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Screening for PS1 mutations in a referral-based late-onset AD cases in Saudi Arabia

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Mutations in PS1 gene were investigated in 58 Saudi patients with late-onset Alzheimer's disease (AD) using PCR and direct DNA sequencing methods. Genomic DNA was extracted from blood of both patients and normal individuals using organic extraction methods. Genomic DNA was amplified for exon 4, 5, 6, 7, 10 and 11 encoding for amino acid 30 to 256 and 320 to 416 of PS1 gene. Electrophoresis was carried out with 1.5% agarose gel and separated fragments were stained with ethidium bromide. Fragments were sequenced and compared with the sequences of the respective exons of normal individuals as well as the data available in GenBank. No mutations were found in the late-onset AD patients understudy. The lack of mutations in exon 4, 5, 6, 7, 10 and 11 of PS1 indicates that the presence of mutations in PS1 gene is not a cause of late-onset AD in Saudi population.

Key words: Mutations, PS1, Alzheimer's disease (AD), Saudi Arabia

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Introduction

Alzheimer disease (AD) is a complex genetic disorder and represents the most common form of dementia in the elderly. Recently, considerable progress has been made in unraveling the complex etiology of AD. To date, 4 loci have been identified contributing to AD: amyloid precursor protein gene (APP) on chromosome 21 (Goate *et al.*, 1991; Mullan *et al.*, 1992), apolipoprotein E gene (ApoE) on chromosome 19 (Pericak-Vance *et al.*, 1991; Saunders *et al.*, 1993; Mulder *et al.*, 2000), the presenilin-1 gene (PS1) on chromosome 14 (Schellenberg *et al.*, 1995; Sherrington *et al.*, 1995) and presenilin 2 gene (PS2) on chromosome 1 (Rogaev *et al.*, 1995; Levy-Lahad *et al.*, 1995).

Approximately 10% of all AD cases are estimated to be early-onset familial AD (FAD) and the disease appears to be transmitted as an autosomal dominant Mendelian trait with age dependent penetrance. Mutations in presenilin-1 (PS1) located on chromosome 14 have been linked with FAD and more than 90% of all genetically determined cases of AD are caused by the mutations in the PS1 (Schellenberg, 1995). Many missense substitutions have been reported in families of different ethnic origins. The products of PS1 mutated genes cause dysfunction/death of vulnerable populations of nerve cells, resulting into clinical syndrome of progressive dementia (Price and Sisidia, 1998; Fraser *et al.*, 2000; Tandon *et al.*, 2000). Up to now, the presence of presenilin mutations have been reported in less than 180 families worldwide (Hull *et al.*, 2000).

PS1 has also been suggested as a potential risk factor for late-onset AD in some studies (Wragg *et al.*, 1996; Theuns *et al.*, 2000). However, those results have not yet been widely replicated and their functional significance is unclear. Moreover, various studies indicated that the chromosome 14 locus (PS1) is not responsible for AD in most late-onset FAD kindreds but could play a role in a subset of these kindreds (Schellenberg *et al.*, 1993; Wavrant-De Vrieze *et al.*, 1999).

Therefore, a study was performed to search for coding sequence variants in exon 4, 5, 6, 7, 10 and 11 of PS1 in a number of late-onset AD patients. In this paper, we report the absence of any mutations at coding sequence of Exon 4, 5, 6, 7, 10 and 11 of PS1 in a series of patients with late-onset AD referred for diagnostic testing at King Fahad National Guard Hospital, Riyadh, Saudi Arabia.

Materials and Methods

Fifty eight Saudi AD patients were involved in the present study. All the patients in the study had a confirmed diagnosis of AD according to DSM IV criteria (1994). The exact information regarding the age at on-set of disease and the family history was not available for few subjects. However, all the patients were diagnosed to have AD at more than 65 years of age indicating the late-onset form of AD. The age of the patients at the time of testing ranges from 67-80 years with a mean age of 70 years. All the patients underwent detailed clinical evaluation, neuropsychological testing, CT Scan or MRI imaging of the brain and electroencephalography (EEG). Metabolic disorder and electrolytes abnormalities were excluded by the appropriate/specific tests.

Table 1: Set of primers used for various exons

Exon	Forward primer	Backward primer
4	5´ - AAC CGT TAC CTT GAT TCT GCT-3´	5´ - TGC TGT AAA GAA AAG CCA CAC-3´
5	5´ - GAT TGG TGA GTT GGG GAA AA-3´	5´ -AAC AGG GTG GAA AGC AAA GA-3´
6	5´ -TTT AAG GGT TGT GGG ACC TG-3´	5´ -GCA AGG AGC AAC AGA AGA ATG-3´
7	5´ -TTG GGA GCC ATC ACA TTA TT-3´	5´ -TGA GGA AAG AAA ACA CTC CAG-3´
10	5´ -GTT TAA GGG CCA GCT AGT TAC AAT-3´	5´ -AGC TAC CTA AAG GAA TCC ATG ACT-3´
11	5´ -CAT TCA TTG TGG GGT TGA GT-3´	5´ -CAC CTG GGG TTA AAA CAG AA-3´

DNA was extracted from the blood with standard procedures utilizing proteinase-K/ phenol/ chloroform extraction. Primers were designed on the basis of the sequence data for exon 4, 5, 6, 7,10 and 11 available in the GenBank to amplify the coding sequence of amino acid 30 to 256 and 320 to 416 of PS1. PCR was performed using PuRe Taq Ready-To-Go PCR Beads (Amersham, USA) with different sets of primers (Table 1).

A 200-300ng of Genomic DNA was used as a template in 25 µl reaction. Genomic DNA was amplified for 40 cycles. Each cycle consisted of 94°C for 30 sec, 53°C for 30 sec, 72°C for 1 min for exon 4,5, 6, 7, 10 and 11.

The PCR products obtained were separated by electrophoresis on 1.5% agarose gel in TAE buffer, visualized by ethidium bromide fluorescence. Fragments with the expected size were cut from the gel, purified using GFX PCR DNA Gel band purification kit (Amersham, USA).

Purified DNA was sequenced using forward or/and backward primers. The DNA sequence was compared with sequence of same exon from the normal individuals and data available in Genbank.

Results and Discussion

No mutations were found at coding regions of exon 4, 5, 6,7,10 and 11 of PS1 in fifty eight Saudi patients with late-onset Alzheimer disease. This could be due to the age factor as all the subjects were more than 65 years old. However, various mutations in PS1 gene are associated with early-onset AD (Schellenberg *et al.*, 1992; Sherrington *et al.*, 1995). Similar results for absence PS1 mutations were reported in older patients (60 ±11 years) (Rogaeva *et al.*, 2001).

Our results support the previous findings that indicate the lack of mutations in PS1 gene of the persons affected with late-onset Alzheimer’s disease or no association of PS1 and late-onset AD (Schellenberg *et al.*, 1993; Wavrant-De Vrieze *et al.*, 1999; Tsuda *et al.*, 1995). Contrary to this, Wragg *et al.* (1996) reported that PS1 accounted for about half as much risk for late-onset Alzheimer’s disease as did ApoE 4 but this view has yet to be tested at a wider level.

Though, our results together with previous similar reports indicated that the mutations in PS1 gene associated with early-onset AD are probably non-existent in late-onset AD, however, the possibility of association of mutations at codons other than tested can not be excluded. Further, studies are certainly required involving more codons. Axelman *et al.* (1998) suggested the existence of other genetic or important environmental factors for the expression of AD phenotype, on the basis of large range at onset in a family with uniform genetic basis for the disease (a His163Tyr mutation in PS1).

According to Chen and Fernandez (2000) several known "risk factors" most likely play a critical role in the late-onset sporadic AD. These can exert their effects either by providing the conditions for ailing neuron to die or by enhancing the individual's vulnerability to natural neurodegeneration. Thereby, suggesting that late-onset sporadic AD would be similar to many other age-related conditions where no single pathogen can be held exclusively responsible for most cases, rather many risk factors are involved in converting initial defect into clinical disease.

The lack of mutations in the coding sequence of exon 4, 5, 6,7,10 and 11 of PS1 in present study indicated that the presence of mutations in coding region of these exons of PS1 was not a major cause of late-onset AD in Saudi population.

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