

HLA-dependent Hypersensitivity Reaction to Nevirapine in Chinese Han HIV-infected Patients

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Abstract

In this study, one hundred and three HIV-positive Chinese Han patients treated with NVP based regimens were investigated for the association between nevirapine hypersensitivity reaction (NVP HSR) and human leukocyte antigen (HLA) allele. HLA-Cw, -DRB1 alleles were determined in Thirty two NVP HSR cases and 71 NVP-tolerant patients. We found that considerable overlap was observed for the clinical and demographic characteristics of the 32 hypersensitive patients and 71 tolerant patients. Twelve out of 32 NVP HSR cases developed allergic hepatotoxicity. More HLA-Cw*04 alleles were observed in NVP HSR cases than in NVP tolerant cases ($p = 0.029$). The frequency of HLA-DRB1*15 in NVP tolerant cases was significant higher than that of in NVP HSR cases ($p=0.018$). Multivariate logistic regression identified that HLA-Cw*04 presence was a risk factor related to NVP HSR ($p=0.030$, OR=3.611, 95%CI of OR: 1.135-11.489). To clearly understanding its value in clinical practice, further studies involving larger cohorts of patients from different races with different level of immune suppression are needed.

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Acquired immunodeficiency syndrome (AIDS) has become a controllable chronic

disease since combined antiretroviral therapy (cART) was introduced^[1,2]. The first line cART regimens are composed of two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitors (NNRTIs) in China^[3]. As expected, cART has decreased the HIV associated mortality dramatically in Chinese HIV population^[4]. Adverse event, such as hepatotoxicity and hypersensitivity reaction (HSR), is one of the common challenges met by HIV-infected patients when they take the advantage of cART. HSR is generally developed in cases of receiving NRTI abacavir (ABC) or NNRTI nevirapine (NVP).

Previous studies have confirmed that the ABC associated HSR is decided by genetic factors. The investigations conducted after 2002 have shown that the ABC associated HSR is closely related to HLA-B*5701 carriage^[5,6]. HLA-B*5701 screening has become a routine practice for patients who are candidates of receiving ABC therapy in developed countries^[7]. This approach is working well in preventing HSR among patients receiving ABC based cART regimens.

NVP is recommended as a component of the first line cART in developing countries. Most of the cART naïve HIV-infected patients in China receive NVP based regimens^[8]. More attention is given to the safe usage of NVP because of the potential HSR similar to that of caused by ABC. Based on the investigation which indicated that female with baseline CD4 cell count above 250 cells/ul or male with baseline CD4 cell count above 400 cells/ul had a higher prevalence of NVP associated HSR, experts have deduced that NVP associated HSR was the result of interactions between NVP as a antigen and CD4 cell special immune reactions, and may be originally associated to the polymorphism of HLA genes^[9]. Martin et al.

has reported that NVP HSR is more prevalent in individuals carrying HLA-DRB1*0101 with higher baseline CD4 cell percentage^[10]. HLA-DRB1*0101 carriage with CD4 cell percentage above 25% may be a high risk factor of NVP associated systematic symptoms or hepatitis. HLA-DRB1*0101 carriage, however, has not been confirmed to be related to solely NVP rash. Moreover, recent studies have shown that additional HLA alleles are associated with NVP hepatotoxicity in Sardinian (HLA-Cw8/HLA-B14) and Japanese (HLA-Cw8) patients^[11,12]. The different results from different regions imply that the different genetic background may play a role in the development of NVP HSR. To our knowledge, there is no report involving the relationship between HLA alleles and NVP related HSR among Chinese HIV-1 infected people by far. Since NVP is widely used in HIV-infected individuals and has been defined as a risk factor related to cART hepatotoxicity in China^[13], we investigated the association between NVP HSR and HLA allele in Chinese Han HIV-infected patients in this study .

cART naïve HIV-1 positive patients, who were Han Chinese mainly from 4 counties and municipalities in Hubei province with the highest concentration of patients with AIDS,, initiated cART during June 2005 and June 2009 under the criteria of National Free cART Guideline^[3]. The first line regimens of the targeted population were NVP plus two additional NRTIs(zidovudine/ stavudine + lamivudine/ didanosine). Patients were divided into two groups according to developing NVP HSR or not after cART initiation. Cases developed NVP HSR within 6 weeks after cART starting were assigned to the HSR group, while those who without NVP HSR after 6 months' cART to the tolerant group.

NVP HSR was evaluated according to the AIDS Clinical Trial Group grading severity

list of adult adverse experiences^[14]. Diagnostic criteria was based on extensive skin rash, vesicular, bullous skin lesions or skin manifestations combined with one or more of the following symptoms: fever ($> 38^{\circ}\text{C}$), liver toxicity^[15,16]. Patients presenting transient skin rash or late side effects (> 6 weeks) were excluded. Alanine aminotransferase (ALT) values elevated to more than five times above the upper limit of normal was defined as NVP allergic hepatotoxicity. Patients with chronic hepatitis B or C who normally had elevated transaminase values, were only included if they had ALT values that were 3.5 times above baseline values^[17]. Most of the patients were asked to shift to EFV from NVP or discontinue cART after developing NVP HSR. A case-control study was carried out. All participants signed informed consent forms. The present study was approved by the ethics committees of Zhongnan Hospital of Wuhan University.

Two hundred μl of whole blood was collected from each of those patients and kept at -20°C until DNA extraction with the QIAamp DNA Blood Mini Kit. Medium resolution typing of the alleles at the HLA-Cw and HLA-DRB1 loci was performed by polymerase chain reaction amplification using sequence-specific primers (PCR-SSP) (PROTRANS, Germany) according to the manufacturer's instructions.

Baseline characteristics were summarized with mean and standard deviation (SD) for continuous variables and proportions for categorical variables. For inferential statistics, parametric or non-parametric tests were used as appropriate. Univariate and stepwise multivariate logistic regression models were used to analyze all variables as potential predictors for the development of NVP HSR. All variables with a $P < 0.10$ in univariate logistic regression models were considered for inclusion in the multivariate logistic

regression model. Odds ratios (OR) and 95% confidence intervals were calculated. All tests were two sided and a P -value < 0.05 was taken as significant. Analyses were performed using SPSS for Windows, version 13.0.

During June 2005 and June 2009, the accumulated number of HIV-1 infected patients received NVP based cART was 430 in the four counties and municipalities in Hubei province. Thirty two out of them were diagnosed NVP HSR after cART initiation. Twelve out of 32 cases of NVP HSR patients developed concomitant allergic hepatotoxicity. We tested 71 samples from NVP tolerant patients which were made available to us with patients' consent for HLA typing. Thus, a total of 103 cases of HIV-infected subjects, whose mean age at the time of starting cART was (41 ± 39.8) years old (range: 16 years-62 years), were enrolled in the study. Most of them (70.9%, 73/103) got HIV infected through improper blood donation or transfusion in the period of 1993–1996. The mean baseline CD4 cell count for all the 103 subjects was (90.8 ± 67.7) cells/ul (range: 1 cells/ul-247 cells/ul). The two groups of NVP HSR and NVP tolerant patients were compared for age, sex, risk group of HIV infection, baseline CD4 cell count, concomitant use of NRTIs, liver function and hepatitis C and/or B virus co-infections. None of the considered variables had significant difference between the two groups (Table 1).

The HLA typing results showed that HLA-Cw*04 allele had a significantly higher frequency in the group of NVP HSR individuals. Twelve point five percent (8/64) of the NVP HSR subjects had the HLA-Cw*04 in comparison with 4.2% (6/142) of the NVP-tolerant group ($P = 0.029$). Nineteen percent (27/142) of the NVP-tolerant subjects had HLA-DRB1*15 in comparison with 6.3% (4/64) of the NVP hypersensitive group ($P = 0.018$,

table 2).

In order to define the risk factors associated with NVP HSR, the related variables listed in Table 1 and Table 2 were brought into binary logistic regression models. Univariate logistic regression showed that HLA-DRB1*15 carriage was a protective factor against the developing of NVP HSR ($p=0.049$, $OR=0.341$, 95%CI of $OR: 0.117-0.995$), and HLA-CW*04 carriage was a risk factor related to NVP HSR ($p=0.030$, $OR=3.611$, 95%CI of $OR: 1.135-11.489$). Multivariate logistic regression identified that HLA-Cw*04 carriage was a risk factor related to NVP HSR ($p=0.030$, $OR=3.611$, 95%CI of $OR: 1.135-11.489$). The remain variables such as gender, age, HBV/HCV co-infection, HIV infection routes, concomitant use of NRTIs, baseline ALT, baseline CD4 cell count, and the rest HLA-Cw/HLA-DRB1 alleles presence showed no association with NVP HSR development among the subjects.

As one of the NNRTIs, NVP is widely used as a component of cART among HIV-1 infected patients in developing countries^[3]. About 5% of cases who exposed to NVP will develop HSR, which characterized by one or more of the following symptoms: extensive skin rash, fever or liver toxicity according to reports^[18,19]. Though most of the HSR cases recovered finally after NVP withdrawn, NVP HSR may influence the patients on their adherence to therapy, and sometimes it may be fatal^[20]. In the univariate and multivariate logistic regression analysis, no demographic or clinical variables were identified as risk factors associated with NVP HSR in the present study. This result together with other consistent reports indicated that there may be something else act as the determinant role in the development of NVP HSR.

As most of the reports showed that the HLA-DRB1 and/or HLA-Cw allele polymorphisms were related to NVP HSR of the patients^[10,12], the two loci were selected to study in the current research. Multivariate logistic regression analyses implicated that HLA-Cw*04 allele carrying was a risk factor related to NVP HSR, while HLA-DRB1*15 allele carrying was a protective factor against NVP HSR development. No associations were found between NVP HSR and demographic or clinical variants, especially baseline CD4 cell count. Our result is similar to that of the report by Sirirat and his colleagues from Thailand^[21], in which they considered HLA-Cw*04 allele presence was a risk factor associated with NVP rash.

We did not find the association between other HLA allele such as HLA-Cw*08 or HLA-DRB1*01 and NVP HSR, the two HLA alleles being identified respectively as risk factors in previous reports^[10,12]. There may be several reasons for the different results. First and maybe the most important, the subjects in our cohort were all Chinese Han originated. It is possible that the ethnic difference lead to a different sensitivity to the same allergen. Moreover, one HLA allele may play different even contrary roles when involved in the same event for persons with different ethnics according to the previous research. For example, HLA-Cw*04 was reported to be associated with rapid AIDS progression in Caucasians^[22] but have a protective effect in African Americans^[23]. Second, our study focused on NVP HSR, which included patients with solely rash and rash with hepatotoxicity, but the previous studies dealt with patients with solely rash or hepatotoxicity. Third, the degree of immune depression was different in different cohort of patients. The mean baseline CD4 cell count of subjects in our cohort was (90.8 ± 67.7) cells/ul (range: 1 cells/ul

-247cells/ul), which was significantly lower than that of the patients in most of other study cohorts. The mean pretreatment CD4 cell count of subjects were all above 250cells/ul in the previous studies except one from Thailand by Likanonsakul et al., in which the mean baseline CD4 cell count was below 100cells/ul^[10,12,19]. Interestingly, our result was similar to that of Likanonsakul's. It is meaningful to a certain extent. It is possible that different HLA allele plays roles in the development of NVP HSR for patients with different immune conditions.

Certainly, our study has several limitations, including small sample size, lacking of cases with higher baseline CD4 cell count (>250 cells/ul) for control, and only two HLA loci typed. Nonetheless, the current study in a cohort of Chinese Han HIV-1 infected patients, whose baseline CD4 cell count were below 250 cells/ul, adds to the growing literature on the genetic determinants of nevirapine hypersensitivity.

In addition to HLA alleles mentioned above, future screening of patients' HLA genes involving NVP HSR may identify other alleles responsible for the development of NVP HSR. Further studies involving larger cohorts of patients from different races with different level of immune suppression are needed for clearly understanding the value of HLA typing in the treatment of HIV infection.

Acknowledgements

The authors are grateful to Laiqiu Xie, shengshuang Xie, Yupin Rong and Yongxi Zhang for substantial contribution in data collection and assembly.

Author Disclosure Statement

No competing financial interests exist.

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Table 1 Demographic and clinical characteristics of patients with or without NVP HSR

	All patients N=103	NVP tolerant n=71	NVP HSR n=32	<i>P</i>
Male [§]	41(39.8)	28(39.4)	13(40.6)	0.909
Age(year) [#]	40.3(10.6)	38.8(10.4)	41.3(9.8)	0.137
HIV risk group				0.105
Blood [§]	73(70.9)	54(76.1)	19(59.4)	
Sex [§]	25(24.3)	13(18.3)	12(37.5)	
Others [§]	5(4.8)	4(5.6)	1(3.1)	
Baseline CD4 (cells/ul) [#]	103.9(72.6)	99.3(63.2)	114.0(90.6)	0.411
Baseline CD4				0.059
<50cells/ul [§]	27(26.2)	17(24.0)	10(31.2)	
50~99cells/ul [§]	31(30.1)	24(33.8)	7(21.9)	
100~199cells/ul [§]	36(35.0)	27(38.0)	9(28.1)	
≥ 200cells/ul [§]	9(8.7)	3(4.2)	6(18.8)	

HBV co-infection [§]	9(8.7)	8(11.3)	1(3.1)	0.268
HCV co-infection [§]	33(32.0)	24(33.8)	9(28.1)	0.568
Baseline ALT(U/L) [#]	33.2(9.1)	32.6(9.0)	31.4(9.2)	0.365
ALT elevation at baseline [§]	11(10.7)	6(8.5)	5(15.6)	0.497
Concomitant NRTI				
AZT [§]	54(52.4)	38(53.5)	16(50.0)	0.741
DDI [§]	24(23.3)	18(25.4)	6(18.8)	0.463
D4T [§]	49(47.6)	33(46.5)	16(50.0)	0.741
3TC [§]	80(77.7)	54(76.1)	26(81.2)	0.558

Note: # mean(standard deviation); § number(%). NVP, nevirapine; HSR, hypersensitive reaction; AZT, zidovudine; DDI, didanosine; D4T, stavudine; 3TC, lamivudine; ALT, Alanine aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus.

Table 2 Occurrence of HLA-Cw and HLA-DRB1 alleles in patients with NVP HSR and NVP-tolerance

HLA-Cw				HLA-DRB1			
Allele	NVP tolerant	NVP HSR	P	Allele	NVP tolerant	NVP HSR	P
Cw*01	32(22.5)	13(20.3)	0.721	DRB1*01	4(2.8)	0	0.586
Cw*02	1(0.7)	0	1.000	DRB1*03	8(5.6)	2(3.1)	0.438
Cw*03	28(19.7)	8(12.5)	0.207	DRB1*04	10(7.0)	7(10.9)	0.339
Cw*04	6(4.2)	8(12.5)	0.029	DRB1*07	9(10.6)	4(6.3)	0.981
Cw*05	29(1.4)	0	1.000	DRB1*08	15(12.5)	7(10.9)	0.936
Cw*06	8(5.6)	3(4.7)	0.780	DRB1*09	34(23.9)	15(23.4)	0.937
Cw*07	34(23.9)	15(23.4)	0.937	DRB1*10	1(0.7)	1(1.6)	0.526
Cw*08	11(7.7)	5(7.8)	0.987	DRB1*11	6(4.2)	5(7.8)	0.289
Cw*12	8(5.6)	2(3.1)	0.438	DRB1*12	18(12.7)	9(14.1)	0.785
Cw*14	9(6.3)	7(10.9)	0.254	DRB1*13	4(2.8)	3(4.7)	0.679
Cw*15	3(2.1)	2(3.1)	0.647	DRB1*14	2(1.4)	3(4.7)	0.175

Cw*16	0	1(1.6)	0.311	DRB1*15	27(19.0)	4(6.3)	0.018
				DRB1*16	4(2.8)	4(6.3)	0.258
Total	142(100.0)	64(100.0)			142(100.0)	64(100.0)	

Note: HLA, human leukocyte antigen; NVP, nevirapine; HSR, hypersensitive reaction.