Research Article

Lack of Association between the GPR3 Gene and the Risk for Alzheimer’s Disease

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Alzheimer’s disease is the most frequent form of dementia and its incidence is rapidly increasing. Genetic factors are important determinants of the individual susceptibility to the disease and many efforts have been made to identify loci and markers involved. Recent finding describes the GPR3 gene as a modulator of β-amyloid production, suggesting that perturbation of its activity and function may contribute to the pathogenesis of AD. Furthermore, the gene is located at chromosome 1, in a region proposed as a susceptibility locus for the disease. We searched for nucleotide variations in the coding sequence and in the region 5 prime of it by dHPLC and analysed their distribution in a group of 104 AD patients and 109 age-matched controls. We identified 5 types of variation, two in the putative promoter region (g.27718954A>G and g.27719102A>T) and the others in exon 2 (c.51C>A, c.80C>G, and c.771C>T). All of them were equally represented in the two cohorts of the study, thus suggesting the absence of an association between GPR3 gene and AD in our population.

1. Introduction

Alzheimer’s disease (AD) is the most common neurodegenerative disease and an important cause of illness and death in the industrialized world [1]. It often initiates with difficulties in remembering new information and gradually progresses to dementia and loss of normal life abilities. The major histopathological features are extracellular senile plaques and intracellular neurofibrillary tangles (NFTs), both related to the deposition of misfolded proteins; beta amyloid (Aβ) is the main component of plaques and hyperphosphorylated tau that of NFTs. A very small proportion of AD cases is directly linked to genetic variations in three genes coding for the amyloid precursor protein (APP) on chromosome 21, presenilin 1 (PSEN1) on chromosome 14, and presenilin2 (PSEN2) on chromosome 1. These genetic forms are collectively defined as early-onset familial AD (EOFAD), since the disease tends to start earlier than the age of 65, sometimes even before 30 years of age. Most cases of AD are sporadic, with less evident familial aggregation and later onset of the symptoms (late-onset AD, LOAD). The causes of sporadic AD are not defined yet, but current thinking suggests that multiple factors, including environment, epigenetics, and genetics concur in determining the individual risk. Advancing age is surely the prominent risk factor for LOAD, but genetics is estimated to determine up to 60–80% of the individual susceptibility [2]. Both candidate gene and genome wide association studies (GWASs) have identified different genes and loci potentially capable of modifying the predisposition to develop AD; the Apolipoprotein E gene (APOE) is by large the most important of these, with carriers of the e4 allele having a 4-fold increased risk [3], while those of the e2 allele benefit of a protective effect [4, 5].
Recent findings have indicated the orphan G protein-coupled receptor 3 (GPR3) has a possible agent in the modulation of Aβ metabolism [6]. Overexpression of GPR3 stimulated the production of the peptide, while its genetic ablation reduced it, both in transduced neural cells and in a mouse model of AD. This effect was exerted through the modulation of γ-secretase, one of the enzymes involved in APP proteolytic processing [7]; overexpression of GPR3 determined an increase in the amount of mature γ-secretase and of its localization at the cellular surface. Interestingly GPR3 is significantly expressed in the central nervous system [8], is elevated in the sporadic AD brain [6], and maps to chromosome 1 p36.1–p35 [9], which has been suggested as a potential AD linkage region in a high-resolution genome screen [10]. On the basis of these data we scanned the putative promoter and coding regions of GPR3 gene by DHPLC and evaluated frequencies and distribution of the identified sequence variations in cohorts of Italian AD patients and age-matched controls.

2. Materials and Methods

2.1. Patients and Controls. AD patients and controls were recruited by the Alzheimer Unit of Ospedale Maggiore in Milan (Italy). All patients (104) underwent a standard battery of examinations and dementia severity was assessed by the Clinical Dementia Rating (CDR) and the Mini Mental Scale Examination (MMSE). The diagnosis of probable AD was made according to NINCDS-ADRDA criteria [11]. The control group consisted of 109 nondemented subjects (MMSE ranging from 27 to 30). All individuals or caregivers provided an informed consent to participate in genetic studies. Table 1 shows the demographic and clinical characteristics of the population sample. Considering the age of onset of the disease, the AD population (78.4 ± 6.7) had similar age than the control one (72.6 ± 7.9) and no differences in gender distribution were present.

2.2. Patients and Controls. Genomic DNA was obtained from whole blood [12] and stored at 4°C until use. APOE genotyping was performed by DHPLC as previously described [13]. Table 1 shows number and percentage of APOE ε4 carriers among patients and controls. The GPR3 gene consists of two exons separated by a single intron.

Table 1: Demographic, clinical, and genetic characteristics of patients with AD and controls.

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>CNT</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>104</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Sex F/M</td>
<td>71/33</td>
<td>74/35</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>78.4 ± 6.7</td>
<td>72.6 ± 7.9</td>
<td></td>
</tr>
<tr>
<td>MMSE score</td>
<td>19.2 ± 5.7</td>
<td>27–30</td>
<td></td>
</tr>
<tr>
<td>APOE ε4+</td>
<td>39.4%</td>
<td>13.2%</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

aData are given as mean ± SD.

bThe age of onset of the disease was considered for AD patients.

AD: Alzheimer’s disease patients; CNT: controls; MMSE: Mini Mental Scale Examination.

Table 2: Nucleotide variants in GPR3 promoter and coding regions and their allele frequencies in AD patients and controls.

<table>
<thead>
<tr>
<th>SNP position</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNPs</td>
<td>AD</td>
</tr>
<tr>
<td>g.2771953A&gt;G</td>
<td>Upstream 5' end</td>
</tr>
<tr>
<td>g.27719102A&gt;T</td>
<td>Upstream 5' end</td>
</tr>
<tr>
<td>c.51C&gt;A</td>
<td>Exon 2</td>
</tr>
<tr>
<td>c.771C&gt;T</td>
<td>Exon 2</td>
</tr>
</tbody>
</table>

The g.2771953A>G (rs2504785) and g.27719102A>T SNPs are in the genomic region (NC_000001.10) upstream of GPR3 exon1 and probably linked in a haplotype (AA or GT). The c.51C>A and c.771C>T (NM_058212.2) are synonymous SNPs and correspond to Rs11586015 and Rs2230880, respectively. SNP: single nucleotide polymorphism; AD: Alzheimer’s disease patients; CNT: controls.

The coding sequence (NM_005281.2, from base 1 to base 1324) and about 500 bp upstream of exon 1 (hereafter referred to as “promoter” region) were analyzed by the Navigator software (Transgenomic Inc, Neb., USA) in order to evaluate the melting profiles in the sequence and optimize the design of primers for PCR amplification. The sequence of interest was subdivided in 5 different fragments, ranging from 280 to 540 bp. About 100 ng of genomic DNA were amplified by primers and settings described in the supplementary (Table 1SD, material available online at doi: 10.4061/2011/576143) DHPLC analysis was performed by a 3500A System (Transgenomic Inc, Neb., USA.); 5 μl of each PCR reaction were loaded on a SepA column and analysed at the temperatures described in Table 1SD. Different elution profiles were obtained and associated to specific genotypes by further DHPLC analysis and sequencing. Finally, homozygous samples always eluted with single-peak, symmetric profiles, with no evident differences in the retention time. In order to distinguish wild-type samples from those carrying mutation in homoyzgosis, each sample was mixed with a known wild-type one, denatured and reannealed, and further analysed by DHPLC; when the elution profile was no more symmetric, sequencing allowed to confirm the presence of a homozygous nucleotide variation.

2.3. Patients and Controls. Allelic and genotypic frequencies were obtained by direct counting. The Hardy-Weinberg equilibrium and differences in the allele frequency distribution between patients and controls were assessed by the χ-squared test.

3. Results and Discussion

The analysis of GPR3 “promoter” and coding regions by DHPLC in about 400 chromosomes evidenced few nucleotide variations, most of which with low frequency (Table 2). We identified a possible haplotype in the “promoter” region, consisting of the SNPs g.27718954A>G (rs2504785) and the g.27719102A>T (NC_000001.10). All the subjects carrying the G variant in the most upstream position were also positive for the second variation. Given the short distance between the two SNPs (150 bp),
they are probably inherited as a haplotype, AA and GT being the two alternative genotypes. The overall allelic frequency was 8.3%; five individuals were homozygous and 19 heterozygous. No frequency is reported in the database (Entrez SNP, http://www.ncbi.nlm.nih.gov/) for rs2504785 and the comparison with our data is impossible. The g.27719102A>T seems to be unreported yet. The analysis of the possible biological effects of the two SNPs was performed by TFSEARCH (http://www.cbrcresearch.com/research/db/TFSEARCH.html) and resulted in no difference in types and positions of putative transcription factor-binding sites among the ancestral sequence and the variant one.

The analysis of the coding sequence (NM_058212.2) revealed only three substitutions, two synonymous (Table 2), c.51C>A (G17G, Rs11586015) and c.771C>T (A257A, Rs2230880) and one nonsynonymous, c.80c>G (A27G), all at the heterozygous state. The frequency of the c.51C>A SNP was 0.024, very close to that described in the database (0.042, pilot 1 CEU sample). That of c.771C>T was 0.010 (database = 0.033, MITOGPOP6 sample), while the third mutation was identified in a single subject.

When we compared the distribution of these variants in the cohort of AD patients versus that of age-matched controls we did not find any statistically significant difference, the frequency being virtually the same in patients and controls (Table 2).

Since we analysed each amplicon at different temperatures, we believe that our search is complete and that the other types of substitution in GPR3 open reading frame (rs34890664, rs34585631, and rs734852) that are described in the database are not present in our population. As a matter of fact their allelic frequency is either not reported or very low (rs734852 = 0.017, HapMapCEU).

4. Conclusions

The number of subjects enrolled in the study together with the low incidence of the nucleotide variations found in GPR3 gene sequence limit the statistical power of the study (between 40 and 50%, http://www.dssresearch.com/toolkit/default.asp); nonetheless the results let us infer that the SNPs identified do not determine modification of the individual risk to develop AD. The analysis of a larger population sample would be useful for the definitive confirmation of the results.

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References


