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ANALYSIS OF GENETICS AND RISK FACTORS OF ALZHEIMER'S DISEASE

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Abstract—Alzheimer's Disease is the leading neurodegenerative cause of dementia. The pathogenesis is not clearly understood yet, is believed to be the complex interaction between genetic and environmental factors. Consequently vascular risk factors and Apolipoprotein E genotyping are increasingly gaining importance. This study aimed at assessing the relationships between Alzheimer's Disease and Apolipoprotein E phenotype and vascular risk factors. Patients diagnosed with ''possible Alzheimer's Disease'' in the Gazi University, Department of Neurology, were included in the study and age-matched volunteer patients who attended the polyclinic were included as a control group. In this study, the risk factors including low education level, smoking, hyperlipidemia, higher serum total cholesterol levels, and hyperhomocysteinemia were found to be statistically significantly more common in the Alzheimer's Disease group in comparison to the Control Group, while all Apolipoprotein E ε4/ε4 genotypes were found in the Alzheimer's Disease group. The presence of the Apolipoprotein E ε4 allele is believed to increase vascular risk factors as well as to affect Alzheimer's Disease directly. The biological indicators which are used in identifying the patients' genes will be probably used in the treatment plan of the patients in the future.

Key words: Alzheimer's Disease, Apolipoprotein E genotyping, vascular risk factors, biological indicators, dementia.

INTRODUCTION

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder of the central nervous system that is characterized by impairment of memory, language and thought (Striepens et al., 2010). AD is the most common form of dementia. The prevalence of AD has been reported as 4.4% in people aged 65 or more in Europe and 9.7% over the age of 70 in the United States of America (Povova et al., 2012).

Issues such as determining the people at risk of developing AD with age, closer monitoring of this people, insistence on early diagnosis are gaining importance (Boustania et al., 2003).

Epidemiologic studies have demonstrated that the risk factors for vascular diseases and stroke are also connected to cognitive impairment, developing AD, and aggravate the severity of clinical symptoms (Breteler, 2000). Risk factors for AD are known as age, sex, dementia in family, genetic factors; Apolipoprotein E (Apo E) ε4, Down Syndrome, head trauma, small head circumstance, history of major depression, atherosclerosis, stroke, hypertension (HTN), hypotension, Diabetes Mellitus (DM), hyperlipidemia (HPL), obesity, plasma homocysteine level, smoking, immunological factors, inflammatory factors, systemic diseases; thyroid disorders, metabolic, infectious factors, and exposure to certain toxic conditions and intoxications (Karaman, 2010).

AD occurs in familial and sporadic forms. The familial form accounts for 5% of the cases in international medical literature, sporadic AD is very common and accounts for 90–95% of the AD cases. Three separate genes were found to be responsible for autosomal transmission, so far: The Amyloid Precursor Protein (APP) gene on the 21th chromosome, presenilin1 gene on the 14th chromosome, presenilin two gene on the 1st chromosome. Although the cause of the sporadic form is not fully understood, it occurs in the aging process as a result of the complex interactions between genetic and environmental risk factors. The leading genetic risk factor in the overall population is the ApoE ε4 allele. There have been some studies demonstrating the contribution of the ApoE ε4 allele to the accumulation of Amyloid-beta (Aβ), to the formation of fibril, plaque (Laws et al., 2003).

The gene encoding Apo E is located on the proximal long arm of the 19th chromosome. Allelic variants of Apo E known as ε2, ε3, ε4 include six Apo E phenotypes which are; homozygous ε2/2, ε3/3, ε4/4, heterozygous ε3/2, ε4/2, ε4/3; ε2 is the most common

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Abbreviations: Aβ, Amyloid-beta; AD, Alzheimer's Disease; Apo E, Apolipoprotein E; ASHD, atherosclerotic heart disease; DM, Diabetes Mellitus; DNA, deoxyribonucleic acid; HPL, hyperlipidemia; HTN, hypertension; MCI, Mild Cognitive Impairment; MMSE, Mini Mental State Examination; NFT, neurofibrillary tangles; NPs, neuritic plaques.

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allele of Apo E. Apo E ε4 has been identified even as a possible indicator of AD. Increased Apo E ε4 allele in AD, in neuritic plaques (NPs), tangles and in the areas of amyloid accumulation suggested that it had an important function in the pathogenesis of the disease. An increased level of Apo E ε4 is associated with an increase in NPs (Vuletic et al., 2008).

EXPERIMENTAL PROCEDURES

44 patients diagnosed with "possible AD" in the Department of Neurology Gazi University Faculty of Medicine were included in the study and 51 volunteers without an intracranial degenerative disorder who attended the Neurology Outpatient Clinic were included in the study as a control group.

The definitive diagnosis of AD is made by neuropathological studies. However, due to its difficulty, the diagnosis is based on clinical, neurological, and psychiatric assessments. The clinical diagnosis of Possible AD was made according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984), Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association, 1994) criteria.

Patients with neurological examination findings, neurological test results which do not comply with these criteria were excluded from the study. Laboratory tests were used to rule out other diseases (to find out the causes of treatable dementia). All patients underwent CT Scan or MRI Scan of the brain within the last month of their admission to the study. The differential diagnosis of other forms of dementia, Mild Cognitive Impairment (MCI) was made. MCI patients were identified by the MCI, Early Detection Parameters of Dementia (Report of the Quality Standards Subcommittee of the American Academy of Neurology) (Petersen et al., 2001).

Hachinski Ischemia Score (HIS) (Hachinski et al., 1975) was used in differentiating AD from severe vascular disorder. NINDS-AIREN (National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neurosciences) criteria were used in the diagnosis of VD to differentiate AD from vascular dementia (Román et al., 1993). Patients, controls were questioned for systemic, brain, heart, psychiatric diseases or symptoms, other diseases (trauma, surgery, habits- medications, intoxication), smoking, alcohol consumption, and current medications.

Tests consisting of Mini Mental State Examination (MMSE) test (Folstein et al., 1975), Clinical Dementia Rating Scale (Hughes et al., 1982), Global Deterioration Scale (Reisberg et al., 1988) were completed by the patients.

Activities of daily living were separately assessed by the Lawton Activities of Daily Living Scale (Lawton and Brody, 1969) as simple and instrumental activities of daily living (Lawton and Brody, 1969). Advanced AD patients, poorly cooperative patients were excluded from the study due to compliance issues with neuropsychological tests. Hamilton Depression Scale (Hamilton, 1960) was completed by the participants. Patients with severe depression were excluded from the study.

The AD assessment scale-Cognitive functions (ADAS-cog) sampling application to the Turkish population was used in the patients (Akca Kalem et al., 2003).

In addition, patients with mixed type dementia (AD-VD) weren't included in the study. Among the patients diagnosed with AD, who developed stroke, epilepsy, severe depression or psychiatric disease, metabolic or endocrine disorders leading to the impairment of mental functions, the participants with a history of brain disease (trauma, infection) weren't included in the study.

Body weight, length, heart rate, and blood pressure measurements of the participants were noted. Blood pressure readings were taken using a standardized method with a manual sphygmomanometer in sitting position from the left arm following a resting period of 5 min. The first and the last Korotkoff sounds were evaluated as systolic, diastolic blood pressures (BP) respectively. According to the blood pressure readings in the categorization of systolic blood pressure (SBP), readings <110 mmHg were categorized as low, readings between 110 and 139 mmHg were categorized as normal, readings between 140 and 159 were categorized as borderline, readings $\geqslant 160$ mmHg were categorized as high. For the diastolic blood pressure; readings <80 mmHg were low, $\leqslant 90$ mmHg were normal, 90–94 were borderline, readings $\geqslant 95$ mmHg were high (Launer et al., 1995).

Patients, controls underwent tests (complete blood count, sedimentation, electrolyte (Ca, Na, K, Cl), biochemical tests concerning kidney function (BUN, creatinine), liver function (AST, ALT, ALP), thyroid function (free T3, free T4, TSH, thyroglobulin), ferritin, folic acid, B12 vitamin, total cholesterol, HDL-C, LDL-C, triglyceride, homocystein, ApoA, ApoB levels, ApoE genotype). In addition, ECG (electrocardiography), EEG (electroencephalography) tests were applied for all the participants.

Control patients were selected among age-matched normal volunteers without dementia and any other neuro-degenerative disease, without a history of any systemic disease that may affect cognitive functions, psychiatric disease affecting mental state. Patients with manageable HTN, DM, atherosclerotic heart disease (ASHD) were included in the study.

10 cc-peripheral venous blood samples were collected in EDTA tubes for the gene analysis, stored at -20 °C until the isolation phase. Deoxyribonucleic acid (DNA) isolation was carried out by a DNA isolation method using high salt concentration in the blood samples of the participants. DNA extraction was performed using the spin column method (MN, Macherey-Nagel, Germany). Isolated DNA samples were controlled by forcing to migrate in the 2% agarose gel matrix during electrophoresis. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were used within the scope of the study (Oner, 2000). In our study Apo E genotypes were