Introduction of pharmacogenetic screening for the human leucocyte antigen (HLA) B*5701 variant in Polish HIV-infected patients

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Objective
Prospective pharmacogenetic screening for the human leucocyte antigen (HLA) B*5701 allele can significantly reduce the number of cases of abacavir-related hypersensitivity among HIV-infected patients treated with this drug. The aim of this study was to establish the frequency of the HLA B*5701 variant in HIV-infected Poles.

Methods
The sequence-specific primer (SSP) test was used to assess the feasibility of the introduction of such testing in clinical practice. For this purpose, 234 randomly selected HIV-positive patients were screened using a low-resolution SSP assay, with HLA B*5701-positive results confirmed using a high-resolution test.

Results and Conclusions
The HLA B*5701 variant was found in 11 of 234 subjects (4.7%). Testing with the selected method proved quick and reliable.

Keywords: pharmacogenetics, abacavir hypersensitivity, HLA B*57, antiretroviral treatment

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Introduction
Despite extensive research in the field of pharmacogenetics, routine genetic marker testing for clinical purposes is not common. One successful example of the implementation of such a test into practice is human leucocyte antigen (HLA) B*5701 testing among people living with HIV, prior to the introduction of the nucleoside reverse transcriptase inhibitor abacavir to antiretroviral treatment. The drug was associated with hypersensitivity reactions (HSRs), which were noted in up to 8% of Caucasian individuals after challenge with the drug [1]. Hypersensitivity can occur within 6 weeks of treatment initiation and most commonly manifests clinically as fever, rashes, respiratory and gastrointestinal symptoms or malaise/lethargy [2]. The symptoms resolve quickly, within 72 hours of drug discontinuation. Re-challenge with the drug in hypersensitive individuals can be fatal, with acute anaphylaxis and hypotension [3].

The hypothesis of a genetic factor-based mechanism for abacavir hypersensitivity was proposed and supported in 2002 by two independent research groups [4,5]. The presence of the HLA B*5701 variant was associated with increased risk of HSR development, which was confirmed in numerous studies [6–9]. Prospective screening was found to significantly reduce the number of HSRs noted, with HLA B*5701 testing having an overall positive prognostic value for clinically diagnosed HSRs of 61.2%, while the negative prognostic value was 95.5% [6].

Many countries introduced prospective HLA B*5701 testing as the standard of care for HIV-infected patients, and this has been particularly successful in Australia and the United Kingdom, allowing reductions in the number of adverse reactions observed, improvements in adherence to therapy and reductions in the number of abacavir discontinuations [10,11]. Testing is cost effective, especially in populations with higher frequencies of the HLA B*5701 allele (e.g. Caucasian populations), allowing reductions in costs related to HSR treatment [12]. For such populations, on
average, only 14 tests would result in the prevention of one case of abacavir HSR [13]. HLA B*5701 testing is included in the European AIDS Clinical Society guidelines for clinical management and treatment of HIV-infected adults in Europe, with abacavir contraindicated if an individual tests positive for this variant (available online at www.eacs.eu).

To avoid costly and time-consuming high-resolution sequencing, screening can be based on the sequence-specific amplification technique. This approach reduces both the cost of the test and the time needed to obtain results [14]. As validated tests become available, it might be expected that this field will develop rapidly in the near future.

In this study, we tested the HLA B*5701 allele frequency in a cohort of 200 HIV-positive individuals from the West Pomeranian region of Poland by means of sequence-specific primer (SSP) polymerase chain reaction (PCR) technology. The aim of the study was not only to provide allele frequency data for this group but also to determine the feasibility of widespread clinical implementation of genetic testing for this pharmacogenetic factor in Poland.

**Material and methods**

**Subjects**

The study group consisted of 234 randomly selected patients with confirmed HIV infection attending the Clinic for Acquired Immunodeficiency Treatment, Department of Infectious Diseases and Hepatology, Szczecin, Poland. Most of the individuals tested were male [male, 169 (72%); female, 65 (28%)]. The mean age (± standard error) of the studied individuals was 40.9 ± 9.5 years (median 39 years). As the majority of patients attending the clinic are of Caucasian origin (99.9%), for this study only Caucasians were selected. All participants voluntarily consented to participate in the study.

**DNA extraction and HLA typing**

Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) from whole blood samples previously collected in tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant. The extraction was performed according to the manufacturer’s protocol. DNA was resuspended in 200 μL of AE buffer (Qiagen) and stored at −20 °C for further analyses.

For HLA B*5701 screening, the SSP HLA-Ready Gene B5/57 Cross low-resolution kit (Inno-Train Diagnostik, Kronberg, Germany) was used to perform an in vitro diagnostics validated, European Economic Area conformity mark (CE) marked test, according to the manufacturer’s protocol. PCR products were electrophoresed on a 3% agarose gel (Sigma, St. Louis, MO, USA) stained with Gel-Star dye (Lonza, Rockland, Switzerland). Results were visualized under UV light (Transilluminator 4000; Stratagene, La Jolla, CA, USA) and recorded with a DS-34 Polaroid Direct Screen Camera.

Additionally, all B*57-positive samples were verified using another CE marked assay performed using the Olerup SSP HLA-B* 57 high-resolution kit (Olerup SSP AB, Saltsjoebaden, Sweden), with subsequent electrophoresis and recording as described above.

**Results**

In the studied group of 234 HIV-1-infected patients, 13 of 234 subjects (5.6%) tested positive for HLA B*5701 in the low-resolution test (corresponding to serological type B57). The results were confirmed by the high-resolution test for 11 of these subjects (4.7%), while one individual was found to carry the HLA B*5703 variant and one patient B*5306. Six of the individuals (54.6%) carrying the HLA B*5701 allele were male.

Example agarose gels demonstrating the presence of the HLA B*5701 variant are shown in Figs 1 and 2.

**Discussion**

The HLA B*5701 allele frequency found in the HIV-1-positive group in this study is higher than the frequency
antiretroviral drugs, or whether another immunological mechanism is involved. The prognostic value of such screening was also noted in a study by Waters et al., [11] with a reduction in HSR from 7.5% prior to the introduction of testing to 2% after the testing was introduced. However, it should be noted that in one case an HLA B*5701-negative individual developed a strong HSR, which was confirmed immunologically by skin-patch testing. Such an event may suggest the involvement of additional immunological mechanisms in the development of symptoms; therefore, even if an individual is negative for HLA B*5701, counselling regarding HSR symptoms is necessary.

In a study by Saag et al., [19] based on retrospective patient record analysis with identification of patients with the skin patch test confirmed abacavir HSR in subsequent HLA B*5701 testing 100% sensitivity in a white population was observed. When HSRs were observed clinically but were immunologically unconfirmed, the sensitivity decreased to 44%, but the specificity remained high at 96%. This study confirms the need for and validity of HLA B*5701 testing in clinical practice. Costs and the time required to provide a valid result must also be considered. Results obtained using SSP assays have been shown to be concordant with those obtained by sequencing [6,14]. The necessity for adequate quality assurance must be emphasized, as the test result is of vital importance not only for HSR risk reduction but also from the perspective of therapeutic options available for the patient [20]. For maximum accuracy, low-resolution HLA B*5701 results should be confirmed with a high-resolution assay using a kit obtained from a different manufacturer, with only confirmed results provided to clinicians. In our opinion, such an approach provides good sensitivity and specificity of the results obtained. The cost of such testing is approximately eight-to-10-times lower than that of testing by HLA-B sequencing or PCR-SSP-based investigation of the entire B locus.

To summarize, we believe that HLA B*5701 testing based on the SSP test, with positive results confirmed by an alternative, high-resolution test, is specific, accurate, fast and cost effective. As it could reduce the number of abacavir HSRs, widespread use of this testing strategy in HIV-positive patients should be encouraged.

Conclusions

- Prospective (prior to the introduction of abacavir-containing therapy) genetic HLA screening for B*5701 in HIV-infected individuals in Poland is feasible and should be performed on a regular basis.
- Sequence-specific testing for HLA B*5701 has been shown to be quick and reliable, especially where low-resolution results are confirmed in a high-resolution test.
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