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

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Abstract

Introduction: Abacavir sulfate 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 with 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 P5 (*HCP5*) gene, is reported to be in linkage disequilibrium (LD) (r^2=1) with the *HLA-B*5701* allele. Genotyping for *HCP5* has been increasingly adopted as a simple, inexpensive method to screen for ABC-HSR. **Objective:** In this study we evaluated the genotype concordance between the *HCP5* SNP and *HLA-B*5701* allele in a large sample set. **Methods**: 1,888 DNA samples were genotyped for the *HCP5* polymorphism by real-time hybridization probe assay and for the *HLA-B*5701* allele by polymerase chain reaction with sequence specific primers (PCR SSP). **Results**: Overall, a good correlation between the two genotyping methods was found with analytical sensitivity (0.99%) and specificity (0.99%). Interestingly, the *HCP5* SNP could not be amplified in two samples, both negative for *HLA-B*5701*. Further investigations with a custom comparative genomic hybridization (CGH) array demonstrated that both samples were homozygous for large deletions that encompassed the *HCP5* gene. The Database of Genomic Variants shows that the *HCP5* gene locus is the site of a recurrent copy number variation (CNV) that does not extend to the *HLA-B* locus. In addition, ten samples with discordant genotyping results between the two methodologies were identified. These included: nine samples *HLA-B*5701* negative/*HCP5* positive, and one sample *HLA-B*5701* positive/*HCP5* negative. **Conclusions**: *HCP5* genotyping results showed a good correlation with *HLA-B*5701* typing by PCR SSP. However we have detected two samples with homozygous deletions of the *HCP5* gene and found the locus is the site of a CNV that does not include the *HLA-B* locus. The fact *HCP5* occurs within a region of 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 considered, particularly when *HCP5* genotyping is performed in patients who are not of European ancestry.

Background

Abacavir sulfate is a nucleoside reverse transcriptase inhibitor (NRTI) used in combination with other antiretrovirals in the treatment of HIV infection. However in the first six weeks of treatment approximately 5-8% of Caucasians and 2-3% of African Americans can develop a serious and sometimes fatal hypersensitivity reaction to Abacavir (ABC-HSR) [1]. ABC-HSR has been strongly associated with 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 (rs2395029T>G) in the HLA complex P5 (*HCP5*) gene, is reported to be in strong LD with the *HLA-B*5701* allele [4]. Several studies have shown the *HCP5*-G (minor) allele is 99.9% predictive for the presence of the *HLA-B*5701* allele, and genotyping for this SNP has been increasingly adopted as a simple, inexpensive method to screen for susceptibility to ABC-HSR [5-7].

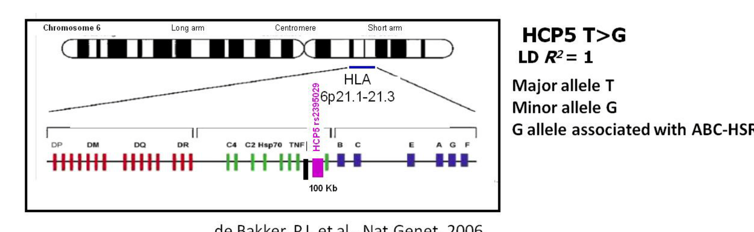

FIGURE 1: HLA and *HCP5* T>C SNP map

Materials and Methods

Samples
The 1,888 DNA samples used in this study were submitted to ARUP Laboratories (Salt Lake City, UT) for routine clinical *HLA-B*5701* genotyping. They were received from throughout the United States, but no information was available about ethnicity in most cases. Samples were de-identified according to protocols approved by the University of Utah Institutional Review Board. Additional samples with known *HLA-B*5701* status were used as controls.

***HCP5* SNP genotyping by melting curve analysis (FRET Probes) on a LightCycler®**
Primers and probes were designed using the Light Cycler Probe design Software v 1.0 (Idaho Technology, Salt Lake City, UT). PCR primers amplify a 122 bp product that includes the position of the SNP. A fluorescein–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.

***HLA-B*5701* allele detection by polymerase chain reaction with sequence specific primers (PCR SSP) and melting analysis**
The *HLA-B*5701* 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 720K custom-designed array on which 5872 tiled probes with a mean spacing of 15 bp spanned g.31429628 to g.31585825 (hg18) on chromosome 6, a region of 156 kb. After scanning, fluorescence intensity raw data was extracted from the scanned images of the array using NimbleScan v2.5 software.

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.

HCP5 SNP Detection

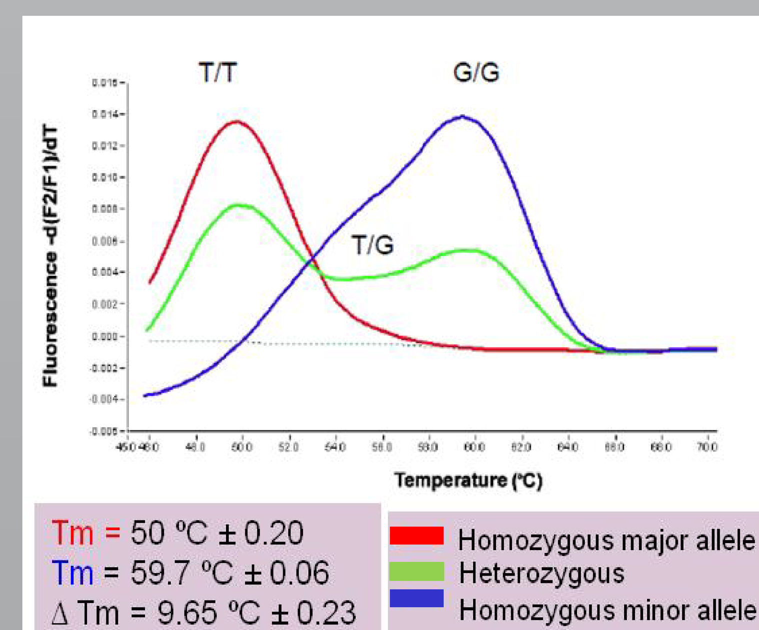

FIGURE 2: *HCP5* genotypes melting peaks

*HLA-B*5701* Detection

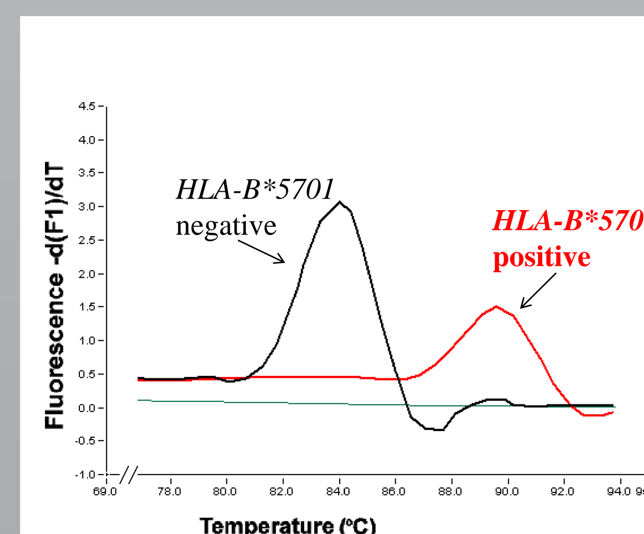

FIGURE 3: *HLA-B*5701* melting peak

Detection of Large Deletion Including the *HCP5* locus

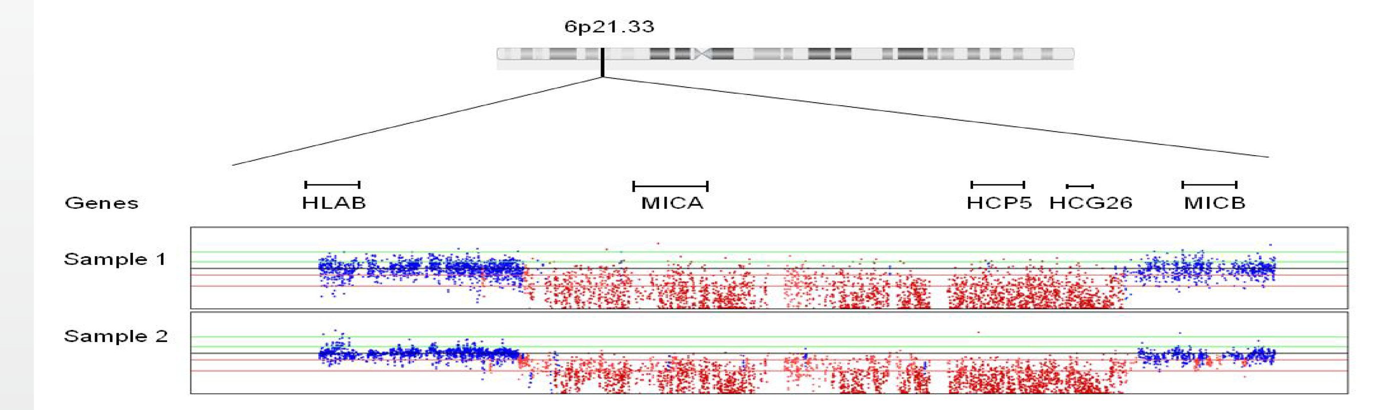

FIGURE 4: CNV analysis

Analysis of samples 1 and 2 revealed homozygous deletions. The red probes indicate deleted probes. The log2 ratio exceeded -1.0 in both patients, indicating homozygous deletions of the region. Several studies provide evidence that deletion of the *HCP5* locus is present at some frequency in certain populations [10,11]. Duplications of this region have also been reported.

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

TABLE 1: *HCP5* rs2395029 T>G and *HLA-B*5701* genotype correlation.

	*HLA-B*5701* positive	*HLA-B*5701* negative	Total
HCP5 TT	1	1766	1767
HCP5 TG	108	9	117
HCP5 GG	2	0	2
HCP5 null allele	0	2	2
Total	111	1777	1888

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): 0.94%-1%], and 99% [95% confidence interval (CI): 0.99%-0.998%] respectively.

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

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