

982 Genetic Factors Predicting Abacavir Hypersensitivity and Tolerance in HLA-B*5701 Positive Individuals: Combined Analysis from PREDICT-1, SHAPE and a Multinational Study

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ABSTRACT

Background: Although multiple studies have confirmed that HLA-B*5701 is highly associated with abacavir hypersensitivity syndrome (ABC HSR), genetic factors predicting abacavir tolerance in patients carrying HLA-B*5701 have not been defined. **Methods:** HLA-B*5701 positive patients were identified from three multi-centre studies: PREDICT-1 (CNA106030), SHAPE (ABC107442), and a collaborative Australian-Swiss-Canadian retrospective study. Genetic characteristics of patients with patch test confirmed ABC HSR (PTC ABC HSR) were compared with patients who tolerated abacavir for >6 weeks with no symptoms (AT). MHC markers associated with the HLA-B*5701 allele and genetic markers related to abacavir metabolism and immune response were examined. Sequence based typing was used for *TNF-238-376*, and PCR-SSP assays were used to detect a nonsynonymous SNP in the HCP5 gene (*rs2395029*), *BAT1-223*, *HSP70Hom 493T*, *C4A6*, *MICA*017*, *KIR3DS1/KIR3DL1* and type 1 alcohol dehydrogenase (ADH) isoforms 1B (*rs1229984*) and 1C (*rs698*), and a RFLP approach was used to identify a functional polymorphism in the promoter region of the CD14 gene (*-159 C/T*). **Results:** Pooled analysis from the three studies identified 95 PTC ABC HSR cases, all of whom carried HLA-B*5701 and 43 HLA-B*5701 positive AT patients. Analysis of 138 patients with complete genotyping across the HLA-B*5701-associated MHC markers HCP5, *BAT1-223*, *TNF-238-376*, *HSP70Hom 493T* showed no significant differences between PTC ABC HSR (n=95) and AT (n=43) (P<NS, Fisher's Exact Test). Furthermore recombination events were identified at multiple sites within the MHC, revealing incomplete linkage disequilibrium between HLA-B*5701 and each of the MHC markers examined including one HLA-B*5701 PTC ABC HSR case with a negative result for the HCP5 (*rs2395029*), and *MICA*017* allele, MHC haplotypic markers close to HLA-B, previously reported to show complete concordance with HLA-B*5701. Pooled analysis revealed an association between *C4A6* (p=.04) and a polymorphism in the promoter region of CD14 [-159 C/T] (p=.025), both of which were overrepresented in the PTC group. There was no significant difference identified between the PTC and AT groups for type 1C (p=.59) or 1B (p=1) ADH genotypes or *KIR3DS1* (p=.7) or *KIR3DL1* (p=1) genotype. **Conclusions:** HLA-B*5701 is necessary but not sufficient for the development of ABC HSR. Genetic variation of markers within the MHC that are co-inherited with HLA-B*5701 appear insufficient to explain abacavir tolerance and the presence of multiple recombination events within the 57.1 MHC haplotype indicate that these haplospecific markers are incomplete surrogates for HLA-B*5701 that cannot be safely used as screening tests for ABC HSR. Although unlikely to improve the positive predictive value of HLA-B*5701 testing, an association between PTC ABC HSR and CD14 [-159 C/T] is intriguing and warrants further investigation, in particular with regards to genetic variation in innate immune responses in HLA-B*5701 positive patients who are tolerant versus hypersensitive to ABC.

Background

- A strong association exists between carriage of the HLA-B*5701 allele within the major histocompatibility complex (MHC) of chromosome 6 and abacavir hypersensitivity syndrome (ABC HSR) across racially diverse populations.
- Recent studies such as PREDICT-1 and SHAPE support a 100% negative predictive value of HLA-B*5701 for immunologically confirmed HSR and its utility as a screening test in clinical practice.
- The positive predictive value of HLA-B*5701 has been identified to be between 50-60% meaning that approximately 40-50% of patients carrying HLA-B*5701 can safely take abacavir without evidence of HSR.
- These findings suggest that HLA-B*5701 is necessary but not sufficient for the immunopathogenesis of HSR.
- Although the genetic basis for the incomplete positive predictive value of HLA-B*5701 for abacavir hypersensitivity remains to be determined, an increasing understanding of the immunopathogenesis of this adverse event identifies a number of putative candidate genetic factors both within the central MHC (57.1 ancestral haplotype) such as HCP5 (*rs2395029*), *BAT1-223*, polymorphisms within the TNF-alpha promoter region (-238, -376), *C4A6* and MHC Class I chain-related gene (MICA) as well as other factors outside the MHC related to the metabolism of abacavir such as alcohol dehydrogenase (ADH) and other genetic factors influencing host innate and adaptive immune responses (Figure 1).

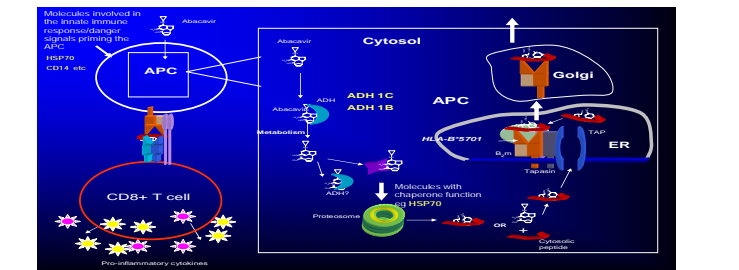


Figure 1: Proposed immunopathogenesis of abacavir hypersensitivity identifying putative candidate genes to explain abacavir tolerance in the presence of HLA-B*5701. A variety of genes involved in the innate immune response, abacavir metabolism and Class I presentation could be implicated. Adapted from McCluskey J, July 25 IAS abstract WEAB3LB

Methods

HLA-B*5701 positive patients who had previously consented to pharmacogenetic research and who were diagnosed with ABC HSR and patch test confirmed (PTC) (using previously described techniques with 1% and 10% abacavir) or who had received abacavir for 6 weeks without evidence of HSR were identified from three multicentre studies: PREDICT-1, a randomized controlled double-blinded study (n=42), SHAPE, a retrospective case control study (n=57) and a multinational collaborative retrospective Canadian-Swiss-Australian Study (n=39). Genetic mapping for alleles that comprise the 57.1 ancestral haplotype (Figure 2) was carried out on PTC and AT patients from all three studies. Genotyping for ADH isoforms, KIR and CD14 was carried out on patients with samples available from all three studies. Sequence based typing was used for *TNF-238-376*. PCR-SSP assays were used to detect a non-synonymous SNP in the HCP5 gene (*rs2395029*), *BAT1-223*, *HSP70Hom 493T*, *C4A6*, *MICA*017*, *KIR3DS1/KIR3DL1* and type 1 ADH isoforms 1B (*rs1229984*) and 1C (*rs698*). A RFLP approach was used to detect a functional polymorphism in the promoter region of the CD14 gene (*-159 C/T*). All methods were based on previously published molecular techniques. Pooled statistical analyses comparing genetic markers between the PTC and AT groups were carried out using Fisher's exact test. Multiple case-control/logistic regression was carried out to ascertain independent associations.

RESULTS

	Patch Test Confirmed N=95 (%)	Abacavir Tolerant N=43 (%)
Median Age (IQR)	44 (38 – 50)	49 (40 – 55)
Race		
Caucasian	89 (94)	41 (95)
Black	6 (6)	2 (5)
Male Gender	83 (87)	37 (86)

Table 1: Demographic Characteristics of HLA-B*5701 Positive Patch Test Confirmed versus Abacavir Tolerant Patients

Genetic Marker	Patch Test Confirmed N=95 (%)	Abacavir Tolerant N=43 (%)	P-value
C4A6	79 (83)	28 (65)	.04
<i>Hsp70Hom 493</i>			
CC	11 (11.6)	5 (11.6)	.33
CT	73 (76.8)	29 (67.4)	
TT	11 (11.6)	9 (20.9)	
<i>TNF-238</i>			
AA	3 (3.2)	1 (2.3)	.61
GA	85 (89.5)	41 (95.3)	
GG	7 (7.4)	1 (2.3)	
<i>TNF-376</i>			
GA	1 (1.1)	1 (2.3)	.53
GG	94 (98.9)	42 (97.7)	
<i>BAT1-223</i>			
AA	2 (2.1)	0	.34
AC	85 (89.5)	42 (97.7)	
CC	8 (8.4)	1 (2.3)	
<i>HCP5(rs2395029)</i>			
GG	2 (2.1)	0	1
GT	92 (96.8)	43 (100)	
TT	1 (1.1)	0	
<i>MICA*017</i>	89 (98.9)	38 (100)	1
<i>ADH 1C (rs698)</i>			
AA	30 (37)	15 (46.9)	.59
GA	33 (40.7)	12 (37.5)	
GG	18 (22.2)	5 (15.6)	
<i>ADH 1B (rs1229984)</i>			
AA	1 (1.25)	0	1
GA	10 (12.5)	4 (12.5)	
GG	69 (86.3)	28 (87.5)	
<i>CD14 [-159]</i>			
CC	18 (20.2)	16 (42.1)	.025
CT	51 (57.3)	13 (34.2)	
TT	20 (22.5)	9 (28.1)	
<i>KIR3DS1</i>	44 (49.4)	17 (44.7)	.70
<i>KIR3DL1</i>	85 (95.5)	37 (97.4)	1

Table 2: Genetic characteristics of PTC vs. AT Patients: no significant differences were seen between PTC and AT for the haplospecific/typic HLA-B*5701 markers at *Hsp70-Hom 493*, *HCP5 (rs2395029)*, *BAT1-223*, *TNF-238/376* and *MICA*017*, or presence of other allelic variants of genes known to interact with HLA-B*5701 such as *KIR3DS1/KIR3DL1* or polymorphisms in enzymes that metabolize abacavir to a putative reactive metabolite such as *ADH 1C* or *ADH 1B*. In multivariate models incorporating all three studies only the presence of *C4A6* (p=.023, OR=2.8) and a functional polymorphism in the promoter of the CD14 gene (CT or TT genotype) (p=.004, OR= 3.2) were independently associated with the presence of patch test confirmed ABC HSR. This presence of CD14 [-159 C/T] although significant when the results of all three studies were pooled was not significant in any one individual study. Although numbers of patients with black race were low, no significant differences existed between black and white race for any of the markers shown or between the AT and PTC groups (data not shown).

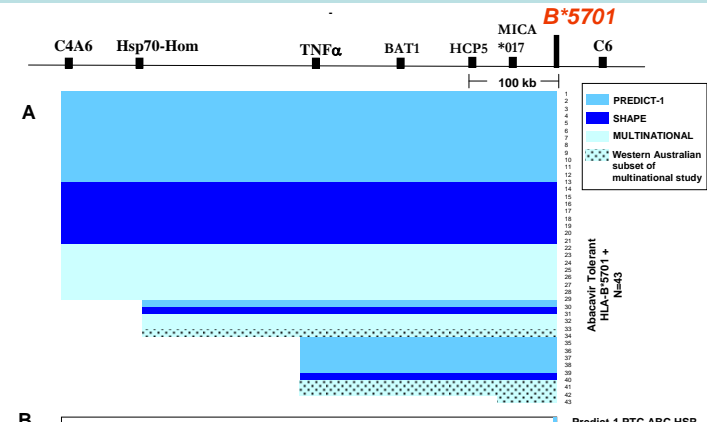


Figure 2: A. Recombinant mapping of the 57.1 ancestral haplotype in 43 HLA-B*5701 positive abacavir tolerant patients showing that the majority of these patients have the "extended" haplotype and also illustrating that multiple recombination events occur. For PTC ABC HSR similar results with multiple recombination events were seen. *C4A6* was overrepresented in the ABC HSR PTC group (Table 2, map not shown here). Although previous studies suggested that the concurrence of HLA-B*5701 and the *HSP70-Hom M493T* allele improved the positive predictive value of HLA-B*5701 for PTC ABC HSR, this strong association appeared to be specific to the Western Australian population and was not replicated across other populations. Although significant in the pooled analysis, absence of *C4A6* also showed the strongest association in the Western Australia AT population. The presence of *MICA*017* was inferred for 5 patients who had missing data for this locus but all other haplospecific markers. Other HLA-B*5701 haplospecific markers are both imperfect surrogates for HLA-B*5701 and predictors of PTC ABC HSR. B. A patch test confirmed HLA-B*5701 positive ABC HSR patient from the PREDICT-1 study lacking all examined haplospecific markers (including *MICA*017* and *HCP5*) supporting that HLA-B*5701 is necessary for the development of ABC HSR and that HLA-B*5701 haplospecific markers are both imperfect surrogates for ABC HSR.

	HLA-B*5701	No HLA-B*5701	PPV/NPV (95% Confidence Intervals)
PTC HSR	23	0	PPV = 48% (33-63%) NPV = 100% (99.5-100%)
No HSR	25	794	
	HLA-B*5701+C4A6	Not (HLA-B*5701+C4A6)	
PTC HSR	17	6	PPV=50% (32-68%) NPV = 99.3% (98.4-99.7%)
No HSR	17	802	
	HLA-B*5701+CD14T	Not (HLA-B*5701+CD14T)	
PTC HSR	16	5	PPV =52% (33-70%) NPV = 99.4% (98.6-99.8%)
No HSR	15	802	
	HLA-B*5701+C4A6+CD14T	Not (HLA-B*5701+C4A6+CD14T)	
PTC HSR	11	10	PPV=50% (28-72%) NPV 98.8% (97.8%-99.4%)
No HSR	11	806	

Table 3: Data from the PREDICT-1 study. The addition of *C4A6* and/or *CD14 [-159 C/T]* to HLA-B*5701 does not significantly improve the positive predictive value of HLA-B*5701 for PTC ABC HSR.

Conclusions

- This study supports previous research in suggesting that HLA-B*5701 is necessary but not sufficient for the development of ABC HSR.
- Specific genetic factors that are associated with the development of ABC HSR despite carriage of HLA-B*5701 have yet to be identified and abacavir tolerance does not seem to be associated with any specific marker or group of markers haplospecific to HLA-B*5701, suggesting that at least some of the factors conferring tolerance to abacavir in those carrying HLA-B*5701 may lie outside the MHC.
- Recombination events were identified at multiple sites within the MHC suggesting incomplete linkage disequilibrium between HLA-B*5701 and each of the MHC markers examined, including *HCP5* and *MICA*017* the two markers closest to HLA-B in one PTC ABC HSR patient. This is further confirmation that these haplospecific markers are both incomplete surrogates for HLA-B*5701 and hence incomplete predictors of PTC ABC HSR and therefore cannot be safely used as screening tests for ABC HSR.
- The suggestion of an association between PTC ABC HSR, *C4A6* and/or *CD14 [-159 C/T]*, although unlikely to impact on the positive predictive value of HLA-B*5701 for ABC HSR is intriguing and suggests further investigation of genetic variation in the innate immune response is warranted with regards to both the immunopathogenesis of ABC HSR and factors conferring abacavir tolerance in the presence of HLA-B*5701.