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Biological and genetic factors associated with ABCB1 and pregnane-X-receptor expressions in peripheral blood mononuclear cells in the STANISLAS cohort

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Abstract

Background: ABCB1 is a membrane transporter ubiquitously expressed particularly in peripheral blood mononuclear cells (PBMCs). Resistance to drugs is associated with genetic variations of its gene and with modulation of its expression through the pregnane-X-receptor (PXR) transcription factor. We have previously shown that *ABCB1* polymorphisms were associated with blood lipid concentrations.
Methods: We wanted to investigate the variation factors and the genetic determinants of ABCB1 and PXR expressions in PBMCs, and their interrelationships with plasma lipid levels. ABCB1 and PXR mRNA were quantified by real-time quantitative RT-PCR in PBMCs of 42 men and 39 women.
Results: ABCB1 and PXR were both expressed in PBMCs of all individuals, but their expressions were not significantly correlated. ABCB1 mRNA was correlated with body mass index (BMI; p=0.01) and age (p=0.03). In women, lymphocyte count also correlated with ABCB1 transcripts (p<0.01). After adjustment for BMI, correlation with age disappears. PXR mRNA expression depends on gender with men expressing higher PXR levels (p=0.01). PXR expression also correlates with γ-glutamyltransferase (GGT; p=0.02), but this disappeared after adjustment.
Conclusions: Neither ABCB1 nor PXR expressions correlate with *ABCB1* gene variants. Finally, association between ABCB1 or PXR expression in PBMCs and lipid or apolipoprotein plasma concentrations were not significant in this subset of healthy subjects. These results should be confirmed in a larger population sample and extended to patients with various cardiovascular risk profiles.

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Introduction

Many drug metabolizing enzymes (DMEs), drug transporters, and transcription factors are related to cardiovascular physiopathology either through cardiovascular drug metabolism or through metabolism of endogenous compounds related to inflammation, blood pressure, vascular regulation or lipid metabolism (1, 2). Only few of them are measurable as phenotype in the plasma. Peripheral blood mononuclear cells (PBMCs) are both easily accessible and involved in cardiovascular events (i.e., through the inflammation process). Some authors have successfully used PBMCs as biosensors for cardiovascular physiopathology or risk studies. For example, PBMCs were a model to explore lipid metabolism associated transcriptome (3, 4) or to determine the gene expression profile of acute ischemic stroke (5). We have previously proposed that PBMCs could be a useful target and tool to investigate relationships between DMEs and metabolic pathways related to cardiovascular physiopathology (2). Indeed, using a microarray approach to measure, in lymphocytes from healthy human subjects, expression of 16 DMEs and 13 transcription factors with high importance in cardiovascular physiopathology, we have observed that the majority of them are expressed in PBMCs without any induction process and that expression of many transcription factors correlates with DME expression (2).

ABCB1, also known as MDR1 or P-glycoprotein, is a membrane transporter ubiquitously expressed, including in PBMCs (6, 7). A well-known function of the protein is the protection of cells against toxic substances or metabolites (6). Resistance to drugs is associated with genetic variations of its gene and with modulation of its expression, in part through the pregnane-X-receptor (PXR) transcription factor (6). PXR is activated by numerous exogenous or endogenous compounds leading to the regulation of several genes including many proteins from Phases I, II or III of drug metabolism and transport (8). PXR is mainly expressed in the liver and the intestine, where it is crucial to ABCB1 expression regulation (6). It is also expressed in other organs such as the PBMCs (8), but it is not clear to date whether or not PXR and ABCB1 expressions in PBMCs are correlated (9, 10). It should be noted that PXR is encoded by a polymorphic gene (*NR1I2*) and variants affecting PXR expression can also alter ABCB1 expression (11).

Interindividual variability of DMEs and transporters, including ABCB1, is not fully understood. Yet, physiopathological variations of expression is an important step to master, in particular to propose pertinent reference values as discussed previously (7). In addition, hereditary variations in genes coding for DMEs or transporters might explain, at least in part, differences among individuals in the pharmacokinetics and the clinical response to numerous drugs. Given the importance of ABCB1 in drug disposition and response, it would be interesting to use the variability of ABCB1 expression in therapeutic drug monitoring or in drug development. However, few studies have dealt with this concern, except effects of *ABCB1* single nucleotide polymorphisms (SNPs) in drug response variability (12). ABCB1 also transports physiological substrates (13) such as many lipids (14) and can play a role in cholesterol homeostasis (15). Indeed, ABCB1 can redistribute cholesterol from the inner leaflet to the outer leaflet of the membrane, or from the membrane to the endoplasmic reticulum enhancing cholesterol esterification (16, 17). In addition, the transporter has been implicated in the intestinal cholesterol reabsorption and susceptibility to atherosclerosis in mice (18, 19). We previously described that some variants in the *ABCB1* gene are associated with plasma lipid or apolipoprotein levels in supposed healthy subjects, such as *ABCB1* C3435T and apolipoprotein A1 concentration (20).

Therefore, here, we wanted to investigate the variation factors and the genetic determinants of ABCB1 and PXR expressions in PBMCs, and their interrelationships with plasma lipid levels.

Materials and methods

Population sample (Table 1)

This study included 81 adults drawn from the STANISLAS cohort: 39 women and 42 men (mean age: 48.4±3.1 and 49.9±3.8 years, respectively). Subjects were of French origin and free from acute or chronic illnesses. Each participant gave written informed consent for participation in the STANISLAS cohort, which was approved by the local ethics committee of Nancy, France (21).

Population characteristics have been described elsewhere (22). Anthropometric measurements and biochemical profiles were assessed for each individual. Genotyping was previously described (23). Briefly, genotypes for T-129C, G1199A, C1236T, G2677T/A and C3435T were determined by PCR-RFLP assays. Genotyping of G-1A, A61G and T-76A variants was performed using the Pre-Made TaqMan SNP Genotyping Assays (Applied Biosystems).

PBMC collection and RNA extraction

In total, 10 mL fresh whole blood was collected on EDTA tubes. PBMCs were isolated by centrifugation on a density gradient of Ficoll (Ficoll-Paque PLUS; GE Healthcare). Percentage of lymphocytes, monocytes, and polynuclear cells were determined by microscopic observation after May-Grunwald-Giemsa staining. RNA was extracted by an automated isolation procedure (MagNA Pure LC

Table 1 Population descriptive clinical, biochemical and genetic characteristics according to gender.

Variables	Men (n=42)	Women (n=41)	p-Value (Mann-Whitney)
Age, years	49.7±3.8	48.4±3.1	NS
BMI, kg/m^2	23.0±3.3	25.8±4.6	0.003
White blood cells, $\times10^9$/L	5.78±1.42	5.86±1.47	NS
Lymphocytes, $\times10^9$/L	1.71±0.37	1.70±0.40	NS
GGT, UI/L	37.3±22.5	17.6±8.5	<0.001
ALP, UI/L	69.6±14.8	62.9±15.5	0.049
AST, UI/L	29±11	21±6	<0.001
ALT, UI/L	33±19	22±11	0.002
Total cholesterol, mmol/L	5.85±0.79	5.67±0.88	NS
Triglycerides, mmol/L	1.21±0.56	0.99±0.52	0.069
HDL cholesterol, mmol/L	1.69±0.33	1.86±0.49	0.067
LDL cholesterol, mmol/L	3.58±0.79	3.34±0.72	NS
Apolipoprotein A1, mmol/L	1.63±0.2	1.69±0.25	NS
Apolipoprotein B, mmol/L	1.07±0.19	0.99±0.16	0.043
ABCB1 T-129C mutated allele, %	2.4	3.8	NS
ABCB1 G-1A mutated allele, %	5.6	7.4	NS
ABCB1 A61G mutated allele, %	15.3	11.8	NS
ABCB1 G1199A mutated allele, %	5.1	6.9	NS
ABCB1 C1236T mutated allele, %	47.6	42.1	NS
ABCB1 T-76A mutated allele, %	45.8	41.2	NS
ABCB1 G2677T/A mutated allele, %	47.6	47.4	NS
ABCB1 C3435T mutated allele, %	45.1	52.6	NS
ABCB1 transcripts, copies number/µL	259.4±93.9	252.3±103.5	NS
PXR transcripts, copies number/µL	16.4±6.4	13.10±4.84	0.010

Arithmetic mean±SD. BMI, body mass index; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

instrument; Roche Applied Science). RNA concentration and quality were determined by the spectrophotometer Nano-Drop ND-1000 (Labtech International) (24).

Quantitative real-time RT-PCR

Total RNA was reverse-transcribed (Quantitect RT kit; Qiagen). After purification, cDNA was used at constant concentration (5 ng/μL) in quantitative real-time polymerase chain reaction using the LightCycler FastStart DNA Master Plus SYBR Green I kit and the LightCycler LC 2.0 instrument (Roche Diagnostics). Primers were from Albermann and colleagues (9). Experiments were carried out in duplicate and results were averaged. Detection of PCR products was monitored by measuring the increase in fluorescence of SYBR Green I dye bound to double-strand DNA. PCR products were quantified using standard curve generated from serial dilutions of a known quantity of purified PCR product. A control sample was run with templates in every PCR assay, in order to determine the analytical coefficient of variation. Inter- and intra-assay variation coefficients were <5% and PCR efficiency was 82% for ABCB1 and 94% for PXR.

Statistical analysis

Statistical analyses were performed using the SAS package program version 9.1 (SAS Institute Inc.). Non-normally distributed data were logarithmically transformed to approximate a normal distribution. A p-value ≤0.05 was considered statistically significant. Pearson correlation coefficient and multiple regression analyses were used to test associations between ABCB1 and PXR expressions and age, gender, body mass index (BMI), white blood cell count, lymphocyte count, activity of γ-glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), oral contraceptives or substitutive hormone use, and *ABCB1* polymorphisms.

Results and discussion

Variation factors of ABCB1 and PXR mRNA expressions in PBMCs

ABCB1 and PXR were both expressed constitutively in PBMCs of all supposed healthy subjects (with a mean±SD of 261±104 and 14.7±5.9 copies/μL, respectively). PXR is a transcription factor known to regulate ABCB1 expression in several tissues, including PBMCs according to some authors (9, 10). However, in our study the expressions of ABCB1 and PXR were not significantly correlated. Among the variation factors tested in bivariate analysis (Table 2), ABCB1 mRNA expression was correlated with age (r=0.24, p=0.03) and BMI (r=0.28, p=0.01) in the whole group of 81 subjects. In addition, ABCB1 expression tended to be associated with GGT and ALT activity. In women, BMI, lymphocyte count, and ALP activity were also significantly associated with ABCB1 transcripts. After adjustment for BMI, correlation between age and ABCB1 mRNA was no longer significant. PXR mRNA expression was significantly higher in men than in women (16.4±6.4 copies/μL vs. 13.1±4.8 copies/μL, respectively; Table 1). In addition, PXR expression correlated positively with GGT activity (p=0.02) and tended to be associated with AST activity (p=0.07; Table 2). After multiple regression analysis, only correlation with gender remains significant. Neither oral contraception nor substitutive hormone use were significantly associated with ABCB1 and PXR mRNA expressions.

In PBMCs, there is an interindividual variability in expression pattern of several genes which depends on many factors such as gender, age or nycthemeral rhythm (25). We reported here that ABCB1 mRNA expression strongly correlates with BMI in PBMCs, which has not been reported to date to our knowledge. Correlation with age has been previously reported in the literature (26). Before adjustment, we found a significant correlation with age. However, it disappeared after multiple regression analysis. This is probably explained by the poor dispersion of our population in terms of age (ranging from 42 to 67 years old). In addition, we observed a strong correlation with lymphocyte count in women. Lymphocytes are essential cells in the inflammation process and a role of inflammation in modulation of ABCB1 mRNA expression in PBMCs has been reported (27). Finally, we also observed that PXR is, weakly, expressed in PBMCs of healthy subjects. This is in agreement with Owen and collaborators who

Table 2 Variation factors and correlates of ABCB1 and PXR mRNA expressions (only Pearson correlations with p≤0.10 were shown).

	ABCB1 mRNA						PXR mRNA	
	All (n=81)		Men (n=42)		Women (n=39)		All (n=81)	
	r	p-Value	r	p-Value	r	p-Value	r	p-Value
Gender (women vs. men)	–	–	–	–	–	–	–0.27	0.01
Age	0.24	0.03	0.26	NS (0.10)	–	–	–	–
BMI	0.28	0.01	–	–	0.31	0.05	–	–
White blood cell count[a]	–	–	–	–	0.31	NS (0.06)	–	–
Lymphocyte count	–	–	–	–	0.42	0.01	–	–
ALP activity	–	–	–	–	0.32	0.05	–	–
GGT activity	0.20	NS (0.08)	–	–	0.30	NS (0.08)	0.27	0.02
AST activity	–	–	–	–	–	–	0.20	NS (0.07)
ALT activity[a]	0.20	NS (0.07)	–	–	–	–	–	–
Triglycerides	0.21	NS (0.05)	–	–	–	–	–	–
HDL cholesterol	–	–	–0.28	NS (0.07)	–	–	–	–

[a]Log transformed values; –, p>0.10; NS, non-significant.

Table 3 Association of ABCB1 mRNA and PXR mRNA in PBMCs with *ABCB1* polymorphisms after adjustment for confounders.

ABCB1 SNPs	ABCB1 mRNA		PXR mRNA	
	Regression coefficient±SE	p-Value	Regression coefficient±SE	p-Value
G-1A	–74.7±82.2	0.37	–0.69±5.12	0.89
A61G	69.9±52.7	0.19	3.28±3.28	0.32
C1236T	49.5±34.4	0.15	0.17±2.04	0.93
T76A	–11.5±36.0	0.75	0.68±2.22	0.76
G2677T/A	29.5±36.1	0.42	0.65±2.12	0.76
C3435T	0.1±36.8	0.99	0.93±2.15	0.66

ABCB1 polymorphisms were expressed as allelic frequencies. Values of ABCB1 mRNA and PXR mRNA were adjusted for sex, age, BMI, lymphocyte count, ALP activity, and GGT activity. p, test for comparison of regression coefficient to zero.

found that PXR is expressed 250 times less in PBMCs than in liver (10). We observed that PXR expression in PBMCs is dependent on gender, which was not reported to date. Expression of many genes differs according to gender (25), but few are known with regard to DMEs, transporters or transcription factors.

Association of mRNA expressions with *ABCB1* polymorphisms

ABCB1 SNPs are correlated because of important linkage disequilibrium and were thus tested separately from other variables. *ABCB1* T-129C and G1199A were not included for regression analysis owing to the low frequency of the mutated allele. We found no significant association between *ABCB1* SNPs and ABCB1 and PXR PBMC transcripts (Table 3). Some studies have searched for effects of *ABCB1* SNPs, mainly C3435T and G2677T/A, on ABCB1 expression and activity in PBMCs of healthy volunteers or patients with various pathologies or treatments (6). In PBMCs of healthy subjects, the 3435T allele seems to be associated with a lower expression of the transporter (28) but we did not reproduce it in our study, in agreement with Storch and colleagues (29). It might be of interest to search for haplotypic effects (12), but this will require a bigger population study. The 2677T allele has been associated with higher ABCB1 expression in few studies but results are rather conflicting (12), and no effects were found in PBMCs (30). Interestingly, among a panel of 95 polymorphisms of 67 genes, the G2677T/A polymorphism has been strongly associated with obesity in Japanese individuals. Indeed, in a candidate gene approach, Ichihara et al. found that individuals with the T allele had a higher BMI than those with a GG genotype (31), which we did not reproduce in our population (data not shown).

Association of ABCB1 and PXR mRNA expressions with serum lipid and apolipoprotein concentrations (Table 2)

PXR expression is not correlated with any of the lipidic parameters. ABCB1 expression tended to correlate with triglycerides (r=0.21; p=0.05). In men, ABCB1 mRNA also tended to correlate negatively with high-density lipoprotein (HDL) cholesterol (r=–0.28; p=0.07). There was no correlation with plasmatic concentrations of total and low-density lipoprotein (LDL) cholesterol and apolipoproteins A1 and B (ApoA1 and ApoB). We have previously shown that some *ABCB1* gene variants, associated with alteration of ABCB1 expression according to the literature, were associated with lower triglycerides levels or higher ApoA1 concentrations in supposedly healthy subjects (20), which would be consistent with trends observed here. However, after multiple regression analysis, trends disappeared and explicative variables are restricted to BMI and oral contraception. ABCB1 is located in lipid-rich membrane microdomains and it has been shown that cholesterol has a regulatory role in ABCB1 expression and activity in vitro (32). Storch and colleagues investigated whether differences in LDL cholesterol levels in humans have an impact on cellular cholesterol content and on ABCB1 activity in PBMCs (29). They did not search for association with other lipids or with apolipoproteins. They observed no correlation between ABCB1 mRNA expression in PBMCs and LDL cholesterol concentration in agreement with our observations.

In conclusion, in this subset of 81 healthy subjects we found no significant correlation, only trends, between ABCB1 or PXR expression in PBMCs and lipid or apolipoprotein plasma concentrations. It could be of interest to reproduce this study in a higher number of subjects, or in people at cardiovascular risk. In the current study, we have also searched for interindividual variability of ABCB1 and PXR expressions. The two major findings are that ABCB1 and PXR expressions correlate with BMI and gender, respectively. Given the importance of ABCB1 in drug disposition and response, it would be interesting to keep in mind these factors of variability in therapeutic drug monitoring and in drug development.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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