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Specific Binding Characteristics of HLA Alleles Associate with Nevirapine Hypersensitivity

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Abstract

Background: Multiple class I and II HLA associations have been described in association with nevirapine (NVP) hypersensitivity reaction (HSR) phenotypes. We tested the hypothesis that peptide binding (PB) properties may be shared between these alleles.

Methodology: HLA-A,-B-,-C, -DR typing was performed on stored DNA from a retrospective case controlled analysis of NVP HSR (ClinicalTrials.gov NCT00310843) using the Roche-454 FLX platform. Univariate and multivariate analyses stratified for race were performed according to HLA class I/II alleles, HLA supertypes and HLA alleles according to PB[3,4], Kir ligand groupings and HLA B/C haplotypes for cutaneous and hepatitis phenotypes of NVP HSR. *In silico* modelling to simulate HLA binding to NVP was performed with the highest ranked candidates.

Results: HLA -A,-B,-C and -DR typing resolved to four digits (794 samples (controls =524, cutaneous NVP HSR cases =170, hepatitis NVP HSR cases = 100)). Multivariate analysis of cutaneous NVP HSR in Southeast Asians (SEA) associated DR4 supertype(OR=2.9, p=0.015) and alleles with HLA-35/18 PB properties(OR=6.4, p=0.002). HLA-DRB1*01:02 was associated with hepatitis NVP HSR in Caucasians (OR=2.7,p=0.01) whereas carriage of alleles of the PB B46 were protective (OR=0.3, p=0.04). HLA-C*04:01 was associated with cutaneous NVP HSR, including SJS/TEN across all races (p<0.0001, Mantel-Haenszel test; Caucasians: OR=2.8 [1.3-5.9], p=0.009; African Americans: OR=4.0 [1.4-13.0], p=0.02; SEA: OR=9.0 [3.2-24.9], p<0.0001). However, haplotype analysis of HLA-B/C showed pairing of HLA-C*04:01 with HLA-B alleles with B35 and B18 like PB (HLA-B*35:01,B*35:05,B*35:08,B*53:01,B*18:01,B*18:02, B*44:02,B*4403), and this effect was strongest in SEA where carriage of HLA-C*04:01 when paired with the HLA-B alleles(OR=11.8,p=0.0003) was more strongly associated with cutaneous NVP HSR than HLA-C*04:01 carried alone(OR=4.8,p=0.047). This suggests that risk of cutaneous NVP HSR attributed to carriage of HLA-C*04:01 may be enhanced by HLA-B alleles which are in strong linkage disequilibrium. An *in silico* and peptide binding model both suggest that NVP non-covalently binds in the F pocket of HLA-B*35:05 and near the B pocket of HLA-C*04:01. In multivariate analyses, Kir ligand groupings Bw4/Bw6 and C1/C2 did not significantly contribute to the modelling of associations with cutaneous hypersensitivity or hepatitis.

Conclusions: Cutaneous and hepatitis phenotypes of NVP HSR associate with different HLA-B and DR-alleles respectively that share PB characteristics. The pairing of these HLA-B alleles with HLA-C*04:01 appears important for the development of cutaneous NVP HSR, providing a testable model for the immunopathogenesis of NVP HSR.

Background

- Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) associated with a hypersensitivity syndrome (HSR) in approximately 5% of patients who begin therapy and is characterised by any combination of fever, rash, hepatitis or eosinophilia. NVP HSR is the treatment limiting toxicity of NVP which is otherwise well tolerated without known short or long-term CNS, metabolic or renal toxicities.
- The evidence from human and rat models is that NVP HSR is dependent on both CD4+ and CD8+ T cell responses.
- NVP HSR has been associated with Class I and Class II alleles that appear to be both phenotype and ethnicity specific. Eg. HLA-DRB1*01:01 with rash-associated hepatotoxicity in Caucasians[1] or HLA-B*35:05 with rash in Asian populations[2] suggesting the role of genetic, immunological and potentially metabolic contributors to the development of NVP HSR.
- We did an extensive analysis to look at peptide binding properties of HLA alleles associated with risk of various NVP HSR phenotypes in a large retrospective case control study to test the hypothesis that peptide binding properties may be shared amongst different HLA risk alleles and explain in part the apparent complexity of these associations across different ethnicities.

Methods

- Patients samples were taken from a case controlled analysis of NVP HSR (ClinicalTrials.gov NCT00310843). Tolerant cases had tolerated NVP for 18 weeks, while NVP HSR was defined as those who experienced clear cutaneous or hepatitis phenotype of NVP within 8 weeks of initiating NVP.
- HLA-A,-B-,-C, -DR typing was performed on stored DNA using the Roche-454 FLX platform.
- CYP2B6 genotyping (rs3745274,rs28399499, rs4803419,rs2687116,rs7251950,rs2279343) was done using massARRAY iPLEX Gold (Sequenom, Inc)
- Predicted peptide binding specificities of HLA alleles were examined using *MHCcluster*[6] and used to group alleles for analysis.
- Univariate and multivariate logistic regression analyses stratified for race were performed according to HLA class I/II alleles, HLA Supertypes[3,4], CREG groups[5], Peptide binding groups[6], KIR ligand groupings and CYP2B6 genotypes.
- HLA B/C haplotypes for cutaneous and hepatitis phenotypes of NVP HSR were also assessed with *HaplotypeBlocks.*
- *In silico* modelling and molecular docking scores was performed to examine HLA binding to NVP with the highest ranked candidates.

Results

Allele Analysis

Phenotype	Race	Allele	P-Value	OR
Hepatitis	Caucasian	DRB1*01	0.003	2.47
		DRB1*01:02	0.010	2.7
		DRB1*04:01	0.083	0.420
	Black	B*53:01	0.075	4.000
Hepatitis Only	Caucasian	DRB1*01	<0.0001	3.290
(no rash)		DRB1*01:01	0.037	2.110
		DRB1*01:02	0.013	4.460
		B*57:01	0.060	2.580
Rash	Caucasian	B*15	0.011	0.210
		Cw*04	0.009	2.80
		DRB1*04:01	0.014	0.160
	Asian	B*15	0.042	0.480
		B*35	0.004	3.550
		B*40:01/02/06	0.052	0.480
		Cw*04	0.007	2.42
		DRB1*04:05	0.008	2.82
	Black	B*53:01	0.048	4.290
		Cw*04	0.020	4.000

Table 1. Significant HLA alleles associated with NVP HSR phenotypes. Protective alleles are blue.

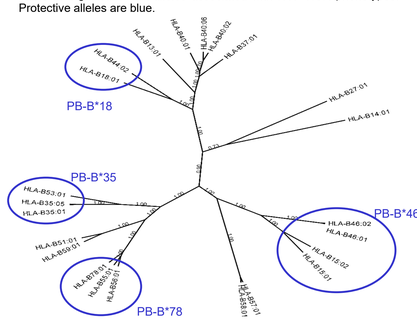


Figure 1. *MHCcluster* analysis of key HLA-B alleles. Allele groups were analysed for NVP HSR associations as indicated based on peptide binding (PB) properties.

Ligand	Absence of Peptide	With Peptide	Allele
Nevirapine	-7.4	-5.0	DRB1*01:01
Nevirapine	-7.0	-4.2	B*35:01
Nevirapine	-8.0	-6.0	B*35:05
Nevirapine	-8.1	-4.5	C*04:01
Abacavir	-7.2	-9.1	B*57:01

Table 3. Docking scores (kcal/mol) for NVP binding to each of the HSR risk alleles with and without peptide. Abacavir which binds to B*57:01 with peptide is shown as a comparison. Stronger binding is indicated by higher negative scores.

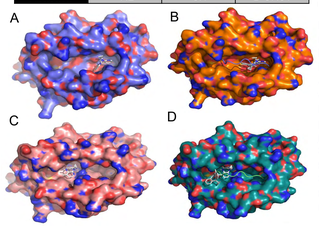


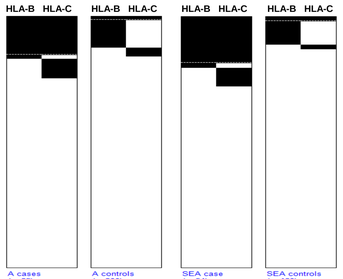
Figure 3. Molecular modelling shows that NVP is predicted to bind in both the **F pocket** of **A.** HLA-B*35:05 without peptide (blue); **B.** HLA-B*35:05 with peptide (orange) and **B pocket** of **C.** HLA-C*04:01 without peptide (red) and **D.** HLA-C*04:01 with peptide (green) (at P9 and P2 respectively).

Supertypes and Predicted Binding Analysis

Phenotype	Race	Group	P-Value	OR
Hepatitis	Caucasian	sDRB1*01	0.001	2.990
		CREG B07	0.016	2.150
		pb-78	0.040	2.180
Rash	Caucasian	sB*62	0.017	0.270
		B05 CREG	0.046	1.760
		pb-B*46	0.009	0.200
	Asian	sB*44	0.053	0.520
		sDRB1*04	0.003	3.230
		sB*07	0.007	2.200
		pb-B*46	0.049	0.560
		pb-B*35	0.004	3.550
	SEA	pb-B*35	0.024	4.670
		pb-B*35/B*18	0.002	6.4
		sDRB1*04	0.015	2.9

sDRB1*01 = **DRB1*0101/02** (*Caucasian*)[4]
sDRB1*04 = **DRB1*0401**/02/**04**/**05**/08/10/21/23/26 (*Asian*)[4]
sB*07 = **B*0702**/03/05,B*1508, **B*3501/03**, B*4201, **B*5101/02/03**, B*5301, **B*5401**, B*5501/**02**, **B*5601**, **B*6701**, B*7801 (*Asian*)[3]
sB*62 = **B*1501**/02/12, **B*4601**, **B*5201** (*Caucasian*)[3]
sB*44 = **B*1801**, **B*3701**, **B*4001/02/06**, **B*4402/03**, B*4501 (*Asian*)[3]
CREG B07 = **B7,8,13**,54,**55,56,27**,60,61,**41**,42,**47,48**,59,67,81,82 (*Caucasian*)[5]
CREG B05 = **B51,52**,62,63,75,76,77,**57,58,18,49,50,35,46,53**,71,72,73,78 (*Caucasian*)[5]
pb-B*46 = **B*4601**#/02, **B*1501**#/**02**[&] (Caucasian#, Asian[&], MHC Cluster assigned)
pb-B*35 = **B*3501/05**/08, B*5301(*Asian*, SEA, MHC Cluster assigned)
pb-B*18 = **B*1801, B*4402/03** (*Asian*, MHC Cluster assigned)
pb-B*78 = B*7801, **B*5501, B*5601** (*Caucasian*, MHC Cluster assigned)

Table 2. Significant HLA groups associated with NVP HSR phenotypes. Protective allele groups are in blue. HLA Alleles within each designated supertype, CREG or PB group are listed. Alleles present within the cohort race of interested shown in bold, with the race listed in brackets.



HLA-B = B*3501, B*3505, B*3508, B*5301, B*1801, B*1802, B*4402, B*4403
HLA-C = C*0401

	ALL ASIANS		SOUTH-EAST ASIANS	
Allele group	OR*	P-value	OR*	P-value
HLA-B	0.16	0.07	0.24	0.2
HLA-C	2.45	0.1	4.75	0.05
HLA-B and HLA-C	11.40	0.0003	11.88	0.0003

* OR, relative to carriage of neither HLA-B nor –C group alleles

Figure 2. Analysis of HLA-C*0401/pb-B*35/B*18 or HLA-C*0401/pb-B*35 haplotypes in Asian and SEA populations show a stronger association with NVP rash than seen for HLA-C*0401 or HLA-B alleles alone.

HLA-B x CYB2B6-516-T interactions

	Without allele		With allele		
Allele	OR	(95% CI)	OR	(95% CI)	P
B07	0.57	(0.3-1.1)	1.80	(1.0-3.4)	0.006
B0702	0.57	(0.3-1.1)	1.85	(1.0-3.5)	0.004
sB07	0.42	(0.2-1.0)	1.33	(0.8-2.2)	0.01
CREG B07	0.31	(0.1-0.8)	1.38	(0.8-2.3)	0.004

CREG B07 = B7,8,13,54,55,56,27,60,61,41,42,47,48,59,67,81,82 (Caucasian)[5]
sB*07 = B*0702/03/05,B*1508, B*3501/03, B*4201, B*5101/02/03, B*5301, B*5401, B*5501/02, B*5601, B*6701, B*7801 (Asian)[3]
OR: Odds ratio for increase in risk per copy T allele of CYP2B-516
P: P-value for difference in CYP2B-516 effect according to absence/presence of B-allele

Table 3. HLA-B x CYP2B-516 interactions: Caucasians with hepatitis vs controls. Amongst Caucasians, carriage of HLA-B*07:02 significantly increased the association of the CYP2B6-516-T genotype with NVP induced hepatitis.

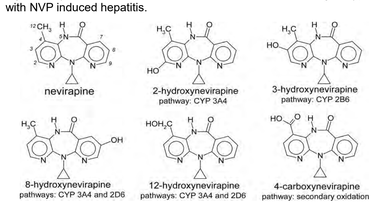


Figure 4. The major products of NVP metabolism. 3-OH-NVP is the major product of the CYP2B6 pathway. The core 3 ring structure of NVP remains intact in all metabolites. (Taken from [7]). 12-OH-NVP and 3-OH-NVP are the most abundant metabolites at steady state after 200mg of NVP twice daily[8] .

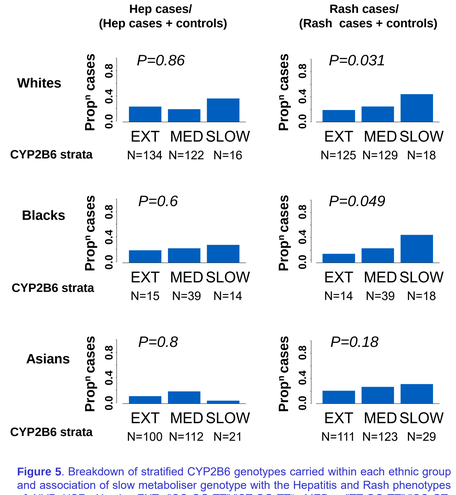


Figure 5. Breakdown of stratified CYP2B6 genotypes carried within each ethnic group and association of slow metaboliser genotype with the Hepatitis and Rash phenotypes of NVP HSR. X-axis: EXT: "CC-GG-TT","CT-GG-TT", MED: "TT-GG-TT","CC-GT-TT","CC-GG-TC","CT-GT-TT","CT-GG-TC", SLOW: "CC-TT-TT","CC-GT-TC" for CYB2B6 "15582-516-983". Y-axis is proportion in each group who are cases. P-value is from Cochrane-Armitage test for increasing trend in proportions.

Conclusions

- Several Class I and Class II alleles are associated with specific NVP HSR phenotypes across ethnic groups eg. HLA-C*04:01 and rash.
- Grouping alleles based on supertypes or additional PB characteristics supports individual allele associations and highlights new candidate alleles which could be significant or protective in NVP HSR based on shared PB characteristics eg.(1) pb-B*35 contains HLA-B*35:01/05 and HLA-B*53:01 associated with rash phenotype in Asians and SEA. (2) pb-B*78 and CREG B07 both contain HLA-B*55:01 and B*56:01 which may be candidates for association with the Hepatitis phenotype in Caucasians. (3) sDRB1*04 contains HLA-DRB1*04:05, and both are positively associated with NVP induced rash in Asians.
- CYP2B6 imputed rate of metabolism associates with NVP HSR rash independently of HLA. Caucasians who have the CYP2B6-516 G→T genotype show a significantly increased association with NVP induced hepatitis when they also carry HLA-B*07:02. This appears to support accumulation of the parent drug and concentration relationships previously described for NVP.
- *In silico* modelling and docking studies show that NVP has the potential to bind non-covalently within the antigen binding cleft of HLA-B*35, HLA-Cw*04 alleles and also HLA-DRB1*01:01/02. The binding is stronger without the presence of peptide. Suggesting (1) direct recognition of drug by TCR or (2) long peptides may bind at the termini pockets of the binding cleft and loop over NVP to interact with the TCR.
- Cutaneous NVP HSR attributed to carriage of HLA-C*04:01 in Asians and South-East Asians may be enhanced by HLA-B alleles which are in strong linkage disequilibrium such as HLA-B*35:05 or HLA-B*35:01.

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