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ScienceDirect
Neuroscience Letters 419 (2007) 195–198
Neuroscience Letters
www.elsevier.com/locate/neulet

The CDC2 I-G-T haplotype associated with the *APOE* ε4 allele increases the risk of sporadic Alzheimer's disease in Sicily

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Received 25 January 2007; received in revised form 3 April 2007; accepted 3 April 2007

Abstract

The cell division cycle 2 (CDC2) gene is a candidate susceptibility gene for Alzheimer's disease (AD). We investigated the CDC2 genotype, and allele and haplotype frequencies in AD patients and matched controls, distinguishing between apolipoprotein E (APOE) ε4 allele carriers and non-carriers. APOE ε4 is an established predictor of AD risk. APOE and CDC2 genotypes were examined in 109 sporadic AD patients and in 110 healthy age- and sex-matched controls from Sicily. The ε4 allele of APOE was predictive of AD risk in our study group (odds ratio: 5.37, 95% CI 2.77–10.41; $P < 0.0001$). Genotype and allele frequencies of the three tested CDC2 polymorphisms (Ex6 + 7I/D, Ex7–15 G > A, Ex7–14 T > A) were not significantly different between AD patients and controls. However, a significant different distribution of a specific CDC2 haplotype (I-G-T) was found between AD patients and controls when analyzing APOE ε4-positive subjects ($P = 0.0288$). Moreover, the combined presence of the I-G-T haplotype and the ε4 allele almost doubled the risk of AD (odds ratio: 10.09, 95% CI 3.88–26.25; $P < 0.0001$) compared to carriers of ε4 alone. This study suggests that the I-G-T haplotype of the CDC2 gene increases the risk of AD in APOE ε4 carriers.
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Keywords: Alzheimer's disease; *APOE*; cdc2; Cell cycle; Genetic polymorphism; Risk factor

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by senile plaques containing β-amyloid protein (Aβ), and neurofibrillary tangles (NFT) riched in hyperphosphorylated tau protein [12]. An aberrant reactivation of the cell cycle seems to play a central role in the pathogenesis of AD [5,6,33]. Degenerating neurons in AD express a repertoire of proteins that are involved in cell cycle activation, such as cyclins and cyclin-dependent kinases (Cdks) [14]. Cell division cycle 2 protein (cdc2) is a cyclin-dependent kinase (also known as Cdk1) [24], which is early expressed in the AD brain, and is also implicated in the phosphorylation of tau protein and NFT formation [19,20,27,32]. Johansson et al. [16] reported that a polymorphism in the CDC2 gene, designated as Ex6 + 7I/D, is associated with AD, and this polymorphism influences total CSF tau levels [17]. More recently, however, a large study carried out by Liang et al. in Caucasian Americans has failed to demonstrate the association of CDC2 gene polymorphisms, including the Ex6 + 7I/D, with AD [21].

To date, the ApoE ε4 allele is the strongest genetic predictor and the major risk factor for sporadic AD. Whereas a protective effect of the ApoE ε2 allele is observed both in familial and sporadic late-onset AD (LOAD) [7,26,35,36], the ApoE ε4 allele is strongly associated with cerebrovascular dementia and Alzheimer's disease [3,8,9,29,36].

To assess whether the ApoE ε4 allele and the CDC2 gene interact in determining the risk to develop AD, we have investigated the association of the CDC2 genotype, allele and haplotype

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doi:10.1016/j.neulet.2007.04.010

frequencies with the disease in a population of patients from Sicily, and with the APOE genotype of both AD patients and matched controls.

A total of 229 subjects, including 109 AD patients (mean age 72.9 ± 12.4), were recruited at the IRCCS Oasi Maria S.S. of Troina (Italy) and the diagnosis of probable AD was made following the NINCDS-ADRDA criteria [22]; no patients had autosomal dominant AD. The 110 control subjects (mean age 73.2 ± 11.8) had no cognitive impairment and/or family history of AD. The mini-mental state examination (MMSE) scores were >28 in all controls. Written informed consent was obtained from the participants or their families. The study was approved by the ethical committee of the Oasi Institute. Patients and controls were all born in Sicily and were of European origin.

Fasting venous blood was collected in EDTA-containing tubes, immediately centrifuged, and stored at −80 °C until analysis. DNA was isolated from a lymphocyte-enriched fraction of whole blood with NUCLEON BACC3 for extraction of genomic DNA kit (Amersham Pharmacia Biotech, Milan, Italy). The procedures for detecting the *112* $C \rightarrow T$ and the *158* $C \rightarrow T$ polymorphism of *ApoE*, were based on polymerase chain reaction (PCR) amplification, restriction cleavage and separation of the DNA fragments by 15% non-denaturant polyacrylamide gel electrophoresis, as previously described [1,2]. These procedures were modified from the original report by Hixon and Vernier [15]. For genotyping of the Ex 6+7 I/D, Ex7–15 $G \rightarrow A$ and Ex7–14 $T \rightarrow A$ polymorphisms of the CDC2 gene, previously described methods were followed [16]. DNA samples corresponding to amplified DNA of the ApoE and CDC2 genotypes were sequenced and subsequently used as controls in all series of genotype determinations. An ABI prism 310 genetic analyzer (Perkin-Elmer, USA) was used for the sequencing the amplified products in both forward and reverse directions. Two independent blinded readers recorded the genotypes and retyped any discrepancies until resolved. Haplotype estimation was based on the method proposed by Niu et al. [25].

For categorical variables, we used a χ^2 test to assess differences; Yates's continuity correction was employed as an approximation in the analysis of 2×2 contingency tables. For continuous variables (age), the Mann–Whitney *U*-test was used. A P-value < 0.05 indicated statistical significance. We used logistic regression analyses to estimate odd ratios (ORs) for being a case, with adjustment for age and sex. The precision of the OR estimates was expressed with 95% confidence intervals (CIs). Data were prospectively collected and analyzed using the Statview for Windows software (Microsoft 1998).

There was no significant difference in sex ratio and age (data not shown) between patients and controls, with 48 (44.0%, 95% C.I.: 35.1–53.4%) males in the AD group compared with 49 (44.5%, 95% C.I.: 35.6–53.9%) in the control group ($P = 0.9396$). The distributions of genotypes from the four polymorphisms were all in Hardy–Weinberg equilibrium. The allele frequencies of the three polymorphisms of the CDC2 gene tested (Ex6 + 7I/D, Ex7–15 G > A, Ex7–14 T > A) were not significantly different between AD patients and controls, as shown in Table 1. However, we found a significantly higher frequency of ApoE ε4-positive subjects in AD patients than in controls (27% versus 8% $P < 0.0001$). Compared to the age- and sex-matched control group, the genotype frequencies of the CDC2 polymorphisms were not significantly different in AD patients (Table 2). In contrast, the difference between AD and control groups regarding the ApoE ε4 genotype frequencies were highly significant ($P < 0.0001$). The odds ratio (OR) in AD cases was 5.37 (95% CI 2.77–10.41) for the presence of the ε4 allele. The ApoE ε4 allele and the Ex6 + 7I allele were not significantly related (data not shown).

We then examined whether specific haplotypes of the CDC2 gene increase the risk to develop AD through an interaction with the ApoE ε4 allele. When examining both controls and AD ε4 allele carriers, we found a significant different distribution of the CDC2 haplotypes by comparing all haplotypes ($P = 0.0288$) (Table 3). Furthermore, we obtained a significance

Table 1
Allele frequency of CDC2 and ApoE polymorphisms in 109 cases with Alzheimer disease (AD) and 110 controls from Sicily

Alleles	Frequency (95% CI)		*P*-value
	Controls	AD patients	
Cdc2 Ex6 + 7I/D			
Allele I	0.66 (0.60–0.72)	0.66 (0.59–0.72)	0.9453
Allele D	0.34 (0.28–0.41)	0.34 (0.27–0.40)	
Cdc2 Ex7–15 G > A			
Allele G	0.72 (0.66–0.77)	0.76 (0.70–0.81)	0.4170
Allele A	0.28 (0.22–0.34)	0.24 (0.19–0.30)	
Cdc2 Ex7–14 T > A			
Allele T	0.72 (0.66–0.77)	0.76 (0.70–0.81)	0.4170
Allele A	0.28 (0.22–0.34)	0.24 (0.19–0.30)	
ApoE			
ApoE ε4 negative	0.92 (0.88–0.95)	0.73 (0.67–0.79)	<0.0001
ApoE ε4 positive	0.08 (0.05–0.12)	0.27 (0.21–0.33)	

Abbreviations: CI, confidence interval; ApoE, apolipoprotein E.

Table 2
Frequency of genotypes from CDC2 and ApoE polymorphisms in 109 cases with Alzheimer disease (AD) and 110 controls from Sicily

Genotypes	Frequency (95% CI)		*P*-value
	Controls	AD patients	
Cdc2 Ex6 + 7I/D			
II	0.41 (0.32–0.50)	0.40 (0.31–0.50)	0.9664
ID	0.51 (0.42–0.60)	0.51 (0.41–0.60)	
DD	0.08 (0.05–0.15)	0.09 (0.05–0.15)	
Cdc2 Ex7–15 G > A			
GG	0.52 (0.43–0.61)	0.58 (0.48–0.67)	0.6546
GA	0.40 (0.31–0.49)	0.36 (0.27–0.45)	
AA	0.08 (0.04–0.14)	0.06 (0.03–0.12)	
Cdc2 Ex7–14 T > A			
TT	0.52 (0.43–0.61)	0.58 (0.48–0.67)	0.6546
TA	0.40 (0.31–0.49)	0.36 (0.27–0.45)	
AA	0.08 (0.04–0.14)	0.06 (0.03–0.12)	
ApoE			
No ApoE ε4	0.86 (0.79–0.92)	0.55 (0.45–0.63)	<0.0001
One ApoE ε4	0.12 (0.07–0.19)	0.38 (0.30–0.48)	
Two ApoE ε4	0.02 (0.00–0.06)	0.07 (0.03–0.13)	

Abbreviations: CI, confidence interval; ApoE, apolipoprotein E.

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