title

Genetic detection of HLA-B*5701 allele for prediction of Abacavir hypersensitivity among HIV-positive patients in Polish population

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summary

Abacavir (ABC) is a potent nucleoside reverse transcriptase inhibitor used in combined antiretroviral therapy (cART) of HIV-positive patients. However, 5 to 8% of patients manifest hypersensitivity reaction to ABC (ABC HSR) during first 6 weeks after therapy initiation. ABC HSR can be fatal if therapy with ABC is continued or ABC is restarted. There is an association between ABC HSR occurrence and a carriage of the Major Histocompatibility Complex class I allele HLA-B*5701. Genetic screening, before ABC initiation, significantly reduces a risk of developing ABC HSR. In accordance with European AIDS Clinical Society's guidelines Molecular Diagnostics Laboratory has been testing towards HLA-B*5701 since 2008.

key words

abacavir, hypersensitivity, HSR, HLA-B*5701, SSP, SBT

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Background

Hypersensitivity reaction (ABC HSR) is associated with initiation of abacavir (ABC), a potent HIV-1 reverse transcriptase inhibitor, as a part of antiretroviral therapy in HIV+ patients (1). Hypersensitivity reaction can be fatal if therapy with ABC is continued or ABC is restarted. The clinical classification of ABC hypersensitivity includes at least two symptoms of fever, rash, nausea, vomiting, headache, respiratory and gastrointestinal symptoms, lethargy, myalgia or arthralgia if they occur less than 6 weeks after exposure and resolve within 72 h of withdrawal of the drug (2). According to worldwide data hypersensitivity reaction affects 5 to 8% of patients (3). In the year 2002, an association between a diagnosis of ABC HSR and carriage of the Major Histocompatibility Complex class I allele HLA-B*5701 was reported independently by two research groups (4, 5). Mallal et al has proposed that in white race genotyping for HLA B*5701 should be performed before prescription of ABC. Improvement of the diagnostics with such testing has resulted in a reduction in the incidence of ABC HSR (4). PREDICT-1 study demonstrated that the presence of the allele HLA-B*5701 had a positive predictive value of 61.2% and a negative predictive value of 95.5% (6). Genetic screening highly reduces the risk of developing ABC HSR (7), but there are evidences that ABC HSR can also develop in HLA B*5701 negative patients (8,9). It seems that not only one genetic marker (HLA-B*5701 allele) is responsible for developing of HRS in HIV+ patients. Mallal et al has proved that presence of HLA-DR7 and HLA-DQ3 alleles along with HLA-B*5701 allele had a positive predictive value for hypersensitivity of 100%, and a negative predictive value of 97% (4).

Genetic screening for HLA-B*5701 typing before ABC initiation is cost-effective in the case of white race (10). In some ethnic groups HLA-B*5701 is not associated with hypersensitivity (11). Park et al had tested 534 Korean patients with HIV infection, no patients had the HLA-B*5701 allele (12). In the group of 320 Taiwanese HIV+ patients there was only one case of HLA-B*5701 presence (13).

The molecular techniques based on PCR are the most sensitive and accurate methods for detecting HLA-B*5701 allele (14), but new alternative tests are introduced, i.a. flow cytometry method (15).

European AIDS Clinical Society (www.europeanaidsclinicalsociety.org) guidelines recommend HLA-B*5701 testing for HIV positive patients at initial visit; U.S. Department of Health and Human Services (www.hhs.gov) recommends HLA-B*5701 testing when ABC use is being considered.

Since the year 2008 our laboratory has been testing HIV-infected patients for the presence of HLA-B*5701 allele. Initially we used sequence based typing method (PCR-SBT, Atria Genetics). SBT is a very accurate and specific technique enabling detection of new alleles, but is also relatively expensive, laborious and time-consuming. Currently we use a method based on PCR with sequence specific primers (PCR-SSP, Inno-Train, Olerup) which is much cheaper and faster. Detection and analysis of PCR product is performed in agarose gel electrophoresis. The detection of HLA-B*5701 is of great clinical value and determines the frequency of presence of this allele in Polish population.

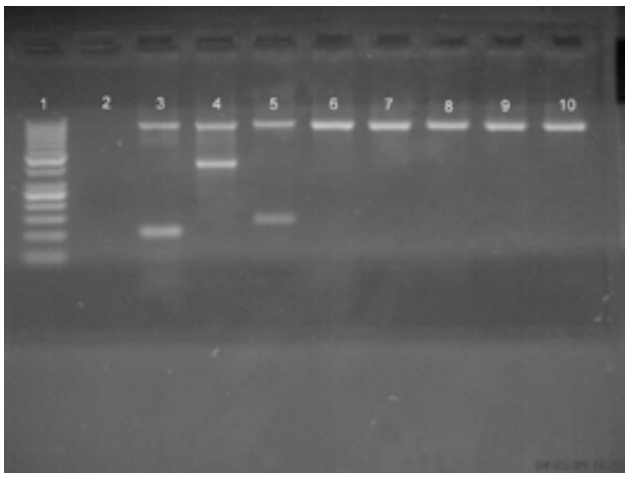
Materials and methods

Blood samples were obtained from HIV-infected patients before ABC-containing therapy was initiated or during first visit. All blood samples were collected from patients of Pediatrics Ward of Warsaw Medical University and Outpatient Clinics Of Warsaw Hospital for Infectious Diseases. Among Pediatrics Ward's patients 70% live outside of Warsaw, among these attending Outpatient Clinics – 25% (dr M. Szczepanska-Putz and dr I. Cielniak – personal information). Genomic DNA was isolated from whole blood by spin column method (NucleoSpin Blood, Macherey-Nagel). Quality of isolated genomic DNA was evaluated in agarose gel electrophoresis. Next, DNA was amplified by PCR (Perkin-Elmer GeneAmp 9600) according to manufacturers' protocols (Inno-Train HLA-B Ready Gene B5/57 cross PCR-SSP, low resolution, Atria Genetics). Finally, PCR products were analyzed as above (PCR-SSP method) or sequenced in ABI Avant 3100 (PCR-SBT, Atria Genetics). Samples suspected for the presence of HLA-B*5701 allele in PCR-SSP low resolution test (Inno-Train) were retested with PCR-SSP high resolution test (Olerup HLA-B*5701 SSP).

Results

Until now 238 patients have been tested for HLA-B*5701 presence – 168 (70,5%) males and 70 (29,5%) females. Majority of tested patients were Caucasian race (98,7%), except 3 patients (Asian, Black and Arab). Twenty patients were tested with PCR-SBT method, the rest of them with PCR-SSP method. Eleven out of 238 (4,6%) patients were positive in low resolution and were further diagnosed by high resolution testing (Fig. 1).

Figure 1. Example of electrophoretic analysis of PCR-SSP product (Inno-Train)



Lane 1 – marker, Lane 2 – negative control, Lanes 3 – 10 – PCR SSP product and internal control

Among these patients, in 10 out of 11 cases the HLA-B*5701 allele was confirmed in PCR-SSP high resolution test; one patient carried HLA-B*5714 allele. Among HLA-B*5701 positive patients there were 9 men. None of non-Caucasian race patients was HLA-B*5701 positive.