Case Report

Novel presenilin 1 mutation associated with early-onset Alzheimer's disease in a Saudi patient

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ABSTRACT

We report a 60-year-old Saudi patient with the clinical diagnosis of Alzheimer's disease (AD) and a novel mutation in the presenilin gene. We investigated mutations in the presenilin-1 gene in Saudi patients with AD using polymerase chain reaction and direct DNA sequencing methods. We extracted genomic DNA from the whole blood of both patients and normal control individuals. We sequenced and compared amplicons with the sequences of the respective exons of normal individuals as well as data available in GenBank. We detected a homozygous mutation (g→c) in exon 12, resulting in the missense mutation (Arg377Thr), in the DNA of a 60-year-old patient. We located this mutation in the cytoplasmic loop near the transmembrane domain 7.

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that occurs predominantly in later life. It is the most common cause of dementia, and has a complex etiology. Four genetic loci have been identified possibly contributing to the pathogenesis of AD. These are the amyloid precursor protein gene (APP) on chromosome 21,[1] the apolipoprotein E gene (ApoE) on chromosome 19,[2] the presenilin-1 gene (PS1) on chromosome 14,[3] and the presenilin 2 gene (PS2) on chromosome 1.[4] Several point mutations in the PS1 gene have been found to segregate with AD onset.[3] Changes in the PS1 gene are thought to cause nearly 10% of all AD cases (familial AD) and, possibly, provoke the development of sporadic AD, which accounts for the remaining percentage of AD cases. Most of the mutations are found in specific coding regions of the gene, mainly in exon number 5, 6, 7, 8, 11 or 12 of PS1. The PS1 point mutations are pathogenic mutations rather than polymorphic variants and co-segregate with the disease with an age difference of up to 20 years with some mutations resulting in an early onset of AD at the age of 30-65 years. However, some mutations result in a later onset (>65 years). The strong evidence suggesting the correlation of the PS1 mutations and the development of AD comes from the observation that cultured fibroblasts of patients with PS1 mutations produce higher amounts of bA4.[5] Moreover, recent findings suggest that some PS1 mutations are likely to provoke the g-secretase cleavages in the Golgi compartment and the trans-Golgi network, which is known to produce the larger form of bA4.[6] This together with the observation of variations present in the effect of

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these mutations with respect to the age of disease onset suggest that changes in the expression or function of PS1 are related to the development of AD, and may be associated with dementia in general. A study was performed to search for coding sequence variants in various exons of PS1 in AD Saudi patients. We report a novel mutation at codon 377 of PS1 cDNA in a 60-year-old Saudi patient with AD.

Case Report. We report a 60-year-old Saudi lady with slowly progressive memory problems over a few years, and when she was initially evaluated she had moderate impairment of her recent memory and had a very poor registration with markedly impaired decision making. She was very alert with no focal neurological finding in her neurological examination. She had no family history of memory problem and specifically of AD. Her parents were distant relatives. This lady had a full work up to look for causes of her memory problem. Her blood count was normal with no evidence of anemia. Serum electrolytes including serum calcium were normal. Complete liver enzyme screening showed no abnormalities. Thyroid stimulating hormone, serum vitamin B12 and folate were all normal. A CT scan of the head showed very mild generalized atrophy. Electroencephalography (EEG) showed very mild non-specific generalized slowing, with theta being the predominant rhythm. Neuropsychological testing was suggestive of AD. This patient was investigated with another 58 Saudi patients with dementia. Only 11 patients were diagnosed to have AD at age younger than 65 years (47-65 years) representing early onset form of AD. An equal number of age and gender matched normal controls were recruited from the same population for the genetic analysis. The DNA was extracted from the blood by standard procedure utilizing proteinase-K/phenol/chloroform extraction. Primers were designed based on the sequence data. Polymerase chain reaction (PCR) was performed using PuRe Taq Ready-To-Go PCR Beads (Amersham, USA) with the following primers: 5´-CAT TCA TTG TGG GGT TGA GT-3´ (sense strand); 5´-CAC CTG GGG TTA AAA CAG AA-3´ (antisense strand). A 200-300 ng of Genomic DNA was used as a template in 25 ml reaction. Genomic DNA was amplified for 40 cycles. Each cycle consisted of 94°C for 30 seconds, 53°C for 30 seconds, and 72°C for 1 min. The PCR products were separated by electrophoresis on 1.5% agarose gel in TAE buffer, visualized after ethidium bromide staining. Fragments with the expected size were cut from the gel and purified using GFX PCR DNA Gel band purification kit (Amersham, USA). Purified DNA was sequenced using sense and antisense primers. The DNA sequence was compared with sequence of the respective coding region from the normal individuals and data available in Genbank. The PCR amplification revealed a DNA fragment of 312 bp **(Figure 1).** The sequence of amplified DNA showed the alteration (g→c) in the coding region of the sequence of a 60-year-old AD patient **(Figure 2)**. This change in DNA was not detected in the controls. This variant indicated a mutation. Further analysis indicated that this alteration in DNA results in a substitution of arginine to threonine at codon 377 of PS1.

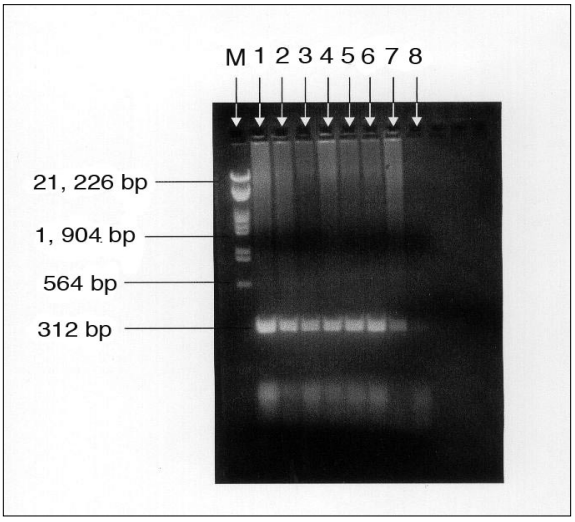

Figure 1 - Showing amplification of genomic DNA from AD patients. Lane M shows Lambda DNA digested with EcoR I and Hind III as DNA size marker. Lane 1, 2, 3, 4, 5, 6, 7 and 8 refer to the amplified DNA from various AD patients.

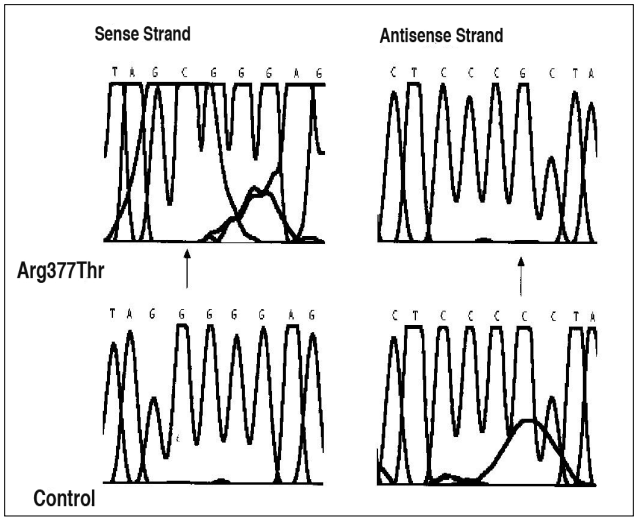

Figure 2 - Comparison of DNA sequences of AD patient and control showing (g→c) change causing a substitution of arginine to threonin (arrow) at codon 377 in PS.

Discussion. A search for mutations in the PS1 gene in Saudi AD patients yielded the discovery of one novel mutation (Arg377Thr). Mutations in the PS1 gene have been associated with early-onset AD and are responsible for ~50% of early-onset AD cases.[3] Whereas 40-50% of the risk for late-onset disease has been attributed to the E4 allele at ApoE locus.[2] Wragg et al,[7] suggested that PS1 also accounted for approximately half as many of the risk factors for late-onset AD as did ApoE. However, out of several late-onset Saudi AD patients studied earlier for PS1 mutations, none had any mutation in any of the exons studied.[8] In the present study, the mutation found was in an early onset AD patient (age at the time of testing was 60 years). Up to this date, more than 70 mutations have been reported in various exons of PS1 in different patients from various ethnic groups.[9] However, no mutations were found in exons 4, 5, 6, 7, 8, 9,10 and 11 of Saudi patients with late-onset AD in an earlier study.[8] Our results revealed a mutation (g→c) altering arginine to threonine at codon 377 of PS1 gene. This is a novel mutation of the PS1 gene occurring in the cytoplasmic loop near the transmembrane 7 region. The amino acid position 377 being highly conserved in different species indicated that this codon is important for PS1 functions. Moreover, the Tmf domain appears to be one of the favored sites for pathogenic mutations as 6 mutations have been reported to cluster in this region at codon, 352, 354, 358, 365 and 378.[9] In addition, the site is near to a functionally important region of hydrophilic loop that contains both the capase recognition site of PS1, consensus sequence for protein kinase A and C and adjacent to the one reported in a french family with early-onset AD (Gly378Glu). This also suggests that the Arg377Thr mutation is likely to be pathogenic rather than innocent polymorphism. The absence of the PS1 Arg377Thr mutation in the general Saudi population (controls) with its detection in an early-onset AD patient also lead to surmise that it is a pathogenic mutation. This mutation is found in one out of 11 (9%) of early-onset AD patients, it confirms the previous findings that nearly 10% of early onset AD cases are caused by mutations in various exons of PS1. However, the cause of disease in the rest of the patients where no mutations/polymorphism could be detected may be the mutations on other loci or other genetic or environmental factors. Further work is required to uncover the causive mutation in other patients.

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