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Lack of Association between Angiotensin-Converting Enzyme and Dementia of the Alzheimer's Type in an Elderly Arab Population in Wadi Ara, Israel

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Abstract: The angiotensin-converting enzyme (ACE), a protease involved in blood pressure regulation, has been implicated as an important candidate gene for Alzheimer’s disease (AD). This study investigated whether the ACE gene insertion–deletion (ID) polymorphism is associated with risk of developing dementia of Alzheimer’s type (DAT) in an Arab–Israeli community, a unique genetic isolate where there is a high prevalence of DAT. In contrast to several other studies, we found no evidence of an association between this polymorphism and either DAT or age-related cognitive decline (ARCD).

Keywords: angiotensin-converting enzyme, dementia of the Alzheimer’s type, age-related cognitive decline, insertion–deletion polymorphism, Arab population

Introduction
Alzheimer’s disease (AD) is a catastrophic affliction of the middle and later years of life. Clinically, it is marked by an irreversible decline in mental abilities, including progressive loss of memory, confusion, and dementia, and culminates in childlike helplessness. In the United States, the incidence of AD is exponentially increasing and is projected to affect 8.64 million people by the year 2047 (Grossberg 2003). Median survival times range from 8.3 years for persons diagnosed at age 65, to 3.4 years for persons diagnosed at age 90 (Brookmeyer et al 2002).

Angiotensin-converting enzyme (ACE) catalyzes the conversion of angiotensin I into a physiologically active peptide, angiotensin II. This reaction is an important process in blood pressure regulation. In addition to this well known physiologic role, ACE is able to cleave other proteins, including bradykinin and substance P, and has been shown to inhibit and cleave amyloid β-peptide (Aβ) aggregation and amyloid plaque formation in vitro (Hu et al 2001). ACE may contribute to the pathogenesis of AD.

To test the hypothesis of association between ACE and DAT, we evaluated an insertion–deletion (ID) polymorphism in the ACE gene as a potential risk factor for dementia of the Alzheimer’s type (DAT) in an Arab community in Wadi Ara, Israel. The population comprised 853 persons over the age of 65 (Bowirrat et al 2001; Bowirrat, Friedland, Farrer, et al 2002). Family studies revealed that more than one-third of DAT cases are members of one extended family. We hypothesized that DAT in Wadi Ara may be due to a founder effect enhanced by consanguinity; thus, a specific disease susceptibility allele may be overrepresented in cognitively impaired subjects compared with cognitively healthy residents. Our results suggest that the
ACE-ID polymorphism is not associated with either DAT or age-related cognitive decline (ARCD) in this Arab community.

Methods and results

Subjects were sourced from a prevalence study of dementia conducted in Wadi Ara, a geographically defined area in northern Israel comprising three Arab villages (Bowirrat et al 2001). Among the original list of 853 residents aged 65 years and older (prevalence date, October, 1995), 823 (363 males and 460 females) were available and consented to participate in the study. Only 30 patients (23 females and 7 males) were excluded from the original list of the study (853) because they did not fulfill the diagnostic criteria for DAT and ARCD (they were classified with other medical conditions). Among the excluded females (n = 23), 19 suffered from vascular dementia (VaD), 1 patient had Parkinson’s disease (PD), and 3 had pseudodementia. Among the excluded males (n = 7), 5 suffered from PD and 2 patients were diagnosed with VaD. The sex-specific prevalence of DAT among males and females was 53/363 = 14.6% and 115/460 = 25%, respectively; and the sex-specific prevalence of ARCD among males and females was 118/354 = 33.3% and 236/354 = 66.6%, respectively. These subjects were interviewed and examined by an Arabic-speaking neurologist (AB). Each subject underwent a battery of standard cognitive tests modified to fit the cultural and linguistic characteristics of this community (Bowirrat et al 2001). The diagnosis of DAT and ARCD was determined using DSM-IV criteria (APA 1994). Those with a medical history and laboratory or cognitive test results that suggested the presence of other illnesses, such as vascular dementia, Parkinson’s disease, normal-pressure hydrocephalus, or pseudodementia (depression), were excluded. Peripheral blood samples were obtained from the 650 subjects for DNA analysis.

To genotype the ACE-ID polymorphism, 2 polymerase chain reactions (PCRs) were performed. In the first reaction, primers that flank the ID polymorphism were used to amplify DNA from each subject (5’-CTGGGACACTCCCATCCTTCT and 5’-TTGATGAGTTCCAGGTATTTCG). The resulting PCR product was analyzed on a 2% agarose gel for the presence or absence of the 287-bp insertion–deletion polymorphism. Because this reaction frequently fails to detect the insertion variant, a second PCR reaction was performed that specifically amplifies the allele containing the insertion (primers 5’-CTGGGATCCACAGGGCGTGATA and 5’-TTGATGAGTTCCAGGTATTTCG). The data from both reactions were used to establish the ACE-ID genotype. For statistical analysis, the Hardy-Weinberg equilibrium (HWE) was assessed for each group (DAT, ARCD, normal). Allele and genotype frequencies were compared among groups by a χ² test.

The results of the ACE (ID) genotyping (Table 1) showed that the frequency of the I allele was 32% in cognitively normal control, 26% in DAT cases (p = 0.2223), and 32% in ARCD cases (p = 0.9559). The genotype frequency distribution in the control groups were also similar to those for DAT cases (p = 0.3731) and ARCD cases (p = 0.9973). Therefore, our results suggest that the ID polymorphism within ACE is not associated with DAT or ARCD in this Arab community (Table 1).

Discussion

AD is the most overdiagnosed and misdiagnosed disorder of mental functioning in older adults. Part of the problem, is that many other disorders show symptoms that resemble those of AD. The crucial difference, however, is that many of these disorders – unlike AD – may be stopped, reversed, or cured. Based on these findings, clinical diagnosis of AD has been referred to as “a diagnosis by exclusion”, and one that can only be made in the face of clinical deterioration over time. There is no specific clinical test or finding that is unique to AD. Hence, all disorders that can bring on similar symptoms must be systematically excluded. The “classical” senile plaques and the neurofibrillary tangles seen in an AD

Table 1 Genotypic and allelic frequencies among ARCD, DAT, and cognitively normal control

<table>
<thead>
<tr>
<th>ACE-ID</th>
<th>Normal</th>
<th>ARCD</th>
<th>DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>8 (7.14)</td>
<td>3 (6.82)</td>
<td>5 (5.88)</td>
</tr>
<tr>
<td>DI</td>
<td>56 (50)</td>
<td>22 (50)</td>
<td>35 (41.18)</td>
</tr>
<tr>
<td>DD</td>
<td>48 (42.86)</td>
<td>19 (43.18)</td>
<td>45 (52.94)</td>
</tr>
<tr>
<td>f(I)</td>
<td>72 (32.14)</td>
<td>28 (31.82)</td>
<td>45 (26.47)</td>
</tr>
</tbody>
</table>

Abbreviations: ACE, angiotensin-converting enzyme; I, insertion allele; D, deletion allele; ARCD, age-related cognitive decline; DAT, dementia of Alzheimer’s type; f(I), frequency of the insertion allele.

Table 1a Genotypic p-value

<table>
<thead>
<tr>
<th>Group</th>
<th>ARCD</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT</td>
<td>0.5745</td>
<td>0.3731</td>
</tr>
<tr>
<td>ARCD</td>
<td>–</td>
<td>0.9973</td>
</tr>
</tbody>
</table>

Table 1b Allelic p-value

<table>
<thead>
<tr>
<th>Group</th>
<th>ARCD</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT</td>
<td>0.3660</td>
<td>0.2223</td>
</tr>
<tr>
<td>ARCD</td>
<td>–</td>
<td>0.9559</td>
</tr>
</tbody>
</table>
brain at autopsy typically are the only definitive diagnosis of the disease.

We did not observe an association between the ACE-ID polymorphism and DAT or ARCD in the elderly Arab population of Wadi Ara. Notably, inherited susceptibility for DAT in Wadi Ara is also not accounted for by the ApoE ε4 allele. Indeed, the frequency of ApoE ε4 among DAT, ARCD, and control was 0.027, 0.033, and 0.029, respectively (Bowirrat et al 2000).

Nonetheless, the relationship between ACE and AD is controversial. Several studies have implicated the ID polymorphism and risk of AD (Narain et al 2000; Cheng et al 2002; Keohoe et al 2003; Elkins et al 2004; Tian et al 2004), while other studies failed to find an association (Myllykangas et al 2000; Taylor et al 2001; Buss et al 2002; Lendon et al 2002; Monastero et al 2002; Harrap et al 2003; Panza et al 2003). Moreover, the pattern of association has been confusing, with some studies showing an association with the I allele (Alvarez et al 1999; Yang et al 2000; Hu et al 2001), while others with the D allele (Amouyel et al 1996; Palumbo et al 1999; Crawford et al 2000; Farrer et al 2000; Richard et al 2001).

The reasons for the disparity in results across studies may be due to differences in ethnic background, different environmental factors, or subject misclassification. To date, few studies have examined the frequency of the ACE genotypes in autopsy-confirmed cases of AD, or investigated the potential effect of these ACE genotypes on the pathology of the disorder. In one autopsy series, the ACE genotype was not associated with the amount of Aβ40, Aβ42, or pathological tau protein deposited within the frontal cortex, or in the extent of amyloid angiopathy in the brain, whether ApoE ε4 allele was present or not (Lendon et al 2002). The only other autopsy-based study of ACE (based on an elderly Finnish population) also failed to detect an association with ACE (Myllykangas et al 2000). In the Arab community, autopsy is prohibited for religious and traditional reasons. Other limitations of our study are the absence of biomarkers and complete biochemical examinations, and the low number of neuroimaging performed. These helpful clinical techniques are necessary to improve our clinical diagnosis of DAT and are required to differentiate it from similar clinical manifestation that mimic the presentation of dementia.

A possible biological explanation for an association between ACE and AD is still unclear. The insertion allele appears to reduce ACE expression, and DD homozygotes and ID heterozygotes have more circulating ACE than II homozygotes: 65%, and 31%, respectively (Rieder et al 1999). This increased activity of the D allele may alter Aβ deposition by a direct proteolytic mechanism (Keohoe et al 2003). Alternatively, ACE may have an indirect role in the progression of AD. The ID polymorphism has also been implicated in various conditions including hypertension (Zaman et al 2002; Hopkins and Hunt 2003), coronary heart disease (Arbustini et al 1995; Kauma et al 1996), cardiomyopathy (Pfohl et al 1998), stroke (de la Torre et al 2002), and juvenile rheumatoid arthritis (Alsaeid et al 2003). Other studies don’t confirm these associations (see Ueda et al 1995; Hung et al 1999; Harrap et al 2003).

Several studies suggest that there may be a relationship between cardiovascular lesions and the severity and progression of AD (Snowdon et al 1997; Zuliani et al 2001; Bowirrat, Friedland, Korczyn 2002; de la Torre 2002; Korczyn 2002). In summary, in the present study, we found a lack of association between ACE and DAT. Our previous studies have not determined any gene that may explain the high prevalence of DAT in this community, but we have found high prevalence of vascular dementia (VaD) following stroke. Indeed, the prevalence of VaD constitutes 5.9% of the total elderly Arab population in Wadi Ara and about 22% of the total dementia population (Bowirrat, Friedland, Korczyn 2002). This prevalence of VaD (22%) is extremely high compared with VaD patients who accounted for only 6% of all dementia patients identified by Fillit and Hill (2002). Given the role of the ACE gene in vascular disease as well as in AD, the ability to detect association with AD may be complex. On the other hand, and in light of the high frequency of VaD in this population, a new study to evaluate the role of ACE in VaD is essential.

Acknowledgments
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