*HLA-B*3505* allele is a strong predictor for nevirapine-induced skin adverse drug reactions in HIV-infected patients of Thailand

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Objective **Investigation of a possible involvement of differences in human leukocyte antigens (HLA) in the risk of nevirapine (NVP)-induced skin rash among HIV-infected patients.**

Methods **A step-wise case–control association study was conducted. The first set of samples consisted of 80 samples from patients with NVP-induced skin rash and 80 samples from NVP-tolerant patients. These patients were genotyped for the *HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DPB1,* and *HLA-DQB1* by a sequence-based HLA typing method. Subsequently, we verified HLA alleles that showed a possible association in the first screening using an additional set of samples consisting of 67 cases with NVP-induced skin rash and 105 controls.**

Results **An *HLA-B*3505* allele revealed a significant association with NVP-induced skin rash in the first and second screenings. In the combined data set, the *HLA-B*3505* allele was observed in 17.5% of the patients with NVP-induced skin rash compared with only 1.1% observed in NVP-tolerant patients [odds ratio (OR) = 18.96; 95% confidence interval (CI) = 4.87–73.44, $Pc = 4.6 \times 10^{-6}$] and 0.7% in general Thai population (OR = 29.87; 95% CI = 5.04–175.86, $Pc = 2.6 \times 10^{-5}$). The logistic regression analysis also indicated *HLA-B*3505* to be significantly associated with skin rash with OR of 49.15 (95% CI = 6.45–374.41, $P = 0.00017$).**

Conclusion **A strong association between the *HLA-B*3505* and NVP-induced skin rash provides a novel insight into the pathogenesis of drug-induced rash in the HIV-infected population. On account of its high specificity (98.9%) in identifying NVP-induced rash, it is possible to utilize the *HLA-B*3505* as a marker to avoid a subset of NVP-induced rash, at least in Thai population. *Pharmacogenetics and Genomics* 00:000–000 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Nevirapine (NVP) is a potent non-nucleoside reverse transcriptase inhibitor which is frequently used as one of the basic components for highly active antiretroviral therapy to HIV-1 infection. NVP is commonly prescribed in resource-limited countries because of the availability of a generic drug at cheap cost. GPO-VIR, a fixed-dose combination of 30/40 mg of stavudine, 150 mg of lamivudine, and 200 mg of NVP has been widely used for scaling up HIV/AIDS treatment in Thailand since 2002 [1]. However, NVP often causes cutaneous adverse drug reactions (cADRs) with an incidence of approximately 15–20% [2–5]. The skin reactions range from mild localized maculopapular rash, with increasing severity, to diffuse maculopapular rashes, and generalized bullous lesions. Severe fatal reactions, Stevens–Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN) were also observed [4–6].

The mechanism of NVP-induced skin rash is unknown. Some clinical features or characteristics that are associated with NVP-induced skin rash are described, such as female sex, pretreatment with antiretroviral drugs before the NVP treatment, a higher level of the number of CD4-positive cells at the beginning of the treatment,

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and the presence of drug-allergy history [4,5,7–9]. The relationship between an exposure to NVP and a risk of adverse reactions has been the subject of several studies. Host genetic factors involved in our immune response have been suspected to play some critical roles; for example, nevirapine may trigger an immunological response that is dependent on CD4-positive T lymphocytes. A majority of patients with NVP-associated rash develop their symptoms between the first and third week after the NVP initiation [3,10], and when the drug is rechallenged to the patients, these symptoms appear earlier and become more serious [10]. The fact that the lower risk of rash development was implicated in patients with a lower number of CD4-positive cells is consistent with other reports indicating a mechanism of immune tolerance in those with severe immunodeficiency. The adverse reactions are more frequent and severe in immunologically uncompromised individuals who took NVP as prophylaxis of HIV infection [10,11]. Recent studies have suggested that the toxicity of some drugs is not dose dependent but more likely because of a hypersensitivity reaction of them or their metabolites that act as specific antigens and trigger a CD4-mediated immune response in individuals who are genetically susceptible [12–17].

Recently, several observations have implicated a possibility that certain genetic factors are involved in the pathogenesis of adverse and hypersensitivity reactions [13,15,18–23]. The hypersensitivity reactions of an antiretroviral drug, abacavir, have been extensively studied and its association with the *HLA-B*5701* allele in Caucasians has been strongly implicated [18–20]. Recent observations of NVP-induced hypersensitivity reaction in Australian [13], French [14], Sardinian [15], and Japanese populations [16] supported the association of the hypersensitivity reaction with various human leukocyte antigen (HLA) markers located within the major histocompatibility complex region.

We aimed to investigate an association between HLA markers and NVP-induced skin rash by means of a case–control study of NVP-receiving HIV patients in Thai population and here report a possible involvement of an *HLA-B*3505* allele in NVP-induced skin rash in Thai population.

Materials and methods

Study population

A case–control study was conducted by using HIV-infected Thai patients who visited (i) Infectious Disease Clinic, Ramathibodi Hospital, Mahidol University, (ii) Bamrasnaradura Infectious Disease Institute, Ministry of Public Health, or (iii) Department of Preventive Medicine, Faculty of Medicine, Srinakharinwirot University, Thailand. The enrollment of 80 skin-rash cases and 80 NPV-tolerant patients for the first set of samples was done between March and December 2006. To verify a possible association in the initial screening, the second set of samples consisting of 67 skin-rash patients and 105 controls was enrolled between January and June 2007.

Inclusion criteria were adult HIV-infected patients (> 15 years old) who were treated with GPO-VIR. Each eligible patient was followed up for at least 6 months after the initiation of NVP treatment or until they developed skin rash. Patients were categorized into case and control groups according to the presence or absence of skin rash. The control group was defined as the patient who had been treated with NVP for at least 6 months and developed no sign of cADRs. The case group was defined to be those with skin rash within 6 months after the beginning of NVP treatment. The diagnosis of the NVP-induced rash was reviewed and given by infectious disease specialists. Severity of rash was categorized according to the Division of AIDS table for grading the severity of adult and pediatric adverse events, National Institute of Allergy and Infectious Disease/National Institutes of Health [24]. Briefly, grade 1: a localized macular rash; grade 2: diffuse macular, maculopapular, or morbilliform rash or target lesions; grade 3: diffuse macular, maculopapular, or morbilliform rashes with vesicles or a limited number of bullae or superficial ulcerations of mucous membrane limited to one site; and grade 4: extensive or generalized bullous lesions, SJS, ulceration of mucous membrane involving more than two distinct mucosal sites, or TEN. Collection of blood samples and clinicopathological information were undertaken with informed consent and approved by the Institutional Review Boards. This study was conducted in accordance with the principles of the Declaration of Helsinki [25].

DNA isolation

Genomic DNA was isolated with a standard phenol-chloroform extraction protocol and resuspended in Tris-HCl buffer (pH 8.5). Their concentration was quantified by using a UV spectrophotometer ND-1000 (NanoDrop Technologies, Wilmington, Delaware, USA). The purity was determined by calculating the ratio of absorbance at 260–280 nm.

Human leukocyte antigen genotyping

Genotypes in *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*, *HLA-DPB1*, and *HLA-DQB* were determined by sequence-based typing using the AlleleSEQR Sequenced Based Typing Kits (Atria Genetics, South San Francisco, California, USA), according to the manufacturer's instructions. Briefly, the primary amplification reaction consists of PCR premix reagent, AmpliTaq, Gold and 20 ng/μl genomic DNA. The PCR products were purified by using ExoSAP-IT and sequenced in the forward and reverse orientations. Finally, the reaction products were reconstituted with 15 μl of Hi-Di formanide (Applied Biosystems, Foster City, California, USA) ran on the ABI 3730*xl* DNA

Analyzer (Applied Biosystems) and genotype calling was made by Assign SBT 3.2.7 software (Conexio Genomics, Perth, Australia). HLA alleles showing a possible association (*Pc* value < 0.05) with ~~NPV~~ NVP-induced skin rash in the first screening were verified in the second set of samples. Allelic frequency of HLA regions in the general Thai population was obtained from the published information [26].

Statistical analysis

Dichotomous variables (presented as frequency, with percentage) between the two groups were compared by using Fisher's exact tests. Continuous variables (presented as median, with interquartile range) were compared by the Mann–Whitney *U*-test. Haplotype reconstruction of the combined HLA loci was performed by using the program PHASE version 2.0 [27]. A comparison of allele and haplotype frequencies between the two groups was made using Fisher's exact tests. Odds ratios were calculated with Haldane's modification, which add 0.5 to all cells to accommodate possible zero counts [28]. All *P* values were two-tailed and *P* values of less than 0.05 were considered to indicate possible statistical significance. The corrected *P* (*Pc*) values were adjusted by applying Bonferroni's correction for 93 tests (14 for *HLA-A*, 20 for *HLA-B*, 16 for *HLA-C*, 18 for *HLA-DRB1*, 12 for *HLA-DQB1*, and 13 for *HLA-DPB1*) to account for the number of alleles observed in more than 1% of the total number of case and control patients.

A multivariate logistic regression model including clinical variables that was shown to be associated with NVP-induced skin rash and *HLA-B*3505* status of the patients was constructed. *HLA-B*3505* was coded according to the presence of *HLA-B*3505* under a dominant model. In accordance with an earlier study of Kiertiburanakul *et al.* [7], the CD4 cell count just before the initiation of the NVP treatment was grouped into intervals of 50 cells/mm^3. The clinical information of prescribed lead-in dosing, the history of drug allergy, and concomitant drugs were also included in the model. The linearity of the model was assessed by residual plot against the log odds. The additive assumption was assessed by introducing possible clinical variables, which interact with *HLA-B*3505* status in the model. The interaction terms with the *P* value of more than 0.05 were considered to be not significant, and then the factors were excluded from the final model. All statistical analyses were performed by using the R statistic program [29].

Results

Characteristics of patients and controls

Demographic variables, immunological status, available liver function tests, history of drug allergy, concurrent medications, time from the first exposure to develop skin rash and their severity of 147 patients with NVP-induced skin rash (80 and 67 patients from the first and second

Table 1 Clinical, immunological, and demographical characteristics of the patients exposed to antiretroviral therapy with or without NVP-induced cutaneous adverse drug reactions

Characteristic	First screening			Second screening			Total		
	NVP-rash ($n=80$)	NVP-tolerant ($n=80$)	*P* value	NVP-rash ($n=67$)	NVP-tolerant ($n=105$)	*P* value	NVP-rash ($N=147$)	NVP-tolerant ($N=185$)	*P* value
Sex, *n* (%)			1.00			0.041			0.15
Male	38 (47.5)	37 (46.3)		25 (37.3)	57 (54.3)		63 (42.9)	94 (50.8)	
Female	42 (52.5)	43 (53.8)		42 (62.7)	48 (45.7)		84 (57.1)	91 (49.2)	
Age (years), median (IQR)	35.7 (31.4–41.1)	35.1 (30.8–41.9)	0.61	37 (32.5–42.8)	35.5 (31.9–41.6)	0.40	36.4 (32.1–42.5)	35.5 (31.6–41.8)	0.46
Body weight (kg), median (IQR)	54 (48.5–60.5)	54.5 (48–64)	0.68	52 (46–58.7)	55 (50–63)	0.039	53 (47–60)	55 (49–64)	0.094
AIDS defining-illness, *n* (%)	34 (42.5)	44 (55)	0.15	45 (67.2)	59 (56.2)	0.20	79 (53.7)	103 (55.7)	0.74
Prescribed lead-in, *n* (%)	47 (58.8)	60 (75)	0.043	33 (50.8)	78 (77.2)	0.00066	80 (55.2)	138 (76.2)	0.000085
CD4 cell value at NVP initiation, median (IQR)									
CD4 cell count (cells/mm^3)	206 (77–367) ($n=80$)	63 (21–203) ($n=78$)	0.00010	76.5 (33.5–178.5) ($n=64$)	69 (20.3–211) ($n=103$)	0.60	154.5 (45.5–249) ($n=144$)	69 (20.8–209) ($n=181$)	0.00040
CD4 percentage	12 (5–17) ($n=80$)	6.5 (2–11.5) ($n=80$)	0.00070	6 (3–10.3) ($n=62$)	6 (2–11) ($n=98$)	0.92	8 (4–14) ($n=142$)	6 (2–11) ($n=178$)	0.0048
Liver function test at NVP initiation, median (IQR)									
AST (mg/dl)	31 (22.5–49.5) ($n=36$)	27.5 (21–43) ($n=42$)	0.30	30 (22.3–39) ($n=39$)	28 (23–44.8) ($n=55$)	0.81	30 (22.3–42.8) ($n=75$)	28 (21.8–44) ($n=97$)	0.61
ALT (mg/dl)	49 (36.3–78.8) ($n=39$)	40.5 (30–58) ($n=46$)	0.071	39.5 (22–53) ($n=39$)	42 (32–52) ($n=58$)	0.31	43 (31–64) ($n=81$)	42 (30.5–56.5) ($n=104$)	0.53

ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range; NVP, nevirapine.
P values were calculated by Fisher's exact test (dichotomous variables) and the Mann–Whitney *U* test (continuous variables) comparing between NVP-induced skin rash patients with those of the NVP-tolerant controls.

recruitments, respectively), and 185 controls (80 and 105 patients from the first and second recruitments, respectively) are summarized in Tables 1 and 2. The median time to develop rash was 12 days (interquartile range, 8–22). Among the 147 NPV-induced rash patients, 13 patients (9%) were classified as grade 1, 51 (35.7%) as grade 2, 68 (47.6%) as grade 3 and 11 (7.7%) as grade 4. The distribution of sex, age, body weight, history of AIDS defining-illness, and liver function tests at the initiation of NVP treatment in the case group showed no difference to those in the tolerant control. However, the proportion of the patient with history of drug allergy ($P = 0.00061$), concurrent medications ($P = 0.0028$), as well as the number of CD4-positive cells at the beginning of the NVP treatment were higher in the case group than the control group ($P = 0.00040$ and 0.0048, respectively), whereas the proportion of the patients with prescribed lead-in of NVP was higher in the control than in the case group.

Association between *HLA-B*3505* and nevirapine-induced skin rash

By DNA sequencing, we determined genotypes at the *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*, *HLA-DQB1*, and *HLA-DPB1* loci in the first set of 80 cases and 80 controls. The results of the first screening are summarized in Table 3; *HLA-A*2407*, *HLA-B*3505*, *HLA-Cw*0401*, and *HLA-DRB1*1202* alleles were observed at the significantly higher frequencies in the case group than the control group. In contrast, *HLA-B*3802* and *HLA-Cw*0702* were found to be significantly lower in the case group than the control group. After the stringent Bonferroni's correction, only *HLA-B*3505* remained significantly different between case and control groups ($Pc = 3.5 \times 10^{-2}$). The *HLA-B*3505* was present in 9.9% of the patients with NVP-induced skin rash, but only 0.7% in the NVP-tolerant group. Guided by the patients who were *HLA-B*3505* carriers, we analyzed the allelic distribution of the combined HLA loci and defined the extended haplotype. The most common haplotype combination, *HLA-Cw*0401–HLA-B*3505*, was present in 7.8% of the patients with NVP-induced skin rash, but only 0.7% in the tolerant group. Although the statistical evidence from the haplotype analysis is not directly comparable with the univariate analysis, the higher effect size of *HLA-B*3505* and robust statistical evidence after Bonferroni's correction supported this allele(s) or those in linkage disequilibrium with this allele as the causative genetic variation.

We further genotyped *HLA-B* locus to confirm the association of *HLA-B*3505* in 67 NVP-induced skin rash patients and 105 tolerant controls. The results are summarized in Table 4. In concordance with the first screening, *HLA-B*3505* also showed an association with NVP-induced skin rash when comparing individuals carrying *HLA-B*3505* among the case and control groups in the second screening ($Pc = 1.4 \times 10^{-2}$). When the combined data set was analyzed, *HLA-B*3505* allele revealed statistically significant increase in frequencies in the NVP-induced skin rash group, compared with the NVP-tolerant group; the *HLA-B*3505* allele was presented in 17.5% (25 of 143 patients) in patients with NVP-induced

Table 2 Clinical, immunological, and demographical characteristics of the patients exposed to antiretroviral therapy with or without NVP-induced cutaneous adverse drug reactions (continued)

	First screening			Second screening			Total		
Characteristic	NVP-rash ($n=80$)	NVP-tolerant ($n=80$)	*P* value	NVP-rash ($n=67$)	NVP-tolerant ($n=105$)	*P* value	NVP-rash ($N=147$)	NVP-tolerant ($N=185$)	*P* value
History of drug allergy[a], *n* (%)	16 (20)	10 (12.5)	0.28	20 (29.9)	9 (8.6)	0.0006	36 (24.5)	19 (10.3)	0.00061
Sulfamethozaxole	14 (17.5)	5 (6.3)	0.048	13 (19.4)	5 (4.8)	0.0039	27 (18.4)	10 (5.4)	0.00033
Dapsone	3 (3.8)	0 (0)	0.25	3 (4.5)	1 (1)	0.30	6 (4.1)	1 (0.5)	0.047
Penicillin	1 (1.3)	0 (0)	1.00	1 (1.5)	0 (0)	0.39	2 (1.4)	0 (0)	0.20
Carbamazepine	0 (0)	0 (0)	1.00	1 (1.5)	0 (0)	0.39	1 (0.7)	0 (0)	0.44
Antituberculous drugs	0 (0)	2 (2.5)	0.50	0 (0)	3 (2.9)	0.28	0 (0)	5 (2.7)	0.069
Others	6 (7.5)	4 (5)	0.75	6 (9)	2 (1.9)	0.057	12 (8.2)	6 (3.2)	0.055
Concomitant drugs[b], *n* (%)	34 (42.5)	55 (68.8)		44 (67.7)	74 (71.2)	0.73	78 (53.8)	129 (70.1)	0.0028
Fluconazole	15 (18.8)	33 (41.3)	0.0014	26 (42.6)	42 (40.4)	0.87	41 (29.1)	75 (40.8)	0.035
Co-trimoxazole	15 (18.8)	18 (22.5)	0.0031	29 (43.3)	36 (34.6)	0.26	44 (29.9)	54 (29.3)	1.00
Dapsone	1 (1.3)	2 (2.5)	0.70	8 (11.9)	5 (4.8)	0.14	9 (6.1)	7 (3.8)	0.44
Antituberculous drugs	5 (6.3)	4 (5)	1.00	5 (7.5)	12 (11.5)	0.44	10 (6.8)	16 (8.7)	0.55
Others	3 (3.8)	2 (2.5)	1.00	2 (3)	6 (5.8)	0.48	5 (3.4)	8 (4.3)	0.78
Time to develop rash (days), Median (IQR)	12 (7–22)	Not observed		14 (10–21)	Not observed		12 (8–22)	Not observed	
Severity of rash, *n* (%)									
Grade 1	0 (0)	Not observed		13 (20.3)	Not observed		13 (9)	Not observed	
Grade 2	28 (35.4)	Not observed		23 (36)	Not observed		51 (35.7)	Not observed	
Grade 3	50 (63.3)	Not observed		18 (28.1)	Not observed		68 (47.6)	Not observed	
Grade 4	1 (1.3)	Not observed		10 (15.6)	Not observed		11 (7.7)	Not observed	

IQR, interquartile range; NVP, nevirapine.
[a]Some patients had more than one drug to cause allergy.
[b]Some patients took more than one concomitant drug.
P values were calculated by Fisher's exact test (dichotomous variables) and the Mann–Whitney *U* test (continuous variables) comparing between NVP-induced skin rash patients with those of the NVP-tolerant controls.

Table 3 First screening association of HLA alleles and haplotypes with NVP-induced cutaneous adverse drug reactions

	All examined alleles		*n* (%)				Odds ratio
MHC marker	NVP-rash	NVP-tolerant	NVP-rash	NVP-tolerant	*P* value	*Pc* value	(95% CI)
HLA-A							
**2407*	128	144	10 (7.8)	1 (0.7)	3.6×10^{-3}	NS	12.12 (1.96–74.24)
HLA-B							
**3505*	152	152	15 (9.9)	1 (0.7)	3.8×10^{-4}	3.5×10^{-2}	16.53 (2.74–98.98)
**3802*	152	152	3 (2)	13 (8.6)	1.8×10^{-2}	NS	0.22 (0.07–0.72)
HLA-Cw							
**0401*	128	144	17 (13.3)	4 (2.8)	1.3×10^{-3}	NS	5.36 (1.83–15.60)
**0702*	128	144	14 (10.9)	34 (23.6)	6.7×10^{-3}	NS	0.40 (0.20–0.78)
HLA-DRB1							
**1202*	160	160	41 (10.9)	19 (11.9)	2.4×10^{-3}	NS	2.56 (1.41–4.61)
Haplotype							
*A*2407, B*3505*	128	144	6 (4.7)	1 (0.7)	NS	NS	7.03 (1.09–44.94)
*Cw*0401, B*3505*	128	144	10 (7.8)	1 (0.7)	3.6×10^{-3}	NS	12.12 (1.96–74.24)
*B*3505, DRB1*1202*	128	144	6 (4.7)	1 (0.7)	NS	NS	7.03 (1.09–44.94)
*A*2407, Cw*0401, B*3505*	128	144	6 (4.7)	1 (0.7)	NS	NS	7.03 (1.09–44.94)
*A*2407, B*3505, DRB1*1202*	128	144	5 (3.9)	1 (0.7)	NS	NS	5.81 (0.88–37.92)
*Cw*0401, B*3505, DRB1*1202*	128	144	6 (4.7)	1 (0.7)	NS	NS	7.03 (1.09–44.94)
*A*2407, Cw*0401, B*3505, DRB1*1202*	128	144	5 (3.9)	1 (0.7)	NS	NS	5.81 (0.88–37.92)

CI, confidence interval; HLA, human leukocyte antigens; MHC, major histocompatibility complex; *n*, number of positive allele; NS, not significant ($P > 0.05$); NVP, nevirapine; *Pc* value, corrected *P* value.
P values were calculated by Fisher's exact test comparing the positive alleles of NVP-induced skin rash patients with those of the NVP-tolerant controls and of the general population controls. *Pc* values were adjusted by using Bonferroni's correction for multiple comparisons (14 for *HLA-A*, 20 for *HLA-B*, 16 for *HLA-C*, 18 for *HLA-DRB1*, 12 for *HLA-DQB1* and 13 for *HLA-DPB1*).

Table 4 Risk HLA allele of NVP-induced cutaneous adverse drug reactions, *HLA-B*3505*

	All patients		*n* (%)				Odds ratio	*n* (%)			Odds ratio
Screening	NVP-rash	NVP-tolerant	NVP-rash	NVP-tolerant	*P* value	*Pc* value	(95% CI)	General population (*n* = 142)[a]	*P* value	*Pc* value	(95% CI)
First	76	76	14 (18.4)	1 (1.3)	5.5×10^{-4}	5.1×10^{-2}	16.94 (2.75–102.82)	1 (0.7)	1.8×10^{-6}	1.7×10^{-4}	31.84 (5.20–192.46)
Second	67	105	11 (16.4)	1 (1)	1.5×10^{-4}	1.4×10^{-2}	20.43 (3.28–125.46)	1 (0.7)	1.8×10^{-5}	1.7×10^{-3}	27.70 (4.45–169.80)
Total	143	181	25 (17.5)	2 (1.1)	4.9×10^{-8}	4.6×10^{-6}	18.96 (4.87–73.44)	1 (0.7)	2.8×10^{-7}	2.6×10^{-5}	29.87 (5.04–175.86)
Severity of skin rash											
mild to moderate	62	181	11 (17.7)	2 (1.1)	7.3×10^{-6}	6.8×10^{-4}	19.30 (4.62–79.84)	1 (0.7)	9.5×10^{-6}	8.9×10^{-4}	30.41 (4.88–186.67)
Severe	77	181	14 (18.2)	2 (1.1)	1.3×10^{-6}	1.2×10^{-4}	19.89 (4.88–80.30)	1 (0.7)	2.1×10^{-6}	1.9×10^{-4}	31.33 (5.12–189.38)

CI, confidence interval; HLA, human leukocyte antigens; *n*, number of patients; NS, not significant ($P > 0.05$); NVP, nevirapine; *Pc* value, corrected *P* value.
[a]Allelic frequency of HLA regions in the general Thai population was obtained from the published information [26]. *P* values were calculated by Fisher's exact test comparing the positive alleles of NVP-induced skin rash patients with those of the NVP-tolerant controls and of the general population controls. *Pc* values were adjusted by using Bonferroni's correction for multiple comparisons (14 for *HLA-A*, 20 for *HLA-B*, 16 for *HLA-C*, 18 for *HLA-DRB1*, 12 for *HLA-DQB1* and 13 for *HLA-DPB1*).

skin rash, but only 1.1% (2 of 181 patients) in the NVP-tolerant group [odds ratio (OR) = 18.96; 95% confidence interval (CI) = 4.87–73.44; $Pc = 4.6 \times 10^{-6}$], and only 0.7% (1 of 142 ~~patients~~) in the general Thai population (OR = 29.87; 95% CI = 5.04–175.86; $Pc = 2.6 \times 10^{-5}$).

We further analyzed an association of *HLA-B*3505* according to the severity of skin rash. Patients with grade 1 and 2 severities were grouped together into a 'mild-to-moderate rash' group and those with grade 3 and 4 into a 'severe rash' group (Table 4). The result revealed

Table 5 Risk factors for rash by logistic regression

Factors	Odds ratio (95% confidence interval)	*P* value
*HLA-B*3505*	49.15 (6.45–374.41)	0.00017
History of drug allergy	2.83 (1.47–5.45)	0.0019
Prescribed lead-in	0.48 (0.28–0.82)	0.0073
CD4 cell count at NVP initiation, each 50 cells/mm^3 increment	1.11 (1.03–1.20)	0.0076

NVP, nevirapine.

significantly higher frequencies of *HLA-B*3505* in both groups than the NVP-tolerant group ($Pc = 6.8 \times 10^{-4}$ and $Pc = 1.2 \times 10^{-4}$, respectively). Thus, the association of *HLA-B*3505* with the NVP-induced skin rash is likely to be independent of the severity, indicating that *HLA-B*3505* may be important for the initiation of the immune response to cause skin rash.

As subsequent multiple logistic regression analysis including the information of concomitant drugs indicated that this variable was not associated with cADRs after adjusting for other variables, we had excluded this variable from the final logistic model. The variables included in the final logistic regression model, the odds ratios, and their significance levels are presented in Table 5. The *HLA-B*3505* status (OR = 49.15; 95% CI = 6.45–374.41; $P = 0.00017$), positive history of drug allergy (OR = 2.83; 95% CI = 1.47–5.45; $P = 0.0019$), and the number of CD4 cells before the NVP treatment (OR = 1.11 for each 50 cells/mm^3 increment; 95% CI = 1.03–1.20; $P = 0.0076$) were found to be significantly associated with the risk of skin rash. Although the regimen of the lead-in dosing of NVP significantly decreased the risk of skin rash (OR = 0.48; 95% CI = 0.28–0.82; $P = 0.0073$), the interactions between the *HLA-B*3505* and other variables were not statistically significant when included in the multivariate logistic regression (data not shown).

Discussion

In this study, we identified an association of the *HLA-B*3505* allele with the risk of the NVP-induced skin rash in Thai population. The majority of rash occurred within 6 weeks after the initiation of NVP treatment. We found that the median time to develop rash was 12 days, as concordant with our earlier report [7]. The *HLA-B*3505* allele was observed in 17.5% of the patients with NVP-induced skin rash, whereas it was observed in only 1.1% in the NVP-tolerant group and 0.7% in the general Thai population that we retrieved from online database (*www.allelefrequency.net* [26]). As the *HLA-B*3505* allele is present in other populations (for examples, 0.2–0.8% in North Americans, 0.7–1.0% in Han Chinese, 2.2% in Brazilian, 6.9% in Malay, 8.5% in Filipino, [30]), this result may also be applied in other ethnic groups and it is urgent and important to examine in patients with NVP-induced skin rash in other populations.

Recent studies also reported an association of NVP hypersensitivity with other HLA loci. *HLA-DRB1*0101* and the status of CD4-positive cells were suggested to render individuals with a risk of NVP hypersensitivity in an Australian study [13] and in a French Caucasian cohort study [14]. In contrast, involvement of the *HLA-Cw*0802-B*1402* haplotype was suggested in a Sardinian population [15]. As *HLA-Cw*0802* and *HLA-B*1402* are in very strong linkage disequilibrium, the authors were not able to establish which alleles were primarily associated with the hypersensitivity reaction to NVP. An additional report using Japanese population suggested the association of *HLA-Cw8* with hypersensitivity to NVP [16]. However, these increased susceptibilities to the NVP hypersensitivity reaction reported in other populations were not confirmed in this study. *HLA-DRB1*0101* was observed in 2 (2.5%) of 80 NVP-tolerant patients, but was found in none of the individuals in the case group. *HLA-Cw*0801* allelic distribution was slightly higher in the case group (13 of 64 patients, 20.3%) than in the tolerance group (10 of 72 patients, 13.9%), but the difference was not statistically significant. *HLA-Cw*0802*, *HLA-B*1402* and *HLA-Cw*0802-B*1402* haplotypes were found in none in our study as well as in the general Thai population reported earlier [26,31]. Although the inconsistency of the association results may be partly explained as their indirect association effect because of the linkage disequilibrium with the truly associated alleles, we had provided convincing statistical evidence that *HLA-B*3505* is the most promising candidate among class I and II major histocompatibility complex molecules as the causative one for NVP-induced skin rash. To consider whether this statistical evidence supported the *HLA-B*3505* itself as the causative gene or other polymorphism(s) in linkage disequilibrium with *HLA-B*3505*, further fine mapping within this region would be required. Some evidence of sequence evolution of *HLA-B* may, however, provide indirect support that the association with various *HLA-B* alleles occurred because of convergence-binding properties of difference *HLA-B* alleles. *HLA-B*3505* and *HLA-B*1402* were categorized into B07/B27 supertype by their peptide-binding properties [32,33]. Convergence of peptide-binding sequences suggested that the substrate(s) of these two HLA molecules might share similar structures. A study of binding capacities of these *HLA-B* alleles should also be explored to confirm their binding properties to the specific peptide(s), metabolite(s), or complex(s) of peptide and metabolite in the serum of NVP-exposed patients that might play a role to be a trigger of a unfavorable immune response.

Recently, several studies identified *HLA-B*1502* and *HLA-B*5801* as the genetic markers for carbamazepine-induced SJS and allopurinol-induced severe cADRs, respectively [21–23]. This study, together with the

earlier reports, suggests that differences in the *HLA-B* alleles play some critical roles in the pathogenesis of immune-mediated cADRs. A specific *HLA-B* molecule may function as antigenic presentation apparatus for a certain drug or its metabolite to HLA-restricted cytotoxic T cell activation. Further studies should be essential to verify our hypothesis of a direct functional involvement of HLA molecules in the pathogenesis of the disease.

Although the *HLA-B*3505* was observed in significantly higher proportion among the NVP-induced skin rash than the tolerant controls, the frequency was still as low as 17.5% in the case group. However, its specificity is extremely high as 98.9%. Hence, *HLA-B*3505* is likely to be one of the critical determinants of NVP-induced skin rash and there are additional yet-unidentified genetic and nongenetic factors involved in NVP-induced skin rash. These factors may include the factors reported earlier, such as female sex [8], pretreated with antiretroviral drugs before the NVP treatment [4], the number of CD4-positive cells [9], and lower levels of HIV RNA [9]. Association of a higher level of the number of CD4-positive cells as a risk factor for NVP-associated rash was also found to be significant in this study although others were not. In addition to these two factors, the presence of drug allergy history was indicated some relation to development of skin rash in the multivariate logistic regression. Although only 20% of the patients with NVP-induced skin rash had *HLA-B*3505*, NVP-treated patients who had *HLA-B*3505* revealed a very high incidence (25 of 27 patients, 93%) of the skin rash, indicating that *HLA-B*3505* can be a very good predictor for a risk of NVP-induced ADRs for Thai HIV-infected patients.

We have clearly demonstrated the association of *HLA-B*3505* with NVP-induced skin rash regardless of the severities of side effects that were categorized into three classes as follows: (i) maculopapular exanthema, (ii) hypersensitivity syndrome, and (iii) SJS/TEN. We considered that analysis on the basis of this phenotype classification would be desirable, but retrospective nature of the study precludes us in obtaining enough clinical information to differentiate patients who presented with muculopapular exanthema from hypersensitivity syndrome. Therefore, the proposed confirmation study of this finding should include clinical information that shall provide additional information regarding the roles of *HLA-B* in various categories of cutaneous adverse reaction.

In summary, the strong indication of the possible involvement of the *HLA-B*3505* genotype in the development of ~~NPV~~ NVP-induced skin rash provides a novel insight into the understanding of the pathogenesis of drug-induced rash in HIV-infected patients of Thailand. On account of its high specificity in identifying NVP-induced rash, it is possible to utilize the *HLA-B*3505* as a marker to avoid NVP-induced rash in Thai population.

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