Role of *ABCB1* and glutathione S-transferase gene variants in the association of porphyria cutanea tarda and human immunodeficiency virus infection

PRISCILA AYELÉN PAGNOTTA[1,2], VIVIANA ALICIA MELITO[1,2], JIMENA VERÓNICA LAVANDERA[3], VICTORIA ESTELA PARERA[1], MARÍA VICTORIA ROSSETTI[1], JOHANNA ROMINA ZUCCOLI[1*] and ANA MARIA BUZALEH[1,2*]

[1]Centro de Investigaciones sobre Porfirinas y Porfirias, Universidad de Buenos Aires, Argentina-National Scientific and Technical Research Council, Hospital de Clínicas José de San Martín, Buenos Aires 1120; [2]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires 1428; [3]Cátedra de Bromatología y Nutrición, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe 3000, Argentina

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Abstract. In Argentina, porphyria cutanea tarda (PCT) is strongly associated with infection with human immunodeficiency virus (HIV); however, whether the onset of this disease is associated with HIV infection and/or the antiretroviral therapy has not been determined. The *ABCB1* gene variants c.1236C>T, c.2677G>T/A and c.3435C>T affect drug efflux. The *GSTT1* null, *GSTM1* null and *GSTP1* (c.313A>G) gene variants alter Glutathione S-transferase (GST) activity, modifying the levels of xenobiotics. The aim of the present study was to evaluate the role of genetic variants in initiation of PCT and to analyze the genetic basis of the PCT-HIV association. Control individuals, and HIV, PCT and PCT-HIV patients were recruited, PCR-restriction fragment length polymorphism was used to genotype the *ABCB1* and *GSTP1* variants, and multiplex PCR was used to study the *GSTM1* and *GSTT1* variants. The high frequency of c.3435C>T (PCT and PCT-HIV) and c.1236C>T (PCT) suggested that the onset of PCT were not specifically related to HIV infection or antiretroviral therapy for these variants. c.2677G>T/A frequencies in the PCT-HIV patients were higher compared with the other groups, suggesting that a mechanism involving antiretroviral therapy served a role in this association. PCT-HIV patients also had a high frequency of *GSTT1* null and low frequency for *GSTM1* null variants; thus, the genetic basis for PCT onset may involve a combination between the absence of *GSTT1* and the presence of *GSTM1*. In conclusion, genes encoding for proteins involved in the flow and metabolism of xenobiotics may influence the PCT-HIV association. The present study is the first to investigate the possible role of *GST* and *ABCB1* gene variants in the triggering of PCT in HIV-infected individuals, to the best of our knowledge, and may provide novel insights into the molecular basis of the association between PCT and HIV.

Correspondence to: Dr Ana María Buzaleh, Centro de Investigaciones sobre Porfirinas y Porfirias, Universidad de Buenos Aires, Argentina-National Scientific and Technical Research Council, Hospital de Clínicas José de San Martín, Av. Córdoba 2351, 1er subsuelo, Buenos Aires 1120, Argentina
E-mail: anamaria@qb.fcen.uba.ar

*Contributed equally

Abbreviations: PCT, porphyria cutanea tarda; HIV, human immunodeficiency virus; P-gp, P-glycoprotein; GST, glutathione S-transferase; RFLP, restriction fragment length polymorphism

Key words: ABCB1, glutathione S-transferase, porphyria cutanea tarda, HIV, genetic variants, personalized medicine

Introduction

Porphyrias are a group of metabolic disorders affecting biosynthesis of heme; each specific subtype of Porphyria is the result of a decrease in the activity of a specific enzyme involved in the biosynthesis of heme (1,2). The specific patterns of overproduction of heme precursors are associated with characteristic clinical features; in particular, porphyria cutanea tarda (PCT) is a hepatic cutaneous Porphyria resulting from an acquired or inherited deficiency of the enzyme Uroporphyrinogen decarboxylase (URO-D) (2-5). PCT is present in two main forms: Type I, sporadic or acquired; and type II, familial or hereditary. The clinical symptoms

of PCT include skin fragility, hyperpigmentation, bullae and hypertrichosis. The onset of PCT is frequently associated with different precipitating agents, primarily hepatotoxic drugs and hepatotropic viral infection (6-8). The prevalence of PCT varies worldwide from 1:5,000 (Czech Republic and Slovakia) to 1:70,000 (Ireland) (9,10) and in Argentina the prevalence is 1:20,000 (2).

In Argentina, PCT patients have a high incidence (16%) of human immunodeficiency virus (HIV) infection (11). However, since almost all HIV-infected patients have additional risk factors for Porphyria manifestation, it is still unclear whether HIV infection is a precipitating factor for development of PCT. Despite this, several reports have mentioned PCT being triggered after or during HIV therapy with antiretroviral drugs, even in the absence of another precipitating agent (12-14).

The human multidrug-resistance gene (*ABCB1*/*MDR1*) encodes for the integral membrane protein P-glycoprotein (P-gp), which is involved in the energy-dependent transport of substances from the inside of cells and/or from membranes to the outside space, acting as a pump that effluxes a wide range of structurally diverse xenobiotics, such as antiretroviral drugs, and protease and integrase inhibitors (15-19). According to the single nucleotide variant (SNV) database of the National Center for Biotechnology Information, the human *ABCB1* coding region has >50 SNVs (ncbi.nlm.nih.gov/gene/5243). The most relevant amongst these are: Exon 12 (rs1128503, c.1236C>T), exon 21 (rs2032582, c.2677G>T/A) and exon 26 (rs1045642, c.3435 C>T), which affect the expression and/or activity of P-gp, and therefore the bioavailability of some drugs (20-22); these three SNVs are the most common in Caucasian populations, and are associated with an increased susceptibility of developing a disease or to modify the effect of drugs used for therapy (17,23-26).

Glutathione S-transferases (GSTs) are a family of enzymes belonging to the Phase II Drug Metabolizing System, which catalyzes the synthesis of thioether conjugates between glutathione and xenobiotics (27,28). These enzymes are also involved in the detoxification of reactive oxygen species (ROS), environmental carcinogens and steroid hormones, as well as in the metabolism of chemotherapeutic agents (27,28). Some genetic variants, including *GSTT1* null, *GSTM1* null and *GSTP1* (rs1695, c.313A>G), are of clinical importance because they alter the activity of GSTs, and may affect the levels of hormones and xenobiotics. An increased susceptibility of developing several different types of cancer (29,30), liver failure due to alcoholism (31) and other diseases (32) has been associated with the presence of non-wild-type variants. Singh *et al* (33) demonstrated the relationship between the variants *GSTM1*, *GSTT1* and *GSTP1* and hepatotoxicity, which was associated with antiretroviral therapy in individuals with HIV.

Based on the above, the aim of the present study was to evaluate the role of genetic variants in triggering PCT, and to analyze the genetic basis of the association between PCT and HIV.

Materials and methods

Subjects. The recruited cohorts consisted of Caucasian individuals of both sexes. The individuals were stratified into four groups: Control group (n=60, 32 males and 28 females, age range 17-77 years, median age 38.5 years), individuals with a negative diagnosis for both HIV and PCT; HIV group (n=35, 30 males and 5 females, age range 20-53 years, median age 27 years), patients infected with HIV; PCT group (n=40, 22 males and 18 females, age range 31-83 years, median age 49 years), patients with acquired PCT without HIV (onset of PCT due to other triggering factors); and PCT-HIV group (n=40, 36 males and 4 females, age range 29-67 years, median age 44.2 years), patients diagnosed with PCT and also infected with HIV. The exclusion criterion was: Individuals of Control and HIV groups related to PCT patients.

Samples were collected from patients attending the Research Center on Porphyrins and Porphyrias (CIPYP), Hospital de Clínicas José de San Martín (Buenos Aires, Argentina) between March 2010 and December 2018. All individuals provided signed consent for participation. The present study conformed with the guidelines stated in the Declaration of Helsinki (34), and was approved by the Institutional Research Ethics Committee of the CIPYP, National Scientific and Technical Research Council, University of Buenos Aires, Argentina.

Biological materials, DNA extraction and genotyping. Genomic DNA was extracted from peripheral blood, using the Illustra blood genomicPrep Mini Spin kit (Invitrogen; Thermo Fisher Scientific, Inc.). PCR was performed using MyTaq HS Red mix, 2x (Bioline); this kit includes the enzyme MyTaq HS DNA Polymerase. Primers were designed using the SeqBuilder and PrimerSelect programs (DNASTAR version 11.0; Lasergene).

ABCB1 gene variants. PCR-restriction fragment length polymorphism (RFLP) was used to analyze the variants in exon 12 (c.1236C>T), 21 (c.2677G>T/A) and 26 (c.3435C>T), according to the protocols described in previous studies (35-37). Fig. 1 shows the representative patterns of the genotypes of each SNV.

To genotype the SNV c.3435C>T of exon 26, the primers used were: *ABCB1* 3435 forward, 5'-GCTGGTCCTGAA GTTGATCTGTGAAC-3' and reverse, 5'-ACATTAGGCAGT GACTCGATGAAGGCA-3', which amplifies a 238 bp fragment. The thermocycling conditions were: Initial denaturation at 95˚C for 5 min; followed by 35 cycles at 94˚C for 30 sec, 61˚C for 30 sec and 72˚C for 30 sec; and a final extension step of 72˚C for 5 min. The PCR products were digested using the restriction enzymes *Sau3*A1 or *Mbo*I. The wild-type allele (allele C) has a cut-off site for these enzymes which generates two fragments with lengths of 178 and 60 bp, whereas the T variant does not possess this site (Fig. 1A).

To genotype the SNV c.1236C>T of exon 12, the primers used were: *ABCB1*-15 forward, 5'-TATCCTGTGTCTGTG AATTGCC-3' and *ABCB1*-15 reverse 5'-CCTGACTCACCA CACCAATG-3', which amplify a 366 bp fragment. The thermocycling conditions were: Initial denaturation at 95˚C for 5 min; followed by 35 cycles at 94˚C for 30 sec, 60˚C for 30 sec and 72˚C for 30 sec; and a final extension step of 72˚C for 5 min. The PCR product was digested with the enzyme *Hae*III, which yields three fragments of 269, 62 and 35 bp in the wild-type gene (allele C). When the variant c.1236C>T was present, a restriction digest site was abolished and only two fragments of 269 and 97 bp were obtained (Fig. 1B).

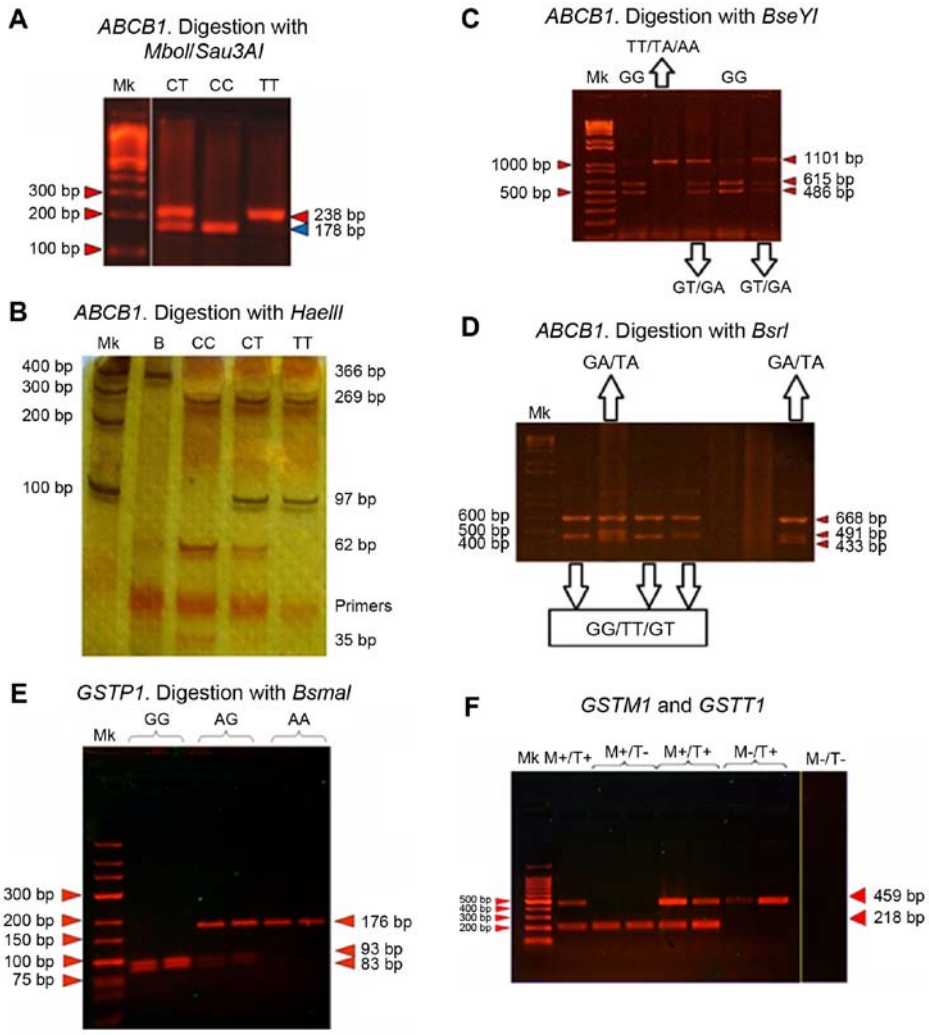

Figure 1. Genotyping band pattern of the variants studied. Representative band pattern of (A-D) the *ABCB1* and (E and F) *GST* gene variants following enzymatic digestion of the PCR products (panels A-E), or PCR-multiplex amplification (panel F). (A) c.3435C>T (exon 26), 3% agarose gel in the presence of ethidium bromide (80 V, 45 min). (B) c.1236C>T (exon 12), 12% polyacrylamide gel with 0.1% silver staining (250 V, 80 min). (C and D) c.2677G>T/A (exon 21), 2% agarose gel with ethidium bromide (80 V, 40 min). (E) *GSTP1*, 3% agarose gel with ethidium bromide (80 V, 60 min). (F) *GSTT1* and *GSTM1*, 2% agarose with ethidium bromide (80 V, 30 min). Mk, marker.

To genotype the SNV c.2677G>T/A of exon 21, the primers used were 21F forward, 5'-GCTTTAGTAATGTTGCCGTGA T-3' and 21R reverse, 5'-ATACCCCTAGCATTTTTCCATA-3', which amplify a 1,101 bp fragment. The thermocycling conditions were: Initial denaturation at 95˚C for 5 min; followed by 35 cycles at 94˚C for 1 min, 58˚C for 30 sec and 72˚C for 2 min; and a final extension step of 72˚C for 5 min. To evaluate the G and T alleles, the PCR products were digested with the restriction enzyme *BseYI*; the pattern of bands for the G allele consists of two bands of 615 and 486 bp, whereas the cut-off site for the T allele is abolished, showing one band of 1,101 bp (Fig. 1C). To genotype the A allele, the PCR product was digested with the restriction enzyme *BsrI*; the resulting pattern for the A allele is three bands of 491, 433 and 177 bp, whereas that for the G or T alleles is two bands of 668 and 433 bp (Fig. 1D).

GST variants. To study the *GSTM1* and *GSTT1* variants, the presence or absence of deletion was evaluated using multiplex PCR; to study the c.313A>G of *GSTP1*, PCR-RFLP was used. Fig. 1E and F shows the characteristic patterns of the different genotypes of each variant.

The thermocycling conditions of multiplex PCR were: Initial denaturation at 95˚C for 5 min; followed by 35 cycles at 94˚C for 30 sec, 60˚C for 30 sec and 72˚C for 30 sec; and a final extension step of 72˚C for 5 min. In the case of *GSTM1*, the primers used were: Forward, 5'-GAACTCCCTGAAAAG CTAAAGC-3' and reverse, 5'-TTGGGCTCAAATATACGG TGGA-3', resulting in an amplification product of 218 bp. For *GSTT1*, the primers were forward, 5'-TTCCTTACTGGTCCT CACATCTC-3' and reverse, 5'-TCACCGGATCATGGCCAG CA-3'. The band patterns were: Two bands of 218 and 459 bp for M+/T+, one band at 218 bp for M+/T-, one band at 459 bp for M-/T+, and the absence of bands for M-/T- (Fig. 1F). It is necessary to consider that this methodology only allows for discrimination between the absence and presence, but not differentiating between heterozygous and non-zero homozygous genotypes, in which the existence of one or two alleles is indistinguishable. Thus, samples with double deletion were amplified again to confirm this aspect.

To genotype the variant c.313A>G of *GSTP1*, the primers used were: Forward, 5'-ACCCCAGGGCTCTATGGGAA-3' and reverse, 5'-TGAGGGCACAAGAAGCCCCT-3', obtaining

Table I. Allelic and genotypic frequencies for c.3435 C>T, c.1236 C>T and c.2677G>T/A variants of the *ABCB1* gene.

	Allelic frequency							Genotypic frequency									
	c.3435 C>T		c.1236 C>T		c.2677G>T/A			c.3435 C>T			c.1236 C>T			c.2677G>T/A			
Groups	C	T	C	T	G	T	A	CC	CT	TT	CC	CT	TT	GG	GT	TT	TA/GA
Control, n=60	0.6	0.36	0.7	0.33	0.5	0.45	0	33	61	5.3	41	51	8	33	40	22.5	5.0/0
HIV, n=35	0.5	0.46	0.6	0.39	0.6	0.37	0	23	63	14.3	26	71	2.9	40	43	14.3	2.9/0
PCT, n=40	0.5	0.52[a]	0.4	0.59[a]	0.5	0.48	0	16	63	20.9[a]	6.9	69	24.1[a]	22	54	19.5	2.4/2.4
PCT-HIV, n=40	0.5	0.55[a]	0.7	0.35	0.4	0.61[a]	0	18	55	27.3[a]	33	59	7.7	5.9	62	29.4[a]	2.9/0

[a]P<0.05. PCT, porphyria cutanea tarda; HIV, human immunodeficiency virus.

a product of 176 bp. The thermocycling conditions were: Initial denaturation at 95˚C for 5 min; followed by 35 cycles at 94˚C for 30 sec, 62˚C for 30 sec and 72˚C for 30 sec; and a final extension step of 72˚C for 5 min. The PCR product was digested with the restriction enzyme *Bsm*AI, and three possible patterns were obtained according to the genotype of the samples: For the homozygous wild type (AA), one band of 176 bp; for the heterozygous genotype (AG), three bands of 176, 93 and 83 bp (AG); and for the homozygous mutant genotype (GG), two bands of 93 and 83 bp (Fig. 1E).

The specific run conditions for analysis of the variants and alleles varied and are described in the figure legend for each specific condition.

Data management and statistical analysis. The results were evaluated using a χ^2 using VCCStats Beta 3.0 (institutodemetodologia.net/propios-c5xu). The frequencies of alleles and genotypes were calculated by directly counting. Haplotype analysis was performed using SNPStats (snpstats.net/start.htm) (38). The association with the risk of developing PCT was estimated using the odds ratios (ORs) and 95% confidence intervals (CIs). P<0.05 was considered to indicate a statistically significant difference.

To compare the variant frequencies in the present study with that reported for other human populations, a literature search was performed and used to develop a quantitative systematic review (meta-analysis) following some of the guidelines stated in the PRISMA (39). The search was performed using the following terms: '*ABCB1*', '*MDR1*', '*GST*', '*GSTT1*', '*GSTM1*', '*GSTP1*', 'genetic variants', 'rs1045642', 'rs2032582', 'rs1128503' and 'rs1695' in PubMed (pubmed.ncbi.nlm.nih.gov) and SciELO (scielo.org/es) in October 2020. The language used was generally English, although searches in Spanish (using SciELO) were also performed to avoid the bias of using only one language. In addition, the references in the studies deemed relevant were also assessed. The criteria used were as follows: i) Studies in humans; ii) investigation of the genetic variants analyzed in the present work (*ABCB1* and *GST*) that included a control group (without any associated pathologies); and iii) studies published since 2000. The data were extracted from each study (19 in total) to construct comparative tables, which included the following information: Name of the first author, and population and allelic (*ABCB1* and *GSTP1* variants) or genotypic (*GSTM1* and *GSTT1* variants) frequency of the control group. The frequencies are expressed as ranges, using the maximum and minimum values found in all studies as the extremes. The data from the literature search was also compared with the values in the 1000 Genomes Browser (browser.1000genomes.org; August 2020).

Results

Allelic and genotypic frequencies of the ABCB1 gene. The allelic and genotypic frequencies of the *ABCB1* gene were calculated and compared between the groups (Table I). For the c.3435C>T variant, the frequency of the T allele in both groups with PCT (PCT and PCT-HIV) was significantly higher than in the Control or HIV individuals. When c.1236 C>T SNV was evaluated, the frequency of the T allele in the PCT group was higher than in the other groups, whereas no differences were detected between the Control, HIV and PCT-HIV groups. The evaluation of the SNV c.2677G>T/A included the analysis of two non-wild-type alleles (A and T); the frequency of the A allele was similar in all the groups evaluated, whereas that of the T allele in the PCT-HIV group was significantly higher than in Control individuals and HIV and PCT patients.

When the genotypic frequency was analyzed, c.3435C>T was more common in the polymorphic variant (TT) in both PCT groups (PCT and PCT-HIV; both P<0.05) compared with the Control group. For the c.1236C>T variant, only the PCT group had an increased frequency (P<0.05) when compared with the other groups. In the case of c.2677G>T/A SNV, the frequency of genotypes that included the presence of A (TA and GA) was similar between the groups, but the TT genotype was higher in the PCT-HIV group compared with the HIV and PCT groups (P<0.05).

Haplotype analysis was performed on the three *ABCB1* SNVs (Fig. 2A). The results indicated that the CGC and TTT haplotype frequencies were >20% in all the groups. In the PCT and PCT-HIV groups, the TTT haplotype was present at a higher frequency than in the Control and HIV groups, and the OR values indicated that it was a risk haplotype for the onset of the disease [PCT group: OR=12.70 (CI, 1.98-81.31), P<0.01; PCT-HIV group: OR=4.64 (CI, 1.16-18.57), P<0.05]. An opposite relationship was observed for the wild-type haplotype CGC, which showed a high frequency in the Control and HIV groups (P<0.05).

Table II. *GSTT1*, *GSTM1* and *GSTP1* (c.313 A>G) frequencies.

	GSTT1		*GSTM1*		c.313 A>G (*GSTP1*)				
	Genotypic frequency				Allelic		Genotypic		
Groups	+/+, +/-	-/-	+/+, +/-	-/-	A	G	AA	AG	GG
Control, n=60	91.67	8.33	58.33	41.67	0.58	0.42	29	58	13
HIV, n=35	93.33	6.67	46.67	53.33	0.53	0.47	28	50	22
PCT, n=40	89.47	10.53	63.16	36.84	0.55	0.45	25	60	15
PCT-HIV, n=40	85.71	14.29[b]	67.86	32.14[a]	0.54	0.46	27	54	19

[a]P<0.05, [b]P=0.075 vs. HIV group. -/-, homozygous genotype for the absence of the gene; +/+, homozygosis genotype for the presence of the gene; +/-, heterozygous genotype.

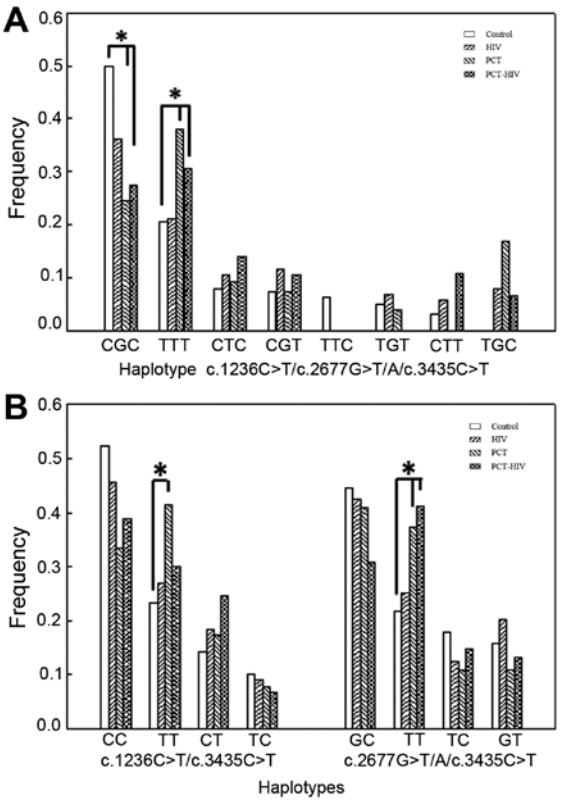

Figure 2. Allelic haplotype study of *ABCB1* variants. (A) Analysis of the three SNVs: c.1236C>T/c.2677G>T/A/c.3435C>T. (B) Analysis of haplotype of the following pairs, c.1236C>T/c.3435C>T and c.2677G>T/A/c.3435C>T. *P<0.05. SNV, single nucleotide variants. *P<0.05 vs. control.

Since the results suggested a possible role of the c.3435C>T variant in the initiation of PCT, a paired haplotype analysis was performed (Fig. 2B). In the PCT group, the TT frequency was increased (P<0.05) for the combination c.1236C>T/c.3435C>T, indicating that this combination is a risk haplotype [OR=6.53 (CI, 1.72-24.70), P<0.01]. When the c.2677G>T/A/c.3435C>T pair was evaluated, there was a higher frequency of the TT haplotype for both PCT populations (PCT and PCT-HIV; P<0.05) when compared with the Control and HIV groups; the OR values revealed a risk haplotype for PCT vs. Control [OR=2.32 (CI, 0.81-6.64), P<0.05] and PCT-HIV vs. Control [OR=3.61 (CI, 1.25-10.32), P<0.05].

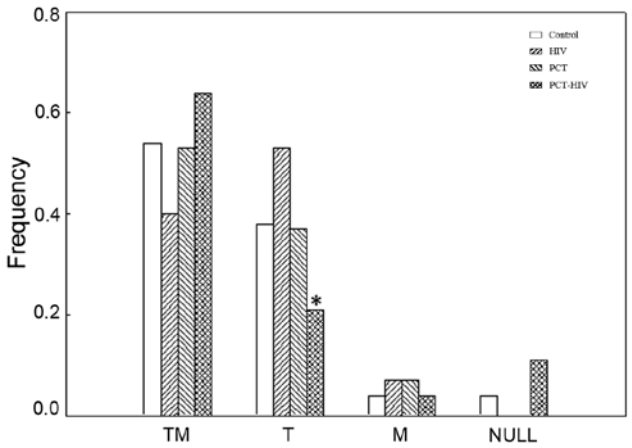

Figure 3. Frequencies of combinations of genotypes of *GSTT1* and *GSTM1*. *P<0.05 vs. control. T, presence of *GSTT1* in at least one allele of the individual; M, presence of *GSTM1* in at least one allele of the individual; Null, absence in homozygosis of both genes.

Analysis of the frequencies of GSTT1, GSTM1 and GSTP1. Results of the frequencies of *GSTT1*, *GSTM1* and *GSTP1* are shown in Table II. The genotypic frequencies of the presence or absence of *GSTT1* showed that the frequency of the homozygous null genotype was increased in the PCT-HIV group when compared with the HIV group, although the differences were not statistically significant. In the case of *GSTM1*, the effect was opposite to that described for *GSTT1*; the PCT-HIV group presented a significantly lower frequency for the null genotype in homozygosis when compared with the HIV group.

When the variant c.313A>G of *GSTP1* was analyzed, no significant differences in the allelic frequencies were observed between the different groups. An almost equivalent distribution was observed between both alleles (A and G), with a slightly higher prevalence of the wild-type variant. The genotypic frequency showed no significant differences between the groups studied, although the presence in heterozygosis (AG) was 2-fold higher than the homozygote genotype (GG).

Considering that the allelic and genotypic frequencies of the variant c.313A>G of *GSTP1* showed no appreciable differences between the groups studied, only the combinations of *GSTM1* and *GSTT1* were further evaluated (Fig. 3). The genotype presence for both genes (TM), either in homozygosis

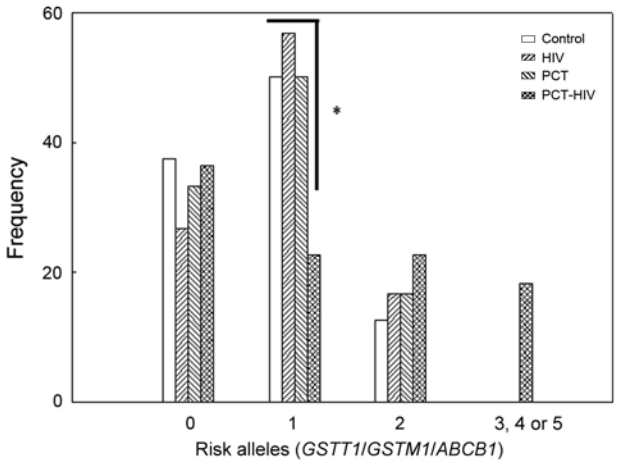

Figure 4. Number of risk alleles taken into consideration the combination of *GSTM1*, *GSTT1* and *ABCB1*. *P<0.05 vs. control.

or in heterozygosis, represented a frequency >40% for all the groups studied. The frequency of individuals with the presence of *GSTT1* in at least one of the alleles and absence in homozygosis of *GSTM1* (T) was lower in the PCT patients infected with HIV (PCT-HIV) compared with HIV infected individuals. Regarding the presence of *GSTM1*, either in homozygosis or heterozygosis, in the absence of *GSTT1* (M), all the groups studied had a similar frequency (<10%). It is noteworthy that no HIV or PCT patients with the null genotype (absence of both genes in homozygosis) were detected in the population analyzed.

Distribution of combined ABCB1, GSTM1 and GSTT1 genotypes. Using the variants studied for *ABCB1*, *GSTM1* and *GSTT1*, the risk alleles of the individuals in each group were determined (Fig. 4). The absence of TT for all *ABCB1* SNVs plus the presence of *GSTT1* plus *GSTM1* was considered as 0 risk alleles; the presence of only one non-wild type variant in homozygosis for *ABCB1* SNVs or absence of *GSTT1* or *GSTM1* was considered as 1 risk allele; and the cases in which 2-5 of the variants were not wild-type in homozygosis was 2-5 risk alleles, respectively. The results showed that the PCT-HIV group had the highest proportion of 2 risk alleles and the lowest proportion of 1 risk allele. Moreover, this group was the only group that had individuals with 3-5 risk alleles.

Discussion

The results of the present study showed that the variants of the *ABCB1* gene may influence the initiation of PCT. It is important to note that Porphyrias are multifactorial diseases, and that PCT in particular can be triggered by alcoholism, estrogens, drug abuse, iron overload or hepatotropic viral infections, through different mechanisms of alterations of heme metabolism (2,4,12,13). In our previous studies (12) and in those from other authors (40,41), the clinical symptomatology and biochemical alterations commonly observed in PCT patients are similar to those seen in HIV patients who develop PCT. Moreover, no differences were observed in terms of response to treatments for PCT (hydroxychloroquine alone or combined with phlebotomies).

When evaluating the c.3435C>T variant in *ABCB1*, its allelic (T) and genotypic (TT) frequencies in the PCT and PCT-HIV groups were significantly higher compared with the Control group, suggesting that the role of this variant in triggering PCT may not be exclusively associated with HIV infection or antiretroviral therapy. The nucleotide position analyzed is located in the second ATP binding domain and although the change is synonymous, there are studies that confirm that it can affect the folding of the protein, insertion to the membrane, the translation process, and the interaction with ATP and substrates/inhibitors (16,42-44). At the hepatic level, alterations in P-gp activity may increase cellular toxicity due to the inefficiency in export of substances and metabolites, resulting in hepatotoxicity and oxidative stress caused by an increase in ROS (17,45). In this context, the administration of substances or drugs that are metabolized in the liver in individuals with the TT genotype for c.3435C>T SNV may promote and contribute to the inhibition of hepatic URO-D and, consequently, to the onset of PCT.

Regarding the SNV c.1236C>T, both allelic (T) and genotypic (TT) frequencies were significantly higher in PCT individuals than in the other groups, and this variant may be associated with triggering the development of PCT as an inducer of hepatotoxicity, independent of HIV infection and antiretroviral treatment. The findings of Fung and Gottesman (16) suggest that the primary impact of this change lies in the presence of a rare codon (GGT instead of GGC) which can lead to a pause or slowdown of ribosomal function, and to a decrease in the activity and/or protein levels of P-gp.

When evaluating the results obtained for the c.2677G>T/A variant, the fact that the frequencies of the A allele and those of genotypes TA/GA were similar in all the groups studied, suggests that there is no evidence to associate this variant with the initiation of PCT in individuals, regardless of HIV infection status. The frequencies of the T allele and the TT genotype were significantly higher for the PCT-HIV group than for the other groups, especially for the patients infected with HIV. This result suggests that the c.2677G>T/A variant may influence initiation of PCT in HIV-infected individuals, possibly through a mechanism that involves antiretroviral therapy based on the fact that anti-HIV drugs are substrates of P-gp and genetic variants alter the expression and activity of the transporter (16,17). Although in the nucleotide position studied there is no functional domain (intracellular loop), biochemical evidence has confirmed that the change in alanine to serine or threonine may alter the transport of drugs, due to irregularities in the ATPase activity of P-gp (46). The P-gp transporter is a key determinant of the bioavailability and penetration of protease inhibitors used as antiretroviral therapies. Taking into account that the deficiencies in drug transporters may increase the risk of hepatotoxicity, a possible explanation for the high incidence of PCT in the Caucasian population of HIV infected patients in Argentina (1:370) compared with the prevalence of PCT in this country (1:20,000) may be linked to the high presence of this variant and the consequent context of hepatotoxicity resulting from the suboptimal transport of antiretrovirals by P-gp, favoring the inhibition of URO-D.

The analysis of haplotypes of the three SNVs of the *ABCB1* gene, the significant increase in TTT in both PCT groups compared with Control and HIV individuals, and the

Table III. Allelic frequencies of c.3435C>T, c.1236C>T and c.2677G>T/A variants of *ABCB1* gene in different populations.

		c.3435C>T		c.2677G>T/A			c.1236C>T		
First author, year	Ethnicity	C	T	G	T	A	C	T	(Refs.)
Wielandt *et al*, 2004	Chilean								(20)
	Mestizo	0.67	0.33	0.65	0.26	0.09	0.59	0.41	
	Mapuche	0.65	0.35	0.69	0.16[a]	0.15	0.4	0.6[a]	
	Pascuense	0.75	0.25	0.78	0.15[a]	0.07	0.7	0.3	
Hoffmeyer *et al*, 2000	Caucasian	0.46-0.48	0.52-0.54	0.53-0.61	0.39-0.43	0.02-0.04	0.54-0.60	0.40-0.46	(15)
Milojkovic *et al*, 2011									(58)
Mhaidat *et al*, 2011	Asian	0.38-0.53	0.47-0.62	0.36-0.62	0.36-0.42	0.02-0.22	0.35-0.44	0.56- 0.65[a]	(57)
Milojkovic *et al*, 2011									(58)
Komoto *et al*, 2006									(59)
Mhaidat *et al*, 2011	African	0.83-0.84	0.16-0.17[a]	0.89-0.96	0.04-0.11[a]	Not determined	0.85-0.86	0.14-0.15[a]	(57)
Milojkovic *et al*, 2011									(58)
Present study	Argentinian	0.64	0.36	0.52	0.5	0.03	0.67	0.33	-

[a]P<0.05 vs. present study.

Table IV. Allelic frequencies of *GSTP1* (c.313A>G) and genotypic frequencies of *GSTM1* and *GSTT1* in different populations.

		GSTM1		*GSTT1*		*GSTP1* (c.313 A>G)		
First author, year	Ethnicity	+/+, +/-	-/-	+/+, +/-	-/-	A	G	(Refs.)
Rossini *et al*, 2002	Brazillian	54	46	87	13	0.69	0.31	(61)
Pinheiro *et al*, 2017								(62)
Weich *et al*, 2017	Caucasian	44-58	45-58	73-88	12-27	0.64-0.71	0.29-0.36	(60)
Klusek *et al*, 2018								(63)
Srivastava *et al*, 2018								(64)
Stamenkovic *et al*, 2018								(65)
ThekkePurakkal *et al*, 2019								(66)
Srivastava *et al*, 2018	Asian	35-58	20-65	49-94	6-51	0.74	0.26	(64)
Musavi *et al*, 2019								(67)
Zehra *et al*, 2018								(68)
Saravani *et al*, 2019								(69)
Farmohammadi *et al*, 2020								(70)
Oshodi *et al*, 2017	African	45-89	11-55	53-88	12-47	0.65-0.72	0.28-0.35	(71)
Srivastava *et al*, 2018								(64)
Idris *et al*, 2020								(72)
Rebai *et al*, 2020								(73)
Present study	Argentinian	58	42	92	8	0.58	0.42	-

inverse relationship in the wild-type haplotype highlight the potential role of *ABCB1* variants in initiation of PCT. On the other hand, the analysis of the SNV pairs c.1236C>T and c.3435C>T indicated that the frequency of the haplotype TT was significantly higher in the PCT individuals, demonstrating the possible influence of this SNV combination on the development of PCT. The variant c.3435C>T has been reported to be of great relevance in the predisposition to various pathologies, such as thyroid cancer, early-onset Parkinson's disease and methotrexate-induced adverse events in rheumatoid arthritis,

amongst others (21,47,48). Regarding the results obtained for the haplotypes of the combination c.2677G>T/A/c.3435C>T, a significant increase in TT was detected for the two PCT groups compared with that observed in the Control and HIV groups, indicating that this haplotype may influence development of PCT mediated by both antiretroviral therapy and other risk factors.

Based on the results of the present study, it can be concluded that the decrease in the expression of *ABCB1* and/or the activity of P-gp, and its role as a predisposing factor in triggering PCT, requires a synergistic combination of changes, altering the molecular and protein structure of the transporters of drugs and xenobiotics.

Since HIV and PCT patients usually present with liver damage, and PCT is a hepatic Porphyria (2,4,49-52), it was of interest to extend this work to study the influence of variants of GST, a marker enzyme involved in cellular detoxification. When the *GSTM1* variant was genotyped, the frequency of the null genotype was significantly lower in the HIV-PCT group, suggesting that the presence of this gene could predispose an individual to development of PCT in HIV-infected patients. Regarding *GSTT1*, the frequency of null homozygotes in PCT-HIV individuals was increased, although this result was not statistically significant; this could be attributed to the fact that the absence of elements of the cellular detoxification system can cause an increase in hepatotoxicity, leading to the onset of PCT in individuals with antiretroviral treatment.

The fact that the null genotype frequencies in homozygosis for *GSTM1* and *GSTT1* showed opposite results indicates the existence of an opposite mechanism and biological implications in the influence of the triggering of the acquired PCT in HIV-infected individuals, without neglecting the multifactorial nature of the pathology.

It was hypothesized that the variant c.313A>G (*GSTP1*) would have some influence on the development of PCT, taking into account that the mutation is located in the active protein site and thus causes suboptimal catalytic activity and, therefore, lower cellular detoxification capacity of xenobiotics and even ROS (26). The allelic and genotypic frequencies between the different groups were similar, although this was probably due to multiple factors, one of which may be that *GSTP1* is not primarily expressed in the liver.

It was considered appropriate to evaluate the combination of variants corresponding to the *GSTT1* and *GSTM1* genes, excluding the *GSTP1* gene, which was similar in all the groups studied. The combination of both genes (*GSTT1* and *GSTM1*) showed there were no PCT or HIV patients with absence in homozygosis of both genes. Moreover, this null genotype for *GSTT1* and *GSTM1* showed a tendency to be increased in the PCT-HIV group, but the results were not statistically different; this condition may predispose individuals to an increased risk of hepatotoxicity, but to a lesser degree than other variants/alleles, that, in combination with other factors, may lead to the development of PCT. The absence in homozygosis of *GSTM1* and the presence of at least one allele of *GSTT1* was significantly lower in the PCT-HIV group, which is consistent with that observed for *GSTT1* and *GSTM1* individually; the condition described could represent a combination that decreases the risk of triggering PCT. It is known that the cDNAs encoded by *GSTM1* and *GSTM2* share a significant amount of sequence identity (~99%) and that following elimination of *GSTM1*, *GSTM2* is overexpressed (53). Thus, *GSTM2* may exhibit more efficient detoxification activity regarding the conjugation of antiretrovirals than *GSTM1*.

Based on the above analysis, it was concluded that the development of PCT in HIV-infected individuals may have a genetic basis regarding GST enzymes via a combination of different genotypes in the *GSTT1* (absence) and *GSTM1* (presence) genes.

When the variants of the *ABCB1* and *GST* genes were evaluated as a whole, only PCT-HIV individuals possessed ≥2 risk alleles. This aspect provides strong evidence that non-wild type variants of these genes contribute to the triggering of PCT in HIV-infected individuals, possibly due to inefficient transport of antiretrovirals and thus increased liver toxicity. External and/or genetic factors that predispose an individual to hepatotoxicity promote the inhibition of URO-D, increasing the probability of the onset of the disease (2,4).

The GST variants analyzed in the present study are related to an increase in oxidative stress markers and ROS in blood samples in individuals exposed to toxic factors or with other pathologies increasing ROS levels (54-56). In this context, HIV-infected individuals carrying GST variants may result in high hepatic toxicity under antiretroviral treatment and/or other triggering factors related to drug metabolism and cellular detoxification.

The allelic frequencies of *ABCB1* found in the Control group were compared with those reported for other countries (Table III). This comparison showed differences between various regions of the world. For example, individuals of African descent were considerably more likely to possess wild-type variants for the three SNVs compared with other ethnicities (57,58). For the c.3435C>T SNV, the mutant variants in the present study was significantly higher than that observed for the African population (57,58). The T frequency of the c.2677C>T variant was significantly higher in the Argentine population than in African individuals (57,58) and in some ethnic groups of Chile (20); for variant A, no significant differences were observed between groups. Regarding the c.1236C>T SNV, the frequency of the T variant in African individuals (57,58) was significantly lower than that found in the present study; in contrast, the frequencies for Mapuche (20) and Asian (57,58) populations were significantly higher than those found in the present study. Results of other studies in Caucasians showed no notable differences with the results of the present study (15,58). The bibliographic data are consistent with that provided by the 1000 Genome Browser.

The frequencies obtained for the variants of the *GST* genes with other populations were also compared (Table IV). No significant differences were detected between our results and another study performed in Argentina (59) or those reported for Brazilian, Caucasian, Asian and African populations (61-73), and were consistent with the 1000 Genome Browser, except for the Asian population, where the database reported a larger range compared to that found in other studies (0.78-0.90 for variant A).

In conclusion, based on the ethnic diversity observed in individuals from different regions of the world compared with the results of the present study, it is important to emphasize that each individual possesses a particular combination of allelic

variants which leads to specific biological inter-individual differences. Therapies and drugs, such as antiretrovirals may be metabolized in slightly different ways between individuals, and thus may exhibit slightly different effects or a per individual basis. Therefore, there are individuals to whom certain substances are innocuous and others to whom the doses may be excessive and cause metabolic damage. The observation that there are combinations of variants and haplotypes that could trigger PCT in HIV-infected individuals highlights the possibility in which chronic therapy with antiretrovirals causes collateral damage, favoring the triggering of this pathology. The study of genetic variants and their impact on drug metabolism must be considered to improve personalized medical therapy, according to the genetic profile of each patient. Pharmacogenetics will optimize the efficiency of xenobiotic action, avoiding harmful effects that lead to collateral damage.

The genetic variants analyzed in the present study, together with other linked genes or marker parameters of liver damage, may improve evaluation of the status of HIV-infected patients, thus providing a powerful therapeutic tool when administering treatments for the background disease to prevent the triggering of PCT or to reduce its impact, protecting the hepatic status via administration of antioxidants.

This is the first study to investigate the possible role of variants of *GST* and *ABCB1* in the development of PCT in HIV-infected individuals and suggests that variants in genes that encode for proteins involved in the removal of xenobiotics and in the Phase II Drug Metabolizing System may have an influence on development of PCT in HIV-infected individuals.

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Availability of data and materials

All data generated or analyzed during the present study is included in the published article.

Authors' contributions

PAP, JRZ, VAM and JVL designed the methodology used, as well as validated and analyzed the data. VEP, JRZ, AMB and MVR conceptualized the study. PAP, JRZ, VAM and AMB wrote and edited the manuscript. VAM and JRZ supervised the study. All authors made substantial contributions to the writing of the manuscript as well as read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Institutional Research Ethics Committee of the CIPYP, National Scientific and Technical Research Council, University of Buenos Aires (Buenos Aires, Argentina). Patients provided signed informed consent for participation in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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