

## RAPID COMMUNICATION

# Frequency of HLA B\*5701 allele carriers in abacavir treated-HIV infected patients and controls from northeastern Brazil

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## INTRODUCTION

In clinical practice, using pharmacogenetics has risen in importance since the technologies for genomic variation searches for different responses to drugs became widespread, easier, more rapid, and affordable for a specialized laboratory. The main pharmacogenetics studies have mostly involved drug resistance monitoring in oncology patients;<sup>1</sup> however, other applications are being developed to detect genetic molecular markers associated with resistance or hypersensitivity/adverse reactions to antiretroviral drug treatment in Human Immunodeficiency Virus (HIV)-infected patients.<sup>2</sup>

HIV treatment is known to be limited by adverse drug reactions and the development of resistance. One significant example is the strong association of the Abacavir hypersensitivity reaction with HLA-B\*5701 in HIV-positive patients.<sup>3</sup> Abacavir, a common drug for treating HIV-infected patients, is an efficient nucleoside reverse-transcriptase inhibitor with a beneficial long-term toxicity profile, often used with other antiretroviral agents. However, Abacavir can yield adverse effects such as immunologically-driven hypersensitivity in 5 to 8% of HIV-positive subjects during the first six weeks of use.<sup>2</sup> Hypersensitivity symptoms immediately reverse after the interruption of Abacavir.<sup>4</sup>

The hypersensitivity reaction to Abacavir has been reportedly associated with the presence of the major histocompatibility complex class I allele HLA-B\*5701 and this association has been confirmed in several replication studies in different ethnic groups of HIV-positive patients.<sup>3</sup>

In our study, we searched for the presence of HLA B\*5701 in 96 HIV-positive patients treated with Abacavir and in 243 healthy subjects from Northeastern Brazil (Recife, Pernambuco) to verify the percentage of HLA B\*5701 allele carriers in HIV patients and in the general population from Northeast Brazil. This area is known to harbor a tri-hybrid

population resulting from contributing African (44%), Caucasian (34%), and native American (22%) genomes.<sup>5</sup>

## MATERIAL AND METHODS

### Subjects

Genomic DNA of 96 HIV-infected patients (50 males, mean age 29.5 years, range 19-48; 46 females, mean age 22.7, range 16-31) treated with Abacavir and 243 healthy blood donors (120 males, mean age 21.3 years, range 18-41; 123 females, mean age 24.2 years, range 19-48) from the Recife metropolitan area stored at the "Laboratório Central de Saúde Pública - Dr. Milton Bezerra Sobral" (LACEN - PE) and the "Laboratorio de Imunopatologia Keizo Azami" (LIKA) were used for the genetic screening. Genomic DNA was previously extracted from peripheral whole blood and stored at -20°C at the LIKA laboratory of Recife using the EZ1 DNA extraction kit and EZ1 robotic device (Quiagen) following the manufacturer's instructions.

Patients and controls were stratified for ethnicity and classified as European- or African-derived according to phenotypic characteristics of individuals and ethnicity data of parents/grandparents reported by the participants in an appropriate questionnaire. The issue concerning the skin color-based classification criteria adopted in Brazil is well-documented and has been already assessed by other research groups in previous studies.<sup>6,7</sup>

### HLA B\*5701 genotyping

HLA B\*5701 genotyping has been performed in triplicate by Melting Temperature Assay using the DUPLIC $\alpha$ -RealTime HLA-B\*5701 Genotyping kit (Euroclone, Milan, Italy). Briefly, 5  $\mu$ l of genomic DNA (50 ng/ $\mu$ l) were added to 10  $\mu$ l of amplification mix (with Hot Start Taq DNA polymerase, nucleotides, MgCl<sub>2</sub>, buffer and SYBR GREEN) and 10  $\mu$ l of oligo mix (with primers to amplify HLA-B\*5701 allele and an internal control, human growth hormone: HGH). Real time PCR reactions were run on the Cepheid (Euroclone, Milan, Italy) platform using the following temperatures and cycles profile: 2' 50°C 1 hold, 10' 95°C 1 hold, then 40 cycles 15" 95°C and 60" 60°C. The melting protocol was as follows: initial hold 2' 65°C with a melting curve from 65°C to 95°C increasing 0.2°C each time. Each specific amplicon (i.e. HLA B\*5701 and HGM) was detectable by a different melting temperature.

## RESULTS

We searched for the presence of HLAB\*5701 allele in 96 HIV-infected patients treated with Abacavir and 243 Northeastern Brazilian subjects from Recife (PE) using a real time allelic-specific PCR approach. The melting temperature assay, performed after real time PCR amplification, allowed us to easily identify the presence of HLA B\*5701, characterized by a melting profile temperature between 91.5 and 92.5°C, while the internal control, the human beta globin gene, showed a melting temperature between 88.8 and 89.8°C. Figure 1 shows the melting profiles of the HLAB\*5701 positive and HLAB\*5701 negative samples.

The real time PCR followed by melting temperature assay were performed in triplicate on three different real time PCR platforms to test the robustness of the genotyping chemistry and its adaptability to different technological platforms. The results obtained by using the ABI 7900 HT Sequence Detection System, the RotorGene 3000 and the Cepheid (Euroclone, Milan Italy) real time PCR platforms were absolutely the same and were highly reproducible, always allowing the detection of the HLA B\*5701 allele in the unknown genotype samples.

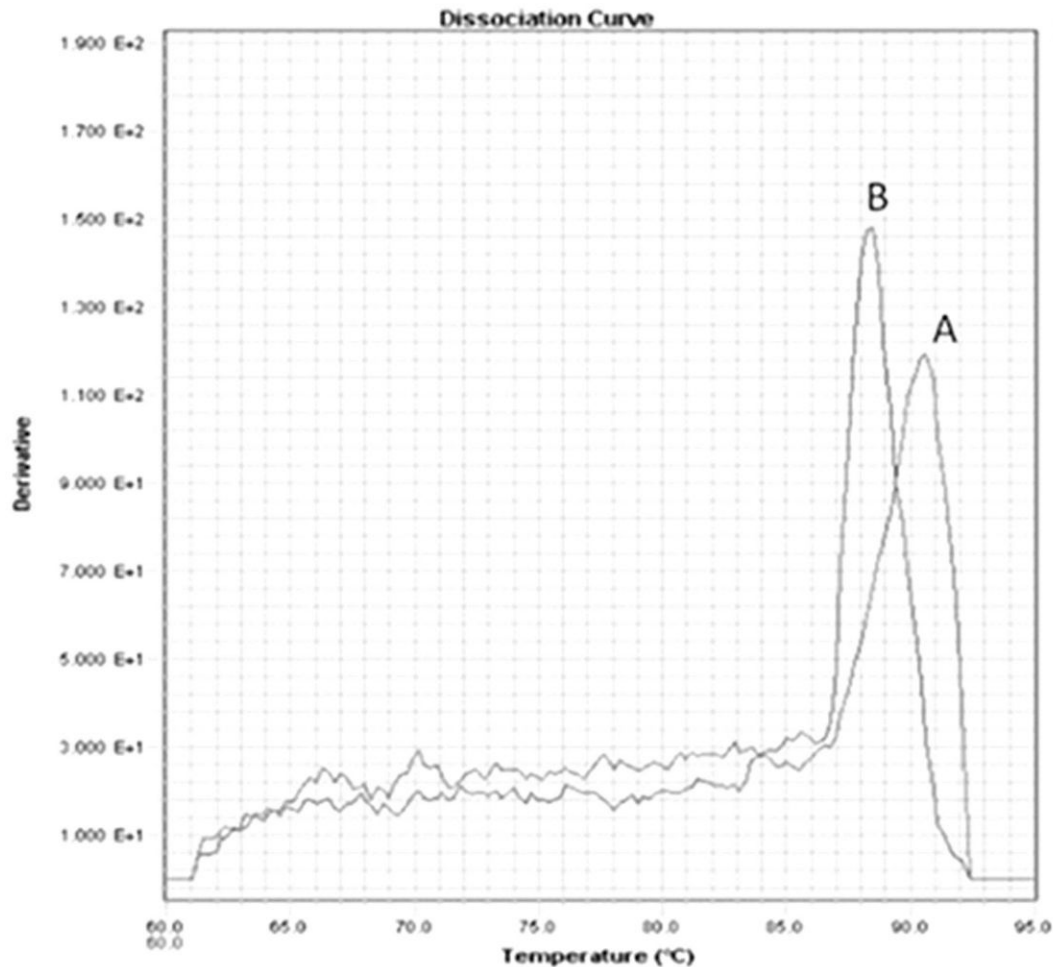
Within the 96 HIV infected patients treated with Abacavir, three (3.1%) were carriers of the HLA B\*5701 allele and presented with hypersensitivity characterized by cutaneous rash and severe gastrointestinal tract symptoms;

in the control population, eight individuals out of the 243 screened (3.4%) were carriers for HLA B\*5701. The HLA B\*5701 allele was heterozygous in all carriers (the results were double-checked using the PCR-SSP Dynal commercial kit). Table 1 summarizes the results for the HIV patients and the controls from Northeast Brazil and reports HLA B\*5701 carriers and allele frequencies for other ethnic groups (reported in the <http://www.allelefreqencies.net>).<sup>8</sup>

When stratifying for ethnicity, within the 96 HIV infected patients 36 were European-derived (two HLA B\*5701 carriers, 5.5%) and 60 were African-derived (one HLA B\*5701 carrier, 1.6%). In the controls group, 90 individuals were classified as European-derived (five HLA B\*5701 carriers, 5.5%) and 153 were classified as African-derived (three HLA B\*5701 carriers, 1.9%).

## DISCUSSION

Recently, the presence of the HLA-B\*5701 allele has been correlated with immunological hypersensitivity to Abacavir in white and black HIV-positive patients from United States.<sup>9</sup> Moreover, HLA-B\*5701 allele carriers in HIV-positive Hispanic patients showed a clinically-diagnosed hypersensitivity reaction to Abacavir.<sup>10</sup> HLA-B\*5701 allele frequency is known to depend upon the HIV-positive patients' ethnicity; in fact, while white Caucasians have an incidence of around



**Figure 1** - Melting temperature assay profiles of two subjects: one carrier of HLA B\*5701 allele (A) and one non-carrier of the HLA B\*5701 allele (B). The two melting profiles are easily distinguishable and allow easy detection of the HLA B\*5701 allele.

**Table 1** - HLA B\*5701 frequencies in HIV-infected patients (HIV\_BRA) and the controls (BRA\_NE) from Northeast Brazil in this study and in previously genotyped populations<sup>9</sup> (<http://www.allelefreqencies.net>).

	HIV_BRA n=96	BRA_NE n=234	BRA_C n=95	HIV_CHI n=492	CHI_P n=300	CHI_M n=70	AUT_C n=200	USA_A n=187	USA_C n=307
HLA B*5701 carriers	3.1%	3.4%	1.1%	2.2%	3.7%	4.0%	5.5%	2.1%	6.2%
HLA B*5701 allele frequency	1.5%	1.7%	0.5%	1.1%	1.8%	2.0%	2.8%	1.1%	3.1%

BRA\_C: Brazil (Belo Horizonte) Caucasian; HIV\_CHI: HIV infected patients from Chile; CHI\_P: Chilean general population; CHI\_M: Chile (Santiago) Mixed; AUT\_C: Austria Caucasian; USA\_A: USA (Bethesda) African-American; USA\_C: USA (Philadelphia) Caucasian.

6%, only about 2.5% present in the black population.<sup>11</sup> Thus, the screening for a genetic marker for hypersensitivity to Abacavir, such as the HLA-B\*5701, is strongly dependent upon the population's racial background.

In our study performed with Northeastern Brazilian individuals, the frequency of HLA-B\*5701 carriers was 3.1% in HIV-infected patients and 3.4% in the general population. These frequencies are between those reported for white Caucasian and black subjects from the United States. The difference can be explained by the ethnic composition of the Pernambuco population, known to be an admixture of Caucasian (34%), Afro-American (44%), and Amerindian (22%) genomes.<sup>5</sup> Moreover, our findings more closely resembled those by Poggi et al.<sup>12</sup> in HIV patients and the controls from Chile than the frequencies reported in a Caucasian population from Belo Horizonte (see Table 1 reporting HLA B\*5701 frequencies in different ethnic groups for comparison).<sup>8</sup>

The importance of HLA-B\*5701 screening for diminishing the frequency of hypersensitivity to Abacavir has been reported in the PREDICT-1 study<sup>13</sup>; the frequency of HLA B\*5701 carriers at high risk of hypersensitivity to Abacavir was 6% in white Caucasian patients.

In our study population from Northeastern Brazil, the frequency of HLA-B\*5701 was between Caucasian and African ethnic groups; however, when stratifying for ethnicity, our findings show that the HLA B\*5701 carriers' frequencies are more similar to those previously reported for Caucasians and Africans.

Finally, considering the clinical importance of hypersensitivity to Abacavir treatment in HIV-infected patients and the frequency of HLA B\*5701 carriers reported in this study (3.1% in patients and 3.4% in the controls), we suggest the preventive use of HLA B\*5701 testing in clinical practice in Abacavir treatment in Northeastern Brazil.

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