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# ABCB1 Genotype and CSF β-Amyloid in Alzheimer Disease

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## Abstract

The ABCB1 gene, coding for the efflux transporter P-glycoprotein (PGP), is a candidate gene for Alzheimer disease (AD). P-glycoprotein is heavily expressed at the blood–brain barrier, where it mediates the efflux of β-amyloid (Aβ) from the brain. In this study, we investigated a possible association between 2 common ABCB1 polymorphisms, G2677T/A (Ala893Ser/Thr) and C3435T, AD, and cerebrospinal fluid (CSF) levels of Aβ. No strong evidence for association was found.

### Keywords

Alzheimer disease; dementia; P-glycoprotein; MDR1; ABCB1; association study

## Introduction

P-glycoprotein (PGP) is the product of the *ABCB1* gene, also known as MDR1. P-glycoprotein is an ATP-dependent efflux transporter located in tissues with excretory function and at blood–tissue barriers. It is a 1280 amino acid long plasma membrane glycoprotein consisting of 2 homologous parts of approximately equal length. Each half contains 6 hydrophobic transmembrane regions and an ATP binding site.[1] P-glycoprotein shields the body from a broad spectrum of potentially toxic substances by limiting their entry through the intestine and promoting their elimination into bile and urine.[2] In the brain, PGP is heavily expressed at the blood–brain barrier and the blood–cerebrospinal fluid barrier, particularly at the luminar surface of brain endothelial cells.[3,4] P-glycoprotein-knockout mice suffer from enhanced neurotoxicity of centrally acting drugs, illustrating the importance of PGP in actively preventing entry of potentially harmful substances into the brain.[5,6]

Several lines of evidence implicate PGP as a candidate gene for Alzheimer disease (AD). P-glycoprotein functions as an efflux pump for β-amyloid (Aβ).[7,8] A possible role of PGP

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dysfunction in the etiology of AD is illustrated by studies in mice showing that amyloid precursor protein-transgenic (APP-transgenic) mice have increased Aβ levels in the brain following treatment with a PGP inhibitor. In addition, PGP-knockout mice have reduced clearance of $A\beta_{40}$ and $A\beta_{42}$ from the brain following intracerebral microinjection compared to wild type animals. Moreover, hybrid APP-transgenic/PGP-null mice show higher levels of brain Aβ deposition than simple APP-transgenic mice with intact PGP.[9] Studies in elderly human participants without dementia have demonstrated cerebral levels of $A\beta_{40}$ and $A\beta_{42}$ to be inversely related to PGP expression.[10,11]

The *ABCB1* gene, located on chromosome 7q21, is 209-kb long and contains 29 exons.[12] Two polymorphisms of ABCB1, G2677T/A (Ala893Ser/Thr) and C3435T, have been extensively studied and linked to PGP expression.[13] A previous small study investigating the distribution of these polymorphisms in patients with dementia and healthy controls found no association.[14] The goal of our study was to investigate a possible association of G2677T/A and C3435T with AD in a larger and well-characterized sample of cases and controls collected as part of the Alzheimer's Disease Research Center (ADRC) at the Puget Sound Veterans Affairs Health Care System (VAPSHCS) in Seattle.[15] In addition, this is the first study to investigate a possible association of ABCB1 genotype with cerebrospinal fluid (CSF) levels of $A\beta_{42}$.

## Materials and Methods

### Participants

All procedures were approved by the institutional review boards of the participating institutions. This study used DNA collected through the Clinical Core of the University of Washington (UW) ADRC. Our sample included DNA from 286 individuals with AD and 240 cognitively intact controls. All participants had normal physical and neurological examinations and laboratory tests (complete blood count, chemistry panel, thyroid stimulating hormone, vitamin B12, and urinalysis). All were free of past or present major psychiatric or neurologic disorders (other than AD) and unstable medical conditions. All participants with AD met clinical diagnostic criteria for probable AD of the National Institute of Neurological and Communicative Disorders and Stroke[16] and Diagnostic and Statistical Manual of Mental Disorders (Third Edition Revised) criteria for dementia of the Alzheimer Type. Cognitively normal participants had a Mini-Mental State Examination (MMSE)[17] score of 26 to 30, Clinical Dementia Rating Scale score of 0, and no evidence or history of cognitive decline. Patients with mild cognitive impairment (MCI) were excluded. For a subset of participants (n = 267), CSF levels of $A\beta_{42}$ from samples previously collected and analyzed as described[18,19] were used in our analysis. The demographics of our sample are described in Table 1.

### ABCB1 Genotyping

We genotyped the G2677T/A (Ala893Ser/Thr) polymorphism in exon 22 (dbSNP: rs2032582) and the C3435T polymorphism in exon 27 (dbSNP: rs1045642). Genomic DNA (100 ng) was amplified in the presence of gene-specific primers and allele-specific fluorescent probes, using inventoried TaqMan SNP genotyping assays (Applied Biosystems, California) and following the manufacturers' instructions.

### Statistical Analysis

Calculations for deviation from Hardy–Weinberg equilibrium and for linkage disequilibrium between G2677T/A and C3435T were performed using $\chi^2$ tests. Haplotype analysis was done using Multiallelic Interallelic Disequilibrium Analysis Software (MIDAS).[20] Bivariate associations between diagnosis (AD vs control) and G2677T/A or C3435T genotype (C/C,

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C/T, or T/T) were assessed using Fisher exact tests. Due to low frequencies for several G2677T/A genotypes, statistical inference for G2677T/A focused on the comparison between T allele carriers vs those with no T-allele. To account for the potential confounding of age, ethnicity, and APOE4 status on the relationship between diagnosis and G2677T/A T allele, logistic regression was used with G2677T/A T allele as the dependent variable and diagnosis, age, ethnicity (white vs. non-white), and APOE4 status (presence of E4 allele vs absence) as independent covariates. Similarly, to account for confounding in the association between C3435T genotype and AD diagnosis, multinomial regression was carried out with C3435T genotype as the dependent variable (C/C as the reference group) and diagnosis, age, ethnicity, and APOE4 status as independent covariates. Results are presented as odds ratios.

Linear regression was used to assess the association between $A\beta_{42}$ (the dependent variable) and G2677T/A T allele or C3435T genotype with covariates age, ethnicity, AD diagnosis, and APOE4 status. Normality of $A\beta_{42}$ was assessed using the Kolmogorov-Smirnov test. Analyses were carried out using Stata 9 (StataCorp) and R 2.9.1.[21]

## Results

### Demographics and ABCB1 Genotype

The demographics of AD participants and controls are shown in Table 1. The control group was significantly younger and had higher minority participation. Genotype distributions for the G2677T/A and C3435T polymorphisms were checked for deviation from Hardy–Weinberg equilibrium and no deviation was observed (AD: G2677T/A $\chi^2 = 6.69$, $P = .08$; C3435T $\chi^2 = 0.15$, $P = .69$. Controls: G2677T/A $\chi^2 = 0.39$, $P = .94$; C3435T $\chi^2 = 0.59$, $P = .44$). G2677T/A and C3435T were in linkage disequilibrium (LD) ($D' = -0.90$, $r^2 = -0.59$). Since vgenotype distributions differed significantly by race (G2677T/A $P = .000$, C3435T $P = .005$, data not shown), we controlled for age and race in the following analyses.

### Association of ABCB1 Genotype With AD

Genotype distributions did not differ significantly by disease status (Table 2). In the dominant model of 2677T (presence of T allele vs other), we observed slightly increased odds for AD among T-allele carriers (OR 1.49, 95% CI 1.03 to 2.16, $P = .034$), however, the effect was no longer significant if we adjusted for age (n = 408, OR 1.23, 95% CI 0.81 to 1.87, $P = .3$). Our results remained essentially unchanged if we adjusted for ethnicity (OR = 1.18, 95% CI 0.77 to 1.80, $P = .4$) or ethnicity plus APOE4 status (OR = 1.23, 95% CI 0.78 to 1.92, $P = .4$) in addition to age. Our study had 65% power ($\alpha = 0.05$) to detect a 10% increase in G2677T/A T-allele frequency among participants with AD, 94% power to detect a 15% increase, and over 99% power to detect a 20% increase using 2-sided tests. No association between C3435T and AD was observed. These results remained the same when age, ethnicity, and APOE genotype were controlled for (data not shown). For participants with AD (n = 285), mean age of disease onset was 67 ± 9.4 years. There were no significant differences in age of AD onset between participants of different races, or between genotype groups for G2677T/A or C3435T (data not shown).

### Association of ABCB1 Genotype With CSF Levels of $A\beta_{42}$

In a subset of 267 participants, CSF levels of $A\beta_{42}$ were significantly lower in participants with AD (n = 54, 109 ± 31 pg/mL CSF) than in controls (n = 213, 163 ± 33 pg/mL CSF), $P < .0001$ (linear regression). Age of entry into the study and APOE genotype were both significantly correlated with CSF levels of $A\beta_{42}$ (linear regression, age: $P = .000$, adjusted $R^2 = 0.21$; APOE4: $P = .000$, adjusted $R^2 = .17$). Neither the C3435T nor the G2677T/A polymorphism were associated with CSF levels of $A\beta_{42}$ (Table 3). These results did not change if we applied a dominant model of 2677T or if participants with AD and controls

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were analyzed separately (not shown). For both polymorphisms, linear regression controlling for age, ethnicity, AD, and APOE4 status showed no significant associations between ABCB1 genotype and CSF levels of $A\beta_{42}$ (data not shown). The Kolmogorov-Smirnov test showed a slight deviation from normality in the distribution of $A\beta_{42}$ ($P = .043$). Using a power of $A\beta_{42}$ derived from the Box-Cox set of transformation did not change the results above.

## Discussion

Our study showed no significant associations of the ABCB1 G2677T/A and C3435T polymorphisms of ABCB1 with AD or with CSF levels of $A\beta_{42}$. This negative result is in keeping with previous conflicting reports about associations of these genetic variants with PGP expression or function.[13,22,23] Our findings also confirm the results of a prior small association study in 113 patients with various types of dementia and 41 controls which reported no association of ABCB1 genotype with dementia.[14] Our results also agree with previous studies that showed CSF $A\beta_{42}$ to be lower in patients with AD than in healthy control participants.[24]

The strength of this study is the simultaneous investigation of ABCB1 genotype and CSF levels of $A\beta_{42}$ in a large cohort of AD participants and controls. We are not reporting here on the influence of APOE genotype on CSF levels of $A\beta_{42}$, as this has previously been described in a subset of our sample.[18,19] A limitation of this study is the lack of detailed clinical information about our AD participants, as it prevents us from making observations about possible effects of ABCB1 genotype on disease severity and progression. Moreover, we investigated only 2 common variants of the ABCB1 gene whose effects on PGP expression remain controversial. A possible effect of rare deleterious variants on AD risk is worthy of further investigation. The ABCB1 gene is subject to complex regulation.[25–27] A disturbance of PGP expression due to disruption of regulatory mechanisms remains a plausible risk factor for AD.

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## Table 1

Comparison of Participants With AD and Controls by Demographic Variables[a]

| | | AD, n (%) | Controls, n(%) |
|---|---|---|---|
| Gender | Male | 148 (52) | 121 (50) |
| | Female | 138 (48) | 119 (50) |
| Race | White | 272 (95) | 205 (87) |
| | African American | 5 (2) | 16 (7) |
| | Asian or Pacific Islander | 5 (2) | 9 (4) |
| | American Indian/Alaskan native | 2 (1) | 3 (1) |
| | Other/unknown | 2 (1) | 4 (2) |
| Age | | 68.2 ± 9.4 | 52.5 ± 19.5 |

Abbreviations: AD, Alzheimer's disease.

[a]For categorical variables (gender and race) the numbers (n) as well as the percentages (%) of participants with AD (n = 286) and controls (n = 237) that met the demographic criteria listed are given. For age at entry into the study, mean ± SD is given. Differences in gender distribution between subjects with AD and controls were not significant, but differences in race distribution (Fisher exact test $P = .004$) and age (*t* test $P = .000$) were highly significant.

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## Table 2

Distribution of Genotype Frequencies for G2677T/A and C3435T by AD Diagnosis[a]

| | | AD | Controls |
|---|---|---|---|
| **Polymorphism** | **Genotype** | **n (%)** | |
| G2677T/A | G/G | 73 (26) | 78 (33) |
| | G/T | 142 (50) | 110 (46) |
| | G/A | 5 (2) | 9 (4) |
| | T/A | 5 (2) | 5 (2) |
| | T/T | 60 (21) | 38 (16) |
| | A/A | 1 (0.4) | 0 |
| C3435T | C/C | 63 (22) | 66 (28) |
| | C/T | 138 (49) | 114 (48) |
| | T/T | 83 (29) | 60 (25) |

Abbreviations: AD, Alzheimer's disease.

[a]Shown are the allele frequencies (%) for subjects with AD (n = 286) and controls (n = 240): 2 AD participants had missing C3435T genotypes. Genotype distributions did not differ significantly between participants with AD and controls (G2677T/A $P = .17$, C3435T $P = .30$, Fisher exact test).

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## Table 3

Cerebrospinal Fluid (CSF) Levels of Amyloid-$\beta_{42}$ by G2677T/A and C3435T Genotype Group[a]

| | | Total | |
|---|---|---|---|
| **Polymorphism** | **Genotype** | **n** | **$A\beta_{42}$** |
| | G/G | 83 | 155 ± 41 |
| | G/T | 129 | 152 ± 38 |
| G2677T/A | G/A | 6 | 175 ± 35 |
| | T/A | 4 | 147 ± 47 |
| | T/T | 45 | 145 ± 40 |
| *P* value | | | 0.44 |
| | C/C | 66 | 152 ± 38 |
| C3435T | C/T | 130 | 155 ± 41 |
| | T/T | 71 | 148 ± 38 |
| *P* value | | | 0.49 |

Abbreviation: AD, Alzheimer's disease.

[a]Shown are the number of participants (n) and mean ± SD CSF levels of $A\beta_{42}$ (pg/mL CSF) per genotype group for all subjects (n = 267). The *P* values (ANOVA) for comparison of $A\beta_{42}$ levels by genotype are shown below. The G2677T/A A/A genotype was not represented in the subset of our sample for which $A\beta_{42}$ CSF levels had been determined.