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Research Article

Association Study of Genetic Variants in *CDKN2A/CDKN2B* Genes/Loci with Late-Onset Alzheimer's Disease

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Alzheimer's disease (AD) is the most common form of dementia clinically characterized by progressive impairment of memory and other cognitive functions. Many genetic researches in AD identified one common genetic variant ($\varepsilon 4$) in Apolipoprotein E (*APOE*) gene as a risk factor for the disease. Two independent genome-wide studies demonstrated a new locus on chromosome 9p21.3 implicated in Late-Onset Alzheimer's Disease (LOAD) susceptibility in Caucasians. In the present study, we investigated the role of three SNP's in the *CDKN2A* gene (rs15515, rs3731246, and rs3731211) and one in the *CDKN2B* gene (rs598664) located in 9p21.3 using an association case-control study carried out in a group of Caucasian subjects including 238 LOAD cases and 250 controls. The role of *CDKN2A* and *CDKN2B* genetic variants in AD is not confirmed in our LOAD patients, and further studies are needed to elucidate the role of these genes in the susceptibility of AD.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia clinically characterized by insidious onset and progressive impairment of memory and other cognitive functions [1], ultimately resulting in complete dependency and death of the patient.

The key features of AD brains are neuronal and synapse loss, extracellular plaques composed of amyloid-β (Aβ) peptides and intraneuronal neurofibrillary tangles consisting of hyperphosphorylated tau protein.

Although most patients develop AD at later age, with a prevalence estimates ranging from 4.4% in persons aged 65 years to 22% at ages 90 and older [2], it is mainly the research performed on the rare autosomal dominant early-onset form of AD that provided valuable insights into disease pathogenesis.

Several penetrant autosomal dominant mutations have been identified (http://www.molgen.ua.ac.be/ADMutations/), leading to early-onset familial AD within three genes: presenilin 1 (*PSEN1*) [3], presenilin 2 (*PSEN2*) [4], and amyloid precursor protein (*APP*) genes [5].

The Apolipoprotein E gene (*APOE*) was identified as a major risk factor contributing to the pathogenesis of late-onset Alzheimer disease's (LOAD) [6]. However, the *APOE* e4 allele is neither necessary nor sufficient for the occurrence of the disease.

Many SNPs have been analyzed to identify new susceptibility candidate genes for the LOAD [7].

Recently, two independent genome-wide studies have demonstrated a new locus on chromosome 9p21.3 implicated in LOAD susceptibility in Caucasians [8, 9].

Züchner and colleagues in their work individuated several SNPs in three different genes: the cyclin-dependent kinase inhibitors (*CDKN2A, CDKN2B*) and methylthioadenosine phosphorylase (*MTAP*), and they conducted an allelic association test to evaluate the genetic effect of these genes [9].

In light of these results, we investigated the role of three SNP's in the *CDKN2A* gene (rs11515, rs3731246, and rs3731211) and one in the *CDKN2B* gene (rs598664) using an association study carried out in a group of Caucasian LOAD patients.

2. Materials and Methods

2.1. Patients. Our study group included 488 Caucasian subjects: 238 LOAD patients (63.9% females, age at onset 72.9 ± 5.6 years, mean ± SD, Mini Mental State Examination score 20.2 ± 5.4 points) and 250 nondemented controls (62.4% females, mean age 71.5 ± 5.7 years; SD) (Table 1).

All subjects were enrolled in the study at the Neurology Unit of the Department of Neurological and Psychiatric Sciences of the University of Florence and at Paul Flechsig Institute for Brain Research, University of Leipzig, Germany.

Patients (19.7% autopsy proven) were clinically evaluated according to published guidelines, and the AD diagnosis fulfilled the Diagnostic and Statistical Manual of Mental Disorders criteria (DSM-IV) [10, 11]. Presence of a family history of dementia was considered an exclusion criterion.

All controls were carefully assessed using a rigorous clinical history evaluation and a general/neurological examination, in order to exclude the presence of any neurological disorder.

The study protocol was approved by the local ethics committee and conducted in accordance with the provisions of the Helsinki Declaration; informed consent for genetic screening was obtained from the study participants or, where appropriate, a relative or legal representative.

2.2. Genotyping. DNA was extracted from white blood cells using the phenol-chloroform procedure and all of the genotyping was performed by KBioscience (http://www.kbioscience.co.uk/). KBiosciences uses a novel form of competitive allele specific PCR system (KASPar) that is a proprietary KBioscience invented genotyping chemistry. The accuracy greater than 99% is achieved and a routine quality control measures is performed on all genotyping.

APOE genotypes were determined in all subjects using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods, as previously described [12].

2.3. Statistics. Differences in distribution between cases and controls were tested using chi-square test (EpiInfo software v. 3.3.2 available free at http://www.cdc.gov/EpiInfo/).

In order to evaluate the association between genotypes and alleles and AD, odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated using logistic regression models (SPSS software ver. 18.0 for Windows (Chicago, ILL, USA)). Further analysis were performed by using logistic regression models adjusted for gender, age (in continuous), and presence of *APOE* e4.

A P-value of <.05 was considered statistically significant.

A power analysis was performed using COMPARE2 software (available free at http://www.brixtonhealth.com/).

Table 1: Main characteristics of all studied subjects.

Characteristics	LOAD[a]	Controls
Total	238	250
Gender, no. (%)		
Female	152 (63.9)	156 (62.4)
Male	86 (36.1)	94 (38.6)
Recruitment site, no. (%)		
Italy	218 (91.6)	230 (92)
Germany	20 (8.4)	20 (8)
Age at onset[b], mean (±SD)	72.9 (±5.6)	71.5 (±5.7)
MMSE points, mean (±SD)	20.2 (±5.4)	28.2 (±1.4)
APOE ε4, no. (%)		
No	133 (55.9)	221 (88.4)
Yes	105 (44.1)	29 (11.6)

[a] LOAD cases were defined by the occurrence of Alzheimer's disease with an onset age ≥65 years.
[b] At examination tests for controls.

Linkage disequilibrium (LD) between the SNPs was analyzed using PowerMarker software, version 3.25) (http://statgen.ncsu.edu/powermarker/) [13].

3. Results

Table 1 gives the distribution of 238 LOAD cases and 250 controls according to gender, recruitment site, age, and other selected characteristics.

Cases and controls had similar recruitment site and age distribution. The mean of the Mini Mental State Examination score (MMSE) points was higher in controls than cases. Conversely, cases reported more frequently the presence of E4 compared to controls (44.1% versus 11.6%).

The frequencies of all SNPs were in Hardy-Weinberg equilibrium in AD patients and controls.

Moreover, no correlation between mean age at onset and all SNPs genotypes was observed and no interaction between *APOE* gene was found (data not shown).

We found no differences in the allelic or genotypic frequencies between AD patients and controls for any of the SNPs (Table 2). We found no association between AD and rs11515 genotype ($\chi^2 = 3.47$; $P = .17$) and allele ($\chi^2 = 3.2$; $P = .07$), rs3731246 genotype ($\chi^2 = 1.13$; $P = .56$) and allele ($\chi^2 = 1.17$; $P = .27$), rs3731211 genotype ($\chi^2 = 0.57$; $P = .75$) and allele ($\chi^2 = 0.0$; $P = .96$), rs598664 genotype ($\chi^2 = 1.11$; $P = .57$), and allele ($\chi^2 = 1.15$; $P = .28$). None of the unadjusted ORs were statistically significant, and when we adjusted for gender, age (in continuous), and *APOE* e4 these results did not substantially change (data not shown).

Moreover, as reported by Züchner and colleagues, we found that rs11515 SNP was in Linkage Disequilibrium (LD) with rs37361246 ($r^2 = 0.64$), rs3731211 ($r^2 = 0.33$) and rs598664 ($r^2 = 0.55$).

4. Discussion

Recent studies reported informative results regarding genomewide association and linkage studies on chromosome 9

Table 2: *CDKN2A/B* polymorphisms: genotype, allele frequencies, and unadjusted odds ratios (OR) and corresponding 95% confidence intervals (CI) for 238 Late-Onset Alzheimer's Disease (LOAD) cases and 250 controls.

	LOAD[a] no. (%)	Controls no. (%)	χ^2 (df, *P*-value)	unadjusted OR (95% CI)
Total	238	250		
CDKN2A				
Rs 11515				
Genotypes			3.47 (2, .17)	
CC	169 (71)	163 (65.2)		1[b]
CG	62 (26)	72 (28.8)		0.83 (0.54–1.27)
GG	7 (3)	15 (6)		0.45 (0.16–1.21)
Alleles			3.20 (1, .07)	
C	400 (84)	398 (79)		1[b]
G	76 (16)	102 (21)		0.74 (0.53–1.04)
Rs 3731246				
Genotypes			1.13 (2, .56)	
GG	183 (76.9)	182 (72.8)		1[b]
GC	50 (21)	61 (24.4)		0.82 (0.52–1.28)
CC	5 (2.1)	7 (2.8)		0.71 (0.19–2.55)
Alleles			1.17 (1, .27)	
G	416 (87.4)	425 (85)		1[b]
C	60 (12.6)	75 (15)		0.82 (0.56–1.2)
Rs 3731211				
Genotypes			0.57 (2, .75)	
TT	105 (44.1)	106 (42.4)		1[b]
AT	99 (41.6)	112 (44.8)		0.89 (0.60–1.33)
AA	34 (14.3)	32 (12.8)		1.07 (0.59–1.93)
Alleles			0.00 (1, .96)	
T	309 (64.9)	324 (64.8)		1[b]
A	167 (35.1)	176 (35.2)		0.99 (0.76–1.31)
CDKN2B				
Rs 598664				
Genotypes			1.11 (2, .57)	
AA	182 (74.5)	181 (72.4)		1[b]
AG	51 (21.4)	62 (24.8)		0.82 (0.52–1.28)
GG	5 (2.1)	7 (2.8)		0.71 (0.19–2.55)
Alleles			1.15 (1, .28)	
A	415 (87.2)	424 (84.8)		1[b]
G	61 (12.8)	76 (15.2)		1.35 (0.89–2.07)

[a] LOAD cases were defined by the occurrence of Alzheimer's disease with an onset age ≥65 years.
[b] Reference category.

as candidate region for LOAD. In particular, Pericak-Vance and colleagues identified the LOAD locus on 9p21.3 in a genomewide microsatellite-based linkage screen on 466 AD families [14] and confirmed in a genetic study of a consanguineous Israeli-Arab community [15]. In 2007 Hamshere and colleagues analyzing 723 affected relative pairs with genomewide linkage analysis showed evidence for disease locus for LOAD on chromosome 9p [8]. In 2008 Züchner identified a chromosomal area under the linkage peak containing several potential AD candidate genes and analyzed *CDKN2A* and *CDKN2B* genes. Among the different SNPs identified, the rs11515 localized in the 3′-UTR of *CDKN2A* was the most significant even if no transcription-factor-binding site, micro-RNA target site, or conserved regulatory potential has been detected; other SNPs are all in intron regions.

Many studies have been carried out on numerous putative gene polymorphisms candidate in AD pathogenesis and, with the exception of *APOE*, that is, the only confirmed genetic risk factor, results are still contrasting.

In our case-control association study we analyzed four SNPs, three in the *CDKN2A* gene (rs15515, rs3731246, and rs3731211) and one in *CDKN2B* (rs598664) in a sample of 488 Caucasian subjects including 238 LOAD and 250 controls.

Our data set of 238 LOAD patients was evaluated as having a power to detect an OR of 2.0 at a 5% significance rate between 86.7 and 96.6 for all SNPs. However, to detect an OR at 1.5—the usual genetic main-effect for complex disorder—our data has a power between 40.4 and 59 for all SNPs. Thus, the study is not sufficiently powered and this is one of the limitations of our study. We did not find any association between these SNPs and AD risk in contrast to Züchner's results: this may be due to the different study design. In fact, Züchner and colleagues reported a linkage and association study in 674 AD families, whilst we conducted a case control association study. Moreover, only in 47 patients (19.7%) of AD cases the autopsy confirmed the diagnosis.

Furthermore no correlation between mean age at onset and all SNPs genotypes, and no interaction between *APOE* gene was observed, suggesting that the genes do not act synergistically.

5. Conclusion

In conclusion, our results did not confirm the hypothesis, suggested by Züchner and colleagues that *CDKN2A* and *CDKN2B* at 9p21 are implicated in the susceptibility in the LOAD.

In fact, our data show no evidence of an association between all the studied SNPs and disease risks, thus a possible role of *CDKN2A* and *CDKN2B* as a genetic risk factor implicated in the susceptibility to AD was not confirmed. In lights of these contrasting results further studies are needed to elucidate the role of these genes in the susceptibility of AD.

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