

HLA-B*5701 frequency in Chilean HIV-infected patients and in general population

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Submitted on: 03/04/2010
Approved on: 06/01/2010

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We declare no conflict of interest.

ABSTRACT

It has been demonstrated that HLA-B*5701 screening reduces the risk for hypersensitivity reaction to abacavir in HIV-infected patients. Since B*5701 prevalence varies among different populations, it is important to determine the carrier frequency prior to its use for the screening of HIV-infected patients. The aim of this study was to determine HLA-B*5701 carrier frequency in Chilean general population and HIV-infected patients referred for B*5701 typing. For that purpose 300 blood bank donors and 492 abacavir-naïve HIV-infected patients from Chile were screened for B*5701 by a sequence specific primer PCR. We detected 14/300 (4.7%) B*57-positive individuals in the Chilean general population, 11 (3.7%) were B*5701 positive, and 3 (1%) had another subtype. All were heterozygous, thus a B*5701 allele frequency of 2% was determined. Eleven of 492 (2.2 %) HIV-patients carried a B*5701 allele. The difference between these frequencies is probably due to slow progression of HIV infection in HLA-B*5701 carriers, thus less patients would require antiretroviral therapy and B*5701 typing. Considering the usefulness of B*5701 screening, its prevalence in the Chilean general population, and the availability of a validated method, we conclude that HLA-B*5701 typing in Chilean HIV-infected patients about to initiate abacavir treatment is strongly recommended.

Keywords: HLA-B*5701, HIV, abacavir, Chile, pharmacogenetics.

[Braz J Infect Dis 2010;14(5):510-512]©Elsevier Editora Ltda.

Abacavir is a nucleoside reverse-transcriptase inhibitor, with few drug interactions and a favorable safety profile, therefore commonly used in combination with other antiretroviral agents as part of highly active antiretroviral treatment regimens. Approximately 5%-8% of human immunodeficiency virus (HIV) infected patients on abacavir treatment develop a hypersensitivity reaction (HSR) which usually occurs within the first 6 weeks of treatment.¹ HSR is characterized by a multisystemic involvement that becomes more severe with continuous dosing, requiring discontinuation of the drug. Subsequent rechallenge with abacavir must be avoided, since there is a high risk of developing a life threatening reaction.² HSR incidence varies among different populations, being more common in Caucasian patients than in those of African or Asian origin.³ Abacavir HSR occurs in approximately 4% of Chilean HIV-infected patients (Chilean AIDS Cohort, Carlos Beltran personal communication).

Since 2002, many studies have reported a strong association between abacavir HSR and the class I human leukocyte antigen (HLA) allele B*5701.^{4,5}

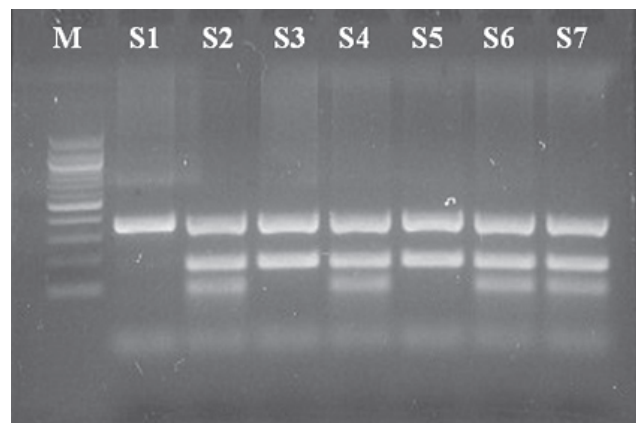
More so, the utility of HLA-B*5701 screening as a pharmacogenetic test to predict the risk of developing HSR has been demonstrated in abacavir-naïve HIV-infected patients.^{6,7} The incidence of HSR may vary substantially among different populations due to the heterogeneity in HLA-B*5701 prevalence across distinct ethnicities.^{3,8} Ideally, before implementing HLA-B*5701 genetic screening in a given population, its prevalence should be ascertained, particularly if it is unknown or poorly described, as in the Chilean population. Serological methods for HLA-B57 detection lack specificity and commercial molecular HLA-B typing methods used for identifying all known HLA-B alleles are expensive and laborious. Therefore the implementation of an alternate cost-effective method for HLA-B*5701 screening would be an appropriate approach to reduce the risk of developing HSR in the HIV positive patient population that is going to receive abacavir treatment. The aim of this study was to determine HLA-B*5701 carrier frequency in Chilean general population and HIV-infected patients after implementing a typing method for HLA-B*5701 screening.

In order to validate the method for B*5701 typing, 28 External Quality Control DNA samples from the College of American Pathologists (CAP) HLA-typing survey (2 B*5701 positive, 26 B*57 negative), and 71 B*57 positive Chilean subjects (previously typed by a low resolution commercial method) were analyzed. To establish the B*5701 carrier frequency in Chilean general population, 300 unrelated anonymised blood bank donors (150 men and 150 women) were typed. After implementation and validation of HLA-B*5701 molecular typing for routine clinical testing, 492 HIV-positive abacavir-naïve patients were referred to our laboratory for HLA-B*5701 screening prior initiating abacavir treatment. DNA from all samples was extracted by a standard “salting out” method.

A touch down PCR was implemented using the sequence specific primers (SSP) and conditions described by Martin *et al.*⁹ with minor modifications. The forward primer was synthesized without the M13 sequence tag, and the PCR cycling conditions were slightly different as the first four cycles at 70°C annealing temperature were omitted. Also, a different target (the alpha-1-antitrypsin gen, AAT) was used as internal control of amplification, using the primers AAT3 (5'-CCC ACC TTC CCC TCT CTC CAG GCA AAT GGG-3') and AAT4 (5'-GGG CCT CAG TCC CAA CAT GGC TAA GAG GTG-3') at a final concentration of 2.5 µM. PCR products were subjected to electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under UV light. This multiplexed SSP-PCR allows the identification of alleles carrying the generic B*57 and those with the subtype B*5701 by their amplicon sizes: the HLA-B*57 group-specific primer pair amplifies a 175 bp fragment, HLA-B*5701-subtype specific primers a fragment of 94 bp, and the internal control primers a 360 bp fragment. Given that this method only assesses the presence or absence of B*57 and B*5701 alleles, zygosity status could not be determined by this mean. Therefore a low resolution commercial typing method (Peel-Freez®SSP-UniTray® Invitrogen™) was used to determine homozygosity or heterozygosity in the B*5701 positive samples. Allele and carrier frequencies for HLA-B*5701 were determined by direct gene counting and the data from both populations (*i.e.* general population and HIV patients) were analyzed by using the Chi-square test (MINITAB®14).¹⁰

The results obtained with the external quality control samples were 100% concordant with those reported by the CAP, and all 71 B*57 samples previously typed were correctly identified by the group-specific primers. Thus, this SSP-PCR method was validated for routine analysis. See Figure 1 for typical results. The B*5701 allele was present in 64 of 71 (90%) B*57 positive subjects, the 7 remaining (10%) carried a different subtype. In the general population, from a total of 300 subjects analyzed, 14 individuals (4.7 %) carried a B*57 allele, from which 11 (3.7 %) were B*5701, and 3 (1.0%) had another subtype. The remaining 286 were negative for the

Figure 1: Typical SSP-PCR results. M: 100 bp DNA size marker, S1: negative control for B*57 and B*5701 alleles, S2, S4, S6, and S7: samples positive for the B*5701 allele, S3 and S5: sample positive for B*57 group-specific amplification, carrying a different subtype.



presence of this allele. All positive samples were heterozygotes, thus an allele frequency of 1.8 % for the B*5701 allele, and of 0.5 % for the B*57 alleles with other subtypes was determined. Eleven (2.2%) out of 492 HIV patients were positive for the generic allele B*57, all of them were of the subtype B*5701 and heterozygotes for this allele as determined by low resolution typing. None of the B*5701 positive HIV patients was subsequently treated with abacavir. The results are summarized in Table 1. The observed B*5701 carrier and allele frequencies were higher in the general population sample than in HIV-infected patients. The statistical analysis performed using the Chi-square test to determine if this difference between both populations was noteworthy, revealed that it was not significant ($p < 0.05$).

A prospective screening genetic test should fulfill several requirements before its introduction into routine clinical practice, such as: improve the clinical outcome, have high predictive values, be cost-effective, and easy to implement technically.³ The clinical utility of B*5701 screening is well demonstrated, so technical aspects and feasibility of an analysis method were important to be evaluated. The PCR-based technique implemented here for HLA-B*5701 screening proved to be 100% sensitive in detecting B*5701 and B*57 positive alleles. It was also a method easy to implement and to validate, as well as technically simple to perform in a clinical routine setting. More so, an “in house” PCR, properly validated as this one, allows cost reduction when compared to commercial kits, thus allowing its use also in developing countries where less financial and technical resources are available.

The observed B*5701 carrier frequency (3.7%) in this general population sample from Chile is in agreement with data (4.0%) from 70 Chilean individuals published at the “allele frequencies in worldwide populations” database.¹¹

Table 1. HLA-B*57, B*5701 and other subtypes carrier and allele frequencies in Chilean population

	General Chilean population n = 300			Chilean HIV patients n = 492		
	n	Carrier frequency	Allele frequency	n	Carrier frequency	Allele frequency
B*57 (generic)	14	4.7%	2.3%	11	2.2%	1.1%
B*5701 (allele specific)	11	3.7%	1.8%	11	2.2%	1.1%
B*57__ (other subtype)	3	1.0%	0.5%	0	-	-

However, in this database there is no information about the status of those Chilean individuals (*i.e.* patients, controls or general population). Also, the B*57 carrier frequency published therein (1.0%) is much different than the one observed by us (4.7%), and unlikely to be correct, since the generic type of an allele cannot have a lower frequency than one of its subtypes. The fact that the B*5701 frequencies we observed in the general population and in the patient population samples are lower than the ones reported for Caucasians and higher than those in populations of Asian origin, is most likely due to our mixed genetic background of Caucasian and Native American of mongoloid ancestry.^{12,13}

To our knowledge, there are no B*5701 carrier or allele frequencies described for HIV-positive patients from other Latin American countries, except for Hispanics in the United States.³ The carrier frequency found in our HIV-infected patients (2.2%) and in Chilean general population (3.7%) were very similar to the ones reported for US-Hispanics.⁸ The difference between HIV-infected patients and general population, although not statistically significant, could probably be related to the finding that HLA-B*5701 carriage has been associated with slow progression of HIV infection, therefore less patients carrying a HLA-B*5701 allele would require antiretroviral therapy and thus B*5701 typing.^{14,15} Considering the vast evidence on the benefits of B*5701 screening, its prevalence in the Chilean general population, and the availability of a proper screening method, B*5701 typing in Chilean HIV-infected patients about to initiate abacavir treatment is strongly recommended.

REFERENCES

- Lucas A, Nolan D, Mallal S. HLA-B*5701 screening for susceptibility to abacavir hypersensitivity. *J Antimicrob Chemother* 2007; 59:591-3.
- Hughes AR, Spreen WR, Mosteller M *et al.* Pharmacogenetics of hypersensitivity to abacavir: from PGx hypothesis to confirmation to clinical utility. *Pharmacogenomics J* 2008; 8:365-74.
- Phillips EJ. Genetic screening to prevent abacavir hypersensitivity reaction: are we there yet? *Clin Infect Dis* 2006; 43:103-5.
- Mallal S, Nolan C, Witt C *et al.* Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* 2002; 359:727-32.
- Hetherington S, Hughes A, Mosteller M *et al.* Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. *Lancet* 2002; 359:1121-1122.
- Mallal S, Phillips E, Carosi G, *et al.* HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med* 2008; 358:568-579.
- Saag M, Balu R, Phillips E *et al.* High sensitivity of human leukocyte antigen-B*5701 as a marker for immunologically confirmed abacavir hypersensitivity in white and black patients. *Clin Infect Dis* 2008; 46:1111-1118.
- Hughes A, Mosteller M, Bansal A *et al.* Association of genetic variations in HLA-B region with hypersensitivity to abacavir in some, but not all, populations. *Pharmacogenomics* 2004; 5:203-211.
- Martin A, Nolan D, Mallal S. HLA-B*5701 typing by sequence-specific amplification: validation and comparison with sequence-based typing. *Tissue Antigens* 2005; 65:571-574.
- Minitab® Statistical Software: <http://www.minitab.com> (last accessed 01/09/09).
- New Allele Frequency Database: <http://www.allelefrequencies.net>. Middleton D., Menchaca L., Rood H., Komerofsky R. *Tissue Antigens* 2003; 61:403-407.
- Encina F. Historia de Chile. Capitulo III. Santiago, Chile: Sociedad editora Revista Ercilla, 1983.
- Cifuentes L, Morales R, Sepúlveda D *et al.* DYS19 and DYS199 loci in a Chilean population of mixed ancestry. *Am J Phys Anthropol* 2004; 125:85-89.
- Miguel SA, Sabbaghian MS, Shupert WL *et al.* HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. *Proc Natl Acad Sci USA* 2000; 97:2709-2714.
- Horton H, Frank I, Baydo R *et al.* Preservation of T cell proliferation restricted by protective HLA alleles is critical for immune control of HIV-1 infection. *J Immunol* 2006; 177:7406-15.