Intronic CYP46 polymorphism along with ApoE genotype in sporadic Alzheimer Disease: from risk factors to disease modulators

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Abstract

Increasing biological and clinical findings argue for a link between brain cholesterol turnover and Alzheimer Disease (AD), high cerebral levels of the former increasing Aβ load. Cerebral cholesterol elimination involves two mechanisms dependent on Apolipoprotein E (ApoE) and cholesterol 24-hydroxylase (CYP46). The aim of this study was to evaluate an intronic variation in CYP46 (intron 2, T → C) along with ApoE genotype as risk factors for AD and to establish the correlation between CYP46/ApoE polymorphism and disease progression. One-hundred and fifty-seven AD patients, who had been followed periodically through 1-year follow-up after enrolment, and 134 age- and gender-matched controls entered the study. The distribution of CYP46 genotypes was significantly different in AD compared to controls (P < 0.004), being CYP46*C allele higher in AD patients (P < 0.002). ApoE ε4 genotype was more frequent in AD (41.4%) than in controls (15.9%, P < 0.0001). The odds ratio (OR) for AD risk in CYP46*C carriers was 2.8, and in ApoE ε4 carriers was 4.05; the OR for having both CYP46*C and ApoE ε4 was 17.75, demonstrating the their synergic effect on AD risk. In AD patients, CYP46*C along with ApoE ε4 genotype were associated with a higher cognitive decline at 1-year follow-up (P < 0.02).

These findings provide direct evidence that CYP46 and ApoE polymorphisms synergically increase the risk for AD development, and influence on the rate of cognitive decline.

Keywords: Alzheimer Disease; CYP46; ApoE; Risk factors; Rate of cognitive decline

1. Introduction

There is growing interest in the potential contribution of cholesterol in the pathogenesis of Alzheimer Disease (AD), several evidences highlighting a role of hypercholesterolaemia in increasing AD risk and worsening disease progression [3,12,19]. In fact, pre-clinical studies have shown an association between elevated mid-life serum total cholesterol levels and late-life AD, and a reduced incidence rate of the disease has been observed in subjects treated with statins, thus suggesting that cholesterol-lowering drugs may exert a protective role on AD onset [6–9]. Linkage between cholesterol and AD has also been suggested by the main recognised genetic risk factor for sporadic late-onset AD, i.e. Apolipoprotein E (ApoE), which is involved in cholesterol homeostasis [18]. In fact, a wide body of data demonstrated that ApoE ε4 carriers are at greater risk of developing AD [22].

Further, it has been demonstrated that cholesterol affects the pathogenic mechanisms of the disease, modulating the amyloid precursor protein (APP) processing, being high cholesterol levels a factor increasing Aβ biogenesis and Aβ toxicity [2,15,20,21]. All together literature findings claim that the excess of brain cholesterol, which is locally synthesised, needs to be eliminated. The blood-brain barrier prevents cholesterol transport from the brain to the blood, thus elimination pathways are necessary [10]. Cholesterol leaves the brain by two different mechanisms: one is ApoE-dependent, ε4 allele being less effective in this process, while the second involves a recently cloned gene cholesterol 24S-hydroxylase (CYP46)
CYP46 is an enzyme almost exclusively located in the brain and catalyses the conversion of excess cholesterol into 24-hydroxycholesterol, which is readily secreted across the blood–brain barrier into the circulation [11]. A putative functional role of CYP46 has been postulated: an increase of 24-hydroxycholesterol in cerebrospinal fluid of AD patients has been shown, and different groups have analysed an intronic variation of CYP46, since genetic polymorphisms might influence functionality of the corresponding protein, but with contrasting results [5,10,16,17]. These observations, arguing for a key-role of genes involved in cholesterol turnover in AD, provide the rationale of the present study, aimed to evaluate an intronic variation in CYP46 (intron 2, T → C) along with ApoE genotype, as risk factors and disease modulators in AD.

2. Materials and methods

2.1. Subjects

Patients consecutively admitted at the “Centre of Ageing Brain and Neurodegenerative Disorders”, University of Brescia, Italy, entered the study. All patients performed a somatic and neurological examination, laboratory analysis, ApoE and CYP46 genotyping, and brain imaging study (Computed Tomography or Magnetic Resonance Imaging). A wide standardised multidimensional assessment evaluating global cognitive functions and behavioural disturbances was performed in each subject. A diagnosis of probable AD was based on National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria [13].

The following exclusionary criteria for the AD group were designed to ensure that participants had probable AD as the cause of their dementia: (a) major depressive disorder, bipolar disorder, schizophrenia, substance use disorder, or mental retardation according to criteria of the DSM-IV; (b) cerebrovascular disorders, hydrocephalus, and intra-cranial mass, documented by CT or MRI within the past 12 months; (c) abnormalities in serum folate and Vitamin B12, syphilis serology, or thyroid hormones’ levels; (d) a history of traumatic brain injury or another neurologic disease (e.g. Parkinson disease, Huntington disease, seizure disorders); (e) significant medical problems (e.g. poorly controlled diabetes or hypertension; cancer within the past 5 years; clinically significant hepatic, renal, cardiac or pulmonary disorders).

Moreover, an age- and gender-matched control sample from the same Italian area from which the patients were drawn was recruited. All controls were found to be cognitively intact, following medical history and neuropsychological examination.

The study was conducted in accordance with local clinical research regulations and an informed consent was required from all subjects and caregivers when indicated.

Polymorphism analyses were performed blinded to diagnosis and genotype.

2.2. CYP46 genotype

Genomic DNA was isolated from whole blood samples by extraction using a salting procedure. A 285 bp polymerase chain reaction (PCR) product containing CYP46 was amplified using specific primers (forward primer: 5′-TGA AAA CGA GTT TCC CGT CC-3′; reverse primer: 5′-GTG TGA CCA GGT AAC AGT CA-3′) PCR was performed using 100 ng of genomic DNA in 50 ml of reaction mixture.

After initial denaturation at 95 °C for 5 min, reaction mixture was subjected to 32 cycles of 30 s denaturation at 95 °C, 30 s annealing at 53 °C, and 30 s extension at 72 °C, followed by 10 min extension step at 72 °C. PCR products were digested by MspI restriction enzyme. CYP46 T allele corresponded to the uncut 285 bp fragment, while CYP46 C allele was characterised by two fragments of 209 and 76 bp.

The results of amplification and the digestion fragments were revealed by 2 and 3% agarose gels with ethidium bromide, respectively.

2.3. ApoE genotyping

Genetic variation at the ApoE locus is determined by restriction isotyping using PCR amplification and subsequent digestion with HhaI (New England Biolabs). The nucleotide substitutions that result in Arg–Cys interchange at position 112 and 158 alter HhaI cleavage sites; each genotype can be distinguished by unique combinations of HhaI fragment sizes in all homozygotic and heterozygotic combinations.

2.4. Study design

AD patients and healthy controls were evaluated at baseline and a blood venipuncture for DNA extraction and polymorphisms’ analysis was performed. In regard to AD patients, current available therapy with Cholinesterase Inhibitors (ChEIs) had been administered at recommended dosage, and they had been followed periodically, and 1-month and 1-year follow-up neuropsychological assessment was recorded.

2.5. Statistical methods

Genotype and allele frequencies between AD patients and control subjects were compared by Pearson χ² test. Hardy–Weinberg equilibrium of the examined population was confirmed by χ² test.

For all the continuous variables, the analysis of variances between groups was performed using the unadjusted ANOVA. In order to account for possible confounds (gender, age), the ANCOVA has been performed as well. Fur-
3. Results

One hundred and fifty-seven demented patients (female: 61.8%; age: 72.3 ± 3.7 years) and 134 age- and gender-matched control subjects entered the study.

The distribution of CYP46 genotypes was significantly different in AD compared to controls (P = 0.004; see Table 1). The presence of at least one C allele (CYP46 C/C or CYP/C) was higher in AD patients (54.6%) compared to control subjects (35.8%; P < 0.0001).

The distribution of ApoE isoforms significantly differed between AD cases and controls, being ApoE ε4 genotype more frequent in patients (AD versus controls: 41.4% versus 15.8%, P < 0.0001). The presence of at least one ApoE ε4 allele (ApoE4) was significantly associated with AD (AD versus controls: 22.6% versus 7.9%, P < 0.0001; see Table 1).

Crude OR for the risk of AD in CYP46 C carriers was 2.56 (95% confidence interval: 1.58 to 4.08), while for ApoE4 carriers it was 3.78 (95% CI: 2.13 to 6.71). Compared to subjects with neither CYP46 ε4 nor ApoE4, the OR for the presence of CYP46 ε4 without ApoE4 allele was 2.81 (95% CI: 1.60 to 4.97), the OR for the presence of ApoE4 allele without CYP46 ε4 was 4.05 (95% CI: 1.95 to 8.39), the OR for having both CYP46 ε4 and ApoE4 was 17.75 (95% CI: 5.83 to 54.06), demonstrating their synergic effect on AD risk.

Eighty-eight patients completed the 1-year follow-up and were re-evaluated. Only patients who have never experienced ChEIs treatment before enrolment were considered at follow-up, to avoid confounds. These patients were grouped according to ApoE and CYP46 genotype. As shown in Table 2, ApoE4/CYP46 ε4 grouped patients, who completed 1-year follow-up, did not differ for demographic or clinical characteristics.

Rate of cognitive decline was calculated as ΔMMSE (MMSE 1 year − MMSE baseline), and factors related to ΔMMSE analyzed. Adjusting for age and gender, neither ApoE4 genotype, nor CYP46 ε4 genotype, nor CYP46 ε4/CYP46 ε4 predicted ΔMMSE (P = 0.0454). In fact, at 1-year follow-up AD patients carrying both ApoE4 and CYP46 ε4 polymorphisms showed a higher rate of cognitive decline compared to the others (F[2, 168] = 2.62, P < 0.02), while no differences among groups were found at 1-month follow-up (see Table 2, and Fig. 1).

4. Discussion

It is well established that sporadic late-onset AD is a polygenic disease, several genetic polymorphisms being suggested as modulators of AD susceptibility [4]. Among others, several proposed genes encode for protein that are
involved in lipid homeostasis, thus claiming that cholesterol plays a key-role in AD pathogenesis [1].

In this study, we reported that an intronic variation in CYP46, CYP46*4, along with ApoE ε4 genotype synergically increase the risk of AD development; further, AD patients carrying the combination of these two polymorphisms showed a higher rate of cognitive decline at 1-year follow-up. CYP46 and ApoE are involved in the pathways by which excess brain cholesterol is transported into circulation [10]. Biological evidences have strengthened the role of these two proteins in AD. In fact, it is well established that ApoE induces cholesterol efflux in an isoform-dependent manner, as the most recognised sporadic AD-related risk factor ε4 allele appears to be less effective in this process [14]. Further, it has been demonstrated that AD is characterised by abnormal induction of CYP46 enzyme, which leads to changes in 24S-hydroxycholesterol from brain to circulation [17,23]. Neuropathological evidences reported that high concentration of CYP46 has been found in glial cells in AD brains, suggesting that an imbalance of cholesterol turnover may be a feature of reactive astroglia in the disease [4].

How CYP46 polymorphisms, such as intronic variation here reported, affect protein function has not been established yet. It could be speculated that intronic variations can either influence on the rate of transcription of gene products by affecting splice sites or modulate nuclear trancriptional factors.

According to this, an imbalance of cholesterol turnover, due to less effective physiological mechanisms, such as that determined by ApoE or CYP46 genetic variations, could represent a risk factor for AD development, and could worsen disease progression once symptomatology is overt. In agreement, AD patients carrying both CYP46*4 and ApoE ε4 progressed much more faster at 1-year evaluation. We acknowledge that our study has some limitations. First of all, a wider sample of subjects is needed as well as hypothesis-confirming sample from other countries. Secondly, further biological studies analysing CYP46 polymorphisms’s effect on cholesterol metabolism are mandatory.

Despite these limitations, this study underlines once more the importance of taking into account the enzymatic pathways which are involved in cholesterol homeostasis to better understand how they influence on AD development. The complex interplay between genetic and environmental factors should be introduced into future trials to fully appreciate individual susceptibility and prognosis’ determinants in AD. In fact, the present findings argue for a double implication of CYP46 and ApoE genotyping, since these genes act both as risk factors and disease modulators in AD. Establishing the real impact of the intricate maze of these genetic polymorphisms and corresponding proteins’ function represents a key-issue in the progress of the pathology’s knowledge, leading to a better definition of the disease and providing many potential opportunities to design preventive therapeutic strategies.

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References


