3.2 kb *Eco*RI–*Pst*I, 2.1 kb *Pst*I, 2.5 kb *Pst*I and 2.2 kb *Hin*dIII–*Eco*RI fragments (Fig. 1A). In total, 9642 bp were sequenced (GenBank accession no. AF205592), of which 6698 bp were upstream of exon 1B.

We systematically screened 3.5 kb upstream of exon 1B (Fig. 1B) in 10 randomly selected control individuals by polymerase chain

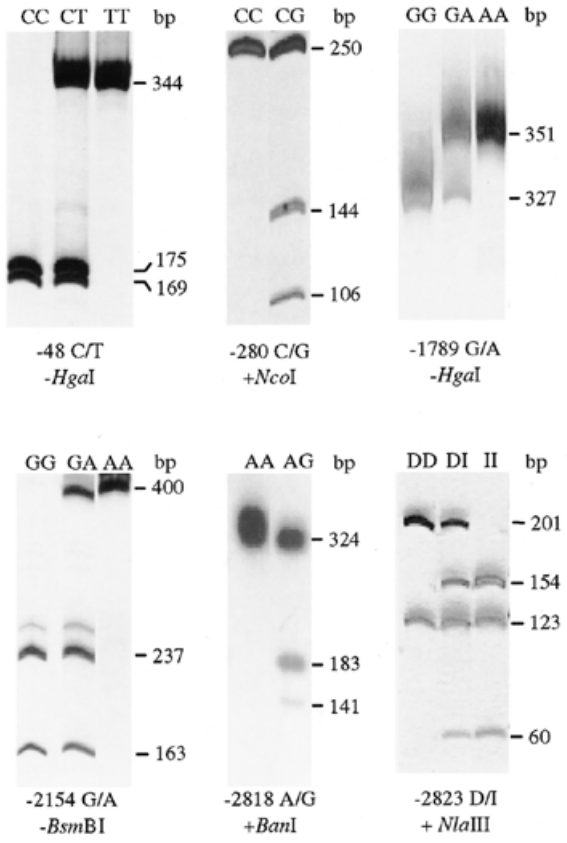

Figure 2. PCR–RFLP analysis of sequence variations in the *PSEN1* regulatory region (Table 1).

reaction–single-strand conformation polymorphism (PCR–SSCP) analysis using 16 overlapping primer sets. Four PCR fragments gave altered SSCP patterns and cycle sequencing revealed five polymorphisms (Table 1). One is –48C→T that we reported previously (3) (Table 1), abolishing an *Hga*I site allowing for its detection by PCR–restriction fragment length polymorphism (RFLP) analysis (Fig. 2). The other four are novel polymorphisms: two single nucleotide changes at positions –1789 (–1789G→A) and –2154 (–2154G→A); and two complex variations, i.e. a T-stretch at –2319 (–2319T_n) and a 13 bp insertion/deletion at –2823 (–2823I/D) (Table 1). –2154G→A and –2823I/D involve restriction enzyme recognition sites for *Bsm*BI and *Nla*III, respectively, and can be detected by a PCR–RFLP assay (Fig. 2). For –1789G→A we developed an *Hga*I mismatch PCR–RFLP assay (Fig. 2). SSCP analysis of –2319T_n was complex, also because the T-stretch is contained in the same PCR fragment comprising the –2154G→A polymorphism. Sequencing indicated that the T-stretch is highly polymorphic although the number of T alleles could not be determined exactly.

Genetic association

We analysed the EOAD case–control sample for genetic association with the three novel polymorphisms: –1789G→A, –2154G→A and –2823I/D. In the same EOAD case–control sample we have previously analysed –48C→T and found positive association with the C-allele and an over-representation of the CC genotype in cases versus controls (Table 2) (11). For all three polymorphisms, genotype frequencies were in Hardy–Weinberg equilibrium in the control population (Table 2). Also, –2154G→A and –2823I/D were in nearly complete linkage disequilibrium with –48C→T ($P < 6.10^{-15}$): the risk allele C of –48C→T is linked to the G allele of –2154G→A and the deletion allele of –2823I/D resulting in similar ORs for these polymorphisms (Table 2). No association was detected with –1789G→A (Table 2).

PSEN1 promoter mutations

When analysing –2823I/D by PCR–SSCP in the EOAD case–control sample, an additional variation was observed in one EOAD patient. Sequence analysis demonstrated a heterozygous A→G transition at position –2818 (–2818A→G) creating a *Ban*I site (Table 1; Fig. 2). PCR–RFLP analysis of the EOAD case–control sample confirmed the presence of –2818A→G in the one patient, whereas all other patients and controls were homozygous for the A allele. In addition, we systematically analysed the –372 to –33 promoter fragment by PCR–SSCP analysis for mutations in the EOAD cases. In our previous study we had already analysed the –134 to +163 fragment revealing –48C→T but no mutations (3). In one patient, we detected a heterozygous C→G transversion at position –280 (–280C→G), creating an *Nco*I site (Table 1; Fig. 2). Again, PCR–RFLP analysis confirmed the presence of the mutation in the one patient with all other patients and controls being homozygous for the C allele.

Reporter gene analysis

We cloned the 1554 bp *Kpn*I–*Hin*dIII fragment of B22 corresponding to the –318 to +1226 region (14) into the luciferase reporter vector pGL3-basic. The G allele of –280C→G was introduced in the construct by *in vitro* mutagenesis and sequence integrity was confirmed by sequencing. The effect of the wild-type (–280C) and mutant (–280G) alleles on the transcriptional activity of the *PSEN1* promoter was studied in transiently transfected cells. In human embryonic kidney (HEK293) cells no significant differences in expression levels were detected between –280G and –280C (Fig. 3). In mouse Neuro2A-neuroblastoma (N2A) cells, however, a 30% decrease was detected for –280G (Fig. 3). Notably, the promoter activity of –280C in N2A cells and HEK293 cells was comparable (data not shown).

DISCUSSION

Following our previous observation of genetic association of the *PSEN1* –48C→T promoter polymorphism with increased risk for EOAD (11), we aimed in this study at analysing systematically the regulatory region of *PSEN1* for genetic variations. Starting from a clone contig of *PSEN1* that we had previously constructed (14), we determined 6.7 kb of genomic sequence upstream of *PSEN1* exon 1B. No TATA box or initiator sequences were found within the 200 bp upstream promoter region. Also, the overall GC content of the 6.7 kb sequence was 48.5%, which is slightly higher than what is expected on average (40%). The GC content was significantly higher (60–70%) in regions upstream of exons 1A and 1B, and highest (73%) in the 100 bp region 5′ of exon 1A. Here the GpC:CpG ratio was 1:1 because of the presence of CpG islands representing SP1 recognition sites. All these features are typical for promoters of housekeeping genes corroborating previous findings showing ubiquitous expression of *PSEN1* (13). Next, we systematically screened 3.5 kb of upstream sequences by PCR–SSCP analysis and

Table 2. Genetic association of polymorphisms in the *PSEN1* 5′ regulatory region in EOAD cases and controls

Polymorphism			Cases		Controls		*P* value[a]	OR (95% CI)[b]
			n	%	*n*	%		
–48 C/T	Allele	C	183	95	209	89	0.04	
		T	9	5	25	11		
	Genotype	CC	88	92	94	80	0.04	2.6 (1.1–6.1)
		CT	7	7	21	18		ref.
		TT	1	1	2	2		ref.
–1789 G/A	Allele	G	130	79	182	81	0.80	
		A	34	21	44	19		
	Genotype	GG	54	66	74	65	0.81	1.0 (0.5–1.7)
		GA	22	27	34	30		ref.
		AA	6	7	5	4		ref.
–2154 G/A	Allele	G	166	95	205	89	0.03	
		A	8	5	25	11		
	Genotype	GG	80	92	92	80	0.04	2.9 (1.2–7.0)
		GA	6	7	21	18		ref.
		AA	1	1	2	2		ref.
–2823 I/D	Allele	D	169	95	206	89	0.03	
		I	9	5	26	11		
	Genotype	DD	81	81	92	79	0.03	3.0 (1.2–7.4)
		ID	7	8	22	19		ref.
		II	1	1	2	2		ref.

[a]Testing allele/genotype distributions in cases versus controls.
[b]Comparing homozygous risk genotype with other genotypes (ref.).

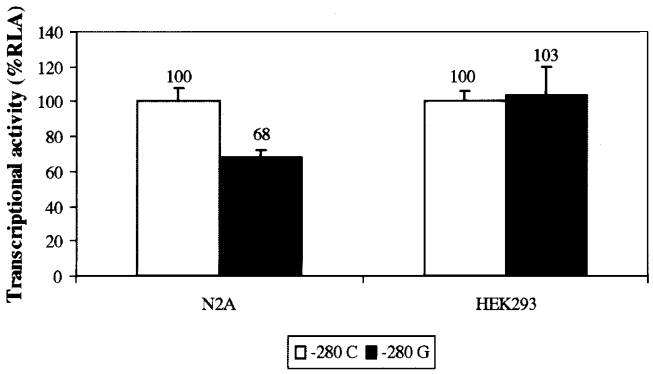

Figure 3. Transcriptional activity of the *PSEN1* –280C/G variants in transient transfection experiments. Bars represent firefly/renilla luciferase ratios for the different constructs [relative luciferase activity (RLA)]. Transcriptional activities are presented as a percentage of the activity of the wild-type construct (–280C, white bars). Values are the means ± SEM of duplicate determinations of at least three experiments of three independent DNA preparations each.

found four novel polymorphisms upstream of exon 1A: two simple base changes (–1789G→A and –2154G→A) and two complex polymorphisms (a T-stretch at –2319T_n and an insertion/deletion at –2823I/D). When we compared our data with the sequence AF109907 published in GenBank, five differences were observed in the 6.7 kb upstream sequence, of which three were in the 3.5 kb systematically screened by us. Two of these represent the poly- morphisms –1789G→A and –2319T_n. The other three (–179C, –3744insT and –4853A) are most likely to be sequencing errors in AF109907 since: (i) they are located in regions that we sequenced at least twice; and (ii) we could not confirm their presence by PCR–RFLP analysis of 10 unrelated individuals.

We analysed the novel polymorphisms, except –2319T_n, in the EOAD cases and controls. The –2154G→A and –2823I/D polymorphisms are in nearly complete linkage disequilibrium ($P < 6.10^{-15}$) with the –48C→T polymorphism that we previously identified (3). Also, strong linkage disequilibrium was found between the *PSEN1* intron 8 and –48C→T ($P = 0.001$). The –2823D and –2154G alleles co-existed with the –48C risk allele. As a result, the genetic association analysis of these two polymorphisms gave almost identical ORs of 3.0 and 2.9, respectively. The –1789G→A polymorphism was not associated with EOAD, suggesting that it arose more recently in history on the risk haplotype.

The association with multiple markers in the region upstream of *PSEN1* described in this study is caused mainly by patients homozygous for the risk haplotype (–48C/–2154G/–2823D). In homozygous patients the effect of a *PSEN1* promoter polymorphism may lead to important changes in *PSEN1* expression levels contributing to the development of AD pathophysiology. Since all three polymorphisms associated with an increased risk for EOAD are located in the regulatory region of *PSEN1*, each one separately or together may be influencing *PSEN1* expression levels. Moreover, all three polymorphisms alter predicted binding properties of potential transcription factor binding sites. The –48C→T alters the matrix similarity of three transcription factor binding sites: c-myb, Th1/E47 and zinc finger protein with interaction domain (ZID) (Fig. 1B). The matrix similarity of c-myb at position –57 is decreased from 0.857 for –48C to 0.853 for –48T, suggesting better binding of the transcription factor to the C allele. The binding of Th1/E47 is predicted to be less strong for –48C (0.882 versus 0.909 for –48T).

E47 is a basic helix–loop–helix transcription factor, which is expressed in various tissues including brain. In brain, E47 forms a complex with brain-specific proteins and is implicated in the development and maintenance of the mammalian nervous system (15–17). The binding site for ZID is only present for the T allele. In the case of –2823I/D, the last 10 nucleotides of the 13 bp insertion sequence are identical to the 10 nucleotides preceding the insertion site. These 10 nucleotides contain an Ets-1 binding site, although the sequence of the core-binding region does not match the consensus sequence completely (0.926 core similarity/ 0.888 matrix similarity). However, it is possible that the two Ets-1 sites in the insertion allele –2823I exert a cooperative binding effect and that deletion of one Ets-1 in the –2823D risk allele decreases *PSEN1* expression by >2-fold. Also, the Ets-1 sites may interact with the Ets site at position –40 influencing basal promoter activity (18). The core similarity of the binding sequence of GKLF at the –2154G→A is decreased from 1 to 0.885 when the –2154G risk allele is present. GKLF belongs to a family of tissue-specific proteins, some of which were shown to influence transcriptional activation by SP1 (19). Since multiple SP1 sites are present in the *PSEN1* regulatory region, a comparable brain-specific scenario may be implicated.

Another possibility is that it is not the polymorphisms *per se* that are biologically relevant, but that they are landmarks for other functionally more important variations in the *PSEN1* regulatory region. In this context, it is interesting that we found two different sequence variations in EOAD patients not present in controls: –280C→G and –2818A→G. Both patients were homozygous for the risk haplotype but heterozygous for the mutation. The mutations were present in patients with onset ages of 63 and 56 years, respectively, and a first degree positive family history of disease, but no family members were available for segregation studies. We studied the effect of –280C→G located in the proximal promoter region on *PSEN1* promoter activity using a luciferase reporter gene analysis and provided evidence that –280C→G modifies transcriptional activity of the *PSEN1* promoter in a cell-type-specific manner. In mouse neuroblastoma cells, a decrease in transcriptional activity of >30% was detected for the G allele compared with the C allele, whereas no effect was seen in human kidney cells. These data suggest that –280C→G alters or creates a cis element important for transcriptional activity in neuron-like cells, but not in kidney cells. The –280G mutation creates a potential NF1-binding site (core similarity 1/matrix similarity 0.872), which may influence the expression of *PSEN1*. It has been shown that NF-I proteins play an important role in the regulation of tissue-specific gene expression during mammalian embryogenesis (20).

In conclusion, our study is the first one systematically analysing the *PSEN1* regulatory region for genetic variability contributing to the genetic risk for AD. In addition to –48C→T, which we identified earlier (3), we found two novel polymorphisms (–2154G→A and –2823I/D) associated with increased risk for developing EOAD independently of *APOE4*, and two potentially EOAD-related mutations (–280C→G and –2818A→G). Both polymorphisms are in nearly complete linkage disequilibrium with –48C→T previously associated with EOAD (3) independently of *APOE4*. However, it will be important to test the polymorphisms for association in other epidemiological studies to confirm that *PSEN1* is a risk gene for EOAD. Also, it will be of interest to determine whether the association previously observed with the intron 8 polymorphism can be explained by genetic variability of the *PSEN1* promoter in LOAD. The genome scan in LOAD reported by Kehoe *et al.* (21) provided significant evidence neither for nor against the notion that *PSEN1* may contribute to the risk of developing LOAD. However, the power of the linkage methods to detect genes of small effect is limited (22), and under the conditions used by Kehoe *et al.* (21) only genes of an effect size equal to or greater than *APOE* were detected.

Each one of the polymorphisms and mutations involve consensus sequences of potential transcriptional regulatory elements (Fig. 1B) and therefore may modulate *PSEN1* transcription. It will be essential to study the functionality of these transcription factor binding sites *in vitro* and *in vivo*. Missense mutations in *PSEN1* were shown to lead to autosomal dominant EOAD by pathways depending on the production of increased amounts of the amyloidogenic peptide Aβ42 (23), a proteolysis product of *APP* deposited in brain. Also, 50% antisense inhibition of *PSEN1* expression in cultured cells resulted in increased Aβ42 production (24). On the other hand, in neuronal cell cultures derived from *PSEN1* deficient mice, *APP* processing into Aβ peptides was prevented (25). Together, these observations suggest that variable expression levels of *PSEN1* may modulate Aβ production. Our finding that the –280G mutation, creating a potential NF1-binding site, decreases *PSEN1* transcriptional activity in neuroblastoma cells by 30%, suggests that the increased risk associated with *PSEN1* can be explained by decreased *PSEN1* expression. However, it is important to note that the –280G mutation appeared in the heterozygous state although the patient was homozygous for the risk haplotype. Therefore, more data on the functionality of the *PSEN1* promoter are needed before concluding that altered *PSEN1* transcription contributes to disease risk. The importance of these studies is demonstrated by recent findings of polymorphisms in the *APOE* promoter modulating risk for LOAD (26,27) by altering *APOE4* expression levels (28). Also, contribution of genetic variability in the *APP* promoter to increased risk for LOAD was recently suggested (29), possibly by increasing *APP* expression leading to increased Aβ production as in Down's syndrome cases. Together, these studies suggest an important role for variability in regulatory elements in the genetic predisposition for developing AD.

MATERIALS AND METHODS

Subjects

Patients were derived from a population-based epidemiological study of EOAD within metropolitan Rotterdam and the four northern provinces of The Netherlands (30). Blood samples for DNA extraction were collected from 102 (52%) of the participating patients. The mean age at onset of the patients was 56.7 ± 5.4 years and the mean age at the time of the study was 63 ± 4.4 years. Patients were compared with an age- and sex-matched control series (n = 118; mean age 63 ± 4.4 years) that was drawn randomly from the Rotterdam Study (30–32). Based on family history up to two degrees, these subjects were not related. None of the control subjects showed symptoms of dementia and none had cognitive test scores suspect for dementia (30,32).

Sequencing of the *PSEN1* 5′ upstream region

Plasmid B22 (14) was subcloned into the pOCUS-2 vector for transposon-based genomic sequencing (Novagen, Madison, WI).

The pOCUS-2 constructs were transferred into chemically competent donor cells, which carry the transposon on an F factor, and one of the resulting colonies was mated with the recipient cells according to the manufacturer's protocol. For each fragment, 96 colonies were randomly selected and stored in a 96-well plate containing LB medium supplemented with 20% glycerol. The transposition site of the different clones was mapped by colony PCR combining one of the two vector-specific primers (POCUSUP or POCUSDOWN) with a transposon-specific primer (GDIR) in separate reactions (33). Colonies were selected based on their site of transposition and DNA was prepared for sequencing using the Wizard Plus SV Minipreps DNA Purification System (Promega, Madison, WI). Plasmid sequencing was performed using the Thermo Sequenase II Dye Terminator Cycle Sequencing kit (Amersham Life Science, Cleveland, OH) according to the supplier's protocol using primers GD1 and GD2 (33). The sequences were assembled using the Lasergene software for Windows (DNASTAR, Madison, WI). Gaps in the sequence were completed by targeted cycle sequencing using primers directed against sequences flanking the gaps.

DNA analysis

Standard PCR was performed using 16 overlapping primer sets covering the 3.5 kb sequence upstream of exon 1B. Two additional primer sets were designed to analyse the two sequence differences –3744insT and –4853G→A. In the SSCP analyses, heat-denatured and renatured PCR products were electrophoresed on precast ExcelGel gels (Pharmacia Biotech, Uppsala, Sweden) for 3.5 h at 600 V using the MultiPhorII electrophoresis system (Pharmacia Biotech) and visualized by silver staining. Products with aberrant SSCP patterns were sequenced as described above. Polymorphisms were analysed by restriction enzyme digestion of the PCR products amplified from genomic DNA, when they produced an RFLP. In case no RFLP was present, mismatch primers were designed or samples were analysed by PCR–SSCP analysis. Fragments were separated on a 1.5–3% agarose gel and visualized on an ultraviolet trans-illuminator after ethidium bromide staining. Alternatively, fragments were separated on precast ExcelGel gels and visualized by silver staining.

Statistical analysis

Allele and genotype distributions in patients and controls, excluding the six patients with *PSEN1* mutations, were compared using Fisher's exact test (34). Hardy–Weinberg equilibrium and linkage disequilibrium were tested using the HWE and EH programs as described by Terwilliger and Ott (34). Since the markers tested were in strong linkage disequilibrium, adjustment for multiple testing is not straightforward, as statistical tests are not independent. Therefore, exact *P* values were calculated. The strength of association between alleles or genotypes and EOAD was evaluated with the OR presented with 95% confidence intervals.

Construction of reporter plasmids

The *PSEN1* 5′ upstream region from –323 to +1231 was cloned into the promoterless pGL3-basic vector (Promega) upstream of the firefly luciferase gene. The Quick-change *in vitro* mutagenesis kit (Stratagene, La Jolla, CA) was used to introduce the –280G mutation using primer 5′-ATTTAGGATGGCCATGGCTTG-TATGCCGGGAGAAGCACACGCTG-3′ and its reverse complement. A mutant clone was selected by *Nco*I digestion and the integrity of the complete insert was confirmed by sequence analysis as described above using vector- and *PSEN1*-specific primers designed for screening of the *PSEN1* 5′ upstream region.

Eukaryotic cell culture and transient transfection

Mouse N2A cells were propagated in a minimal essential medium with Earle's salt, 10% fetal bovine serum, 2 mM L-glutamine, 200 IU/ml penicillin, 200 g/ml streptomycin and 0.1 mM non-essential amino acids (Life Technologies, Gaithersburg, MD). HEK293 cells were propagated in Optimem with 10% fetal bovine serum, 200 IU/ml penicillin and 200 g/ml streptomycin (Life Technologies). For transient transfection, N2A and HEK293 cells were seeded in 6-well tissue culture dishes, at 9×10^4 and 7×10^5 cells/well, respectively, and allowed to recover for 24 h. Cells were co-transfected with 20 ng of pRL-TK plasmid containing the herpes simplex virus thymidine kinase promoter upstream of the renilla luciferase gene (Promega) and 1 g of either one of the *PSEN1* promoter constructs or one of the control plasmids, using the Lipofectamine procedure (Life Technologies) as described in the manufacturer's protocol. Empty pGL3-basic vector was used as a negative control, pGL3-promoter plasmid containing the SV40 promoter upstream of the firefly luciferase gene (Promega) as a positive control.

Relative luciferase activity measures

Transfected cells were cultured for 48 h, washed with 1 ml of phosphate-buffered saline (Life Technologies), and lysed with Passive lysis buffer (Promega). Firefly luciferase activities (LA_F) and renilla luciferase activities (LA_R) were measured sequentially using a Dual-Luciferase reporter assay system (Promega) and a model TD-20E Luminometer (Turner Design, Sunnyvale, CA). To correct for transfection efficiency and DNA uptake, the relative luciferase activity (RLA) was calculated as: $RLA = LA_F/LA_R$. To compare the RLA of a sample in one cell line with another, relative RLA was calculated as a percentage of the RLA of the wild-type construct: $\%RLA = (RLAmt/RLAwt) \times 100$.

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