**CNV and incomplete linkage disequilibrium interfere with the HCP5 genotyping assay for Abacavir hypersensitivity**

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**Abstract**

Introduction: Abacavir sulfone is an effective antiretroviral drug used to manage HIV infection, but 5-8% of patients develop abacavir hypersensitivity reaction (ABC-HSR). ABC-HSR is a life threatening condition that is ethnic-dependent and associated in the human leukocyte antigen HLA-B*5701 allele. Current guidelines for antiretroviral treatment recommend screening for HLA-B*5701 prior to initiating abacavir therapy. However, HLA typing or sequencing remains prohibitively expensive for routine screening. In Caucasians a SNP (rs2395029) in the major histocompatibility complex (MHC) Class I human leukocyte antigen (HLA) specifically the HLA-B*5701 allele [2]. Current guidelines for antiretroviral treatment recommend screening for HLA-B*5701 prior to initiating abacavir therapy [3]. However, HLA typing or sequencing remains prohibitively expensive for routine screening. In Caucasians a SNP (rs2395029 T>G) in the HLA complex P5 (HCP5) gene, is reported to be in linkage disequilibrium (LD) (r2=1) with the HLA-B*5701 allele. Genotyping for HCP5 has been increasingly adopted as a simple, inexpensive method to screen for ABC-HSR.

**Materials and Methods**

**Methods**

The 1,888 DNA samples used in this study were submitted to ARUP Laboratories (Salt Lake City, UT). PCR primers amplify a 122 bp product that includes the position of the SNP. A fluorescent-labeled probe and an LC-Red 640-labeled probe hybridize side-by-side on the PCR product to generate a fluorescent signal through FRET. The presence of the variant HCP5-G (minor) allele produces a melt peak at higher melting temperature than the major HCP5-T allele.

**HCP5 SNP genotyping by melting curve analysis**

The HCP5-T allele was detected using PCR primers that amplified a 94 bp product as previously published. [8,9]

**Copy number variation (CNV) analysis**

CGH microarray was used to characterize suspected CNVs in the HCP5 region. Sample DNA was labeled with 5'-Cy3 tagged nanomers while the control was labeled with Cy5 nanomers (Roche NimbleGen, Madison, WI). After purification, labeled patient and reference DNA were combined. The mixture was hybridized to a NimbleGen 270K custom-designed array on which 5872 tiled probes with a mean spacing of 15 bp spanned a large sample of genomic CNV and the possibility that linkage disequilibrium between the HLA-B*5701 and HCP5 SNP may vary between ethnicities is a concern. The possibility of incomplete disequilibrium and CNV should be considered, particularly when HCP5 genotyping is performed in patients who are not of European ancestry.

**Results**

Detection of T/T, T/G and G/G HCP5 SNP genotypes using the LightCycler FRET Probes assay is shown in Figure 2, and detection of the HLA-B*5701 allele by PCR SSP and melting analysis is shown in Figure 3.

**Conclusions**

Consistent with prior reports, we found a good overall concordance between the two markers; however, the LD was incomplete. In accordance with previously published studies, we found samples negative for HLA-B*5701 but positive for the HCP5-G (minor) allele, and one sample that was HLA-B*5701-positive and homozygous for the HCP5-T (major) allele. Importantly, the discovery that the HCP5 SNP is located within a CNV that is deleted with some frequency in certain populations raises concerns about its use in non-Caucasian populations, and suggests additional studies of LD between HLA-B*5701 and HCP5 are warranted.

**References**


**TABLE 1:** HCP5 rs2395029 T>G and HLA-B*5701 Genotype Correlation

<table>
<thead>
<tr>
<th>HCP5 rs2395029 T&gt;G</th>
<th>HLA-B*5701 positive</th>
<th>HLA-B*5701 negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/T</td>
<td>1</td>
<td>1766</td>
<td>1767</td>
</tr>
<tr>
<td>T/G</td>
<td>108</td>
<td>9</td>
<td>117</td>
</tr>
<tr>
<td>G/G</td>
<td>2</td>
<td>0</td>
<td>2</td>
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<tr>
<td></td>
<td></td>
<td>111</td>
<td>1777</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1888</td>
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</table>

The analytical sensitivity and specificity of the HCP5 SNP for the detection of HLA-B*5701 was estimated to be 99% [95% confidence interval (CI): 99.4%-99.9%], and 99% [95% confidence interval (CI): 0.99%-0.998%] respectively.