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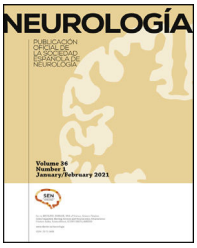

ORIGINAL ARTICLE

ABCB1 gene variants as risk factors and modulators of age of onset of demyelinating disease in Mexican patients

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Abstract
Introduction: The C1236T, G2677T/A, and C3435T variants of the *ABCB1* gene alter the functioning of P-glycoprotein and the transport of endogenous and exogenous substances across the blood-brain barrier, and act as risk factors for some neurodegenerative diseases. This study aimed to determine the association between demyelinating disease and the C1236T, G2677T/A, and C3435T variants of *ABCB1* and its haplotypes and combinations of genotypes.
Methods: Polymerase chain reaction with restriction fragment length polymorphism analysis (PCR-RFLP) and Sanger sequencing were used to genotype 199 patients with demyelinating disease and 200 controls, all Mexicans of mixed race; frequencies of alleles, genotypes, haplotypes, and genotype combinations were compared between patients and controls. We conducted a logistic regression analysis and calculated chi-square values and 95% confidence intervals (CI); odds ratios (OR) were calculated to evaluate the association with demyelinating disease.
Results: The TTT and CGC haplotypes were most frequent in both patients and controls. The G2677 allele was associated with demyelinating disease (OR: 1.79; 95% CI, 1.12-2.86; $P = .015$), as were the genotypes GG2677 (OR: 2.72; 95% CI, 1.11-6.68; $P = .025$) and CC3435 (OR: 1.82; 95% CI, 1.15-2.90; $P = .010$), the combination GG2677/CC3435 (OR: 2.02; 95% CI, 1.17-3.48; $P = .010$), and the CAT haplotype (OR: 0.21; 95% CI, 0.05-0.66; $P = .001$).
TTTTTT carriers presented the earliest age of onset (23.0 ± 7.7 years, vs 31.6 ± 10.7; $P = .0001$).

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Conclusions: The GG2677/CC3435 genotype combination is associated with demyelinating disease in this sample, particularly among men, who may present toxic accumulation of P-glycoprotein substrates. In our study, the G2677 allele of *ABCB1* may differentially modulate age of onset of demyelinating disease in men and women.

PALABRAS CLAVE
Enfermedad desmielinizante;
Esclerosis múltiple;
Análisis de asociación;
Análisis de haplotipos;
Gen ABCB1;
Glicoproteína P

Variantes del gen *ABCB1* como factores de riesgo y factores moduladores de la edad de inicio en pacientes mexicanos con enfermedad desmielinizante

Resumen

Introducción: Las variantes *C1236T*, *G2677T/A* y *C3435T* del gen *ABCB1* alteran la función de la Glicoproteína P y el transporte de sustancias endógenas y exógenas en la barrera hemato-encefálica, además actúan como factores de susceptibilidad para algunas enfermedades neurodegenerativas.

El objetivo del estudio fue determinar la asociación de polimorfismos *ABCB1* (*C1236T*, *G2677T/A* y *C3435T*), sus haplotipos y sus combinaciones de genotipos con la enfermedad desmielinizante.

Método: Se genotipificaron 199 pacientes con enfermedad desmielinizante y 200 controles mestizo mexicanos mediante PCR-RFLP y secuenciación Sanger para comparar las frecuencias de alelos, genotipos, haplotipos y combinaciones de genotipos entre pacientes y controles. El análisis estadístico se realizó con regresión logística y χ^2 de Pearson al 95% de confianza; se calculó el OR y se evaluó la asociación con enfermedad desmielinizante.

Resultados: Los haplotipos *TTT* y *CGC* fueron los más frecuentes en pacientes y controles. El alelo *G2677* (OR = 1.79; IC 95%: 1,12-2,86; $p = 0{,}015$) muestra asociación con enfermedad des-mielinizante, así como los genotipos *GG2677* (OR = 2,72; IC 95% = 1,11-6,68; $p = 0{,}025$) y *CC3435* (OR = 1,82; IC 95%: 1,15-2,90; $p = 0{,}010$) y su combinación *GG2677/CC3435* (OR = 2,02; IC 95%:1,17-3,48; $p = 0{,}010$) y el haplotipo *CAT* (OR = 0,21; IC 95%: 0,05-0,66; $p = 0{,}001$).

Los portadores *TTTTTT* presentaron la edad de inicio más temprana ($23{,}0 \pm 7{,}7$ vs. $31{,}6 \pm 10{,}7$; $p = 0{,}0001$).

Conclusiones: La combinación de genotipos *GG2677/CC3435* está asociada al desarrollo de enfermedad desmielinizante en esta muestra, principalmente en sexo masculino, en el cual puede darse acumulación tóxica de sustratos de glicoproteína P. En este estudio, la edad de inicio de la enfermedad desmielinizante podría ser modulada diferencialmente entre sexos por el alelo *G2677* del gen *ABCB1*.

Introduction

P-glycoprotein (P-gp) is a blood-brain barrier (BBB) transport protein that extracts endogenous (peptides, amino acids, steroid hormones, lipids, and phospholipids, among others) and exogenous substances (xenobiotics, including drugs) from the nervous system (CNS) into the blood in order to maintain brain homeostasis.[1,2]

P-gp is encoded by the *ABCB1* gene, located on chromosome 7q21.12; several genetic variants can potentially alter P-gp function,[3] with 3 being of particular interest due to their considerable interindividual and interethnic variability: C1236T (rs1128503), G2677T/A (rs2032582), and C3435T (rs1045642), whose CGC haplotype (1236/2677/3435) is most frequent in white and African populations, whereas the TTT haplotype is more frequent in Asian populations.[3–6] Normally, P-gp function is gradually lost with age, predisposing to a range of diseases[7]; this process is more marked in carriers of certain P-gp genotypes.

ABCB1 is expressed in a wide range of immune cells, such as monocytes, dendritic cells, and T and B lymphocytes (including astroglial and microglial cells). P-gp mediates the flow of steroids, prostaglandins, and cytokines in reactive astrocytes involved in lesion formation and the inflammatory process in multiple sclerosis (MS), which indicates that the protein has an immunomodulatory function[8,9]; however, its action mechanism during the immune response is unknown.

Oxidative and inflammatory stress may cause alterations to the tight junctions of the BBB, which probably occurs in numerous neurodegenerative diseases.[10] Alterations to the expression and function of P-gp in the BBB may contribute to the pathogenesis of such neuroinflammatory disorders as MS.[8,9]

Several variants of *ABCB1* have been linked to diseases with an inflammatory component, such as epilepsy,[11] cancer,[12] neurodegenerative diseases[7] (Alzheimer disease,[13] Creutzfeldt-Jakob disease,[14] Parkinson's disease,[15] etc[7]), and such autoimmune diseases as systemic lupus erythematosus, inflammatory bowel

disease, hepatic cirrhosis, autoimmune thrombocytopaenia, and rheumatoid arthritis, in which they cause resistance to antineoplastic, antidepressant, and immunosuppressant drugs.[16]

Diseases affecting healthy myelin, generally due to metabolic disorders causing its destruction, are known as demyelinating diseases (DD). MS is a chronic, multifactorial, inflammatory, degenerative, demyelinating disease of the CNS, with both environmental and genetic factors playing an important role in susceptibility to the disease. MS is the most frequent DD (followed by neuromyelitis optica and optic neuritis, among others) and constitutes a significant cause of disability in young adults.[17]

The disease predominantly affects women, although male patients tend to develop more severe symptoms[18,19]; studies conducted in Asian populations have reported different treatment responses associated with *ABCB1* genotype, in addition to high prevalence in women.[16,20]

This study aimed to determine how *ABCB1* variants (C1236T, G2677T/A, and C3435T) and their haplotypes and combinations of genotypes are associated with and involved in DD.

Material and methods

We selected 199 unrelated patients with DDs (Table 1), including 104 with MS (relapsing-remitting, secondary progressive, or primary progressive), 43 with neuromyelitis optica, 37 with optic neuritis, and 15 with clinically isolated syndrome, attended at the demyelinating diseases clinic at the Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez in Mexico City (Mexico). Patients with family history of DD were excluded.

We also included 200 controls (129 women and 71 men) with no clinical evidence of neurological or autoimmune disease and no family history of DD, with a mean (standard deviation [SD]) age of 31.0 (10.3) years, from our centre's DNA bank.

The study protocol was approved by the centre's scientific and research ethics committees. All patients and controls were descendants of Mexican parents and grandparents. After obtaining informed consent, we collected blood samples for DNA extraction by the standard method.

Genotyping of the C1236T and C3435T variants was conducted with the polymerase chain reaction-restriction fragment length polymorphism method, as described by Cascorbi et al.[21] Genotyping of the G2677T/A variant was conducted by Sanger sequencing with an ABI PRISM 3130 genetic analyser and oligonucleotides designed by Cascorbi et al.,[21] using the ABI PRISM BigDye Terminator® v3.1 kit (Applied Biosystems; USA).

Haplotypes and combinations of genotypes are characterised in accordance with previous reports, following the sequence of the variants in coding DNA (1236/2677/3435),[22,23] obtaining 54 combinations of genotypes.

Statistical analysis was performed using the SPSS statistics software, version 20 (SPSS Inc.; Chicago, IL, USA); the SNPStats tool was used to analyse haplotypes.[24] Genotype and allele frequencies in the control group conformed to Hardy-Weinberg equilibrium. To determine differences between groups, we used the *t* test for parametric statistical analysis and logistic regression and the chi-square test for non-parametric statistics, and calculated odds ratios (OR) and 95% confidence intervals to determine the association with DD.

We also gathered the following clinical data on the patients with MS: age of onset of the first compatible symptom, Expanded Disability Status Scale[25] (EDSS) scores at baseline (first consultation) and at the time of blood sample collection, and disease progression time (onset to sample collection), in order to calculate the mean annualised progression rate or progression index (PI: final EDSS/years of progression). Patients with all clinical forms of MS were included in a single group to preserve statistical power. We used linear regression analysis and the *t* test to analyse these variables as a function of *ABCB1* genotype, combinations of genotypes, and haplotype. The threshold for statistical significance was set at $P < .05$.

Results

We present clinical and demographic data (Table 1); genotype and allele frequencies; an association analysis of *ABCB1* variants in patients and controls (Table 2); logistic regression models for *ABCB1* genotypes and alleles (Table 3); an association analysis of patients and controls who were carriers and non-carriers of combinations of *ABCB1* genotypes (Table 4); an association analysis of haplotypes in patients and controls (Table 5); and linear regression models of age of DD onset in carriers and non-carriers of *ABCB1* variants (Table 6).

Genotypes and alleles

No differences were identified between patients and controls in genotype and allele frequencies of the C1236T variant (Table 2).

The distribution of the G2677T/A variant (Table 2) was heterogeneous, with patients showing a higher frequency of the G2677 allele, which was identified as a risk factor for DD in both sexes (the GG2677 genotype in men and the GT2677 genotype in women). The TT2677 genotype and the A2677 allele were associated with protection against DD.

The CC3435 genotype of the C3435T variant was more frequent in patients than in controls, although the risk of DD was greater in men. The C3435 allele was identified as a risk factor in men, whereas the T3435 allele had a protective effect in both sexes.

Table 3 presents the final logistic regression models for the *ABCB1* alleles and genotypes studied. The first part shows the analysis of alleles, which were sequentially excluded until the only remaining alleles were G2677, associated with risk of developing DD (OR = 2.07), and T3435, associated with protection against DD (OR = 0.57); statistical significance was lost when the C3435 allele was excluded from the analysis.

The second part of the table shows the analysis of genotypes, which were sequentially excluded until we obtained the final model, including genotypes GT2677, associated with risk of DD (OR = 1.95), and CT3435, which had a protective effect (OR = 0.51).

Combinations of genotypes

We identified 32 of the 54 possible combinations of genotypes in our sample. The most frequent in both patients and controls was CTGTCT (52 patients [25.6%] and 47 controls [23.5%]), followed by TTTTTT (24 [12.1%] and 29 [14.5%]) and CCGGCC (24 [12.1%] and 15 [7.5%]), with no significant differences between groups.

The CTGGCC combination (12 [6%] and 7 [3.5%]) was more frequent in male patients (4 [6.7%]) than in male controls (0%), with no such difference observed in women (8 patients [5.8%] and 7 controls [5.4%]). The risk calculation included carriers of the GG2677 and CC3435 genotypes (CTGGCC; CCGGCC, in 0 patients and 0 controls; and TTGGCC, in 7 patients [3.5%] and 2 controls [1%]), the combination designated ''GGCC,'' which was associated with increased risk of DD (OR = 2.02), particularly in men (OR = 3.65) (Table 4).

The CTGTTT combination of genotypes (12 patients [6%] and 6 controls [3%]) presented similar frequencies among men (1 patient [1.7%] and 1 control [1.4%]) and was more frequent among female patients than female controls (11 [7.9%] and 5 [3.9%], respectively); thus, we grouped together carriers of similar combinations (CTGTTT; CCGTTT, in 0 patients and 0 controls; and TTGTTT, in 7 patients [3.5%] and 3 controls [1.5%]) to obtain the combination ''GTTT,'' which was associated with risk of DD in women (OR = 2.67) (Table 4).

The TTTTCT combination (2 patients [1%] and 12 controls [6%]) protects women against DD (OR = 0.11) as it includes the TT2677 genotype and the T3435 allele, which are protective; similarly, the

Table 1 Clinical and demographic characteristics of the sample.

Variable	Patients		
	Women	Men	Total
Demyelinating disease	139	60	199
Age of onset, years (mean [SD])	31.0 (10.9)	29.7 (10.2)	30.6 (10.7)
MS	64	40	104
Age of onset, years (mean [SD])	30.3 (10.8)	28.7 (10.5)	28.3 (9.9)
Baseline EDSS	3.2 (1.9)	3.6 (2.2)	3.3 (2.1)
Final EDSS	3.9 (2.6)	4.3 (2.6)	4.0 (2.6)
Progression index	0.62 (0.7)	0.71 (0.8)	0.66 (0.8)
Disease progression, years (mean [SD])	8.9 (5.9)	8.2 (4.7)	8.6 (5.5)
NMO	38	5	43
Age of onset, years (mean [SD])	31.7 (12.5)	32.0 (10.6)	31.7 (12.3)
Optic neuritis	28	9	37
Age of onset, years (mean [SD])	33.4 (9.4)	27.6 (7.8)	31.9 (9.3)
CIS	9	6	15
Age of onset, years (mean [SD])	25.7 (7.7)	38.3 (9.2)	30.7 (10.3)

CIS: clinically isolated syndrome; EDSS: Expanded Disability Status Scale; MS: multiple sclerosis (relapsing-remitting, primary progressive, and secondary progressive); NMO: neuromyelitis optica; SD: standard deviation.

CCGACT combination (1 patient [0.5%] and 8 controls [4%]) protects both sexes (OR = 0.12) as it contains the protective A2677 and T3435 alleles (Table 4).

The remaining combinations of genotypes presented similar frequencies in patients and controls.

Haplotypes

Table 5 shows the 12 haplotypes identified in patients and controls. TTT is the most frequent in both groups (36.6% and 38.5%, respectively), followed by CGC (32.7% and 26.1%). The CAT haplotype was associated with protection against DD in both sexes. The CTC, CTT, TAC, and TAT haplotypes were present in less than 1% of individuals in both groups.

Age of onset

The clinical parameters evaluated presented differences between patients with different genetic variants and between sexes. The differences found are presented in Table 6, which shows that *ABCB1* variants modulate the age of onset of DD.

Compared to non-carriers, disease presented 3.2 years later in carriers of the C1236T variant when the CT1236 genotype was present in heterozygosis.

The G2677 allele also delays age of onset by 6.3 years, with a more pronounced effect in women (10 years). This allele's modulatory effect is preserved when it is combined with the T2677 allele in GT2677-heterozygous women, delaying onset by 4.6 years compared to non-carriers.

Heterozygous presence of CT3435 is associated with a similar delay to that observed for CT1236 (3.1 years).

The heterozygous presence of all 3 genotypes (CTGTCT) delays onset of DD by 5.4 years with respect to carriers of any other combination of genotypes, with a mean (SD) of 29.2 (10.6) years.

The TTTTTT combination, on the other hand, is associated with onset 8.6 years earlier than in non-carriers.

Women carrying the CCGGCT combination presented a mean age of onset of 42.2 years, 11.8 years later than the remaining female patients.

In men, the linear regression model identified practically no differences in age of onset, with the exception of one carrier of the TTTTCT combination, with age of onset of 62 years, 32.7 years later than the mean.

Progression index

Regarding the progression index, we found significant differences between carriers and non-carriers of the G2677 allele (mean [SD] of 0.72 [0.90] vs 0.44 [0.30]; $P = .029$), between men and women presenting the CCGGCC combination of genotypes (0.90 [0.50] vs 0.27 [0.30]; $P = .043$), and between male carriers and non-carriers of the CGC haplotype (1.01 [1.10] vs 0.41 [0.30]; $P = .043$).

Discussion

Several studies have reported that the combination of the C1236T, G2677T/A, and C3435T variants plays a key role in modifying the expression of *ABCB1* and that the function of P-gp[16] is associated with the development of disease,[23] consequently influencing the response to treatment with substrates of P-gp.[16]

Vascular P-gp expression in the BBB is reduced in MS lesions: disappearance of this protein coincides with the presence of perivascular infiltrates of lymphocytes.[9] This reduced function of P-gp during neuroinflammation may alter brain homeostasis and accelerate disease progression by exposing vulnerable CNS cells to damaging compounds.[8,9]

The different combinations of risk genotypes identified in this study (GGCC and GTTT) suggest that P-gp functions differently in men and women, supporting the theory that transport proteins are expressed differently in each sex and play an important role in drug distribution and in toxicity.[26] The G2677T/A variant is known to cause an amino acid substitution (Ala893Ser/Thr), with Ser893 and Thr893, but not Ala893, being vulnerable to phosphorylation and glycosylation, altering the structural conformation, proper folding, degradation, trafficking, localisation, and function of P-gp[26,27]; on the other hand, the C1236T and C3435T variants cause conformational changes in mRNA that lead to instability and alter the half-life of P-gp.[3,6] This influences the protein's metabolism and elimination of toxic or carcinogenic substances due to intracellular accumulation of metabolites, cell damage, impaired apoptosis, deficient immune response, and development of cancer.[12]

Table 2 Association between genotypes and alleles of the C1236T, G2677T/A, and C3435T variants of *ABCB1* in patients with demyelinating disease and controls.

Variant	Controls						Patients						Association analysis		
	Women		Men		Total		Women		Men		Total				
	n	%	n	%	n	%	n	%	n	%	n	%	OR	95% CI	*P*
C1236T															
CC	21	16.3	15	21.1	36	18.0	27	19.4	10	16.7	37	18.6	1.04	0.63-1.73	.898
CT	63	48.8	26	36.6	89	44.5	64	46.0	29	48.3	93	46.7	1.09	0.74-1.62	.688
TT	45	34.9	30	42.3	75	37.5	48	34.5	21	35.0	69	34.7	0.88	0.59-1.33	.603
Total	129	100	71	100	200	100	139	100	60	100	199	100			
C	105	40.7	56	39.4	161	40.2	118	42.4	49	40.8	167	41.9	1.13	0.75-1.70	.603
T	153	59.3	86	60.6	239	59.8	160	57.6	71	59.2	231	58.1	0.96	0.58-1.60	.878
G2677T/A															
GG	35	27.1	9	12.7	44	22.0	39	28.1	17	28.3	56	28.1	2.72	1.11-6.68	.025
GT	50	38.8	35	49.3	85	42.5	70	50.4	31	51.7	101	50.8	2.11	1.29-3.45	.003
GA	8	6.2	5	7.0	13	6.5	5	3.6	0	0.0	5	2.5	0.37	0.13-1.06	.089
TT	28	21.7	19	26.8	47	23.5	20	14.4	10	16.7	30	15.1	0.58	0.35-0.96	.033
TA	8	6.2	3	4.2	11	5.5	5	3.6	1	1.7	6	3.0	0.53	0.19-1.47	.322
AA	0	0.0	0	0.0	0	0.0	0	0	1	1.7	1	0.5			
Total	129	100	71	100	200	100	139	100	60	100	199	100			
G	128	49.6	58	40.8	178	46.5	153	55.0	65	54.1	218	54.7	1.79	1.12-2.86	.015
T	114	44.2	76	53.5	190	47.5	115	41.4	52	43.4	167	42.0	0.90	0.59-1.38	.663
A	16	6.2	8	5.6	24	6.0	10	3.6	3	2.5	13	3.3	0.47	0.23-0.97	.037
C3435T															
CC	31	24.0	8	11.3	39	19.5	42	30.2	19	31.7[a]	61	30.7[b]	[a]3.65	1.46-9.11	.004
													[b]1.82	1.15-2.91	.010
CT	69	53.5	43	60.6	112	56.0	63	45.3	29	48.3	92	46.2	0.68	0.45-1.00	.051
TT	29	22.5	20	28.2	49	24.5	34	24.5	12	20.0	46	23.1	0.93	0.58-1.47	.745
Total	129	100	71	100	200	100	139	100	60	100	199	100			
C	131	50.7	59	41.6	190	47.5	147	52.8	67	55.8	214	53.8	1.77	1.09-2.91	.022
T	127	49.2	83	58.4	210	52.5	171	47.2	53	44.2	184	46.2	0.27	0.11-0.68	.004

95% CI: 95% confidence interval; OR: odds ratio.

Table 3 Logistic regression models of *ABCB1* variants.

Final model for alleles

	B	Standard error	Wald	df	*P*	Exp (B)	95% CI for Exp (B)	
							Lower limit	Upper limit
G2677	0.727	0.282	6.663	1	.010	2.069	1.191	3.592
C3435[a]	−0.536	0.287	3.491	1	.062	0.585	0.334	1.027
T3435	−0.559	0.247	5.135	1	.023	0.572	0.353	0.927
Constant	0.380	0.535	0.505	1	.477	1.462		

Final model for genotypes

	B	Standard error	Wald	df	*P*	Exp(B)	95% CI for Exp(B)	
							Lower limit	Upper limit
GT2677	0.670	0.230	8.496	1	.004	1.954	1.245	3.065
CT3435	−0.678	0.229	8.730	1	.003	0.508	0.324	0.796
Constant	−0.101	0.361	0.078	1	.780	0.904		

95% CI: 95% confidence interval; df: degrees of freedom; Exp(B): odds ratio.
[a] Needed to preserve statistical significance of G2677 and T3435.

Table 4 Association between combinations of genotypes of the C1236T, G2677T/A, and C3435T variants of *ABCB1* in patients with demyelinating disease and controls.

Combination 1236/2677/3435	Women				Men				Association analysis		
	Patients		Controls		Patients		Controls				
	n	%	n	%	n	%	n	%	OR	95% CI	*P*
GGCC					13	21.7	5	7.0	3.65	1.22-10.94	.015
GGCC	30	21.6	19	14.7	13	21.7	5	7.0	2.02	1.17-3.48	.010
GTTT	16	11.5	6	4.7	3	5.0	3	4.2	2.67	1.01-7.04	.041
TTTTCT	1	0.7	8	6.2					0.11	0.01-0.89	.016
TTTTCT	1	0.7	8	6.2	1	1.7	4	5.6	0.16	0.03-0.72	.011
CCGACT	1	0.7	4	3.1	0	0.0	4	5.6	0.12	0.01-0.98	.037

95% CI: 95% confidence interval; OR: odds ratio.

Table 5 Association between haplotypes of the C1236T, G2677T/A, and C3435T variants of *ABCB1* in patients with demyelinating disease and controls.

	rs1128503	rs2032582	rs1045642	Patients (n = 199)		Controls (n = 200)		Association analysis		
Haplotype	C1236T	G2677T/A	C3435T	n	%	n	%	OR	95% CI	*P*
1	T	T	T	73	36.6	77	38.5		1	
2	C	G	C	65	32.7	52	26.1	0.72	0.51−1.04	.078
3	T	G	C	27	13.4	20	9.9	0.71	0.44-1.15	.170
4	C	G	T	11	5.6	14	7.2	1.12	0.58-2.14	.740
5	T	T	C	9	4.5	15	7.6	1.60	0.81-3.13	.170
6	T	G	T	6	3.1	7	3.3	1.00	0.39-2.56	1.000
7	C	A	C	5	2.8	4	2.1	0.79	0.30-2.04	.620
8	C	A	T	0	0	7	3.5	0.21	0.07-0.62	.003
9[a]	C	T	C	0	0.2	3	1.4	0.93[a]	0.25-3.44	.910
10[a]	C	T	T	1	0.7	0	0			
11[a]	T	A	C	0	0.2	1	0.4			
12[a]	T	A	T	1	0.2	N/A	N/A			

95% CI: 95% confidence interval; N/A: not applicable; OR: odds ratio.
[a] Rare haplotypes with frequency under 1%.

Table 6 Linear regression model of *ABCB1* variants and age of onset of demyelinating disease.

Variable 1236/2677/3435	n = 199	Mean (SD)	NSC		95% CI for B		SC	t	*P*
			B	Standard error	Lower limit	Upper limit	Beta		
CT1236	94	32.3 (9.8)	−3.246	1.503	−6.21	−0.28	−0.152	−2.160	.032
Complement	105	29.1 (11.3)	35.587	2.419	30.82	40.36			
G2677	163	31.8 (10.5)	−6.299	1.903	−10.05	−2.55	−0.229	−3.311	.001
Complement	36	25.5 (10.1)	38.084	2.373	33.41	42.76			
CT3435	93	32.3 (10.8)	−3.062	1.506	−6.03	−0.09	−0.143	−2.033	.043
Complement	106	29.2 (10.5)	35.320	2.431	30.53	40.11			
CTGTCT	52	34.6 (10.0)	−5.399	1.687	−8.73	−2.07	−0.222	−3.200	.002
Complement	147	29.2 (10.6)	40.015	3.027	34.04	45.98			
TTTTTT	24	23.0 (7.7)	8.612	2.254	4.17	13.06	0.262	3.821	.000
Complement	175	31.6 (10.7)	14.430	4.300	5.95	22.91			
Women									
G2677	115	32.8 (11.0)	−9.974	2.263	−14.45	−5.50	−0.351	−4.408	.000
Complement	24	22.8 (5.3)	42.748	2.804	37.20	48.29			
GT2677	71	33.2 (10.1)	−4.558	1.810	−8.14	−0.98	−0.210	−2.518	.013
Complement	68	28.7 (11.2)	37.798	2.850	32.16	43.43			
CCGGCT	5	42.4 (18.8)	−11.830	4.885	−21.49	−2.17	−0.202	−2.421	.017
Complement	134	30.6 (10.4)	54.230	9.639	35.17	73.29			
Men									
TTTTCT	1	62.0	−32.797	9.486	−51.78	−13.81	−0.413	−3.458	.001
Complement	59	29.2 (9.4)	94.797	18.852	57.06	132.53			

NSC: non-standardised coefficients; SC: standardised coefficients; SD: standard deviation.

The GT2677 genotype encodes the Ala/Ser893 phenotype; according to our results, only expression of G, translated as Ala893, is associated with risk, whereas expression of T, translated as Ser893, improves the function of P-gp in women. On the other hand, the GG2677 genotype in men produces the Ala/Ala893 phenotype; thus, the structure and function of P-gp is always altered, ultimately leading to increased risk of complications due to disease and deficient function of the protein. Our results for the progression index support this idea, as men who were carriers of the G2677 allele, the CCGGCC combination of genotypes, and the CGC haplotype presented the most severe disease.

Furthermore, the combination of G2677T/A and C3435T appears to induce variable release of the cytokines associated with these genotypes,[1] which would affect the inflammatory process and the severity of DD.

The prognosis of DD is influenced by sex, with female sex considered a risk factor for developing MS,[18] and by age of onset, which varies between sexes and influences disease characteristics and progression.[18,28–30]

Sex hormones may modulate the processes of inflammation, neurodegeneration, and neuronal repair involved in MS.[18,19] Oestrogens and progesterone may increase levels of P-gp and act as substrates of this protein,[31] whereas testosterone inhibits P-gp[32] and increases oligodendrocyte vulnerability to excitotoxicity[19]; during MS relapses, testosterone levels decrease in women, but not in men.[19] Thus, female sex is considered a good prognostic factor, as women often present less aggressive forms of MS. Likewise, male sex is associated with poorer prognosis, with men tending to present more severe symptoms.[19] Our results support these findings, as the *ABCB1* variants studied stratified both sexes: in both sexes, carriers of risk genotypes may present complications in terms of disease symptoms and treatment response, whereas carriers of protective genotypes may present better recovery and treatment response. This suggests that the function of P-gp and its interaction with sex hormones also depend on *ABCB1* genotype. Our findings also indicate that age of onset is also affected by *ABCB1* variants, confirming the importance of sex-specific variants of transport proteins in the study of DD.[29,33]

These results may be useful in treating these diseases, as late onset of treatment is usually less effective than earlier interventions; early treatment often even delays the conversion of clinically isolated syndrome to MS.[34]

The risk factors identified in this study (G2677, GG2677, CC3435, GGCC, and GTTT) have been considered predictive factors of the onset of cancer and autoimmune diseases in populations from China, Japan, and Spain, among other countries[12,23,35–37]; thus, they may be used as predictive markers for DD in Mexican patients. Allele and genotype frequencies in our sample were consistent with those previously reported in healthy Mexican populations[38] and in Chinese, Japanese, and Russian populations.[3,5,16,35,39]

The GG2677 and CC3435 genotypes favour slow transportation of P-gp substrates in Asian and European populations,[4,5,16,22,37,40] resulting in slower transportation of endogenous and exogenous substrates of P-gp, as reported by Cotte et al.[40] in patients with MS under treatment with mitoxantrone. This indicates that carriers of these genotypes may develop toxic accumulation of these substrates, with a similar response to that observed in Asian populations, in which these combinations of genotypes have been shown to influence the pharmacokinetic properties of drugs transported by P-gp.[5,22,37]

The similarity between Mexican and Asian populations was confirmed by our results for the TTT, CGC, and TGC haplotypes in patients and controls (Table 5),[5,16,22] with different frequencies from those reported in white and African populations.[4–6] According to a study of a Chinese cohort of patients with autoimmune disease, the TTT haplotype was more common than TAT, and CAC was more frequent than CTC[16]; this was also the case in our sample (Table 5).

Several studies highlight the importance of analysing combinations of *ABCB1* genotypes, which would be highly valuable for predicting P-gp activity,[6,22] pharmacokinetics,[22] treatment response,[1,16,22,40] and association with disease. Our results are consistent with these findings, and lead us to consider that P-gp is involved in the physiological and pathological mechanisms of DD and also other autoimmune diseases,[10] in which the CNS probably presents a sufficiently toxic environment to cause neurodegeneration, although further studies are needed. *ABCB1* risk variants are probably present in patients whose DD may result from the accumulation of substances over a period of years,[7] which may eventually damage cells expressing deficient P-gp due to stress and weakening of the BBB,[10] with the immune response being activated in an attempt to repair this damage.[30]

The higher frequency of risk variants observed in patients with DD indicates that the function of P-gp in the CNS is affected by the combinations of *ABCB1* variants; early genotyping may contribute to the indication of a suitable treatment according to each patient's genotype, improving their health status.

These findings are even more important as they may constitute the basis for the development of protocols to identify patients at risk of DD and biomarkers assisting in early, accurate diagnosis; correlation of these biomarkers with clinical progression and response to disease-modifying therapies may enable personalised treatment and the extrapolation of findings from basic research to clinical practice with improved models for the diagnosis and treatment of DD.

Conclusions

The GGCC combination of genotypes in men and the GTTT combination in women may be associated with increased risk of developing DD; carriers of these genotypes may present toxic accumulation of P-gp substrates.

The TTT haplotype was the most frequent in our sample, with similar prevalence to that observed in Asian populations.

The G2677 allele of *ABCB1* may differentially modulate age of onset of DD in men and women.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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