Full title: Prevalence of *ABCB1* 3435C>T polymorphism in the Cuban population.

Short title: *ABCB1* 3435C>T polymorphism in Cubans

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Abstract

Background: *ABCB1* gene polymorphisms can modify P-glycoprotein function with clinical consequences.

Methods: The 3435C>T polymorphism prevalence was analyzed using oligonucleotide probes and next-generation sequencing in 421 unrelated healthy individuals living in Cuba. Data were stratified by gender, ethnic background and residence. The genotype and allelic frequencies were determined.

Results: The genotype distribution met the Hardy-Weinberg equilibrium assumption. The allelic frequency was 63.5% for the 3435C variant. The genotype frequencies were 41.1% for CC, 44.9% for CT and 14.0% for TT. The allele and genotype distributions differed between individuals living in La Habana and Santiago de Cuba ($p<0.05$) when ethnic background was analyzed. The allelic distribution was similar among Admixed and Black subjects, and they differed from Caucasians. The CC genotype was equally distributed among Admixed and Black subjects, and they differed from Caucasians. The TT genotype frequency differed between Caucasians and Admixed. The CT genotype was distributed differently among the three groups. Similar distribution was obtained in Brazilians, whereas some similarities were observed in African, Spanish and Chinese populations, consistent with the mixed Cuban ethnic origin.

Conclusions: This is the first report on allele and genotype frequencies of the 3435C>T polymorphism in Cuba, which may support personalized medicine programs.

Key words: *ABCB1*, 3435C>T polymorphism, Cuban population, pharmacogenetics.

Introduction

P-glycoprotein (P-gp), the most studied member of the ATP-binding cassette (ABC) transporters, acts as a drug efflux protein pump to protect cells against xenobiotics [1]. However, in tumor cells, it causes reduced uptake of cancer drugs and hence promotes drug resistance and a worse prognosis for different cancer types. Thus, P-gp function has been suggested to be a major determinant for efficacy and toxicity of cancer drugs [2].

Several studies have shown differential expression of P-gp across populations due to polymorphisms in the *ABCB1* gene, which encodes this protein [3-5]. Around 50 single nucleotide polymorphisms (SNP) have been described for this gene, and attention has mainly been focused on a base change at position 3435 within exon 26 [6-10]. The presence of the *ABCB1* 3435 TT genotype has been related to decreased drug bioavailability and a less favorable cancer treatment [11-13]. Meanwhile, the CT genotype seems to produce an intermediate phenotype with slow metabolism for some drugs [1, 3, 14, 15]. Ethnicity related P-gp polymorphisms also contribute to inter-ethnic differences in drug responses [16,17]. Caucasians and Asians show a higher frequency of the 3435T allele than Africans [18,19]. The higher C allele frequency in Africans is thought to be a consequence of evolution, thus providing a selective advantage against gastrointestinal infections [8,20].

The Cuban population is the result of a complex process of migration and admixture. Cuba's autochthonous residents (aborigines) were practically exterminated after 1492. Since then, the population evolved as the admixture between Spanish, West African and its original population, and a small contribution of immigrated Asians [21]. The main ethnic groups are Caucasians (64.1%, from different regions of Spain), Blacks (9.3%, from sub-Saharan West Africa), and Admixed or “Mestizos” (26.6%) with intermediate phenotypic characteristics [22]. Some studies have been reported on the genetic variability in Cubans [16,17, 23-30]. For instance, mutations of the low-density lipoprotein (LDL) receptor gene have been found in

families with hypercholesterolemia that live in Havana [28]. Associations between polymorphisms in the catechol-O-methyl transferase (*COMT*) gene and schizophrenia [29] were also identified. There is also the MESTIFAR project, developed by the RIBEF-CEIBA Consortium, which describes the allele frequencies of metabolic genes with pharmacogenetic relevance [31]. A recent study on the Hispanic Community (HCHS/SOL) who reside in the USA, reports the prevalence of the 3435C allele of the *ABCB1* gene to be around 0.60 in Cuban-Americans [32]. However, to date, there is no information about the genotype and allele frequencies of this gene among Cubans living on the island. The present study aimed to characterize the prevalence of the 3435C>T variant of the *ABCB1* gene in a random sample cohort representative of the Cuban population.

Materials and Methods

Study population. Unrelated and healthy Cuban subjects living in the country were randomly selected to participate in this study. Most of the recruited subjects came from two provinces: La Habana, the capital and located to the west of the island and Santiago de Cuba, on the East, both with more than one million inhabitants, as documented in the last National Census [22]. At the start, a sample cohort size of approximately 450 subjects was planned, stratified by gender and ethnic background. Most of the participants were recruited via blood banks, and their general health was assessed by doing a physical examination and blood pressure measurements. Four hundred and twenty-one participants were finally recruited during a six-month time period, and they were between 19 and 90 years old (mean: 47.7, SD 17.0).

The participants completed a questionnaire about their ethnic background and current residence. The admixture level of Cubans is high, including European and African roots. This makes ethnic classification a challenging task. For this study, we conducted stratification by

self-perception, the same criteria used in the census, which states that the Cuban population can be clustered by skin color and facial features into three categories: ‘Blanco’’ (Caucasian), ‘‘Mestizo’’ (Admixed) and ‘‘Negro’’ (Black) [22]. This study was conducted in agreement with the recommendations of the Ethics Committee from the National Institute of Oncology and Radiobiology, Havana, Cuba. Each Individual was informed about the study aim and scope, and each participant signed a consent document for the use of their samples in this research study.

Genotyping procedure. Five mL of venous blood was collected from each subject. DNA was isolated from peripheral leukocytes using the QIAamp DNA Mini Kit (QUIAGEN, Germany), according to the manufacturer’s protocols. Each DNA sample was tested for *ABCB1* 3435C>T SNP using oligonucleotide probes designed to target and capture the region of interest, followed by next-generation sequencing (NGS). Samples (10 ng/μl) were loaded into 96 well plates, and PCR amplification was done. The following primers were used, GCAGTGACTCGATGAAGGCA for the forward direction and CCATCCTGTTTGACTGCAGC for the reverse direction. Primer quality was assessed using capillary electrophoresis in the QIAxcel Advanced System (Qiagen). The library was sequenced in a Hiseq X Ten System (Illumina) using a reversible terminator-based method, incorporated into DNA template strands, which produce single nucleotide resolution sequencing results.

Statistical analyses. The allelic and genotype frequencies were calculated by gene counting. The Hardy-Weinberg equilibrium (HWE) was tested by comparing the genotype frequencies with the expected values using the X^2 statistic with Yates’s correction. The frequency distribution of observed genotypes in our study was compared to the frequencies published in the 1000 Genomes project database [33] and reports from the literature by using the Fisher exact test (Fisher-Freeman-Halton format) and the z-test for independent

proportions [34,35]. The frequency of the allele and genotype was shown as a 95% confidence interval, $p < 0.05$ was considered to be statistically significant. The statistical analyses were carried out using the R Studio Programming Environment for Data Analysis, Version 1.2.1335 and the Fmsb package [36,37].

Results

This study evaluated the prevalence of the 3435C>T SNP of the *ABCB1* gene within the Cuban population. The sample cohort was representative of the current distribution of the population, in terms of gender (Table 1, male: 48.0%, female: 52.0%, $p = 0.42$ against the reported Cuban distribution, Fisher exact test) and census category (White: 52.7%, Admixed: 26.1%, Black: 21.2%, $p = 0.06$, Fisher exact test). Subjects were also stratified within two provinces of the country: La Habana (population, 2 131 480) and Santiago de Cuba (1 049 256) [22].

The genotype distribution for the *ABCB1* 3435C>T SNP in the study population was 41.1% CC, 44.9% CT and 14.0% TT, frequencies were in HWE ($p = 0.52$). Upon gender stratification of the genotype distribution (HWE: male $p = 0.28$, female $p = 0.83$), no significant differences were observed ($p = 0.42$, Fisher exact test). Furthermore, the *ABCB1* 3435C allele frequency was 63.5% and *ABCB1* 3435T frequency was 36.5% within the study population. The distribution of the allelic frequencies was similar between males and females and among the three ethnic groups from the study population ($p > 0.05$, Fisher exact test).

Subjects were further clustered according to ethnic background, into Caucasians (n = 248, 58.9%), Admixed (n = 123, 29.2%) and Blacks (n = 50, 11.9%) and according to residence into two groups: La Habana (n = 314, 74.6%) and Santiago de Cuba (n = 79, 18.8%). Subjects (n = 28) that lived in the rest of the provinces were not taken into account for further residence-related analyses. Distribution of the *ABCB1* 3435C>T SNP showed no deviation

from HWE and genotype and allele distribution were neither related to ethnic background ($p = 0.19$, Fisher exact test) nor to residence ($p = 0.73$, Fisher exact test) when both taxa were assessed individually.

However, significant differences were revealed by combining both effects (Table 2). Subjects from La Habana that had the *ABCB1* 3435CC genotype were distributed differently according to their ethnic backgrounds than subjects from Santiago de Cuba ($p = 0.00$, Fisher exact test). Similar results were found in subjects that had the *ABCB1* 3435CT and *ABCB1* 3435TT genotypes (respectively, $p = 0.00$ and $p = 0.01$, Fisher exact test). The distribution of *ABCB1* 3435 C and T alleles were also different ($p = 0.00$, Fisher exact test). Post-hoc analysis revealed that the *ABCB1* 3435CC genotype was similarly distributed among Admixed and Black subjects from the two provinces ($p = 0.80$, Fisher exact test), but Caucasians showed a different distribution ($p = 0.00$, Fisher exact test). The *ABCB1* 3435TT genotype was differentially distributed only among Caucasian and Admixed subjects from La Habana and Santiago de Cuba ($p = 0.01$, Fisher exact test), while the *ABCB1* 3435CT genotype was distributed differently among the three ethnic groups ($p < 0.05$, Fisher exact test). The allelic distribution was similar in Admixed and Black subjects from La Habana and Santiago de Cuba (respectively, $p = 0.46$ for C allele and $p = 0.08$ for T, Fisher exact test), but they were different to Caucasians ($p = 0.00$, Fisher exact test). These data revealed a significant dependence of the genotype and allele frequencies among Caucasians, Admixed and Black subjects when geographical distribution was taken into account.

Discussion

Here, we provide the first data on the prevalence of the *ABCB1* 3435C>T SNP in Cuba. The C variant of this SNP was observed in 63.5% of participants in this study. A significant dependence of the allelic and genotype frequencies among individuals with different ethnic

backgrounds was found between inhabitants from La Habana and Santiago de Cuba (Table 2), thus suggesting that drug responses may vary across the country, probably due to the mixed influence of environmental and genetic factors. Therefore, identifying differences in genotype distribution should be important for establishing public health policies.

Interestingly, the C variant frequency observed was slightly different from the reported allelic frequency among Cuban-Americans from the HCHS/SOL study ($p = 0.04$, z test for independent proportions) [32]. Stratification by ethnicity revealed differences between Admixed subjects from the present study and Cuban-Americans, but no significant differences were found when this latter group was compared to Caucasians or Black subjects living on the island (respectively $p = 0.80$ and $p = 0.06$, z test for independent proportions). However, no detailed demographic information is available on the HCHS/SOL study participants to carry out further analyses.

Skin color self-perception was used here to determine ethnicity, but this source of information has been widely debated [25, 38-42]. Ethnic criteria have evolved from skin color and classical anthropologic traits to molecular markers. Ancestry Informative Markers (AIMs) and SNPs located in *SLC24A5* and *SLC45A2* genes have been suggested to be informative and robust [43]. However, Dumitrescu *et al.* [40] report that self-reported ethnicity and ethnicity determined from genetic traits using AIMs were in agreement in European-Americans and African-Americans. Additionally, it has been shown that skin pigmentation levels in Cubans strongly correlate to ancestry, when this is determined using AIMs [25]. Marcheco *et al*. [25] demonstrate that the melanin index averages higher values in individuals who self-reported as ‘‘negro” than those measured in individuals that self-reported as “blanco”, intermediate values is observed in subjects who self-reported were described as ‘‘mestizo’’. The ethnic distribution in our study (Caucasian: 58.9%, Admixed: 29.2% and Black: 11.9%, Table 1) did not differ from the ones reported by Marcheco *et al.* ($\chi^2 = 5.8$, $p = 0.06$) [25], nor from the

Cuban population ($\chi^2 = 3.4$, $p = 0.19$). Thus, we used skin color self-perception for our study. However, as molecular markers of ancestry provide robust ethnicity assessments [42], future studies should be performed to obtain more precise data.

The main genetic background in Cubans came for Spaniards, Africans, and to a lesser extent Asians (Chinese mainly), whereas native Amerindian genes could also be traced [21]. The *ABCB1* 3435 TT genotype was more frequent in Caucasians than in Black Cubans, which was in agreement with the low abundance of this genotype observed in Africans [20, 33, 44, 45]. However, our data differed ($p < 0.05$, Fisher exact test) from the reported frequencies among African populations, which show even lower frequencies (Table 3). The African background of our population is not unique because of the European contribution and in particular, the Hispanic one is also important for explaining our genetic architecture. Therefore, it was expected that *ABCB1* 3435C>T SNP would exhibit intermediate values in Cubans, compared to African and Hispanic populations. Indeed, our results agreed with data obtained from Spanish Caucasians, particularly from the northern region of Spain and to the European "super population" [33,46-47]. These studies show that the CT genotype is the most frequent one (around 50%), similarly to the one reported here (Table 3).

Furthermore, it was expected that the Cuban population results would be comparable to those in Brazil [48,49] as a consequence of common demographic origins with a larger proportion of African genetic ancestry than other Latin American populations. The observed *ABCB1* 3435C allele frequency seemed to be higher in Cubans than in other Latin American populations (Table 3). Surprisingly, genetic variability of this SNP within Peruvian, Mexican, Ecuadorian, Colombian populations and Admixed Americans when considered as a "super population" [33, 50-52] did not entirely reflect the frequencies that we observed in our results for Cuba. Interestingly, upon datamining in the 1000 Genomes Project [33] our SNP distribution results seemed to be most comparable with city subpopulations from Lima

(Peru), and Medellin (Colombia). One possible explanation for this different outcome could be the subtle regional variations in the underlying ethnic structure of the selected samples. It has been demonstrated that differences in the genotype and allele frequencies in *ABCB1* 3435C>T SNP were strongly associated with the ethnic component [53]. For instance, Peruvian populations are composed of 83% Amerindian, 7.4% European, 1.7% African and 3.5% Asian [54], which is remarkably different from the Cuban population composition. However, as was expected, cosmopolitan and coastal cities such as Lima or Medellin exhibit higher European and African ancestry proportions (about two-fold higher compared to the whole national population), which makes them more similar to the Cuban or Brazilian populations. In this regard, larger randomized cohort studies across different regions in Cuba may more accurately reflect the polymorphism distribution in general Latin-American country populations.

Similarities in the *ABCB1* 3435C>T SNP distribution were present when our data was compared to the “East Asia super population” and specifically to Chinese individuals. However, simililarities were not found in regard to South Asia populations [33, 55] and the “South Asia super population”, according to data available at the 1000 Genomes Project database (Table 3). Chinese ancestry is known to be present within the Cuban population, but to a lesser extent than African and Spanish backgrounds. However, the observed degree of homogeneity in *ABCB1* 3435C>T SNP distribution in Chinese individuals was remarkable and showed even higher values ($p > 0.20$, Fisher exact test) in comparison to previous studies [56-58].

In short, the frequency distribution showed no significant differences in relation to gender, ethnic background, or geographical distribution between La Habana and Santiago de Cuba provinces when data were stratified by only one taxon. Differences in genotypic and allelic frequencies did exist between individuals from the two regions when ethnic background was

also taken into consideration. Nevertheless, we are aware that our study had some limitations. As subjects were recruited mainly from two urban centers, future studies should take into account more extensive participant recruitment from other regions as well. Skin color self-perception was used to describe ethnicity, which was suitable for achieving our goals, but the high degree of admixture of the Cuban population recommends the additional use of molecular ancestry markers to obtain a more objective ethnic classification. In addition, *ABCB1* 3435C>T SNP is often studied in conjunction with other polymorphisms, and the next step should be to determine frequencies of other SNPs in order to provide a more comprehensive clinical interpretation of this data. However, our results hold promise for supporting precision medicine programs, for example by allowing the calculation and optimization of the SNP specific dosage of P-gp substrate drugs.

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