Research Article

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

Andrea Tedde,1 Irene Piaceri,1 Silvia Bagnoli,1 Ersilia Lucenteforte,2 Uwe Ueberham,3 Thomas Arendt,3 Sandro Sorbi,1 and Benedetta Nacmias1

1 Department of Neurological and Psychiatric Sciences, University of Florence, Viale Morgagni, 85 - 50134 Florence, Italy
2 Department of Preclinical and Clinical Pharmacology, University of Florence, 50139 Florence, Italy
3 Paul Flechsig Institute for Brain Research, University of Leipzig, 04109 Leipzig, Germany

Correspondence should be addressed to Andrea Tedde, andrea.tedde@unifi.it

Received 15 November 2010; Revised 31 January 2011; Accepted 22 February 2011

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 (ε4) 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 ε4 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 SNPs 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 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 (Epilinfo software v. 3.3.2 available free at http://www.cdc.gov/Epilinfo/).

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 | Controls |
|-----------------|------|----------|
| Total           | 238  | 250      |
| Gender, no. (%) |      |          |
| Female          | 152  | 156      | (63.9) | (62.4) |
| Male            | 86   | 94       | (36.1) | (38.6) |
| Recruitment site, no. (%) |      |          |
| Italy           | 218  | 230      | (91.6) | (92) |
| Germany         | 20   | 20       | (8.4)  | (8)  |
| Age at onseta, mean (±SD) | 72.9 (±5.6) | 71.5 (±5.7) |
| MMSE points, mean (±SD) | 20.2 (±5.4) | 28.2 (±1.4) |
| APOE e4, no. (%) |      |          |
| No              | 133  | 221      | (55.9) | (88.4) |
| Yes             | 105  | 29       | (44.1) | (11.6) |

*LOAD cases were defined by the occurrence of Alzheimer’s disease with an age onset ≥65 years.

a 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 e4 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 Z¨uchner 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.

|                  | LOADa no. (%) | Controls no. (%) | \( \chi^2 \) (df, P-value) | unadjusted OR (95% CI) |
|------------------|---------------|------------------|-----------------------------|------------------------|
| Total            | 238           | 250              |                             |                        |
| CDKN2A           |               |                  |                             |                        |
| Rs 11515 Genotypes |               |                  |                             |                        |
| CC               | 169 (71)      | 163 (65.2)       | 3.47 (2, .17)               | 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          |               |                  |                             |                        |
| C                | 400 (84)      | 398 (79)         | 3.20 (1, .07)               | 1\(^b\)                |
| G                | 76 (16)       | 102 (21)         |                             | 0.74 (0.53–1.04)       |
| Rs 3731246 Genotypes |           |                  |                             |                        |
| GG               | 183 (76.9)    | 182 (72.8)       | 1.13 (2, .56)               | 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          |               |                  |                             |                        |
| G                | 416 (87.4)    | 425 (85)         | 1.17 (1, .27)               | 1\(^b\)                |
| C                | 60 (12.6)     | 75 (15)          |                             | 0.82 (0.56–1.2)        |
| Rs 3731211 Genotypes |           |                  |                             |                        |
| TT               | 105 (44.1)    | 106 (42.4)       | 0.57 (2, .75)               | 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          |               |                  |                             |                        |
| T                | 309 (64.9)    | 324 (64.8)       | 0.00 (1, .96)               | 1\(^b\)                |
| A                | 167 (35.1)    | 176 (35.2)       |                             | 0.99 (0.76–1.31)       |
| CDKN2B           |               |                  |                             |                        |
| Rs 598664 Genotypes |           |                  |                             |                        |
| AA               | 182 (74.5)    | 181 (72.4)       | 1.11 (2, .57)               | 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          |               |                  |                             |                        |
| A                | 415 (87.2)    | 424 (84.8)       | 1.15 (1, .28)               | 1\(^b\)                |
| G                | 61 (12.8)     | 76 (15.2)        |                             | 1.35 (0.89–2.07)       |

---

aLOAD cases were defined by the occurrence of Alzheimer’s disease with an onset age ≥ 65 years.
bReference 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.

Acknowledgments

This study was supported by Grant from the Cassa di Risparmio di Pistoia e Pescia 2009.0220; the Ministry of Health RFPS-2007-1-643522, and the Ministry of Health RFPS-2007-2-39.

References

[1] G. McKhann, D. Drachman, and M. Folstein, “Clinical diagnosis of Alzheimer’s disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s disease,” Neurology, vol. 34, no. 7, pp. 939–944, 1984.
[2] A. Lobo, L. J. Launer, L. Fratiglioni et al., “Prevalence of dementia and major subtypes in Europe: a collaborative study of population-based cohorts,” Neurology, vol. 54, no. 11, supplement 5, pp. S4–S9, 2000.
[3] E. I. Rogae, R. Sherrington, E. A. Rogaea et al., “Familial Alzheimer’s disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer’s disease type 3 gene,” Nature, vol. 376, no. 6543, pp. 775–778, 1993.
[4] R. Sherrington, E. I. Rogae, Y. Liang et al., “Cloning of a gene bearing missense mutations in early-onset familial Alzheimer’s disease,” Nature, vol. 375, no. 6534, pp. 754–760, 1995.
[5] A. Goate, M. C. Chartier-Harlin, M. Mullan et al., “Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer’s disease,” Nature, vol. 349, no. 6311, pp. 704–706, 1991.
[6] W. J. Strittmatter, A. M. Saunders, D. Schmechel et al., “Apolipoprotein E: high-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease,” Proceedings of the National Academy of Sciences of the United States of America, vol. 90, no. 5, pp. 1977–1981, 1993.
[7] L. Bertram, M. B. McQueen, K. Mullin, D. Blacker, and R. E. Tanzi, “Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database,” Nature Genetics, vol. 39, no. 1, pp. 17–23, 2007.
[8] M. L. Hamshere, P. A. Holmans, D. Avramopoulos et al., “Genome-wide linkage analysis of 723 affected relative pairs with late-onset Alzheimer’s disease,” Human Molecular Genetics, vol. 16, no. 22, pp. 2703–2712, 2007.
[9] S. Züchner, J. R. Gilbert, E. R. Martin et al., “Linkage and association study of late-onset Alzheimer disease families linked to 9p21.3,” Annals of Human Genetics, vol. 72, no. 6, pp. 725–731, 2008.
[10] American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders. DSM-IV, American Psychiatric Association, Washington, DC, USA, 4th edition, 1994.
[11] The Dementia Study Group of the Italian Neurological Society, “Guidelines for the diagnosis of dementia and Alzheimer’s disease,” Neurological Sciences, vol. 21, no. 4, pp. 187–194, 2000.
[12] S. Sorbi, B. Nacmias, P. Forleo et al., “ApoE allele frequencies in Italian sporadic and familial Alzheimer’s disease,” Neurology Letters, vol. 177, no. 1–2, pp. 100–102, 1994.
[13] K. Liu and S. V. Muse, “PowerMaker: an integrated analysis environment for genetic maker analysis,” Bioinformatics, vol. 21, no. 9, pp. 2128–2129, 2005.
[14] M. A. Percik-Vance, J. Grubber, L. R. Bailey et al., “Identification of novel genes in late-onset Alzheimer’s disease,” Experimental Gerontology, vol. 35, no. 9-10, pp. 1343–1352, 2000.
[15] L. A. Farrer, A. Bowirrat, R. P. Friedland, K. Waraska, A. D. Korczyn, and C. T. Baldwin, “Identification of multiple loci for Alzheimer disease in a consanguineous Israeli-Arab community,” Human Molecular Genetics, vol. 12, no. 4, pp. 415–422, 2003.