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Original article

CYP46A1 variants influence Alzheimer's disease risk and brain cholesterol metabolism

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Abstract

Background. – Cholesterol 24S-hydroxylase (CYP46) catalyzes the conversion of cholesterol to 24S-hydroxycholesterol, the primary cerebral cholesterol elimination product. Only few gene variations in CYP46 gene (*CYP46A1*) have been investigated for their relevance as genetic risk factors of Alzheimer's disease (AD) and results are contradictory.

Methods. – We performed a gene variability screening in *CYP46A1* and investigated the effect of gene variants on the risk of AD and on CSF levels of cholesterol and 24S-hydroxycholesterol.

Results. – Two of the identified 16 SNPs in *CYP46A1* influenced AD risk in our study (rs7157609: $p = 0.016$; rs4900442: $p = 0.019$). The interaction term of both SNPs was also associated with an increased risk of AD ($p = 0.006$). Haplotypes including both SNPs were calculated and haplotype G–C was identified to influence the risk of AD ($p = 0.005$). AD patients and non-demented controls, who were carriers of the G–C haplotype, presented with reduced CSF levels of 24S-hydroxycholesterol ($p = 0.001$) and cholesterol ($p < 0.001$).

Conclusion. – Our results suggest that *CYP46A1* gene variations might act as risk factor for AD via an influence on brain cholesterol metabolism.

Keywords: Cholesterol 24S-hydroxylase; Alzheimer's disease; CSF; Cholesterol metabolism

1. Background

Alterations of cholesterol metabolism have been detected in patients suffering from Alzheimer's disease (AD), the most common cause of dementia world wide. Presence of the apolipoprotein E *(APOE)* 4 allele is the strongest known genetic risk factor for late-onset AD [35]. Brain and peripheral cholesterol metabolism are disturbed in AD, as indicated by altered cholesterol, 24S-hydroxycholesterol and 27-hydroxycholesterol levels [17,19]. Brain cholesterol is synthesized locally and thus independent from nutritional intake. Excess brain cholesterol derived from increased membrane turnover or neuronal loss is eliminated via cholesterol hydroxylation catalyzed by a cytochrome P-450-dependent enzyme, cholesterol 24S-hydroxylase (CYP46). This process leads to the formation of 24S-hydroxycholesterol. 24S-Hydroxycholesterol is the major cholesterol elimination product of the brain [24]. It is assumed to pass the blood brain-barrier (BBB) via ATP-binding cassette transporters (ABCA1), which probably mediate an apolipoprotein A1 (apo A-1) dependent transfer of cerebral 24S-hydroxycholesterol into the plasma [30]. More than 90% of plasma 24S-hydroxycholesterol originates from the brain. Previous studies revealed that 24S-hydroxycholesterol acts neurotoxic *in vitro* [21].

Cholesterol 24S-hydroxylase is encoded by *CYP46A1* gene, which is primarily expressed in neurons [23]. Expression of

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CYP46A1 is decreased in AD brains; even though increased expression has been detected in some degenerating neurites, senile plaques and glial cells [2,3].

Different polymorphisms in *CYP46A1* have been described to influence AD risk and levels of cholesterol or Aß42 [7,12,31]. However, there are also publications which did not find an association of *CYP46A1* polymorphisms with AD [5,34]. Thus, the role of *CYP46A1* as a genetic risk factor of AD is unclear. Most studies have investigated only two polymorphisms in *CYP46A1*, located in intron 2 (rs754203) and 3 (rs4900442). Other variations have only been investigated in single publications without replication [10,25].

It might be suggested, that other gene variations in *CYP46A1* that are in putative linkage disequilibrium with the investigated ones, are responsible for the association of this gene with AD. Thus, we performed a gene variability screening of *CYP46A1* in AD patients and controls and investigated, if the detected polymorphisms influenced the risk of AD and CSF levels of cholesterol and 24S-hydroxycholesterol.

2. Methods

2.1. Participants

AD patients ($n = 455$, mean age: 73.1 ± 8.6; range: 53–101; years; female: 67.7%) were recruited from the Department of Psychiatry, University of Bonn, Germany, and from the Division of Neuroradiology of the Central Institute of Mental Health, Mannheim, Germany. Patients were diagnosed according to DSM-IV, supported by clinical examination, detailed structured interviews, neuropsychological testing, cognitive screening including MMSE [8] and neuroimaging studies. Age matched healthy controls ($n = 327$, mean age: 73.9 ± 7.5; range: 65–100 years; female: 53.8%) were recruited with the support of the local Census Bureau and the regional Board of Data Protection (Nordrhein-Westfalen, Germany) and from the Central Institute of Mental Health, Mannheim, Germany. The cognitive status was assessed by neuropsychological testing and structured interviews. All participants of the study gave informed written consent. The study protocol has been approved by the Ethics Committee of the Faculty of Medicine of the University of Bonn and of the institutional ethics committee, Mannheim, Germany.

2.2. Screening for variations in CYP46A1 and genotyping

Leukocyte DNA was isolated with the Qiagen® blood isolation kit according to the instructions of the manufacturer (Qiagen, Hilden, Germany). To verify SNPs in *CYP46A1* given in databanks in our sample and to detect putative new variants, the exonic regions of *CYP46A1* and flanking introns were investigated by direct sequencing. PCRs of the 15 exons and flanking intronic regions were performed using primers as outlined in Table 1 designed from the sequence of chromosome 14 (GenBank acc.no.: NW_925561). The resulting amplification products were investigated by cycle sequencing with the ABI Prism Genetic Analyzer 310A (PE Biosystems, Weiterstadt, Germany) in 40 probands (20 AD patients and 20 controls). This number of probands is sufficient to detect frequent sequence variations (>5% of the population) with a likelihood of >95%. We selected only gene variants with a frequency of >5% for our study, and by this followed the common disease-common variant hypothesis [22,32].

Table 1
Primers used for the screening of *CYP46A1* exonic regions and flanking introns.

Exon	Primer sequences	Product sizes
1	F: 5′-AAGAGGTGGGGGCGGAGCCT-3′ R: 5′- CCAGCTGTGCGCGGTGGG-3′	695 bp
2 + 3	F: 5′-GAGCCAGTGCTGTAACCTCC-3′ R: 5′- CCATTCCTTTCTGGATCTTTGG-3′	1058 bp
4 + 5	F: 5′- GCACACTGCGGCTGAGACT-3′ R: 5′- CCCACTTGGTCAGCCTGTCT-3′	945 bp
6 + 7	F: 5′- GGGGTTGCCATTTTTGTGAG-3′ R: 5′- CCATGGAGCCATCATAGTCATC-3′	1906 bp
8 + 9	F: 5′- AGTCTCCCTGGCTGCTTCAT-3′ R: 5′- GGTGAGCTCTGCTCAGATAGC-3′	950 bp
10	F: 5′- CCTCATAGAACCTCAGGGTC-3′ R: 5′- GGAAGCAGGGCATGTTGAG-3′	532 bp
11 + 12	F: 5′- GGCGACAGAGCAAGACTTTG-3′ R: 5′- CTTCTGCTGGACAGTGTGAGG-3′	1197 bp
13	F: 5′- CAGTGCAATGGACACCAACC-3′ R:5′- GCTGTGATTTCCTCCACAACC-3′	390 bp
14 + 15	F: 5′- CCCTCGGCAGAGTCAGCG-3′ R: 5′- TGGTTCCAGGTCATTCATGC-3′	1290 bp

For genotyping of all detected 16 SNPs in an enlarged sample, genotyping methods using RFLP or allele specific PCR (Polymerase chain reaction-CTPP [11]) were established. Details are given in Table 2.

The *APOE* genotype was studied as already described by Hixson and Vernier [13].

2.3. Analysis of cholesterol and 24S-hydroxycholesterol in CSF

A subgroup of patients and controls received lumbar puncture during clinical routine, and CSF from these persons could be used for the determination of cholesterol and 24S-hydroxycholesterol. After an overnight fast CSF samples of 118 AD patients (mean age: 68.5 ± 7.9; female: 61.9%) and 62 age matched, non-demented controls (mean age: 70.4 ± 7.1; female: 57.9%) were obtained by lumbar puncture. AD patients were diagnosed according to the criteria described above. Non-demented controls were patients referred to the Department of Neurology, University of Bonn, who underwent lumbar puncture during clinical routine diagnosis of neurological disorders for indications such as unclear causes of headache, exclusion of CNS inflammation, exclusion of aneurismal subarachnoid haemorrhage or exclusion of meningitis. Subjects with symptomatic cardiac disease, renal or hepatic dysfunction, insulin-dependent diabetes mellitus, untreated thyroidal dysfunction, blood brain-barrier-disturbance, inflammatory disease, other neurodegenerative disorders (i.e. multiple sclerosis) or alcohol