The HCP5 Single-Nucleotide Polymorphism: A Simple Screening Tool for Prediction of Hypersensitivity Reaction to Abacavir

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The HLA-B*5701 allele is predictive of hypersensitivity reaction to abacavir, a response herein termed “ABC-HSR.” This study of 1103 individuals infected with human immunodeficiency virus assessed the usefulness of genotyping a HCP5 single-nucleotide polymorphism (SNP), rs2395029, in relation to ABC-HSR. In populations with European ancestry, rs2395029 is in linkage disequilibrium with HLA-B*5701. The HCP5 SNP was present in all 98 HLA-B*5701-positive individuals and was absent in 999 of 1005 HLA-B*5701-negative individuals. rs2395029 was overrepresented in 25 individuals with clinically likely ABC-HSR, compared with its frequency in 175 ABC-tolerant individuals (80% vs. 2%, respectively; P<.0001). Therefore, HCP5 genotyping could serve as a simple screening tool for ABC-HSR, particularly in settings where sequence-based HLA typing is not available.

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The nucleoside-analogue reverse-transcriptase inhibitor abacavir (ABC) is a widely used antiretroviral drug. Although ABC has a favorable long-term toxicity profile, it is associated with hypersensitivity reaction—a response herein termed “ABC-HSR”—in 5%–8% of ABC recipients [1]. Retrospective studies indicate a strong association between ABC-HSR and the presence of the major histocompatibility complex (MHC) class I allele HLA-B*5701 [2, 3] in chromosome 6. The usefulness of genetic screening for the purpose of reducing the incidence of ABC-HSR has been demonstrated in white populations [4], and it has been confirmed in the large randomized PREDICT-1 trial [5]. In the latter study, the negative predictive value of HLA-B*5701 was 96% for clinically suspected ABC-HSR and 100% for immunologically confirmed ABC-HSR. Therefore, screening for HLA-B*5701 before initiation of ABC therapy is recommended in settings where HLA typing is available [5].

The current gold standard in screening for HLA-B*5701 is the sequence-based genotyping method. Its universal use is limited because it requires specialized laboratories and is labor intensive; in addition, its relatively high costs are not always covered by health insurance. Alternative HLA-typing methods include polymerase chain reaction sequence-specific primer (PCR-SSP) assay and flow cytometry [6, 7]. One report has suggested that sequence variation in HIV-1 reverse transcriptase be used as a marker of HLA-B*5701 carriage [8]. However, none of these alternative techniques shows 100% concordance with the results of sequence-based HLA typing, and they cannot reliably differentiate between HLA-B*5701 and closely related HLA-B alleles (e.g., HLA-B*5702, HLA-B*5703, and HLA-B*5801) that are not associated with ABC-HSR.

Recently, de Bakker at al. described a perfect linkage disequilibrium (r² = 1.0) between the rs2395029 SNP in the HLA complex P5 gene (HCP5) located 100 kb centromeric of HLA-B on chromosome 6 and HLA-B*5701 [9]; this degree of association also had been found by a previous genomewide association analysis [10]. Prompted by these findings, we assessed, in a larger population, the pattern of linkage disequilibrium between rs2395029 (herein also referred to as “HCP5 SNP”) and HLA-B*5701, and we analyzed the usefulness of HCP5 genotyping in providing an alternative marker that would allow cheaper and less labor-intensive screening of individuals at risk for ABC-HSR.

Patients and methods. The HCP5 SNP genotype and the HLA-B alleles were analyzed in 1103 participants in the Swiss HIV Cohort Study (http://www.shcs.ch). All patients gave informed consent for genetic testing. HCP5 genotyping was performed by use of either custom TaqMan SNP genotyping assays (Applied Biosystems) or the HumanHap550 BeadChip (Illumina), as we have described elsewhere [10]. High-resolution
HLA typing was performed by sequence-based methods, as described elsewhere [11].

The specificity and sensitivity of HCP5 genotyping for the prediction of ABC-<i>HSR</i> was assessed by comparison of ABC-tolerant subjects versus individuals whose ABC treatment had been discontinued because of presumed ABC-<i>HSR</i>. Individuals with presumed ABC-<i>HSR</i> were identified within the Swiss HIV Cohort Study database, which reports the reason for discontinuation of antiretroviral therapy in all participants. The clinical diagnosis of ABC-<i>HSR</i> was reassessed in 108 individuals, on the basis of standardized clinical criteria [1–3]. A diagnosis of ABC-<i>HSR</i> required that at least 2 of the following symptoms occur <6 weeks after initial exposure to ABC: fever, rash, and gastrointestinal (nausea or vomiting), respiratory, or constitutional symptoms. On the basis of the characteristics and the time at onset of these symptoms, as well as the use of comedication, 2 experienced HIV clinicians blinded to the HLA-typing results independently classified suspected ABC-<i>HSR</i> on a scale between +3 (definitive ABC-<i>HSR</i>) and −3 (ABC-<i>HSR</i> highly unlikely). The mean score was used for analysis; cases were classified as clinically unlikely ABC-<i>HSR</i> (mean score ≤ −2), clinically uncertain ABC-<i>HSR</i> (mean score ≥ −1 and ≤ +1), and clinically likely ABC-<i>HSR</i> (mean score > +2). ABC tolerance was defined as ABC treatment for ≥6 weeks without signs of ABC-<i>HSR</i>.

Results and discussion. Of the 1103 study participants, 98 were HLA-B*5701 positive, and 104 carried the HCP5 SNP (table 1). All HLA-B*5701–positive individuals were HCP5 SNP positive. The HCP5 SNP was present in 6 of 1005 HLA-B*5701–negative individuals. Discrepant results were confirmed by independent analysis. The sensitivity of the HCP5 SNP for the carriage of HLA-B*5701 was 100% (95% confidence interval [CI], 96%–100%); its specificity was 99% (95% CI, 99%–100%). In this study population, the HCP5 SNP had a negative predictive value of 100% (95% CI, 99%–100%), and a positive predictive value of 94% (95% CI, 88%–98%), for carriage of HLA-B*5701.

### Table 1. Overall correlation between the HCP5 rs2395029 single-nucleotide polymorphism and the HLA-B*5701 allele.

<table>
<thead>
<tr>
<th>HLA-B*5701 status</th>
<th>HCP5 rs2395029 status</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>98</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
<td>99.9</td>
<td></td>
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</table>

**NOTE.** Data are % of correlation between HCP5 rs2395029 minor allele and HLA-B*5701. The mean (95% confidence interval) values for the HCP5 single-nucleotide polymorphism as a marker for HLA-B*5701 are as follows: sensitivity, 100% (95.3%–100%); specificity, 99.4% (98.7%–99.8%); positive predictive value, 94.2% (87.9%–97.9%); and negative predictive value, 100% (99.6%–100%).

In the evaluation of the 6 discrepant results, we first assessed the HLA alleles that are closely related to HLA-B*5701 (i.e., HLA-B*5702, HLA-B*5703, and HLA-B*5801), for linkage disequilibrium with the HCP5 SNP. The HCP5 SNP was found in 1 of the 6 HLA-B*5703–positive individuals and in 0 of the 24 HLA-B*5801–positive individuals; there were no HLA-B*5702–positive individuals in this cohort. For the additional 5 tests that had results that were discrepant for HCP5, the associated HLA types were B*1801-4901, B*4102-7301, B*0702-1501, B*0702-4901, and B*4415-4415. It is expected that HCP5 SNP–positive HLA-B*5701–negative individuals will not be at risk for ABC-<i>HSR</i>, because carriage of HLA-B*5701 is necessary—although not sufficient—for susceptibility to immunologically confirmed ABC-<i>HSR</i> [5]. Indeed, since the completion of the current study, 3 patients with discordance (HLA-B*5701 negative and HCP5 positive) have started treatment with ABC and have not experienced HSR.

Of the 108 individuals whose treatment with ABC had been discontinued because of presumed ABC-<i>HSR</i>, the latter was classified as being clinically likely in 25 (23%), clinically unlikely in 33 (30%), and clinically uncertain in 50 (46%). In the subset of 283 ABC-exposed individuals, the HCP5 SNP and HLA-B*5701 were perfectly correlated ($r^2 = 1.0$) (table 2). The HCP5 SNP was significantly overrepresented in individuals with likely ABC-<i>HSR</i>, compared with it frequency in those with clinically uncertain or clinically unlikely ABC-<i>HSR</i> (80% vs. 28% and 3%, respectively; $P < .0001$, by $\chi^2$ test). Of the ABC-tolerant individuals, 2% carried the HCP5 SNP—a frequency that compares well with the results of the PREDICT-1 trial, which found that 2.4% of the individuals whom it studied were HLA-B*5701 positive and ABC tolerant [5].

Although the present study did not identify any HLA-B*5701–positive HCP5 SNP–negative individuals, such a discordance could potentially result in ABC exposure in patients who are at increased risk for ABC-<i>HSR</i>, if screening were based on HCP5 genotyping alone. Resequencing of the MHC region of 138 ABC-exposed individuals in a combined analysis of various

### Table 2. Frequency of carriers of the HCP5 single-nucleotide polymorphism in the subset of 283 abacavir (ABC)–exposed patients.

<table>
<thead>
<tr>
<th>Response to ABC</th>
<th>Frequency of HCP5 rs2395029</th>
</tr>
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<tbody>
<tr>
<td>Tolerance (n = 175)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Hypersensitivity reaction</td>
<td></td>
</tr>
<tr>
<td>Unlikely (n = 33)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Uncertain (n = 50)</td>
<td>14 (28)</td>
</tr>
<tr>
<td>Likely (n = 25)</td>
<td>20 (80)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients. In the overall population in the present study, the HCP5 single-nucleotide polymorphism and the HLA-B*5701 allele were perfectly (100%) correlated.
studies identified recombination events at multiple sites, suggesting that there is incomplete linkage disequilibrium between HLA-B*5701 and other MHC markers examined, including HCP5. 12. Specifically, the PREDICT-1 trial identified 2 HLA-B*5701–positive individuals who did not carry the HCP5 SNP; 1 of them experienced ABC-HSR (as evidenced by clinical symptoms and a skin-patch test reaction positive for ABC), and the other 1 was excluded, because of HLA-B*5701 status, from treatment (A. R. Hughes, personal communication).

The possibility of discordance between the HCP5 SNP and HLA-B*5701 should be discussed in the context of (1) the reliability of HLA-typing results in routine settings, (2) the general availability of HLA typing in the various countries in which the studies are conducted, (3) turnaround time, and (4) cost considerations. Sequence-based HLA typing remains the gold standard for identification of HLA-B*5701; however, its widespread use is limited by relatively high costs and by the need for specialized laboratories. A recent quality assessment and proficiency testing of 7 laboratories showed accurate reporting of HLA-B*5701 status by PCR-SSP [13]. Although the interlaboratory variance did not affect the accuracy of PCR-SSP, inspection of the agarose-gel images provided by the various laboratories illustrates the necessity for thorough quality-control procedures. Flow-cytometry assays [7] offer a cheap alternative with a short turnaround time; however, because they cannot reliably differentiate between HLA-B*5701 and closely related HLA alleles, subsequent molecular HLA typing is necessary. Variation in the HIV-1 sequence provides a cheap way to identify HLA-B*5701, but its positive predictive value in this regard is only 20% [8].

HCP5 SNP genotyping based on allelic discrimination offers several advantages over other approaches to HLA-B typing. Various broadly used technologies (e.g., Taq Man platforms) allow the standardized identification of 2 distinct sequences in 1 reaction tube, limiting the risk of contamination and allowing high-throughput genotyping that has high sensitivity and specificity. In addition, the test is largely independent of both the performance of and interpretation by laboratory personnel. SNP genotyping is also less time consuming and cheaper than sequence-based HLA typing, and it does not require specialized laboratories.

In conclusion, the presence of HCP5 rs2395029 shows very high concordance with HLA-B*5701 positivity, and, in ABC-exposed individuals, the HCP5 SNP is highly associated with ABC-HSR. If the high sensitivity that HCP5 SNP genotyping has for both HLA-B*5701 and ABC-HSR can be confirmed by other studies, this method could serve as a simple and cheap screening tool for the prediction of ABC-HSR, particularly in settings where sequence-based high-resolution HLA typing is not available. However, it is important to note that, in the present study’s cohort, neither the presence of the HCP5 SNP nor the presence of the HLA-B*5701 allele identified all individuals with clinically likely ABC-HSR. Clinicians should therefore be aware that genetic screening to assess the risk for ABC-HSR should never be considered to be a substitute for appropriate clinical vigilance regarding patients who are starting ABC treatment.


**Acknowledgments**

We thank Simon Mallal, Mina John, and David Nolan (Centre of Clinical Immunology and Biomedical Statistics, Perth, Western Australia), for HLA typing and helpful comments on the manuscript; Arlene R. Hughes, for providing genetical information from the GlaxoSmithKline-data set; the patients, for participation; and the physicians and study nurses of all clinical centres, for excellent patient care.

**References**

