Neuroscience 269 (2014) 215–222

NOVEL PRESENILIN MUTATIONS WITHIN MOROCCAN PATIENTS WITH EARLY-ONSET ALZHEIMER'S DISEASE

N. EL KADMIRI,[a*] N. ZAID,[c] Y. ZAID,[c] A. TADEVOSYAN,[c] A. HACHEM,[c] M.-P. DUBÉ,[c] K. HAMZI,[a] B. EL MOUTAWAKIL,[a,b] I. SLASSI[a,b] AND S. NADIFI[a]

[a] *Laboratory of Medical Genetics and Molecular Pathology, Faculty of Medicine and Pharmacy, University Hassan II, Casablanca, Morocco*

[b] *Department of Neurology, CHU IBN ROCHD, Casablanca, Morocco*

[c] *Montreal Heart Institute and Université de Montréal, Montreal, Canada*

Abstract—Alzheimer's disease (AD) is a progressive brain disorder that causes gradual and irreversible loss of higher brain functions and is the most common cause of dementia in the elderly, as assessed by autopsy and clinical series. Furthermore, it has an annual incidence of approximately 3% in the 65–74-year-old age group. This incidence rate doubles with every increment of 5 years above the age of 65. In Morocco, AD affects almost 30,000 individuals and this number will possibly increase to 75,000 by 2020 (projections of the World Health Organization (WHO)). Genetically, AD is caused by a mutation in one of at least 3 genes: presenilin 1 (PS1), presenilin 2 (PS2) and the amyloid precursor protein (APP). Most cases are late onset and apparently sporadic, most likely as a result of a combination of environmental and non-dominant genetic factors. In Morocco, the genes predisposing individuals to AD and predicting disease incidence remain elusive. The purpose of the present study was to evaluate the genetic contribution of mutations in PS1 and PS2 genes to familial early-onset AD cases and sporadic late-onset AD cases. Seventeen sporadic late-onset AD cases and eight familial early-onset AD cases were seen at the memory clinic of the University of Casablanca Neurology Department. These patients underwent standard somatic neurological examination, cognitive function assessment, brain imaging and laboratory tests. Direct sequencing of each exon in PS1 and PS2 genes was performed on genomic DNA of AD patients. Further, we identified 1 novel frameshift mutation in the PS1 gene and 2 novel frameshift mutations in the PS2 gene. Our mutational analysis reports a correlation between clinical symptoms and genetic factors in our cases of Early-Onset Alzheimer's Disease (EOAD). These putative mutations cosegregate with affected family members suggesting a direct mutagenic effect.

Key words: Moroccan patients, Alzheimer's disease, frameshift mutations, PS1 PS2 genes.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of neurodegenerative dementia of the human central nervous system and remains the leading unmet medical need in neurology. AD is clinically characterized by a progressive loss of cognitive function and by the onset of slowly progressive memory impairment. Pathological changes underlying AD process in the brain is characterized by two types of lesions: beta-amyloid (Aβ) peptide deposits (known as senile plaques) and accumulation of neurofibrillary tangles (Bertram et al., 2010). Three genes have been identified to date in which mutations result in early-onset familial AD, inherited in an autosomal dominant fashion. These genes are the amyloid precursor protein (APP) on chromosome 21 (more than 30 pathogenic mutations have been described), presenilin-1 gene (PS1) on chromosome 14 (associated to more than 170 mutations), and presenilin-2 gene (PS2) on chromosome 1 (only 18 potentially pathogenic mutations have subsequently been reported, making this the least common genetic cause of AD) (available at: http://molgen-www.uia.ac.be/ADmutations). Apolipoprotein E alters the risk for, and the age of onset of the common late-onset type of AD (Lendon et al., 1997). Cell-based studies and mouse models have shown that mutations in genes encoding APP, PS1 and PS2 cause an increased production of the neurotoxin Aβ42 (Theuns and Van Broeckhoven, 2000), indicating that unbalanced APP processing may be the primary event leading to the neurodegenerative brain pathology in AD patients carrying these mutations.

In Morocco, AD has emerged as a serious public concern with the number of people suffering from AD expected to increase as the elderly population continues to grow. Genes predisposing individuals to AD and predicting disease incidence remain elusive and prevent health care professionals from identifying AD in its early stages, with the goal of slowing down the progression.

*Corresponding author. Address: Laboratory of Medical Genetics and Molecular Pathology, Faculty of Medicine and Pharmacy, University Hassan II, 19 Rue Tarik Ibnou Ziad, B.P. 9154, Casablanca 20000, Morocco. Tel: +212-6-41-61-98-35.

E-mail address: elkadmiri1979@gmail.com (N. El Kadmiri).

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, beta-amyloid; EOAD, Early-Onset Alzheimer's Disease; FAD, familial Alzheimer's disease; MRI, Magnetic Resonance Imaging; MMSE, Mini-Mental State Examination; PS1, presenilin 1; PS2, presenilin 2; WHO, World Health Organization.

http://dx.doi.org/10.1016/j.neuroscience.2014.03.052

Accordingly, the aim of the present study was to evaluate the genetic contribution of mutations in PS1 and PS2 genes to familial early-onset AD cases and sporadic late-onset AD cases.

EXPERIMENTAL PROCEDURES

Patient recruitment

Patients were followed since 2004 by the Memory Consultation group at the CHU IBN ROCHD Neurology Department in Casablanca, Morocco. The protocol was approved by the human ethics committee of the CHU IBN ROCHD in accordance with the declaration of Helsinki for experiments involving humans and written consent was obtained from the patients or their guardians prior to the study.

Seventeen sporadic late-onset AD cases and 8 familial early-onset AD cases were seen at the memory clinic of the Neurology Department of the University of Casablanca Hospital IBN ROCHD. A family history was obtained by a clinical interview of the patient and a ''yes'' or ''no'' self-reporting questionnaire from the guardian or other family members. The disease was considered familial if at least one additional first-degree relative suffered from early-onset AD-type dementia. All patients underwent standard somatic neurological examination, cognitive function assessment, brain imaging and laboratory tests.

Assessment of cognitive function varied according to the education level of the subject and it included at least one Mini-Mental State Examination (MMSE), as recommended by the public health high authority. The examination was quoted on thirty points, including investigation of orientation, learning, attention, calculation, immediate memory, language and the ability to execute simple orders (Folstein et al., 1975). The deterioration stages are termed: ''light'' for scores between 20 and 26; ''moderate'' for scores between 15 and 19; ''moderate severe'' for scores between 10 and 14; and ''severe'' for scores below 10. The MMSE remains the most used test because it allows for a quick evaluation of cognitive functions and it is required for insanity diagnosis according to the NINCDS-ADRDA criteria. Several elements may modify the assessment tests results, such as the patient's age and sociocultural status, which are important factors to take into consideration when interpreting the test results. In our cases, the questions of the Folstein test were translated orally into the Moroccan dialect, regardless of sociocultural level, to be easily understood. In addition to the MMSE, according to the level of patient education, the clinician used alternative tests that do not require necessarily a level of education such as (BEC96) (Signoret et al., 1988), visual short-term or digital memory assessment, work memory assessment, language assessment test (DO80) (Deloche and Hannequin, 1997) and apraxia.

Brain Magnetic Resonance Imaging (MRI) was routinely performed on all patients. The biochemical assessment consisted mainly of blood analysis for complete blood count, liver, renal and thyroid function, as well as vitamin B12 and B9 serum levels. However, depending on the clinical context, other tests were also performed.

Genomic studies

Genomic DNA extraction and amplification of PS1 and PS2 genes.

Genomic DNA was isolated from peripheral blood leukocytes using the salting-out procedure (Miller et al., 1988). In this conventional technique, proteins and other contaminants are precipitated from the cell lysate using high concentrations of salt such as potassium acetate or ammonium acetate. The precipitates are removed by centrifugation and the DNA is recovered by alcohol precipitation. A PCR was performed for PS1 and PS2 genes (Table 1).

Genomic DNA sequencing and analysis

Direct sequencing was performed for each exon in PS1 and PS2 genes in 8 familial early-onset AD cases (as below) and 17 sporadic late-onset AD cases:

- The first family case harbors a female (ID P20) with a disease onset age of 60 and a positive family history of EOAD.
- The second family case harbors a female (ID F1) with an onset age of 63 and a positive family history of EOAD
- The third family case harbors a female (ID P32) with a disease onset age of 64 and a family history of AD.
- The fourth family case harbors a male (ID P30) with an onset age of 63 and a family history of AD.
- The fifth patient harbors a male (ID P24) with a disease onset age of 49 and a family history of AD. In addition, the patient's mother, 75 years of age, was likely afflicted with AD since she showed signs of generalized severe cognitive impairment. Her disease onset age was 66, which was followed by severe dementia 9 years later. Both of the patient's sisters (non affected members) and the patient's mother (ID P25) (the sixth affected member) agreed to participate in the study.
- The seventh patient harbors a male (ID F2) with an onset age of 60 and a family history of AD.
- The eighth family case harbors a male (ID F3) with a disease onset age of 55 and a family history of AD.

The other non affected members of families did not agree to participate which limited our study.

Briefly, Sephadex-purified PCR products were sequenced using the Big Dye terminator v3.1 Cycle Sequencing kit (Applied Biosystems). 1000 Genomes were used to study human genetic variations. ANNOVAR, PYTHON and BLAT UCSC programs were used to evaluate these mutations.

RESULTS

Sequence analysis of PS1 and PS2 genes revealed 3 novel frameshift mutations in 3 out of 8 familial early-onset AD cases and none in sporadic cases and healthy controls (Table 2).