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SHORT REPORT

Germline mutational analysis of *presenilin 1* and *APP* genes in Jewish-Israeli individuals with familial or early-onset Alzheimer disease using denaturing gradient gel electrophoresis (DGGE)

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Germ line mutations in three genes have been detected in patients with familial Alzheimer's disease (FAD) and sporadic, early onset disease: amyloid precursor protein (*APP*), presenilin 1 (*PS-1*), and presenilin 2 (*PS-2*). The relative proportions in which mutations in these genes occur among AD patients in Israel has not been evaluated. To that end, we screened 52 Jewish-Israeli patients with AD: 22 with sporadic, early-onset disease (below 65 years), and 30 with FAD. Mutation screen employed denaturing gradient gel electrophoresis (DGGE) of exon-specific PCRs and restriction enzyme digest. Five patients from three different families displayed mutations within the *PS-1* gene: three patients of one family showed a mis-sense mutation in codon 120 (Glu 120Lys), and two other unrelated patients showed an identical mis-sense mutation in codon 318 (Glu318Gly). No patient showed an abnormal migration on DGGE (for *APP*) or mutant restriction digest pattern (for *PS-2*) genes. These data may indicate the existence of another familial Alzheimer disease (FAD) gene locus in the Israeli Jewish population.

Keywords: Alzheimer's disease; genetic predisposition; mutation analysis

Introduction

Alzheimer disease (AD), is a neurodegenerative disease affecting up to 15% of the octogenarian population.[1]. The ε*4* allele of the *APOE* gene has been associated with increased AD risk.[2] A subset of AD patients has a family history of the disease, where inheritance follows an autosomal dominant pattern and at times, early age at onset (under 65), with three identified genes involved in familial AD predisposition. A handful of germ line mutations occur within exons 16 and 17 of the amyloid precursor protein (*APP*) gene on chromosome 21.[3–6] Germline mutations in presenilin 1

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(*PS-1*), on chromosome 14[7] presumably account for the majority of familial and early onset AD,[8] with more than 30 different mis-sense mutations reported in ethnically diverse AD families.[8–13] Finally, in a well defined population of Volga Germans, a single mis-sense mutation (*N141*I) within presenilin 2 (*PS-2*) on chromosome 1 accounts for the majority of phenotypically affected individuals.[14,15] Anecdotal reports of germ line mutation in *PS-1* in Jewish FAD patients[9, 16] were reported, but no systematic characterization of germ line mutations in these genes in Jewish FAD patients was published. To that end, we screened 52 Jewish-Israeli patients with either early-onset or FAD for mutations within these genes, using denaturing gradient gel electrophoresis (DGGE), and restriction enzyme digest. In addition, *APOE* genotype was determined in the same population. (Tables 1a and Table 1b.)

Table 1a Clinical details on familial AD patients

Patient no.	*Origin*	*Sex*	*Age at onset*	*Additional affected first degree family members*
1	Libya	Female	40	–
2*	Romania	Female	43	Two more siblings
3	Germany	Female	44	–
4	Romania	Female	44	–
5*	Romania	Male	45	Two more siblings
6	Iran	Female	47	–
7	Russia	Male	48	–
8*	Romania	Male	48	Two more siblings
9	Yemen	Female	51	–
10	Ashkenazi	Female	53	–
11	Poland	Female	53	–
12	Egypt	Male	54	–
13*	Romania	Female	55	–
14	Ashkenazi	Male	55	–
15	Egypt	Female	57	–
16	Poland	Female	58	–
17	Lebanon	Female	58	–
18	Sephardic	Male	59	–
19	Iraq	Male	60	–
20	Romania	Female	62	–
21	Poland	Female	64	–
22	Romania	Female	66	–
23	Ashkenazi	Female	67	–
24	Poland	Male	68	–
25	Germany	Male	70	–
26	Poland	Male	72	–
27	Romania	Male	72	–
28	Ashkenazi	Female	78	Two brothers
29	Poland	Female	78	Two sisters
30	Poland	Female	80	–

*Denotes a mutation carrier; age at disease onset was based on standard criteria: clinical and objective diagnosis of AD, exclusion of other reasons for dementia, with onset of symptoms prior to age 65 or at least one additional first degree relative with AD. Whenever the origin is Ashkenazi both parents originated from East-European countries. Sephardic, non-Ashkenazi origin. From each family, only one individual was tested, and all testees were unrelated to each other.

Materials and Methods

Clinical Characteristics

Fifty-two patients from 49 families formed the basis of this study, which was approved by the Human Ethics Committee. All participants had AD based on standard, accepted criteria.[17,18] Early-onset and familial AD were diagnosed, if clinical diagnosis of AD with exclusion of other causes of dementia,[19] with onset of symptoms prior to age 65, or at least one additional first degree relative with AD, respectively.

Mutation Analysis and Allelic Determination

DNA extracted from peripheral blood leukocytes using standard protocols, served as a template in the polymerase chain reaction (PCR). The final reaction volume was 50 μl (except for *APOE* genotyping of 25 μl), containing the standard PCR components. The primers used in DGGE analysis (*PS-1* and *APP*) were based on the melting profiles,[20] predicting that the amplified fragment region of interest would be contained in a single, low-melting domain, increasing the likelihood of mutation detection.

Between 100 and 250 ng of DNA were used per PCR, or in the case of 'nested PCR', 3–5 μl of the 'outer PCR'. Thermal cycling was achieved in a PTC 100-60 thermocycler (M.J. Research, Watertown, MA). An initial 94°C denaturation step for 5' was followed by 30 cycles of denaturation at 94°C (1'), annealing at a temperature of 52–66°C and extending to 72°C (1'), with a final extension step of 5' at 72°C. The cycling profiles and PCR conditions are available. All products (5 μl/reaction), were analysed on a 2% agarose gel.

Of the PCR products of the *PS-2* gene or *APOE* 10 μl were subjected to a Sau3A or HhaI digest respectively (New England Biolabs) at 37°C overnight, and were run on a 2% or 8% polyacrylamide gel respectively, at 250V for 2 hours, and visualized with ethidium bromide.

Parallel denaturing gradient gels were cast and run using the Hoffer Scientific instruments model SE620 (San Francisco, CA) as described.[21] The optimal denaturant range yielding focused bands for the *APP* and *PS-1* genes are summarized in Table 2.

Of the PCR reaction, 20 μl were loaded onto the gel and, following electrophoresis for 16 hours at 80 volts or 3–4 hours at 160 volts (Table 2) in 60°C on 7% denaturing acrylamide gel, the gels were silver stained. DNA sequencing was performed using a biotinylated primer, as previously described.[22]

Results

Patient Characteristics

Fifty-two patients (22 men, 30 women) were analysed. Thirty cases (11 men, 19 women) were designated as familial if there was at least one additional first degree

Table 1b Clinical details on sporadic early onset AD patients

Patient no.	*Origin*	*Sex*	*Age at onset*
1	Egypt	Male	42
2	Buchara	Female	45
3	Slovania	Female	50
4	Poland	Female	51
5	South Africa	Female	52
6	Ashkenazi	Female	53
7*	Morocco	Male	55
8	South Africa	Female	55
9	Ashkenazi	Male	55
10	Egypt	Male	56
11	Turkey	Male	56
12	Iran	Male	56
13	Morocco	Male	56
14	Ashkenazi	Male	57
15	Tunisia	Male	59
16	Ashkenazi	Female	60
17	Ashkenazi	Female	60
18	Morocco	Female	61
19	Poland	Female	61
20	Ashkenazi	Male	63
21	Poland	Male	63
22	Uzbekistan	Female	63

*Denotes a mutation carrier; age at disease onset was based on standard criteria: clinical and objective diagnosis of AD with onset of symptoms prior to age 65. Whenever the origin is Ashkenazi, both parents originated from East-European countries.

Table 2 DGGE conditions for running PCR fragments

Gene	*Exon no.*	*Denaturant range (%)*	*Running time (h)*	*Voltage*
APP	16	20–60	3.5	160 v.
	17	30–70	3.5	160 v.
PS–1	3	20–80	ON	80 v.
	4a	40–70	2.75	160 v.
	4b	45–85	ON	80 v.
	5	35–75	ON	80 v.
	6	20–80	ON	80 v.
	7a	20–80	ON	80 v.
	7b	35–75	ON	80 v.
	8	25–55	3.0	160 v.
	9a	35–75	ON	80 v.
	9b	30–50	ON	80 v.
	10	35–75	ON	80 v.
	11	20–80	ON	80 v.
	12	20–80	ON	80 v.

*Denotes an overnight run, which typically lasts 15–18 hours.

family member with probable AD. Age range at disease onset for these patients was 40–80 years (median 58). The rest (11 men, 11 women) were sporadic, early onset cases (disease onset prior to age 65), and the age range was 42–63 years (median 56), with five patients between 60 and 65 years old. There were 32 patients of Ashkenazi (East European) origin and 20 non-Ashkenazis (Table 1).

Mutation Analyses and APOE Genotypes

Of 52 patients, 47 did not display any DGGE migration abnormalities in the 10 coding exons *PS-1*. Three patients from a single Ashkenazi family were found to carry a mis-sense mutation Glu120Lys.[16] Two additional patients, one Ashkenazi and one Moroccan, displayed migration abnormalities in exon 9 in the *PS-1* gene (Figure 1a). Direct DNA sequencing showed a mis-sense mutation: A to G change at codon 318 altering Glutamine to glycine (Figure 1b).

No patient showed any abnormal migration pattern on DGGE in exons 16 and 17 of the *APP* gene, whereas a known mutation in exon 16 (*K670N/M671L*)[23] was easily detected (Figure 2). Furthermore, no patient displayed the N141I mutation of the *PS-2* gene.

APOE genotyping revealed E3 – 66.6%, E4 – 25% and E2 – 8.3% for the familial cases, and E3 – 77.2%, E4 – 20.4% and E2 – 2.2% for the sporadic cases. Notably, two patients were homozygous for the *APOE4* allele – one familial case (age at onset 70 years) and a sporadic case (age – 53 years).

Case Reports of Patients Displaying *PS-1* mutations

The clinical details of the Glu120Gly mutation carriers have been previously reported,[16] and an identical mutation has independently been reported.[10] Two patients carried an identical mutation in codon 318 of the *PS-1* gene (Glu318Gly). One is a Jewish-Moroccan male, with slow progressive dementia starting at age 55. Notably, no cases of AD in his family were reported, and his parents died of cancer at 67 (mother) and 72 (father) years of age. The second mutation carrier is a Jewish-Romanian woman, in whom progressive memory impairment started at age 55, with depression being the predominant accompanying manifestation and slow progression of cognitive dysfunction over a 10-year period. The patient's father who died at 84 years suffered from dementia starting at his late seventies.

Discussion

In this study we screened for germ line mutations in three genes known to be associated with AD predisposition in 52 patients with FAD or early-onset AD, representing 49 families, and only five patients from

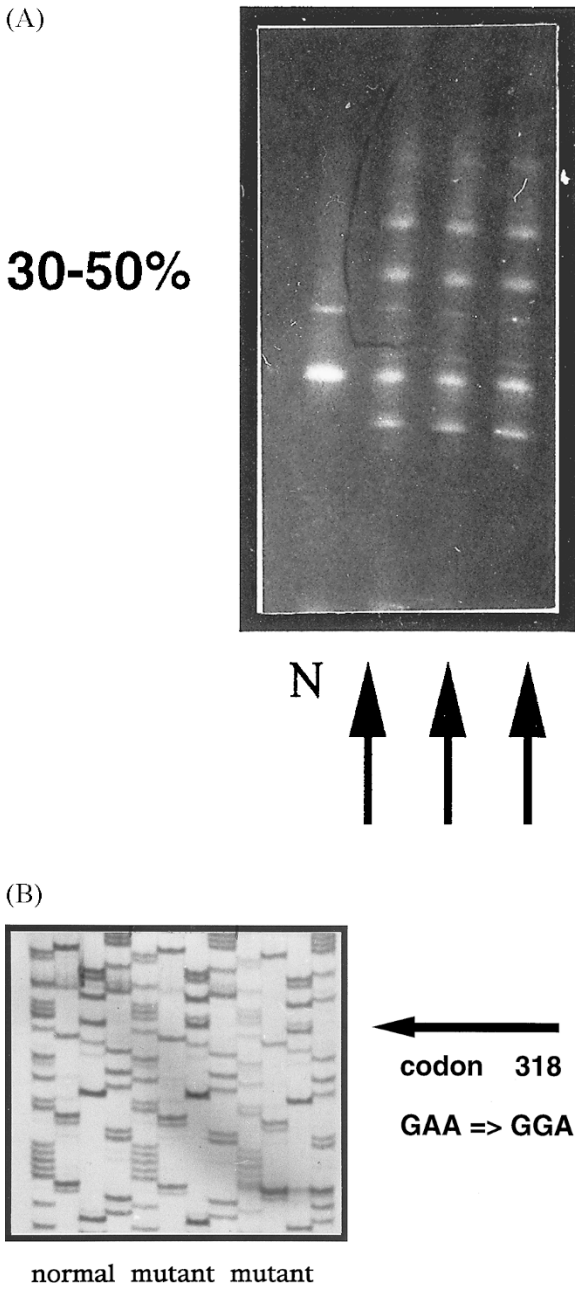

Figure 1a *Denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR)-amplified fragments of exon 9 of the presenilin 1 (PS-1) gene. The numbers on the left side denote the percentage range of DGGE. Primer sequences were: upstream primer - 5′ TTC-TAA-ATA-TTA-GAG-CTG3′; downstream primer - 5′ GC clamp-GAT-AAT-GTA-GCT-ACC-TAA-AGG 3′. Clear-cut migration abnormalities are visible for two patients (one of the samples was loaded twice), both affected individuals from different families, marked with arrows at the bottom of the gel.* **Figure 1b** *Direct DNA sequencing of PCR-amplified fragment of exon 9 from the two patients and normal control. The arrow points to the position of the heterozygous mutation; the order of loading is A C G T from left to right*

three families were identified as carriers of two distinct mis-sense mutations in *PS-1*. One mutation, Glu120Lys, was found in a Jewish Ashkenazi family with early onset AD accompanied by seizures.[16] Another mutation, Glu318Gly, was found in two Jewish families from different ethnic backgrounds, Ashkenazi (Romanian) and Moroccan. This mutation was previously reported in one German family.[11] No mutations were found either within exons 16 and 17 of the *APP* gene, nor was the predominant *PS-2* mutation (*N141I*) detected in any sample.

Low rates of *PS-1* mutations were found in Israeli AD patients: for the familial cases mutation rate was 7.4% (2/27) and for the sporadic cases 4.5% (1/22). In all mutation carriers disease manifestations started before the age of 60 years. This low rate of *PS-1* mutation may stem from several causes. First, the phenotypic ascertainment of AD may have been erroneous, since no histopathological proof of the diagnosis is available. However, a unidirectional error in diagnosis of AD by independent experienced physicians, in three medical centres, is highly unlikely. Secondly, the definition of 'familial' AD cases might have been too lax. Given the Jewish Israeli reality of an immigrant population, with truncated families because of the holocaust, and extrapolating from other inherited predispositions, it is acceptable to designate these individuals as FAD.

Lastly, the possibility that existing mutations may have escaped detection seems unlikely given the specificity and sensitivity of DGGE,[21] and its proven ability to identify known and novel mutations in the *APP* and *PS-1* genes. Thus it seems that the paucity of mutations detected in our patients truly reflects a low

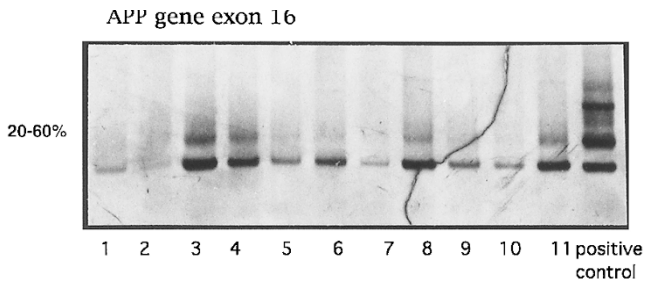

Figure 2 *Denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR)-amplified fragments of exon 16 of the APP gene. The numbers on the left side denote the percentage range of DGGE. The numbers below indicate patients' number. Positive control (C) is DNA amplified from patient with a known mutation in exon 16 (K670N/M671L). (Generously supplied by Dr L Lannfelt, Huddinge Hospital, Stockholm, Sweden)*

occurrence rate. It is possible that mutations exist in the gene regions which were not analysed; the non-coding exons of the *PS-1* gene, the other exons of the *APP* gene, promotor and regulatory regions.

In summary, the majority of FAD and sporadic, early onset AD cases in Israel do not display mutations within the *PS-1*, *APP* or *PS-2* genes, despite the adequacy of the mutation detection technique used. This may indicate the existence of other gene loci responsible for AD predisposition in Jewish Israeli families with AD. Moreover, a single mutation, Glu318Gly, seems to occur in ethnically diverse patients, perhaps indicating the significant role that this residue plays in protein function.

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