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## Abstract

**Objectives:** The *HLA-B\*5701* allele is highly predictive of Abacavir (ABC) hypersensitivity reactions (HSR). However, HLA-typing is relatively expensive, labor-intensive and requires specialized laboratories. We assessed the utility of a single nucleotide polymorphism in the HLA Complex P5 gene (*HCP5* SNP) in the setting of ABC-HSR as this SNP is in high linkage disequilibrium with *HLA-B\*5701*.

**Methods:** The *HCP5* T>G SNP (rs2395029) was determined in 108 participants of the Swiss HIV Cohort Study (SHCS) who had stopped ABC due to suspected ABC-HSR and in 175 ABC-tolerant controls. ABC-HSR-reactions were reassessed by two experienced HIV-physicians blinded to the genotyping results and classified as ABC-HSR-likely, uncertain and unlikely based on the presence of rash, fever, concomitant symptoms, onset of symptoms and co-medication use. In addition, we determined the *HCP5* SNP in a random sample of 820 SHCS-participants with available HLA-typing results. Genotyping of the *HCP5* SNP rs2395029 was done by TaqMan allelic discrimination. High-resolution HLA-typing was performed by sequence-based methods.

**Results:** The *HCP5* SNP was present in 34 of the 108 individuals with suspected ABC-HSR and 34/34 of these individuals carried the *HLA-B\*5701*-allele. One individual was homozygous for the rare allele (*HCP5*-GG) and also carried two copies of *HLA-B\*5701*. Conversely, none of the *HLA-B\*5701*-negative individuals carried the *HCP5* SNP. The *HCP5* SNP was significantly more frequent in the 25 individuals with likely ABC-HSR as compared to the 50 individuals with uncertain diagnosis, to the 33 with unlikely ABC-HSR and to 175 ABC-tolerant controls (80%, 28%, 3% and 2% respectively, p<0.001). Among 1103 individuals including the random sample (n=820), all 98 *HLA-B\*5701*-positive individuals carried the *HCP5* T>G variant. Only 6 (0.6%) of the 1005 *HLA-B\*5701*-negative individuals carried the *HCP5* SNP (1 of these carried the *HLA-B\*5703* allele). Overall, the sensitivity of the *HCP5* SNP for carriage of *HLA-B\*5701* was 100% (95% CI 96-100%) and the specificity 99% (95% CI 99-100%).

**Conclusion:** The *HCP5* SNP was highly associated with ABC-HSR. This SNP identified all *HLA-B\*5701*-positive individuals and was absent in 99% of *HLA-B\*5701* negative individuals. If the high sensitivity of this *HCP5* SNP genotyping for *HLA-B\*5701* can be confirmed in larger populations, it could serve as a simple and cheap screening tool for predicting ABC-HSR particularly in settings where high-resolution HLA-typing is not available.

## Background

- Abacavir (ABC) use is associated with drug hypersensitivity reactions (ABC-HSR) in 5-8% of ABC-receptants [1].
- Retrospective studies indicated a strong association of ABC-HSR with the presence of the major histocompatibility complex class I allele *HLA-B\*5701* [2;3].
- The usefulness of genetic screening for reducing the incidence of ABC-HSR has been demonstrated recently in predominantly Caucasian populations [4, 5].
- Screening for *HLA-B\*5701* before initiation of ABC-therapy is recommended in current guidelines (e.g. EACS Guidelines 2007) in settings where HLA typing is available.
- The current gold-standard for *HLA-B\*5701* screening is through sequence-based genotyping methods. However, its universal use is limited because the test requires specialised laboratories and is labor-intensive; in addition, its relatively high costs are not always covered by health insurances.
- Alternative HLA typing methods (e.g. Polymerase Chain Reaction Sequence-Specific Primer (PCR-SSP) assays, Flow Cytometry [6;7]) do not have 100% concordance with sequence-based HLA typing results and the tests cannot always differentiate between *HLA-B\*5701* and closely related HLA-B alleles not associated with ABC-HSR.
- Recently, several studies described a perfect linkage disequilibrium ( $r^2=1.0$ ) between the rs2395029 SNP in the HLA Complex P5 gene (referred herein as *HCP5* SNP) located 100kb centromeric from *HLA-B* on chromosome 6 and *HLA-B\*5701* [8,9].

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3) Mallat S, et al. Association between presence of HLA-B\*5701, HLA-DQB1, and HLA-DQA1 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. Lancet 2002 Mar 23;359(9308):727-32.  
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5) Mallat S, et al. PREDICT-1 results. Abstract WES2101, 4th IAS Conference, Sydney, Australia, July 22-25  
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8) de Bakker PI, et al. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. Nat Genet 2006 Oct;38(10):1166-72.  
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• *HCP5* SNP genotyping based on allelic discrimination can be performed by broadly used assays, allows high-throughput and cheap genotyping with high sensitivity and specificity, and is largely independent of the performance and interpretation by the laboratory personnel.

## Aims

To assess the pattern of linkage disequilibrium between *HCP5* SNP and *HLA-B\*5701* in a larger population and to analyze the utility of *HCP5* genotyping as an alternative marker that would allow cheaper and less labor-intensive screening for individuals at risk for ABC-HSR.

## Patients and Methods

1103 participants of the Swiss HIV Cohort Study (SHCS, www.shcs.ch) were included. All patients gave informed consent for genetic testing.

The specificity and sensitivity of *HCP5* genotyping for predicting ABC-HSR was assessed comparing individuals who had stopped ABC due to presumed HSR (n=108) and ABC-tolerant subjects (n=175). Individuals with presumed ABC-HSR were identified within the SHCS-database, which reports the reason for stopping antiretroviral therapy in all participants.

The clinical diagnosis of ABC-HSR was re-assessed by retrospective patient chart review based on ABC-HSR related symptoms (fever, rash, gastrointestinal, respiratory or constitutional symptoms), on onset of symptoms and on comedication use.

Two experienced HIV-clinicians (CF, CT) blinded to the HLA typing results independently classified suspected ABC-HSR on a scale between +3 (definitive ABC-HSR) and -3 (ABC-HSR highly unlikely). The mean score was used for analysis. Cases were classified as clinically unlikely ABC-HSR (mean score  $\leq -2$ ), clinically uncertain (mean score  $\geq -1$  &  $\leq +1$ ), and clinically likely (mean score  $\geq +2$ ). ABC-tolerance was defined as receiving ABC for at least 6 weeks without signs of ABC-HSR.

*HCP5* SNP genotyping was performed using custom TaqMan<sup>®</sup> SNP genotyping assays (Applied Biosystems, Switzerland) or by the HumanHap550 BeadChip (Illumina, San Diego, CA, USA) as described previously[9]. High-resolution HLA typing was performed by sequence-based methods.

## Results

**Table 1:** Overall correlation between the *HCP5* rs2395029 variant and the *HLA-B\*5701* allele. Sensitivity, specificity, positive and negative predictive values [95% confidence interval] refer to the *HCP5* SNP as a marker for *HLA-B\*5701*.

	HLA-B*5701 present	HLA-B*5701 absent
<i>HCP5</i> rs2395029 present	98	6
<i>HCP5</i> rs2395029 absent	0	999

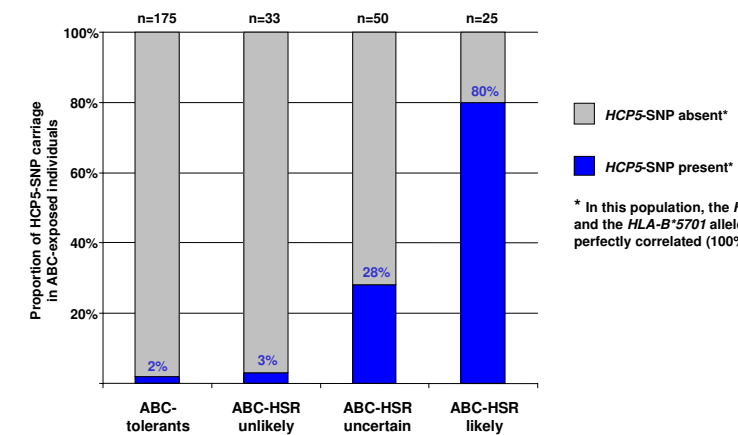
sensitivity = 100% [0.963-1.00]  
specificity = 99.4% [0.987-0.998]  
positive predictive value = 94.2% [0.879-0.979]  
negative predictive value = 100% [0.996-1.00]

The 6 discrepant results (*HCP5* SNP present, *HLA-B\*5701* absent) were confirmed by independent analysis. Of these, 1 individual carried the *HLA-B\*5703*-allele. For the additional five *HCP5*-discrepant tests, the associated HLA types were: *B\*1801-4901*, *B\*4102-7301*, *B\*0702-1501*, *B\*0702-4901*, *B\*4415-4415*.

We also assessed the HLA-alleles that are closely related to *HLA-B\*5701* (*HLA-B\*5702*, *HLA-B\*5703*, and *HLA-B\*5801*) for linkage with the *HCP5* SNP. The *HCP5* SNP was

found in one of sixteen *HLA-B\*5703*-positive individuals, but in none of the 27 *HLA-B\*5801*-positive individuals. There were no *HLA-B\*5702*-positive individuals in this cohort.

Figure 1 Proportion of *HCP5*-SNP carriage in 283 ABC-exposed individuals



\* In this population, the *HCP5* SNP and the *HLA-B\*5701* allele were perfectly correlated (100%)

## Conclusions

- The presence of the *HCP5* SNP showed very high concordance with *HLA-B\*5701*-carriage.
- In ABC-exposed individuals, the *HCP5* SNP was highly associated with ABC-HSR.
- If the high sensitivity of *HCP5* SNP genotyping for *HLA-B\*5701* and for ABC-HSR can be confirmed in other studies, it could serve as a simple and cheap screening tool for predicting ABC-HSR, particularly in settings where sequence-based high-resolution HLA-typing is not available.
- All *HLA-B\*5701*-positive individuals in our cohort carried the *HCP5* SNP. Nevertheless, we can not exclude the rare occurrence of *HCP5* SNP-negative, *HLA-B\*5701*-positive individuals at risk for ABC-HSR.
- It is important to note that neither the presence of the *HCP5* SNP nor of the *HLA-B\*5701*-allele identified all individuals with clinically likely ABC-HSR in our cohort. Clinicians should therefore be aware that genetic screening to assess the risk for ABC-HSR should never substitute for appropriate clinical vigilance in patients starting ABC treatment.

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