Association of the genetic marker for abacavir hypersensitivity HLA-B*5701 with HCP5 rs2395029 in Mexican Mestizos

Prospective screening for HLA-B*5701 decreases or abolishes abacavir hypersensitivity reaction. In Caucasians, the HLA complex protein 5 gene (HCP5) rs2395029(G) allele is in complete linkage disequilibrium (LD) with HLA-B*5701 (r2 = 1). Aim: To assess the frequency of HLA-B*5701 and its LD with HCP5 rs2395029(G) allele, to extend our knowledge of genetic variants that are of critical relevance for the development of pharmacogenetics in Mexico. Materials & methods: We genotyped 300 Mexican Mestizos from the Mexican Genome Diversity Project. HLA-B*5701 genotyping was performed using a DNA sequencing method. HCP5 rs2395029 was genotyped using a custom TaqMan® SNP genotyping assay and confirmed by direct sequencing. Genotypes for 14 SNPs in the HCP5 region were retrieved from the Mexican Genome Diversity Project database for LD analysis. Results: HLA-B*5701 carrier frequency was 2% and the allelic frequency was 0.010. Haplotype analysis revealed that HLA-B*5701 and the HCP5 rs2395029(G) allele are in complete LD (r2 = 1) in this Mexican Mestizos sample. Conclusion: It is feasible to have a pharmacogenetic program based on HCP5 rs2395029 genotyping as a screening tool with confirmation of HLA-B*5701 carriage by sequencing, to prevent abacavir hypersensitivity reaction in Mexican patients before initiating abacavir therapy.

KEYWORDS: abacavir HCP5 HLA-B*5701 hypersensitivity pharmacogenetics

Abacavir is a nucleoside reverse-transcriptase inhibitor antiretroviral indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in adult and pediatric patients. Abacavir is associated with potentially serious hypersensitivity reactions in 3–9% of Caucasian patients that require immediate and permanent discontinuation of the drug [2–4]. It has been shown that carriers of the allele 5701 of the HLA locus B (HLA-B*5701) are at high risk for developing abacavir hypersensitivity reaction (AHR) [2–4] and that prospective genetic screening for HLA-B*5701 greatly diminishes or abolishes the occurrence of AHR [5–7].

In July 2008, the US FDA approved a label change for abacavir (Ziagen) to include a boxed warning indicating that patients who carry the HLA-B*5701 allele are at high risk for experiencing a hypersensitivity reaction to abacavir and a recommendation to screen for HLA-B*5701 prior to the initiation of abacavir therapy [10].

In Caucasians, rs2395029 (c.335T>G) in the HLA complex protein 5 gene (HCP5), located 100 kb centromeric of HLA-B, is in complete linkage disequilibrium (LD) with HLA-B*5701 (r2 = 1) [8–10]. Further studies have identified recombination events at multiple sites within the MHC [11], revealing a high but incomplete LD between HLA-B*5701 and HCP5 rs2395029 [11–13].

Mexico’s population is mainly composed of Mestizos, who, as with other Latin American populations, are a recently admixed population composed of Amerindian, European and, to a lesser extent, African ancestors. The aim of our study was to assess the prevalence of HLA-B*5701, HCP5 rs2395029(G) and their LD pattern in the Mexican Mestizo population by analyzing the samples from the Mexican Genome Diversity Project (MGDP), to extend our knowledge of genetic variants of critical relevance for the development of pharmacogenetics in Mexico.

Materials & methods

The panel of 300 Mexican Mestizo samples from the MGDP consists of nonrelated, self-identified Mestizo individuals having four grandparents not self-recognized as recent immigrants from six states in geographically distant regions of Mexico [14]: Sonora and Zacatecas in the north, Guanajuato in the central region, Guerrero in the central/Pacific region, Veracruz in the central/Gulf region and Yucatan in southwest Mexico. From those, 268 samples were available for genotyping.

HLA-B*5701 genotyping was performed by DNA sequencing using the AlleleSEQR HLA-B PCR/sequencing Kit (Abbott Laboratories, IL, USA) and the genetic analyzer 3130xL

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DOI: 10.2217/PGS.11.31 © 2011 Future Medicine Ltd
Pharmacogenomics (Epub ahead of print)
Sequence analysis was carried out with Assign-SBT software (Conexio Genomics, Freemantle, Australia). To genotype HCP5 rs2395029, we used a custom TaqMan® SNP genotyping assay designed with File Builder 3.0 (Applied Biosystems) and TaqMan GT Master Mix (Applied Biosystems). To confirm the genotype determined by the allelic discrimination, 15% of the samples were sequenced using locus specific primers (forward: TACCCTCATTGTGTGACAGCA, reverse: GTCGTGGGATTTTGCACT) and the SNP's PCR amplification protocol, which is available at [102]. We amplified a 252-bp fragment containing the rs2395029 SNP. Amplicons were sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing products were purified by BigDye XTerminator® (Applied Biosystems) prior to loading on the ABI 3730×L DNA Analyzer. ABI files were analyzed with the Lasergene SeqMan sequence analysis software (DNASTAR, WI, USA).

The MGDP database contains genotypes from 300 Mexican Mestizo samples that were analyzed with three different platforms with a total of nearly 1.4 million SNPs, including rs2395029. Genotypes of HCP5 rs2395029 and 13 other SNPs in the HCP5 region (Supplementary Table 1, www.futuremedicine.com/loi/pgs) were retrieved from the MGDP database. We created a dummy variable to represent the HLA-B*5701 genotype. The variant allele represented the presence of HLA-B*5701, while the wild allele represented the absence of HLA-B*5701. Retrieved genotypes and the dummy variable were used for imputation of missing HLA-B*5701 genotypes (n = 50) by haplotype reconstruction with PHASE software version 2.1 [15]. Phased genotypes and the dummy rs were included for LD analysis with the Haploview software [16]. Individual ancestry estimates were calculated using STRUCTURE as previously described [14].

## Results & discussion

Of the original 300 samples in the MGDP, 268 samples were available for testing and were used

<table>
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<th>Haplotype content</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
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<td>33.0</td>
</tr>
<tr>
<td>11212421422333</td>
<td>147</td>
<td>24.5</td>
</tr>
<tr>
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<td>96</td>
<td>16.0</td>
</tr>
<tr>
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</tr>
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<td>4.7</td>
</tr>
<tr>
<td>11311424124333</td>
<td>18</td>
<td>3.0</td>
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<td>113322222122331</td>
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<td>13332421222331</td>
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<tr>
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<tr>
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<td>0.5</td>
</tr>
<tr>
<td>11331421224333</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>1131142412333</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>11112421224333</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>1131242122331</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*Bold numbers at positions 1 and 9, respectively, indicate that the haplotype contains both the rs dummy allele, which represents the presence of HLA-B*5701 and G allele of HCP5 rs2395029.*

Table 1. List of 15 haplotypes found in the best reconstruction with PHASE v2.1.

![Typical sequencing results](image-url)
to characterize the prevalence of HLA-B*5701 and HCP5 rs2395029. Of these, 250 samples were successfully genotyped for HLA-B*5701 and 18 samples could not be genotyped because of DNA degradation. HCP5 rs2395029 was successfully genotyped in all 268 samples. We found six individuals who were heterozygote carriers of HLA-B*5701 and were also heterozygote carriers of the G allele of rs2395029. There was complete agreement between the rs2395029 genotype by allelic discrimination and the genotype retrieved from the MGDP database. Haplotype reconstruction with PHASE software found 15 different haplotype, only one of which had both the rs dummy allele representing the presence of HLA-B*5701 and the G allele of HCP5 rs2395029 (Table 1). The missing HLA-B*5701 genotypes were imputed by haplotype reconstruction. None of the imputed genotypes carried HLA-B*5701; thus, only six out 300 individuals were heterozygote carriers for both HLA-B*5701 and the G allele of HCP5 rs2395029. Sequencing confirmed the six heterozygote genotypes as well as 44 randomly chosen wild-type genotypes. Typical sequencing results of the heterozygous HCP5 rs2395029 and wild-type are compared in Figure 1. The carrier frequency for HLA-B*5701 and the HCP5 rs2395029 G allele was 2.0%; whereas, their allelic frequency was 0.010 (Table 2). The LD analysis of 600 phased haplotypes showed that HLA-B*5701 and HCP5 rs2395029(G) allele are in complete LD ($r^2 = 1$) (Figure 2). Ancestry analysis of HLA-B*5701 carriers showed that their average ancestry estimates for European, Amerindian, African and East Asian contributions (33, 63, 3.5 and 0.05%, respectively) are similar to the mean contributions observed in the 300 Mexican Mestizos analyzed (0.418 ± 0.155, 0.552 ± 0.154, 0.018 ± 0.035 and 0.012 ± 0.018, respectively) [14]. European contribution in HLA-B*5701 carriers was not significantly different (t-test, p = 0.125) from the mean European contribution.

The aim of our study was to asses the prevalence of HLA-B*5701, HCP5 rs2395029 and their LD pattern in the Mexican Mestizo population, to extend our knowledge of genetic variants of critical relevance for the development of pharmacogenomics in Mexico. We found a 2.0% carrier and 0.010 allelic frequencies that are similar to those previously reported in Hispanic subjects living in the USA [17]. Mexican Mestizos have a lower frequency of HLA-B*5701 compared with the 5–8% reported in Caucasians, but a higher frequency than the 0% reported in China, Japan [18] and Korea [19], which is most likely related to our Caucasian and Amerindian ancestries.

In this population, HLA-B*5701 and HCP5 rs2395029(G) alleles were in complete LD ($r^2 = 1$); this should be interpreted cautiously, as previous reports demonstrate that the LD is high but not complete [11–13]. We did not observe individuals that were HCP5 rs2395029(G) positive and HLA-B*5701 negative, this may be due to the relatively low HLA-B*5701 allele frequency observed in this study and the small number of individuals in the MGDP panel.

There are various reports of successful implementation of prospective HLA-B*5701 genetic screening programs for patients initiating abacavir therapy. These programs resulted in significant decrease of AHR [5–7, 20]. The sequencing-based genotyping method is the gold standard in screening for HLA-B*5701; however, its use is limited because it is labor intensive, costly and requires an instrument not easily available in clinical laboratories. The PCR sequence-specific primer (SSP) assay is a reliable alternative for HLA-B*5701 genotyping. This method is currently used by several laboratories for HLA-B*5701 screening in patients initiating abacavir [21]. Colombo and Rodríguez-Nóvoa showed that in HIV patients, the HCP5 rs2395029 had a positive predictive value of 100%; however, the negative predictive value for carriage of HLA-B*5701 was 94 and 93%, respectively, due to the presence of HLA-B*5701 negative but HCP5 rs2395029(G) positive individuals, relying exclusively on HCP5 rs2395029, those individuals would be unnecessarily excluded from abacavir therapy. Both authors agree that HCP5 rs2395029 could be an alternative in situations where HLA-B*5701 testing is not easily available [12, 13].

Mexico is the Latin American country with the second largest number of people living with HIV. The 2009 statistics on HIV–AIDS from the National Center of Prevention and Control of HIV-AIDS (CENSIDA) from the Secretary of Health reports that in November 2009, there were 220,000 people living in Mexico with HIV [103]. Based on UNAIDS

<table>
<thead>
<tr>
<th>SNP</th>
<th>HLA-B*5701</th>
<th>Other HLA-B alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs2395029 (G)</td>
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<td>0</td>
</tr>
<tr>
<td>Rs2395029 (T)</td>
<td>0</td>
<td>594</td>
</tr>
</tbody>
</table>

Prevalence 2.0%, allele frequency 0.010.
Mexico Epidemiological Fact Sheet on HIV and AIDS 2008 Update [104], we estimate that 84,000 patients need antiretroviral treatment; however, only approximately 47,000 are receiving it. There are no published data on the number of patients receiving abacavir in Mexico, but using the figure reported by Lalonde in Canada [22], we estimate that 15,600 patients (a third) have been exposed to abacavir and have thus been at risk of presenting an AHR. 

*HLA-B*5701 is one of the best examples of the clinical utility of a genetic marker to identify individuals at risk for an adverse drug reaction. The clinical application of *HLA-B*5701 as a genetic marker to reduce the number of cases of AHR has been comprehensively described [20]. Studies evaluating the cost–benefit ratio of prospective *HLA-B*5701 genetic screening showed that the benefit will depend on the cost of the treatment, the cost of the genetic test and the prevalence of the genetic marker [23,24]. Our study reveals the prevalence of *HLA-B*5701 in a Mexican Mestizo population and the potential use of a single SNP genotyping method as a low cost screening tool that, together with epidemiological data, can be used for future, cost-effective, public interventions.

Single SNP genotyping is an easier method with a much lower cost compared with sequencing or SSP. This lower cost could facilitate the implementation of prospective genetic screening programs for AHR, especially in countries such as Mexico and other Latin American countries with limited access for sequencing-based HLA typing or limited resources. However, samples positive for *HCP5 rs2395029(G)* allele must be confirmed as carriers of *HLA-B*5701 by either a sequencing or SSP method because of the high but not complete LD between those two genetic markers. The clinical utility of such a program must be confirmed by a clinical trial in Mexican Mestizo HIV patients that will initiate treatment with abacavir.

In conclusion, the prevalence of *HLA-B*5701 carriers in the Mexican Mestizo population is 2.0%, and the allelic frequency of *HLA-B*5701 is 0.010. There was a complete LD between *HLA-B*5701 and the *HCP5 rs2395029(G)* allele in the population studied. We estimate that in Mexico there is a significant number of people exposed to abacavir, according to the prevalence of *HLA-B*5701 found in this study and the estimated number of AHR that may have occurred; we believe that it would be of
clinical relevance to have a pharmacogenetic program for Mexican patients who will initiate abacavir therapy, using HCP5 rs2395029(G) as a screening tool and confirmation of HLA-B*5701 carriage by either sequencing or SSP methods. A clinical trial in Mexican Mestizo HIV patients to confirm the clinical relevance is needed. Considering that a single SNP genotyping will be more accessible and affordable, the implementation will be favored, increasing patient’s safety. It is important to note that genetic screening to prevent AHR should never substitute for clinical vigilance in patients who start abacavir treatment.

This information, novel in Latin America, could also be useful for other populations that share ancestral history with the Mexican Mestizo population. The present study is also an example of the use of the MGDP database for pharmacogenomic studies in the Mexican Mestizo population.

Future perspective
A single SNP genotyping together with a confirmation method will favor the implementation of pharmacogenetic programs through accessible and affordable tests that will increase patient’s safety. Pharmacogenetic tests will be increasingly available at clinical laboratories and will enable the practice of personalized medicine.

Acknowledgements
The authors are grateful to Haydee Miranda Ortiz and Salvador Hernandez Morales who did the sequentiation.

Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research
The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Introduction
- Abacavir is an antiretroviral indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection. It is associated with potentially serious hypersensitivity reactions in 3–9% of Caucasian patients.
- Carriers of HLA-B*5701 are at high risk for developing abacavir hypersensitivity reaction (AHR). Prospective genetic screening for HLA-B*5701 greatly diminishes or abolishes the occurrence of AHR.
- In Caucasians, HCP5 rs2395029(G) is in high but not complete linkage disequilibrium (LD) with HLA-B*5701.

Results & conclusion
- Analysis of the 300 samples from the Mexican Genome Diversity Project revealed a prevalence of HLA-B*5701 carriers of 2.0%, and the allele frequency is 0.010. There was a complete LD between HLA-B*5701 and the HCP5 rs2395029(G) allele in this population studied.
- It would be of clinical relevance to have a prospective pharmacogenetic program implemented for Mexican patients who will initiate abacavir therapy, using HCP5 rs2395029(G) as a screening tool and confirmation of HLA-B*5701 status by either a sequencing or PCR sequence-specific primer methods. The clinical utility of such a program must be confirmed by a clinical trial in Mexican Mestizo HIV patients that will initiate treatment with abacavir. Genetic screening to prevent AHR should never substitute for clinical vigilance in patients who start abacavir treatment.

Bibliography
Papers of special note have been highlighted as: ** of considerable interest


** This study included approximately 2000 patients from 19 different countries. It demonstrates the effectiveness of a HLA-B*5701 screening program to prevent abacavir hypersensitivity reaction.


** Describes the complete linkage disequilibrium between HLA-B*5701 and HCP5 rs2395029 in Caucasians.


** Evidence of genetic recombination within MHC and thus of incomplete linkage disequilibrium between HLA-B*5701 and HCP5.


** Clinical application of HCP5 rs2395029 to screen for abacavir hypersensitivity reactions.


** Websites

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