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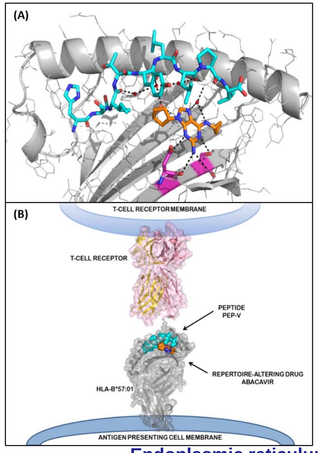
Variation in ERAP influences risk for HLA-B*57:01 Abacavir Hypersensitivity

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Background



- **Abacavir hypersensitivity syndrome (**ABC HSS) is characterized by skin rash, fever, malaise, gastrointestinal and respiratory symptoms in patients during the first 6 weeks of antiretroviral therapy.
- ABC HSS develops when HLA-B*57:01 binds ABC in the F pocket of the peptide binding groove affecting the shape and chemistry of the groove, subsequently altering the repertoire of peptides bound and presented by HLA-B*57:01 to T cells (Figure 1) (1-3).
- 55% of individuals who carry HLA-B*57:01 will develop ABC HSS and 100% of ABC HSS cases carry HLA-B*57:01. However, it is not understood why the remaining 45% of HLA-B*57:01 carriers are ABC tolerant and previous studies have not associated additional factors within the major histocompatibility complex with ABC HSS or tolerance.

Figure 1. (A) Crystal structure of the ABC-peptide-MHC complex reveals intermolecular contacts within the antigen-binding cleft of HLA-B*57:01. Diagram of HLA-B*57:01 in grey. The synthetic peptide-V is shown in cyan carbons. ABC is shown as orange for carbon, blue for nitrogen, and red for oxygen. The residues that distinguish the ABC-sensitive allele HLAB*57:01 from ABC-insensitive HLA-B*57:03 are shown in magenta. ABC forms hydrogen-bond interactions (black dashes) with both the peptide and HLA-B*57:01 (1). (B) Model of ABC-peptide-MHC complex interacting with the T-cell receptor. HLA-B*57:01 is depicted in grey. The Peptide-V is shown in cyan carbons. ABC is shown as spheres: orange for carbon, blue for nitrogen. The T cell receptor is shown in pink (1,4).

Endoplasmic reticulum aminopeptidases

- ERAP1 and ERAP2 are critical components for the final processing of MHC-1 ligands in the ER, trimming peptides to the ideal length for MHC-1 binding.
- ERAP prefers substrates that are 9-16 residues in length with a C-terminal hydrophobic site which allows rapid trimming of N-extended peptides to 8-9 residues via a "molecular ruler" mechanism (Figure 2) (5,6).
- ERAP function determines patterns of immunodominance, epitope processing and T-cell responses during infection.
- ERAP can also influence MHC-class I expression on the cell surface.
- ERAP1 and ERAP2 genes are located on chromosome 5 and genetic variants and haplotypes of ERAP are associated with Ankylosing spondylitis (AS), psoriasis, and Behçet's disease (ERAP1) and birdshot retinopathy (ERAP2). In each of these diseases, disease-associated variants of ERAP have been shown to interact with disease-associated class I HLA alleles to influence disease risk (7).

Objective

- **We examined genetic polymorphisms across ERAP1 and ERAP2 to investigate whether the ERAPs may contribute to the risk of developing ABC HSS for individuals who are HLA-B*57:01.**

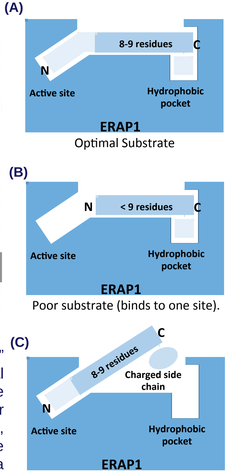


Figure 2. Model to explain the substrate preference of ERAP1 via the "molecular ruler" mechanism. (A) High-affinity binding of optimal substrates through the C-terminal hydrophobic residue and active site. Antigenic products (eight to nine residues) are released after removal of additional N-terminal residues. (B) Poor substrates (fewer than nine residues) have low affinity because they can interact only with the active site, or if they bind to the hydrophobic pocket, their N-terminal residues cannot reach the active site. (C) A poor substrate has low affinity because its C-terminal residue has a charged side chain (Figure adapted from (5)).

Methods

- 3'UTR, intron and exon encoded SNPs which characterise ERAP1 allotypes significant in AS were examined in HLA-B*57:01+ ABC patch test positive (PT+) ABC HSS patients [n=53] and HLA-B*57:01+ ABC tolerant controls [n=22] with sequence-based typing.
- rs2248374, a tag SNP for functional ERAP2 haplotypes was also examined. Haplotype A is tagged by the (A) allele, while haplotype B is tagged by rs2248374(G). ERAP2 expressed from haplotype B undergoes differential splicing and encodes a truncated protein, leading to nonsense-mediated decay of the mRNA. Haplotype B homozygotes express lower levels of MHC class I on the surface of B cells (7).
- Fisher exact tests and multiple logistic regressions were used to compare genotypes between HLA-B*57:01+ ABC HSS PT+ and HLA-B*57:01+ ABC tolerant groups.
- Significant SNPs were mapped to the functional domains in the solved crystal structure of ERAP1 (PDB ID:3QNF) using Cn3D.

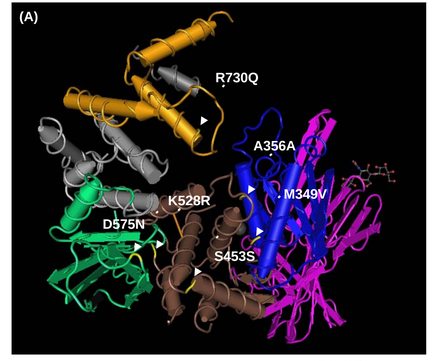
Results

SNP	Nucleotide (Major to minor)	AA (Major to minor)	Global MAF	PT + ABC HSS MAF	HLA-B*57:01 ABC Tolerant MAF	P value (carriage minor allele PT+ HSS v Tolerant)	P value (PT+ MAF v global MAF)	P value (Tolerant MAF v global MAF)
rs26653	G > C	R127P	C=0.4375	0.33	0.2	0.30	0.018	0.0005
rs27543	T > G	Intron variant	G=0.2274	0.18	0.28	0.26	0.26	0.50
rs26618	T > C	I276M	C=0.2266	0.23	0.25	0.42	0.31	0.73
rs27895	C > T	G346D	T=0.1036	0.047	0.045	1	0.0075	0.078
rs2287987	T > C	M349V	C=0.1050	0.17	**0.32**	**0.04**	0.11	**0.0062**
rs27434	G > A	A356A	A=0.3492	0.30	**0.09**	**0.005**	0.26	**<0.0001**
rs27529	G > A	S453S	A=0.4040	0.47	0.28	0.09	0.18	0.072
rs27710	G > A	Intron variant	A=0.4040	0.47	0.28	0.06	0.17	0.072
rs30187	C > T	R528K	T=0.4038	0.49	**0.27**	**0.03**	0.063	**0.052**
rs10050860	C > T	D575N	T=0.1062	0.15	**0.28**	0.08	0.32	**0.030**
rs469758	T > C	Intron variant	C=0.4042	0.42	0.29	0.42	0.69	0.12
rs17482078	C > T	R725Q	T=0.1006	0.14	**0.30**	**0.03**	0.21	**0.0055**
rs27044	C > G	Q730E	G=0.3207	0.34	0.20	0.20	0.74	0.079
rs1065407	T > G	Intron variant	G=0.1741	0.32	0.38	1	0.0031	0.034
rs27689	T > G	3' UTR Intron variant	G=0.1382	0.08	0.06	1	0.057	0.045
rs13167972	A > G	3' UTR Intron variant	G=0.2879	0.40	0.40	0.30	0.022	0.23
rs2248374	G > A	ERAP2 intron variant A = Haplotype A* G = Haplotype B#	A=0.4473	0.41	0.60	0.08	0.50	0.065

Table 1. ERAP SNPs and association with PT+ ABC HSS or ABC tolerance in HLA-B*57:01 patients (Areas of Linkage disequilibrium are shaded). MAF = Minor Allele Frequency. * Haplotype A = full length ERAP2 protein. #Haplotype B = non-functional truncated ERAP2 protein. Both ERAP 1 / 2

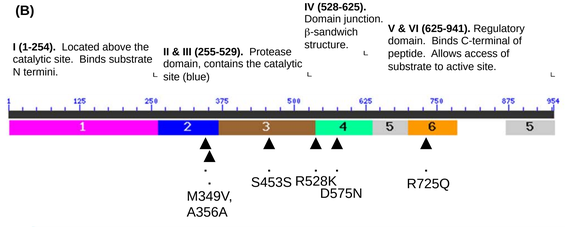
- Several SNPs were significant in a comparison of PT+ ABC HSS versus ABC tolerant HLA-B*57:01 patients (Table 1).
- Strong linkage was evident between rs27543 and rs26618 ($p < 0.001$), as well as in the block of SNPs extending from rs2287987 to rs17482078 which included rs27434 ($p = 0.03 < 0.001$).
- Significant SNPs in the LD block differed from global MAF for ABC tolerant HLA-B*57:01 patients while PT+ ABC HSS patients had MAFs equivalent to the global MAF.
- In joint models with rs27434. None of the SNPs were significantly associated with outcome after accounting for rs27434, due to linkage disequilibrium,
- In a multivariable model with rs27434(GG), the ERAP2 SNP (rs2248374(G)) that tags haplotype B, characterized by a truncated protein, was decreased in tolerant individuals ($p = 0.04$).

Results



Residues	Possible Functionality
349	Close to the active site.
528 and 575	Located in or close to inter-domain regions, and might affect the conformational transition between the open and closed states.
730	Located in the substrate binding cavity and might modulate peptide binding.

Figure 3.
(A) Crystal structure of ERAP1 in open conformation with significant SNPs in B*57:01 ABC tolerance indicated (yellow). (B) Domain map of ERAP protein with colour corresponding to crystal structure in (A).



Tolerant SNP	Tolerant AA	Alternate AA
Rs2287987(C)	349V	349M
Rs27434(G)	356A	356A
Rs27529(G)	453S	453S
Rs30187(C)	528R	528K
Rs17482078(T)	730Q	730R

Table 2.
Summary of ERAP SNPs with significant association with tolerance in HLA-B*57:01 patients, where the MAF differs to the global MAF.

Conclusions

- Representation of SNPs in ERAP1 and ERAP2 in part explain and differentiate HLA-B*57:01+ ABC HSS from HLA-B*57:01+ ABC tolerance
- Distinct SNP locations suggest putative functional influences on ERAP activity among co-occurring polymorphisms.
- ERAP activity may influence the repertoire of peptides presented by HLA-B*57:01 or influence early changes in immunodominant epitope selection. This provides a potential pathogenic mechanism for the development of ABC HSS or ABC tolerance in HLA-B*57:01 carriers.

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