Polymorphism in ABCA1 influences CSF 24S-hydroxycholesterol levels but is not a major risk factor of Alzheimer's disease

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Abstract. The ATP-binding cassette transporter A1 (ABCA1) mediates reverse cholesterol transport, polymorphisms have been shown to influence the levels of cholesterol and of HDL and the risk of coronary artery disease. Since altered cholesterol metabolism is also involved in Alzheimer's disease (AD), the effects of two ABCA1 polymorphisms (G-395C promoter polymorphism (rs 2246293) and exonic R219K) on the risk of AD in 241 AD patients and 294 non-demented controls, and on CSF cholesterol and 24S-hydroxy-cholesterol in 74 AD patients and 42 non-demented controls were investigated. None of the investigated ABCA1 poly-morphisms influenced the risk of AD. However, the ABCA1 G-395C polymorphism influenced CSF levels of 24S-hydroxy-cholesterol, but not of cholesterol, whereas the R219K influenced neither CSF levels of 24S-hydroxycholesterol nor cholesterol. Our data support the observation that ABCA1 polymorphisms influence cholesterol metabolism of the brain, but might not act as a major risk factor in AD.

Introduction

Alzheimer's disease (AD) is the most common neuro-degenerative disorder of the elderly. Mutations of the genes encoding the ß-amyloid precursor protein and the presenilins 1 and 2 are risk factors for early-onset AD (1,2). The only confirmed and often replicated risk factor for late-onset AD is the ε4-allele of apolipoprotein E (APOE). However, the APOE4 allele is neither a necessary nor a sufficient risk factor for AD (3). Thus, the presence of additional genetic risk factors is suggested.

Research over the last ten years revealed the involvement of cholesterol metabolism in the pathogenesis of AD: plasma cholesterol levels are increased in AD patients (6,7), levels of cholesterol metabolites such as 24S-hydroxycholesterol and 27-hydroxycholesterol are decreased in AD patients (8), depletion of cholesterol inhibits the production of ß-amyloid (Aß) *in vitro* (9), cholesterol lowering 3ß-hydroxy-3ß-methyl-glutaryl-CoA reductase inhibitors (statins) reduce the levels of Aß *in vivo* and *in vitro* (10), and possibly reduce the risk of AD (11,12).

A region showing possible linkage with AD was found on chromosome 9q22 (4,5). A putative candidate gene that lies about five megabases distal to the identified markers at chromosomal locus 9q21 is the gene encoding the ATP-binding cassette transporter 1 (ABCA1). ABCA1 is mainly involved in reverse cholesterol transport. Different sequence variations and mutations in the ABCA1 gene have been identified (13,14). ABCA1 polymorphisms might influence levels of HDL and the risk of coronary heart disease (15,16). The G-395C polymorphism in the promoter of ABCA1 [also denoted G-402C (14)], has been shown to be more prevalent in carriers of low HDL-blood levels (13). Furthermore, an exonic non-synonymous (R219K) polymorphism influenced CSF cholesterol levels and risk of premature coronary heart disease in patients with hypercholesterolemia (17).

Due to the chromosomal location of the gene and due to its function in relation to AD, it is speculated that ABCA1 polymorphisms influence the risk of AD and brain cholesterol metabolism. Different studies investigated the effect of ABCA1 polymorphisms on the risk of AD; however, conflicting results have been reported (18-20). To further explore the effect of ABCA1 G-395C and R219K polymorphisms on AD risk and on the CSF levels of cholesterol and 24S-hydroxycholesterol, we investigated genotype distribution in a homogeneous age matched sample of German origin.

Patients and methods

AD patients and controls. AD patients (n=241, mean age: 73.2±9.4 years, 71.5% female) were recruited from the Department of Psychiatry of the University of Bonn. Patients were diagnosed according to DSM IV, supported by clinical examination, detailed structured interviews [Composite International Diagnostic Interview (CIDI) (21), SIDAM (22)] and neuropsychological testing applying MMSE (23).

Healthy controls from the general population (n=294, mean age: 71.8±8.1 years, 54.5% female) were recruited with the

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Table I. Allele frequencies and genotype distributions of ABCA1 polymorphisms in AD patients and healthy controls.

ABCA1 G-395C		Allele frequency		Genotype			χ^2 test		
	n	G	C	G/G (%)	G/C (%)	C/C (%)	χ	df	p
AD patients	241	0.55	0.45	74 (30.7)	115 (47.7)	52 (21.6)	0.82	2	0.67
Controls	294	0.53	0.47	80 (27.2	149 (50.7)	65 (22.1)			
ABCA1 R219K		Allele frequency		Genotype			χ^2 test		
	n	R	K	R/R (%)	R/K (%)	K/K (%)	χ	df	p
AD patients	241	0.72	0.28	130 (53.9)	88 (36.5)	23 (9.5)	1.89	2	0.39
Controls	294	0.72	0.28	149 (50.7)	123 (41.8)	22 (7.5)			

support of the local Census Bureau and the regional Board of Data protection (Nordrhein-Westfalen, Germany). Neuro-psychological testing, detailed structured interviews and clinical examination was performed. All patients and control subjects gave informed consent for participation in the study. The study protocol was approved by the Ethics Committee of the Faculty of Medicine of the University of Bonn.

Genetic analysis. Genomic DNA was isolated with the Qiagen® blood isolation kit (Qiagen, Hilden, Germany). The G-395C promoter polymorphism [rs 2246293 (13)] and the R219K polymorphism in ABCA1 were determined by RFLP. In brief, for the G-395C polymorphism the whole product was amplified using the following primers: 5'-TGAGGGAGAT TCAGCCTAGA<u>T</u>C-3' (forward) and 5'-AGTGGAATTTGC TTCCTCTAGATC-3' (reverse), the forward primer contained a nucleotide change (underlined), to generate a control restriction site specific for BstY I. The amplification products of the G- and the C-alleles were 72, 24 and 18 bp or 96 and 18 bp. The R219K polymorphism was investigated by using the following primers: 5'- TCAGAAGAGATGATTCAACT TGG -3' (forward) and 5'-TTTCTACAAAACAAAGTCAT <u>C</u>CTG-3' (reverse), the reverse primer contained a nucleotide change (underlined), to generate a control restriction site specific for EcoN I. The amplification products of the R- or the K-alleles were 190 and 27 bp or 135, 55 and 27 bp. The APOE genotype was studied as described (24).

Analysis of cholesterol and 24S-hydroxycholesterol in CSF. CSF samples of 74 AD patients (67±9.7 years, 60.8% female) and 42 non-demented neurological controls (67±9.5 years; 52.4% female) were obtained by lumbar puncture. Probands with a history of atherosclerotic disorder or taking statins were excluded from this study. AD patients were again recruited from the Department of Psychiatry of the University of Bonn. Non-demented controls were neurological patients recruited from the Department of Radiology of the University of Bonn. Diagnosis was supported by clinical examination and cognitive screening applying MMSE. CSF samples were frozen in aliquots and stored at -80˚C until assay. CSF concentrations of 24S-hydroxycholesterol and cholesterol were measured by

a modified sensitive method using combined gas-chroma-tography/mass spectrometry as described previously (25).

Statistical analysis. Allele frequencies and genotype distri-butions of ABCA1 polymorphisms in the diagnosis groups were compared using χ^2-statistics. Logistic regression analyses were used to examine the simultaneous effect of these poly-morphisms, the APOE4 allele, age and gender on the risk of AD. The power to detect a relative risk of 2.0 with $\alpha=0.05$ was 98% for the R219K polymorphism and 92% for the G-395C polymorphism. The effects of ABCA1 polymorphisms on cholesterol and 24S-hydroxycholesterol CSF and plasma levels were investigated by MANOVA including the co-variates age, sex, APOE4 allele and diagnosis. p-values were set at two-sided $p \leq 0.05$. Statistical tests were carried out using SPSS (10.07).

Results

The Hardy-Weinberg equilibrium was confirmed for cases and controls. Genotype distribution and allele frequencies of both polymorphisms in AD patients and controls are given in Table I. Logistic regression analysis revealed that the two investigated ABCA1 polymorphisms did not influence the risk of AD (G-395C, presence of C-allele: $\chi^2=0.31$, d.f.=1, p=0.59; R219K, presence of K-allele: $\chi^2=0.65$, d.f.=1, p=0.42). There was also no interaction between both ABCA1 poly-morphisms on the risk of AD ($\chi^2=0.81$, d.f.=1, p=0.37), and no interaction between ABCA1 polymorphisms and the APOE4 allele (G-395C, presence of C-allele vs. presence of APOE4: $\chi^2=1.61$, d.f.=1, p=0.21; R219K, presence of K-allele vs. presence of APOE4: $\chi^2=0.01$, d.f.=1, p=0.95). As to be expected, presence of the APOE4 allele and female gender were associated with an increased risk of AD (presence of APOE4: $\chi^2=48.3$, d.f.=1, p<0.001, OR=3.73, 95% CI: 2.57-5.40; sex: $\chi^2=15.84$, d.f.=1, p<0.001, OR=2.18, 95% CI: 1.49-3.20). Age did not significantly influence the risk of AD in our population ($\chi^2=3.73$, d.f.=1, p=0.054), due to the low age variance of the investigated sample.

Investigation of the effect of ABCA1 polymorphisms on CSF cholesterol and 24S-hydroxycholesterol revealed that
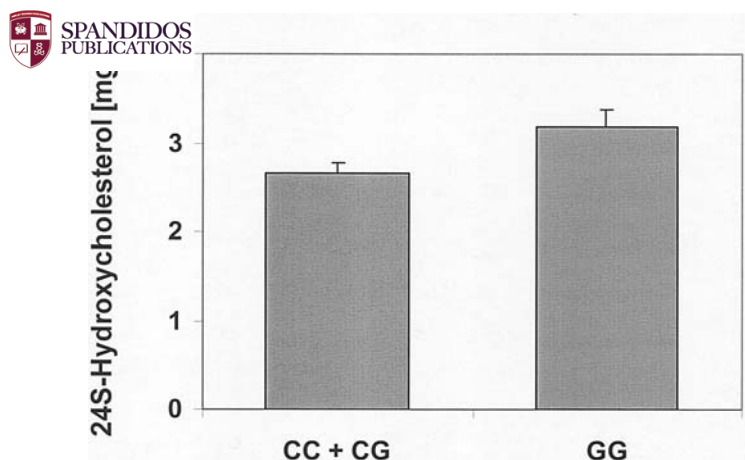
Figure 1. CSF cholesterol levels in carriers and non-carriers of at least one C-allele of the ABCA1 G-395C polymorphism (n=116).

24S-hydroxycholesterol concentrations were lower in probands who were carriers of at least one C-allele of the ABCA1 G-395C polymorphism than in carriers of the GG-genotype (F=5.74, d.f.=1, p=0.018; Fig. 1); however, levels of cholesterol were not influenced by this polymorphism (F=0.87, d.f.=1, p=0.35). We did not find any effect of the ABCA1 R219K polymorphism on the CSF levels of cholesterol (F=0.68, d.f.=1, p=0.41) or of 24S-hydroxycholesterol (F=0.01, d.f.=1, p=0.91) in our whole proband sample. CSF 24S-hydroxy-cholesterol levels were influenced by the APOE4 allele (F=8.69, d.f.=1, p=0.004) whereas; the APOE4 allele did not influence CSF cholesterol levels in this model (F=0.18, d.f.=1, p=0.67). Diagnosis influenced CSF levels of 24S-hydroxy-cholesterol and cholesterol in that these levels were higher in non-demented controls than in AD patients (24S-hydroxy-cholesterol: F=14.9, d.f.=1, p<0.001; cholesterol: F=23.9, d.f.=1, p<0.001). We found age to influence CSF levels of 24S-hydroxycholesterol and cholesterol in that levels increased with increasing age; however, for cholesterol this did not reach statistical significance (24S-hydroxycholesterol: F=11.51, d.f.=1, p=0.001; cholesterol: F=3.21, d.f.=1, p=0.076). Gender did not influence levels of 24S-hydroxycholesterol and cholesterol in our analysis (24S-hydroxycholesterol: F=0.37, d.f.=1, p=0.55; cholesterol: F=0.37, d.f.=1, p=0.54). Since a previous publication (19) reported altered levels of cholesterol depending on ABCA1 polymorphisms only in non-demented probands, statistical analysis assessed the interaction between ABCA1 polymorphisms and diagnosis: we did not find any significant interaction of ABCA1 polymorphisms and diagnosis either on cholesterol levels (G-395C, presence of C-allele vs. diagnosis: χ^2=0.56, d.f.=1, p=0.45; R219K, presence of K-allele vs. diagnosis: χ^2=0.07, d.f.=1, p=0.94) or on levels of 24S-hydroxycholesterol (G-395C, presence of C-allele vs. diagnosis: χ^2=3.2, d.f.=1, p=0.08; R219K, presence of K-allele vs. diagnosis: χ^2=0.27, d.f.=1, p=0.61).

Discussion

We investigated the ABCA1 G-395C and R219K polymorphisms for their influence on the risk of AD and on CSF levels of cholesterol and 24S-hydroxycholesterol. Different polymorphisms in ABCA1 have been described, and influences on HDL and total cholesterol plasma levels and on the risk of disorders such as coronary artery disease (26) or AD (20) have been detected. However, only few of these findings have been replicated and it is unclear which polymorphism might act as the relevant functional one. The exonic R219K polymorphism in ABCA1 has been shown to influence CSF cholesterol levels in healthy probands (19), while our study did not detect any effect. This polymorphism was also shown to influence HDL-cholesterol plasma levels (16) while these effects were not detected in another study (14).

We also did not find the exonic R219K or the promoter G-395C polymorphisms to influence the risk of AD; thus, we cannot support the suggestion that polymorphisms in ABCA1 might act as risk factors of AD. One might argue that the lack of a significant effect of ABCA1 polymorphisms on the risk of AD in our study might be due to a lack of power; however, our study was adequately powered to detect relevant associations with an OR of ≥2.

However, we found the G-395C promoter polymorphism to influence CSF levels of 24S-hydroxycholesterol in that carriers of at least one C-allele presented with decreased levels. However, this polymorphism did not influence CSF cholesterol levels. Up to now, the functional relevant polymorphism in ABCA1 promoter has not been clearly identified. One study described that a C-565T ABCA1 promoter polymorphism influences expression of ABCA1 (27). A further study described that another promoter polymorphism G-273C influences plasma HDL cholesterol levels (14); however, the authors suggest that this polymorphism is not located in a putative transcription factor-binding site and thus the relevance for gene expression and thus protein activity needs to be explored. It was also described that this G-273C polymorphism is in absolute linkage disequilibrium with the C-565T [denoted also as C-559T in (14)] and G-395C polymorphisms [denoted also as G-402C in (14)]. The latter was found to influence CSF 24S-hydroxycholesterol levels in our study. Corresponding to the program 'TFSEARCH: Searching Transcription Factor Binding Sites' (28), the ABCA1 G-395C polymorphism is located in a putative transcription factor-binding site for c-rel with a threshold score of 85.1 in carriers of a C-allele. However, it needs to be clarified if c-rel is involved in the regulation of ABCA1 expression. These data together suggest that the relevant ABCA1 promoter poly-morphism which might influence gene expression and protein activity, and by this, cholesterol levels has not yet been identified.

Further studies that identify the relevant functional ABCA1 promoter polymorphism are needed. These results will help to clarify the role of ABCA1 polymorphisms in the regulation of cholesterol metabolism and as putative risk factors in AD and atherosclerotic diseases.

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