CHARPTER IV

RESULTS AND DISCUSSIONS

Since there are 3 anti-HIV drugs in this research work. We shall divide this section into 3 separate parts as follows: 1) Section I: Abacavir; 2) Section II: Nevirapine, and; 3) Section III: Stavudine.

Section I: Abacavir

Result of section I (Abacavir)

Study selection

Of the 687 articles identified, 11 articles met the inclusion criteria [6, 27, 28, 29, 30, 31, 32, 33, 66, 67, 68]. The studies of Mallal, et al. [31] and Martin, et al. [6] contained the same data so the former was excluded. Another study was excluded because it provided insufficient information for the statistical analysis, leaving nine that were included in the meta-analysis [6, 29, 30, 31, 32, 33, 66, 67, 68] (Figure 10). No additional articles were identified in the bibliographies of the included studies.

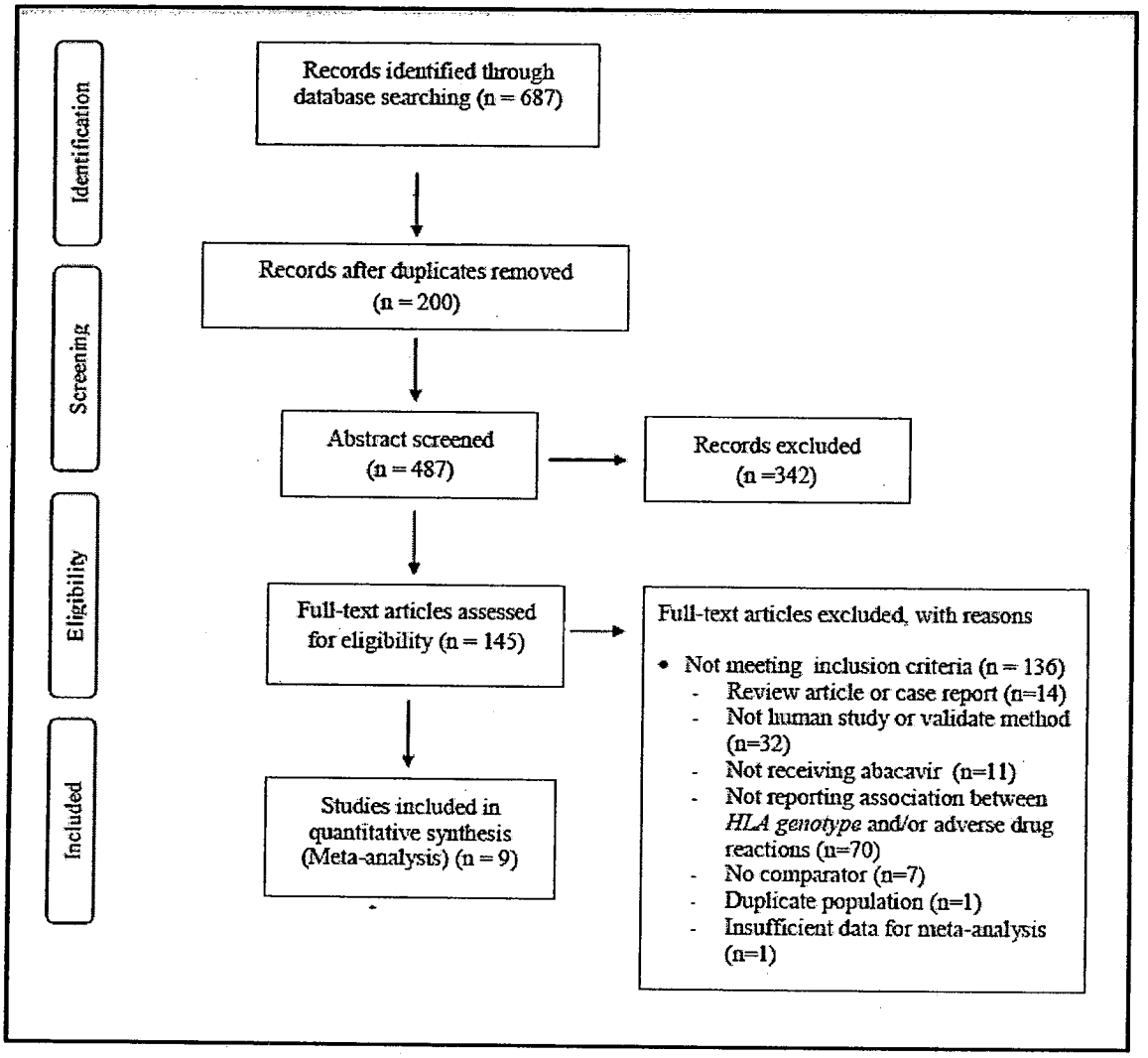

Figure 10 A flow diagram of our systematic searching procedure for the studies related to the association of abacavir included hypersensitivity reactions and HLA-B*5701.

Study characteristics

Characteristics of the included studies are summarized in Table 4 and 5. All of the studies investigated the association between HLA-B*5701 genotype and abacavir-induced hypersensitivity. The studies included 229 cases of abacavir-induced HSRs and 2,594 control subjects. All studies were case-control studies [6, 29, 30, 31, 32, 33, 66, 67, 68]. Six studies were conducted in white populations, three studies in black populations [29, 32, 33], and one study investigated Asians [30]. The mean ages of the patients were 40.7 and 40.8 years in cases and controls, respectively. Males comprised 87% of the cases (117/135) [6,33,66,68] and 82% of controls (712/866) [6, 33, 63, 66, 67]. The starting dose and average dose of abacavir were not reported. In five studies [30, 32, 33, 66, 67] patients using abacavir for 6-52 weeks without HSRs were included in the control group, whereas patients who demonstrated hypersensitivity within 6-10 weeks after commencing the abacavir therapy were classified into the case group [6, 30, 31, 32, 66, 68]. Four studies [30, 32, 66, 68] defined abacavir-induced HSRs by the occurrence of at least two of classic symptoms (fever, rash, gastrointestinal symptoms, malaise, fatigue, respiratory symptom) within 6 weeks after starting the drug. To confirm abacavir-induced HSRs, four studies used the skin patch test [6, 30, 32, 68]. One of the included studies reported the time to onset of the HSR, noting that white patients and black patients demonstrated hypersensitivity within 17 days and 32 days, respectively, after the initiation of abacavir therapy. The studies identified HLA-B*5701 using several distinct techniques: a high resolution DNA-based sequencing method [29, 32, 33], polymerase chain reaction (PCR) as a sequence based typing technique [30, 68], PCR sequence-specific oligonucleotides primers [6, 31, 67], or PCR-restriction fragment length polymorphism [66]. No study provided enough information to calculate HWE.

Quality assessment

The methodological quality of case-control studies was summarized as a mean NOS score of 4.8 (range: 3 to 6) (Table 4, last column).

Table 4 Characteristics of abacavir studies meeting the selection criteria.

No.	Study [reference]	Study design	Study country	Population	Case		Control		Diagnosis of HSR	NOS
					Positive	Negative	Positive	Negative		
1	Hetherington, et al., 2002 [33]	Case-control	UK	White (case 100%, control 100%)	36	29	2	78	Clinical manifestation	4
				Black (case 100%, control 100%)	2	7	1	17		
2	Hughes, et al., 2004 [66]	Case-control	UK	White (case 100%, control 100%)	6	7	5	46	Clinical manifestation	4
3	Martin, et al., 2004 [6]	Case-control	Australia	White (case 100%, control 85%)	17	1	4	226	Skin patch test	5
4	Stekler, et al., 2006 [67]	Case-control	America	White (case 78%, control 95%)	2	5	0	41	Clinical manifestation	6
5	Sun, et al., 2007 [30]	Case-control	Taiwan	Asian (case 100%, control 100%)	1	16	0	215	Clinical manifestation	4
6	Mallal, et al., 2008 [31]	Randomized double-blind	Australia	White (case + control = 84.4%)	23	0	25	794	Skin patch test	6
7	Saag, et al., 2008 [32]	Case-control	United States	White (case 100%, control 100%)	42	0	8	194	Skin patch test	6
				Black (case 100%, control 100%)	5	0	2	204		
8	Munderi, et al., 2011 [29]	Case-control	Uganda	Black (case 100%, control 100%)	0	6	0	241	Clinical manifestation	3
9	Berka, et al., 2012 [68]	Case-control	Canada	White (case 84.2%, control 65.3%)	18	2	0	468	Skin patch test confirm	5

Table 5 Patient demographic information in abacavir included studies for the meta-analysis.

NO.	Author	Year	N		% Male		Mean age, year (range)		ABC Start Dose, mg/day (range)		ABC Mean Dose, mg/day (range)		Actual Duration of ABC Exposure	
			Cases	Control	Cases	Control	Cases	Control	Cases	Control	Cases	Control	Cases	Control
1	Hetherington, et al. [33]	2002	85	115	93	91	40.3 (29-63)	39.8 (24-65)	NR	NR	NR	NR	NR	NR
2	Hughes, et al. [66]	2004	13	51	69	90	34 (22-45)	37 (27-66)	NR	NR	NR	NR	NR	NR
3	Martin, et al. [6]	2004	18	230	83	86	45	42.6	NR	NR	NR	NR	NR	NR
4	Stekler, et al. [67]	2006	9	41	NR	NR	30.6 (24.8-38.8)	33.5 (21.7-45.2)	NR	NR	NR	NR	9 (1-60)	NR
5	Sun, et al. [30]	2007	17	215	91.4		37 (9-80)		NR	NR	NR	NR	NR	NR
6	Mallal, et al. [31]	2008	23	819	71.1		42 (18-72)		NR	NR	NR	NR	NR	NR
7	Saag, et al. [32] (White population)	2008	42	202	82	93	45 (22-73)	41 (19-72)	NR	NR	NR	NR	Within 17 days	NR
	(Black population)		5	206	89	71	45 (22-76)	41 (19-73)	NR	NR	NR	NR	Within 32 days	NR
8	Munderi, et al. [29]	2011	6	241	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
9	Berka, et al. [68]	2012	19	470	73.3	77.4	45 (19-84)	81 (27-69)	NR	NR	NR	NR	NR	NR

Note: NR = Not reported

Quantitative synthesis

Characteristics of the included studies are summarized in Table 5. Nine studies [6, 29, 30, 32, 33, 66, 67, 68] were case-control studies with 219 cases and 2,579 controls were included in the comparisons of carrier frequency to examine the gene association between cases and abacavir-tolerant controls. In these studies, the number of HLA-B*5701 carriers was 152/219 in the cases and 55/2,579 among and the controls. We found a clear association between HLA-B*5701 and abacavir-induced hypersensitivity across the included (OR = 157.91; 95% CI = 31.99-779.40, I^2=77.4%, p = 0.00) (Figure 11 and 12). We explored heterogeneity by performing subgroup analysis according to race. We found that the risk of abacavir-induced HSR was significant in the populations with HLA-B*5701. The summary ORs were 191 (95% CI = 22.83-1587.63; I^2 = 82.6%, p = 0.00) for whites [6, 32, 33, 66, 67, 68] and 39.18 (95% CI = 1.54-999.98; I^2 = 0.0%, p = 0.00) for Asians [30], respectively (Figure 12). However, the summary OR for blacks [29, 32, 33] was not statistically significant (OR = 60.89; 95% CI = 0.34-10,953.99; I^2 = 84.7%, p = 0.01) (Figure 12). However, the heterogeneity was high among white populations [6, 32, 33, 66, 67, 68]. Therefore, subgroup analysis by the method used to diagnose the HSR was performed. By clinical manifestation [33,66,68], the association between abacavir-induced HSR had a summary 20.28 (95% CI = 5.30-77.53; I^2 = 39.1%). By skin patch test [6, 32, 68], the association was far stronger: OR = 1921.65; 95% CI= 414.84-8901.54, I^2 = 0.00) (Figure 13). Therefore the heterogeneity might be explained by racial and diagnostic variations among studies.

There was publication bias as revealed by an asymmetric funnel plot (Figure 14). However, the publication bias was not observed by Begg's test (p = 0.47) and Egger's test (p = 0.13). After correcting the publication bias using the trim-and-fill method, the overall ORs remained unchanged [124.44 vs. 124.39; I^2 = 35.31%, p=0.00). Thus asymmetry of the funnel plot suggests other factors operate within the populations.

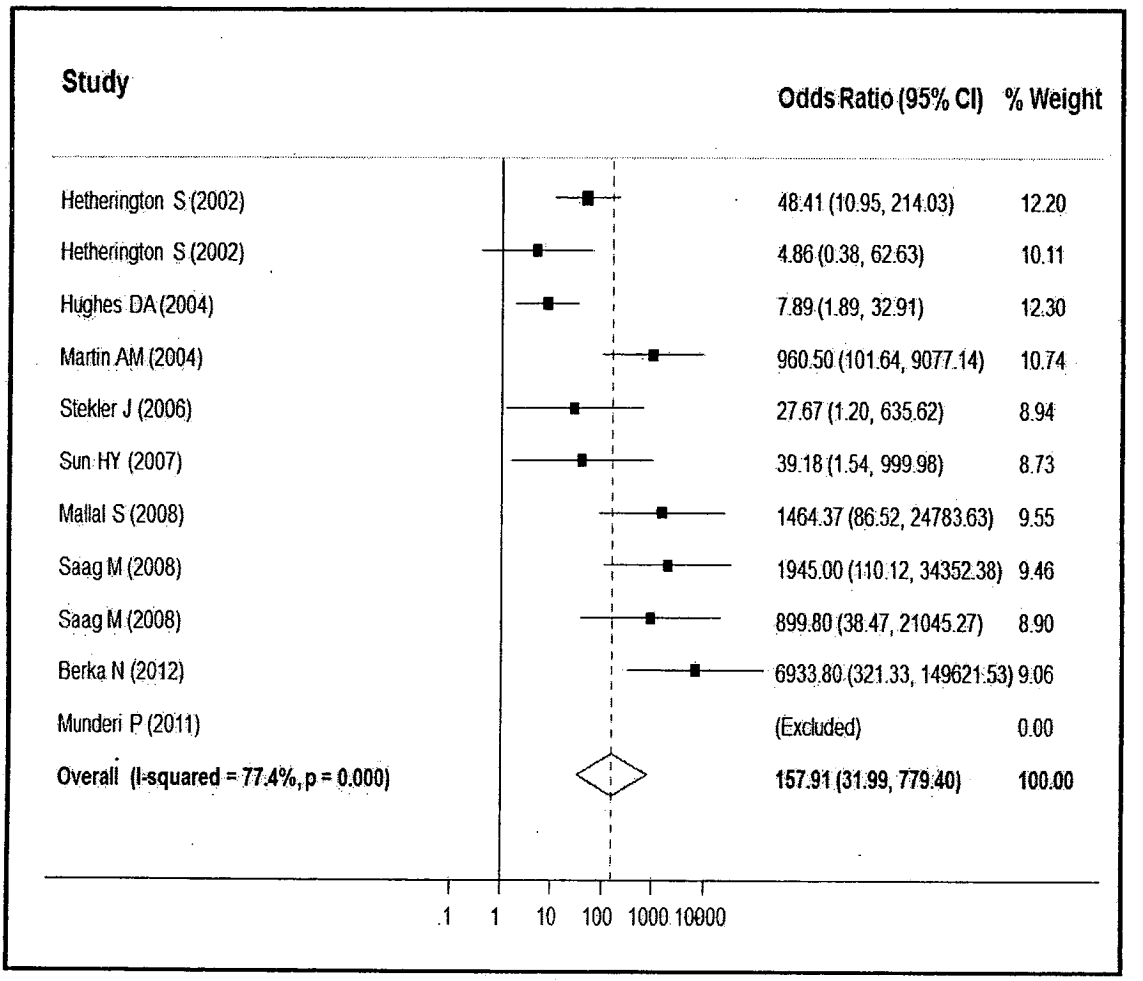

Figure 11 Random effects meta-analysis of abacavir-induced hypersensitivity among HLA-B*5701 positive

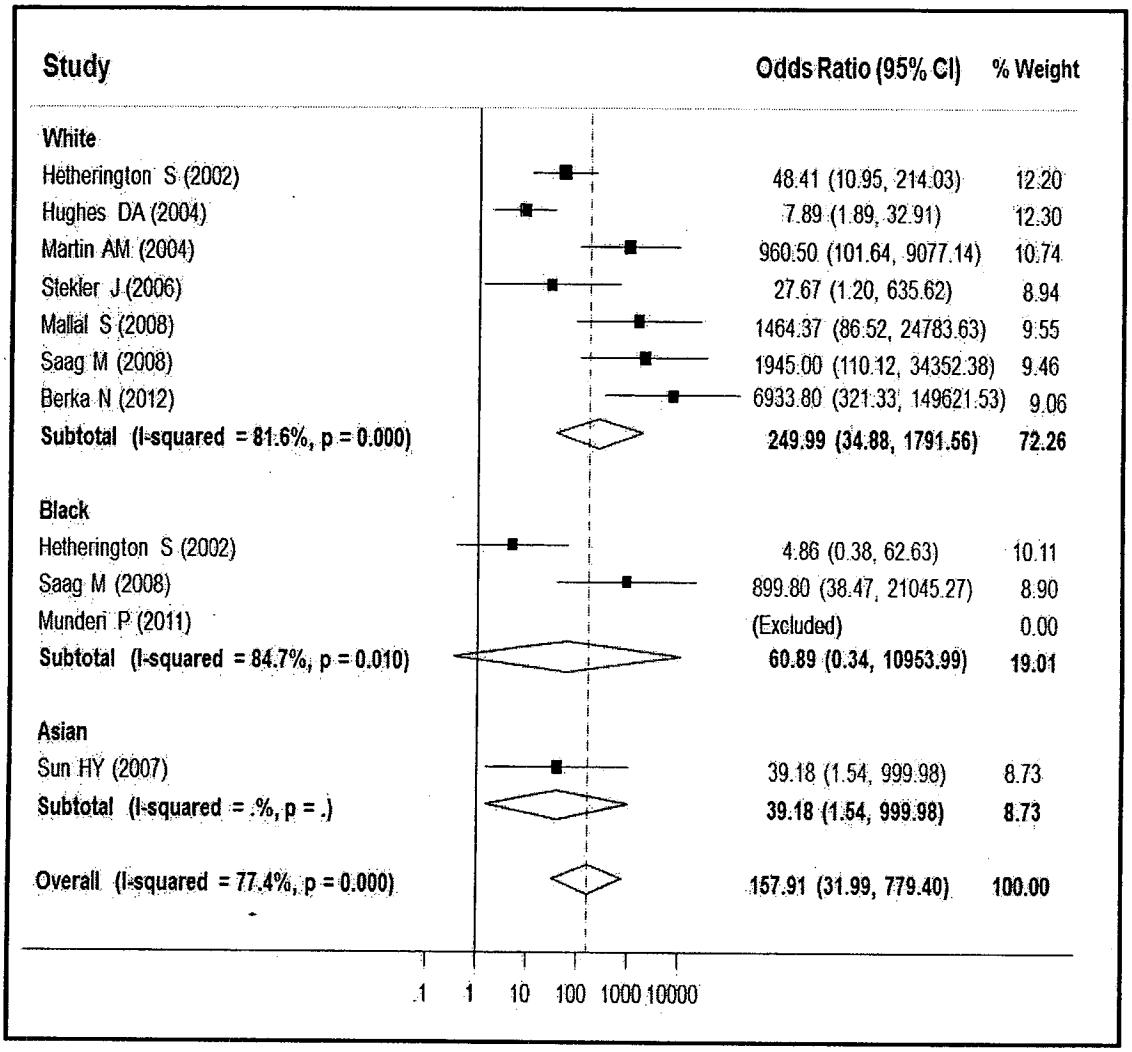

Figure 12 Random effects meta-analysis of abacavir-induced hypersensitivity among HLA-B*5701 positive according to ethnicity

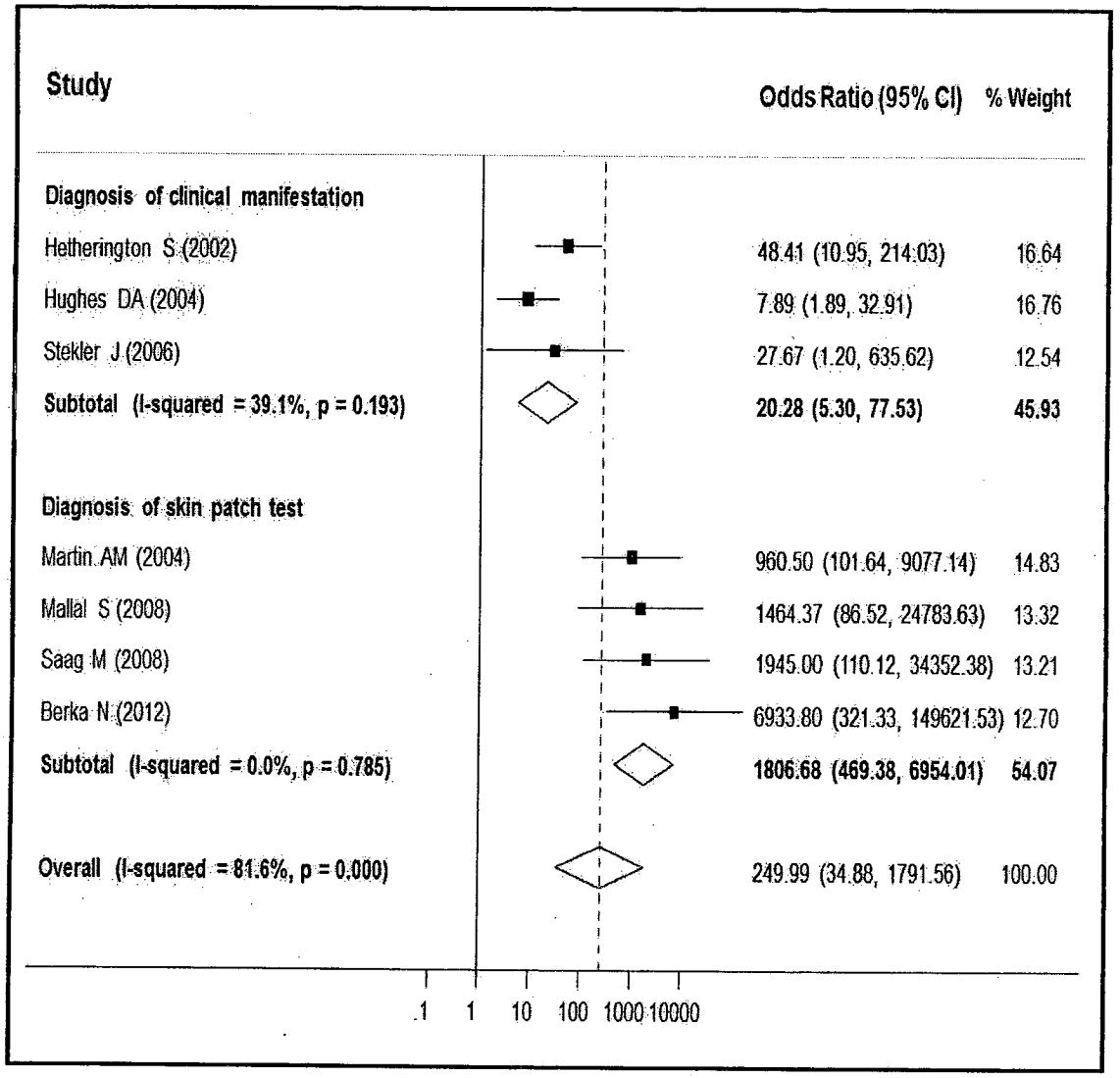

Figure 13 Random effects meta-analysis of abacavir-induced hypersensitivity among HLA-B*5701 positive whites, according to the diagnostic method of detecting hypersensitivity

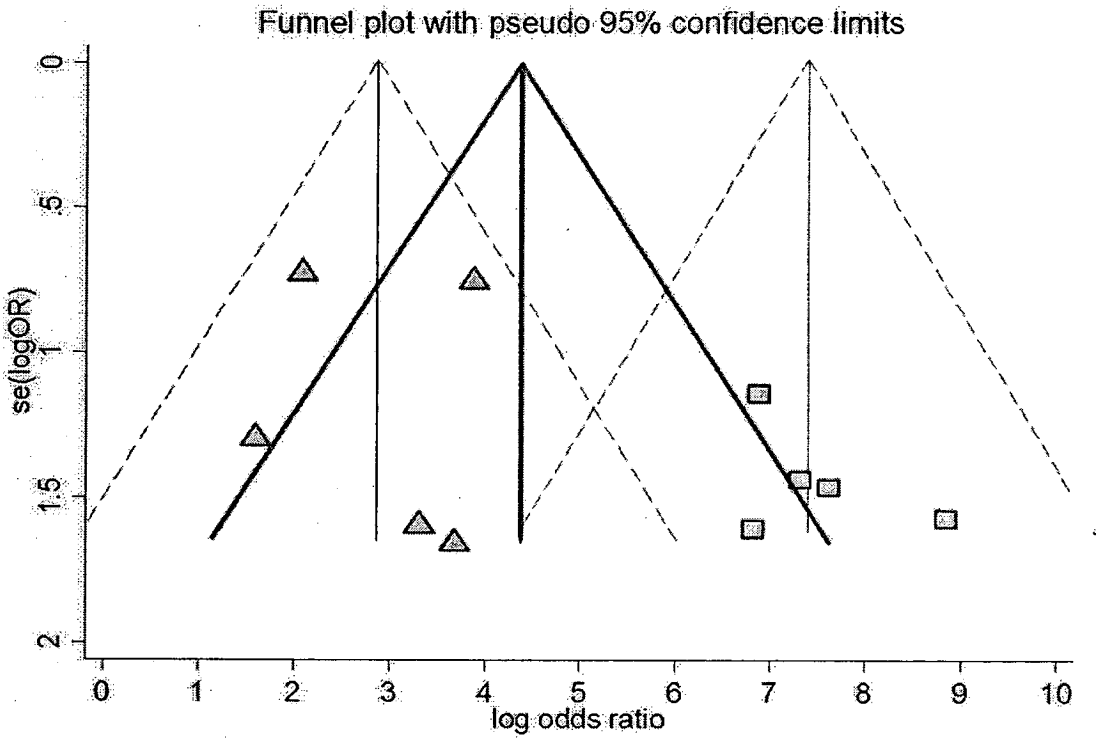

Figure 14 A funnel plot of the abacavir studies

Discussion of section I (Abacavir)

In this study, our results showed a strong association between the HLA-B*5701 allele and the abacavir-induced HSR (OR = 157.91; 95% CI = 31.99-779.40). We also found a significant association between the HLA-B*5701 allele and abacavir-induced HSR in whites (OR = 190.64; 95% CI = 22.83-1,587.63) and Asians (OR=39.18; 95% CI = 1.54 – 999.98), whereas an association among pooled black populations was not proven (Table 6). These results suggested that race plays an important role in the risk of abacavir-induced HSR. Therefore, a genetic screening of HLA-B*5701 allele is considered in patients in susceptible populations before starting an antiretroviral therapy containing abacavir.

We hypothesize that a high level heterogeneity across the studies among white populations (Figure 12 and Table 6) might be due to differences in the diagnostic approaches. Interestingly, using a subgroup analysis by methods of diagnosis in white population, we computed ORs of 20 and 1,900 in patients who received their final diagnosis using clinical manifestations and on the more reliable skin patch test, respectively (Figure 13 and Table 6). This difference suggests that false-positive diagnosis is an important factor in misclassifying abacavir-induced HSRs. In Mellal, et al. [31] study, sensitivity of skin patch test was 100% (85.2–100) and specificity was 96.9% (95.5–98.0), whereas, positive predictive value and negative predictive value was 47.9% (33.3–62.8) and 100% (99.5–100), respectively.

No association between HLA-B*5701 and abacavir-induced HSR in black populations was detected in a pooled analysis (Figure 12 and Table 6). However, each of the studies by Hetherington, et al. [33] and Saag et al. [32] showed a significant association in black populations. The ORs in Hetherington, et al. [33] studying British African Caribbean was about 5-fold increase. Whereas, among African-Americans, the risk of developing HSR among abacavir users carrying HLA-B*5701 was approximately a 900-fold increase [32]. Nonetheless, we did not find the association in Ugandan population [29]. These inconsistencies among black populations may be due to differences in the diagnostic approaches and prevalence rates of HLA-B*5701. Thus, the rates of HLA-B*5701 in African-American and Sub-Saharan-Africans are about 2.5% and <1%, respectively [69, 70] , which demonstrates

that the 'black' classification is an oversimplification. More studies in the prevalence rate of HLA-B*5701 in other black populations are needed.

We found a publication bias as demonstrated by an asymmetric funnel plot (Figure 14). However, the trim-and-fill procedure could correct for the observed publication bias. The odds ratio remained unchanged. Therefore, an asymmetry in our funnel plots may genuinely reflect the differences in the diagnostic tests used to determine which patients were allocated to case or control groups (Figure 14).

An emerging mechanism for abacavir action is by its specific steric binding via two unique amino-acid residues in the 'F-pocket' of HLA-B*5701 protein, which results in the protein complexing with [54,71,72] self-peptides of which about 20% become recognized as non-self by CD8+T-cells and which elicits an ensuing autoimmune activation. A similar modification of HLA-B*1502 also explained carbamazepine-induced hypersensitivities. Nevertheless, abacavir-HLA-B*5701 penetrance was not 100%, and we show that the actual abacavir-induced hypersensitivity varied between white, Asian and black HLA-B*5701 carriers and a plausible explanation for this is maybe differing spectrums of self-tolerant peptides in the different populations or more likely different T-cell clonotypes which express a restricted range of the receptors responding to non-self antigens [73]. The penetrance of abacavir-induced hypersensitivity in HLA-B*5701 individuals was about 50%, indicating that additional factors determine whether abacavir treatment induces abacavir-induced hypersensitivity. Thus, a polymorphism in HLA-B*5701 among the populations is possible. To prove this hypothesis, genetic sequencing studies of this particular gene in each population may be able to provide a better understanding in the difference in the abacavir-induced HSR.

Table 6 Reported odds ratio of the abacavir included studies and summary odds ratio categorized by ethnicity

Study	Year	HLA-B*5701 Positive/Total		Odds Ratio (OR)	95% Confidence Interval
		case	control		
• **White**					
- **Clinical manifestation**					
Hetherington, et al. [33]	2002	36/65	2/80	48.41	10.95 - 214.03
Hughes, et al. [66]	2004	6/13	5/46	7.89	1.89 – 32.91
Stekler, et al. [67]	2006	2/9	0/41	27.67	1.20 – 635.62
Sub-total (I-squared=39.1%, P=0.193)				**20.28**	**5.30 – 77.53**
- **Skin patch test**					
Martin, et al. [6]	2004	17/18	4/230	960.50	0.44 – 22.40
Mallal, et al. [31]	2008	23/23	25/819	1,464.37	86.52 – 24,783.63
Saag, et al. [32]	2008	42/42	8/202	1,945.00	110.12 – 34,352.38
Berka, et al. [68]	2012	18/18	2/470	6,933.80	321.33 – 149,621.53
Sub-total (I-squared= 0.00%, P=0.595)				**1,806.68**	**469.38 - 6954.01**
Sub-total (I-squared=81.6%, P=0.000)				**249.99**	**34.88 - 1791.56**
• **Black**					
- **Clinical manifestation**					
Hetherington, et al. [33]	2002	2/9	1/18	4.86	0.38 – 62.63
Munderi, et al. [29]	2011	0/6	0/241	NA	NA
- **Skin patch test**					
Saag M, et al. [32]	2008	5/5	2/206	899.80	38.47 – 21,045.27
Sub-total (I-squared= 84.7%, P=0.010)				**60.89**	**0.34 – 10,953.99**
• **Asian**					
Sun, et al. [30]	2007	1/17	0/215	39.18	1.54 – 999.98
Overall OR (I-squared=77.4%, P=0.000)				**157.91**	**31.99 - 779.40**

Section II: Nevirapine

Result of section I

Study selection

A schematic diagram of our systematic searching is shown in Figure 15. A database search identified 2,201 articles. After following removal of duplicates, 1,779 abstracts were assessed and 619 articles fitted with our inclusion criteria for further review. Full-text articles of these 619 records were reviewed, 9 articles [24, 25, 43, 74, 75, 76, 77, 78, 79] met eligibility requirements for our systematic review and 5 studies [43, 71, 72, 74, 75] were included in 2 meta-analyses (Figure 15). Nonetheless, 610 studies were excluded because of for the following reasons: 1) being a review article or case report (264 studies) 2) Not human study or validated method (33 studies) 3) Not receiving nevirapine (40 studies) 4) Not reporting association between HLA genotype and/or ADRs (272 studies), and 5) Insufficiency data (1 study). No additional articles was identified in the bibliographies of the included studies.

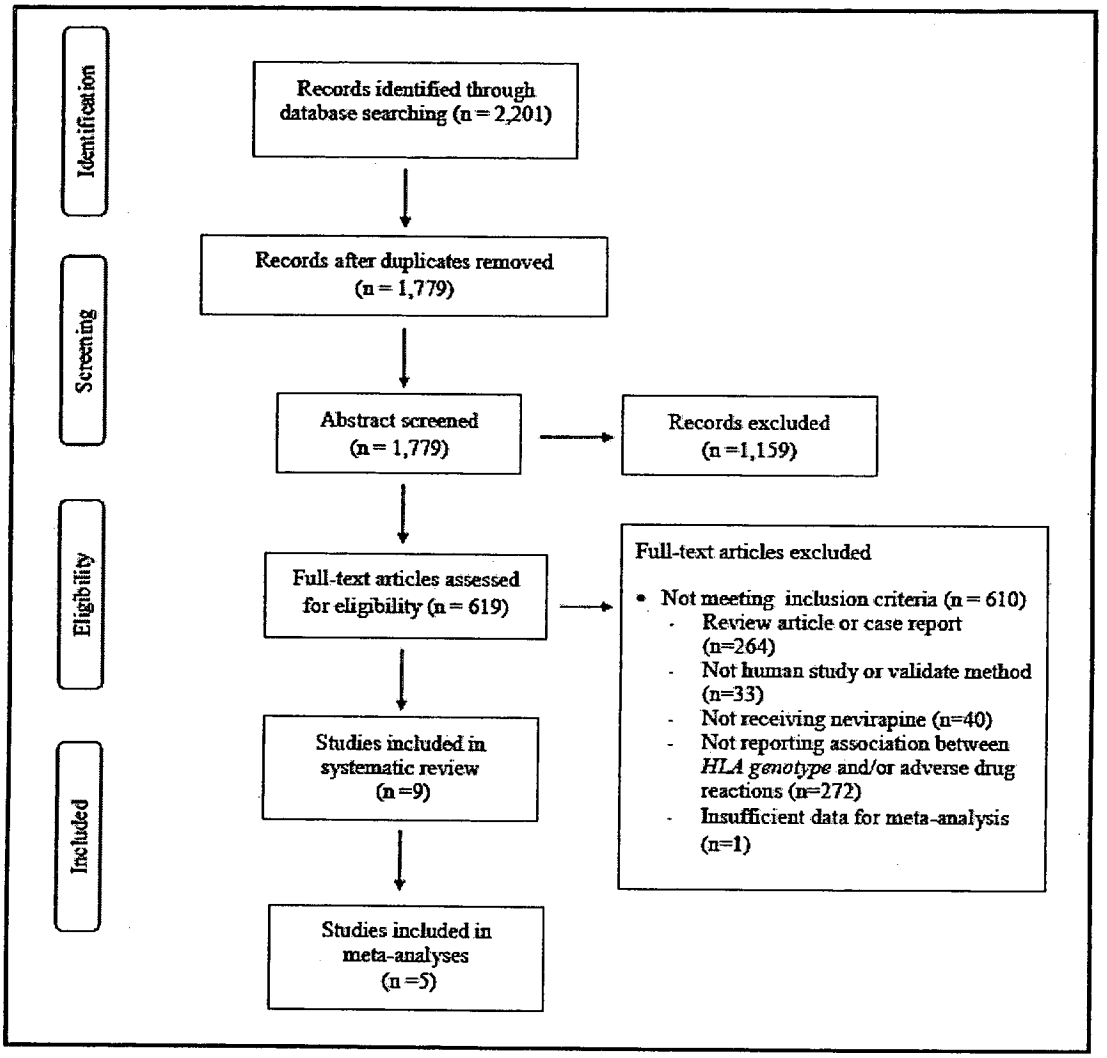

Figure 15 A flow diagram of our systematic searching procedure for the studies related to the association of nevirapine included adverse drug reactions and HLA genotypes.

Study characteristics

The characteristics of the included studies are summarized in Table 7. Seven studies were case-control studies [24, 25, 43, 74, 75, 76, 77, 78, 79], whereas, 2 studies were cohort studies [71, 73].

Outcomes of the selected studies were HSR (4 studies) [74, 75, 76, 78], hepatotoxicity (2 studies) [43,79], skin rash (3 studies) [24, 43, 77] and (1study) [25]. This study included 615 patients with nevirapine-induced ADRs [Those ADRs included HSR (57cases), hepatotoxicity (155 cases), skin rash (367 cases) and SJS/TEN (36 cases)] and 1,829 nevirapine tolerant controls. Seven studies investigated the association between HLA class I and nevirapine-induced ADRs were HLA-A genotypes (3 studies) [24, 74, 79], HLA-B genotypes (4 studies) [24, 43, 74, 79], HLA-C genotypes (8 studies) [24, 25, 43, 74, 75, 77, 78, 79]. Nonetheless, seven studies [24, 25, 43, 74, 76, 78, 79] investigated the association between HLA class II.

There were 5 studies [43, 71, 72, 74, 75] that included in our meta-analysis. Two studies [43, 74] investigated an association between HLA-Cw*04 and nevirapine-induced HSR. While three studies [71, 72, 75] investigated an association between HLA-Cw*08 and nevirapine-induced skin reaction.

Table 7 Characteristics of nevirapine studies which were met with the selection criteria.

No	Author	Ethnicity	Clinical outcome (case/control)	Diagnostic criteria		MHC class I			MHC class II	NOS
				Case	Control	HLA-A	HLA-B	HLA-C		
1	Littera R, et al., 2006 [74]	Sardinian	HSR (13 cases, 36 controls)	Patients who experienced skin rash, vesicular, bullous or scaling skin lesions or skin manifestations combined with one or more of the following symptoms: fever (> 38°C), myalgia, arthralgia, visceral impairment, liver toxicity	Patients who had been treated with standard doses of nevirapine (NVP) for a period of more than 6 months but did not develop any HSR	HLA-A*33	HLA-B*14	HLA-Cw*08	HLA-DR1	7
2	Gatanaga H, et al.,, 2007 [75]	Japanese	HSR (12 cases, 29 controls)	Patients who experienced extensive skin rash and/or fever >38 °C, and/or liver toxicity	Patients who had been treated with NVP for a period of more than 6 months and did not develop any HSR	ND	ND	HLA-Cw*08	ND	5
3	Vitezica Z, et al., 2008 [76]	French	HSR (6 cases, 15 controls)	Patients who had developed cutaneous ADRs	Patients who received antiretroviral treatment for at least 6 months without any evidence of cutaneous adverse reactions (cADRs)	ND	ND	ND	HLA-DRB1*01	5

Table 7 (cont.)

No	Author	Ethnicity	Clinical outcome (case/control)	Diagnostic criteria		MHC class I			MHC class II	NOS
				Case	Control	HLA-A	HLA-B	HLA-C		
4	Chantarangsu S, et al., 2009 [24]	Thai	Skin rash (147 cases, 185 controls)	Patients who were followed up at least 6 months after the initiation of NVP treatment or until they developed skin rash.	Patients who had been treated with NVP for at least 6 months and developed no sign of cADRs	HLA-A*2407	HLA-B*3505, HLA-B*3802	HLA-Cw*0401, HLA-Cw*0702	HLA-DRB1*1202	6
5	Likanonsakul S, et al., 2009 [77]	Thai	Skin rash (39 cases, 60 controls)	Patients who developed apparent skin rash anywhere on the body after NVP containing highly active anti-retroviral therapy and had to change their NVP containing regime to efavirenz-containing ones were diagnosed as rash.	Patients who reasonably good adherence to NVP and did not develop rash at all or developed only mild rash	ND	ND	HLA-Cw*01, HLA-Cw*03, HLA-Cw*04, HLA-Cw*05, HLA-Cw*06, HLA-Cw*07, HLA-Cw*08, HLA-Cw*12, HLA-Cw*14, HLA-Cw*15	ND	5

Table 7 (cont.)

No	Author	Ethnicity	Clinical outcome (case/control)	Diagnostic criteria		MHC class I			MHC class II	NOS
				Case	Control	HLA-A	HLA-B	HLA-C		
6	Yuan J, et al., 2011 [43]	Asian , Thai, Black, White	Skin rash (175 cases, 587 controls)	Patients who experienced symptomatic NVP -associated severe (grade III/IV) cutaneous and/or hepatic adverse events within 8 weeks of initiating NVP	Patients who did not experience adverse events during more than 18 weeks of NVP therapy	ND	HLA-B*35	HLA-Cw*04	-	4
			Hepatic (101 cases, 587 controls)			ND	ND	ND	HLA-DRB1*01	
7	Gao S, et al., 2012 [78]	Han- Chinese	HSR (32 cases, 71 controls)	Patients who developed NVP HSR after 6 months of combined antiretroviral therapy. Diagnostic criteria were based on extensive skin rash, vesicular, bullous skin lesions, or skin manifestations combined with one or more of the following symptoms: fever (> 38 °C) or liver toxicity	Patients who did not develop NVP HSR after 6 months of combined antiretroviral therapy	ND	ND	HLA-Cw*01, HLA-Cw*02 HLA-Cw*03, HLA-Cw*04, HLA-Cw*05, HLA-Cw*06, HLA-Cw*07, HLA-Cw*08, HLA-Cw*12, HLA-Cw*14, HLA-Cw*15, HLA-Cw*16	HLA-DRB1*01, HLA-DRB1*03, HLA-DRB1*04, HLA-DRB1*07, HLA-DRB1*08, HLA-DRB1*09, HLA-DRB1*10, HLA-DRB1*11, HLA-DRB1*12, HLA-DRB1*13, HLA-DRB1*14, HLA-DRB1*15, HLA-DRB1*16	7

Table 7 (cont.)

No	Author	Ethnicity	Clinical outcome (case/control)	Diagnostic criteria		MHC class I			MHC class II	NOS
				Case	Control	HLA-A	HLA-B	HLA-C		
8	Carr D, et al., 2013 [25]	Malawian	SJS/TEN (36 cases, 153 controls)	Patients who developed SJS/TEN	Patients who were followed up for at least 6 months while taking NVP without developing any signs of HSR	ND	ND	HLA-C*0401	HLA-DRB1*05:01:01	4
9	Phillips E, et al., 2013 [79]	African	Hepatotoxicity (54 cases, 106 controls)	Patients who had Hepatotoxicity [defined as treatment-emergent grade 3 (5–10 times upper limit of normal) or grade 4 (greater than 10 times upper limit of normal) elevation in ALT, with or without associated symptoms].	NR	HLA-A*6801	HLA-B*0705, HLA-B*1510, HLA-B*1801, HLA-B*3501, HLA-B*3505, HLA-B*4101, HLA-B*5801, HLA-B*5802	HLA-C*0210, HLA-C*0302, HLA-C*0304, HLA-C*0602, HLA-C*1203	HLA-DRB1*0101, HLA-DRB1*0102, HLA-DRB1*0301, HLA-DRB1*1401	6

Note: ND = Not determined

NR = Not reported

Systematic review and meta-analysis

HLA class I and nevirapine-induced ADRs

HLA-A genotypes

There were 3 studies [24, 74, 79] that investigated HLA-A genotypes.

In Littera R, et al. [74], an association between HLA-A*33 and nevirapine-induced hypersensitivity in Sardinian population was investigated. The number of HLA-A*33 carriers was 1/13 in the cases and 1/36 in controls. However, there was no statistically significant association between HLA-A*33 and the nevirapine-induced hypersensitivity. Chantarangsu S, et al. [24] reported an association between HLA-A*2407 and nevirapine-induced skin rash in Thai population. The number of HLA-A*2407 carriers was 10/147 in the cases and 1/185 in controls. Thus, according to this study, a clear association between HLA-A*2407 and the nevirapine-induced skin rash in Thai population was identified. A reported OR using Haldane's modification method was 12.12 (95% CI 1.96-74.24). Phillips E, et al. [79] observing an association between HLA-A*6801 and nevirapine-induced hepatotoxicity. In these studies, the number of HLA-A*6801 carriers was 0/53 in the cases and 8/103 controls. There was no statistically significant association between HLA-A*6801 and nevirapine-induced hepatotoxicity.

HLA-B genotypes

There were 4 studies [24, 43, 74, 79] that investigated an association between HLA-B genotypes and nevirapine-induced ADRs.

In Littera R, et al. [74], an association between HLA-B*14 and nevirapine-induced hypersensitivity in Sardinian population was investigated. The number of HLA-B*14 carriers was 3/13 in the cases and 1/36 in controls. However, there was no statistically significant association between HLA-B*14 and the nevirapine-induced hypersensitivity. In HLA-B*35, there were 3 studies [24, 43, 74] that investigated an associations between HLA-B*35 and nevirapine-induced ADRs. Chantarangsu S, et al. [24], investigated an association between HLA- B*3505 and nevirapine-induced skin reaction in Thai population. The number of HLA-B*3505 carriers was 15/147 in the cases and 1/185 in controls. A statistically significant association between HLA-B*3505 and nevirapine-induced skin reaction in Thai population (overall OR = 16.53; 95% CI = 2.74-98.98) Whereas, Yuan J, et al. [43] investigated an association between

HLA-B*35 and nevirapine-induced skin reaction in Thai, Asian, Black and white populations. The number of HLA-B*35 carriers was 38/175 in the cases and 83/581 in controls. In subgroup analysis, a significant association between HLA-B*35 and nevirapine-induced skin reaction in Thai populations. The ORs were 5.65 (95% CI = 2.03-15.71). Nonetheless, Chantarangsu S, et al. [24] was investigated an association between HLA-B*3802 and nevirapine-induced skin reaction in Thai population. The number of HLA-B*3502 carriers was 3/147 in the cases and 13/185 controls. The OR was 0.28 (95% CI = 0.08 – 0.99). In Phillips E, et al. [79], the association between HLA-B*0705, HLA-B*1510, HLA-B*1801, HLA-B*3501, HLA-B*3505, HLA-B*4101, HLA-B*5801 and HLA-B*5802 and nevirapine-induced hepatotoxicity in African population were observed. There was no statistically significant association between these HLA-B genotypes and nevirapine-induced hepatotoxicity. However, an association between HLA-B*5801 and nevirapine-induced hepatotoxicity was identified (OR = 3.15; 95% CI = 1.23-8.06).

HLA-C genotypes

There were 8 studies [24, 25, 43, 74, 75, 77, 78, 79] that investigating the association between HLA-C genotypes and nevirapine-induced ADRs.

Chantarangsu S, et al. [24] reported an association between HLA-Cw*0401 and nevirapine-induced skin rash in Thai population. The number of HLA-Cw*0401 carriers was 17/147 in the cases and 4/185 in controls. Thus, a clear association between HLA-Cw*0401 and the nevirapine-induced skin rash in Thai population was identified. A reported OR using Haldane's modification method was 5.36 (95% CI 1.83-15.60). Nonetheless, in this study, an association between HLA-Cw*0702 and nevirapine-induced skin rash in Thai population was investigated. The number of HLA-Cw*0702 carriers was 14/147 in the cases and 34/185 in controls. The association between HLA-Cw*0702 and nevirapine-induced skin rash in Thai population was found (OR = 0.47; 95% CI = 0.24-0.91).

In Gao S, et al. [78] study an association between several HLA-C genotypes and nevirapine-induced HSR in Han-Chinese were investigated (Table 8). Nonetheless only an association between HLA-Cw*05 and nevirapine-induced HSR in Han-Chinese was statistically significant (OR= 0.06; 95% CI 0.003 -0.97).

In Carr D, et al. [25] study an association between several HLA-Cw*0401 and nevirapine-induced SJS/TEN was investigated (Table 8). The number of HLA-Cw*0401 carriers was 23/36 in cases and 39/153 in controls. According to this study, a clear association between HLA-Cw*0401 and nevirapine-induced SJS/TEN was found. (OR= 5.17; 95% CI 2.39-11.18).

Phillips E, et al. [79], an association between HLA-Cw*0210, HLA-Cw*0302, HLA-Cw*0304, HLA-Cw*0602 and HLA-Cw*1203 and nevirapine-induced hepatotoxicity in African population were observed. There was no statistically significant association between these HLA-C genotypes and nevirapine-induced hepatotoxicity. However, an association between HLA-Cw*0302 and HLA-Cw*1203 and nevirapine-induced hepatotoxicity was identified (OR = 4.29; 95% CI = 1.03-17.89 and OR = 4.44; 95% CI = 1.27-15.47, respectively).

According to several associations between HLA-C genotypes and nevirapine-induced ADR, there were 2 associations have been observed by meta-analyses technique.

1. There were 2 studies that observed an association between HLA-Cw*04 and nevirapine-induced skin reaction [74, 75]. In these 2 studies, the number of HLA-Cw*04 carriers was 82/214 in the cases and 123/647 in controls. We found a statistically significant association between HLA-Cw*04 and nevirapine-induced skin reaction in pooling analysis (overall OR = 2.66; 95% CI = 1.88 – 3.76; I^2 = 0%, p = 0.416) (Figure 16). In subgroup analysis, we found a significant association between HLA-Cw*04 and nevirapine-induced skin reaction in Asian, Black and White populations. The ORs were 2.88 (95% CI = 1.74 – 4.76; I^2 = 0.0%, p = 0.723, 5.17 (95% CI = 2.01 – 13.30) and 1.92 (95% CI = 1.11 – 3.35) for Asians, Black and White population, respectively (Figure 16).

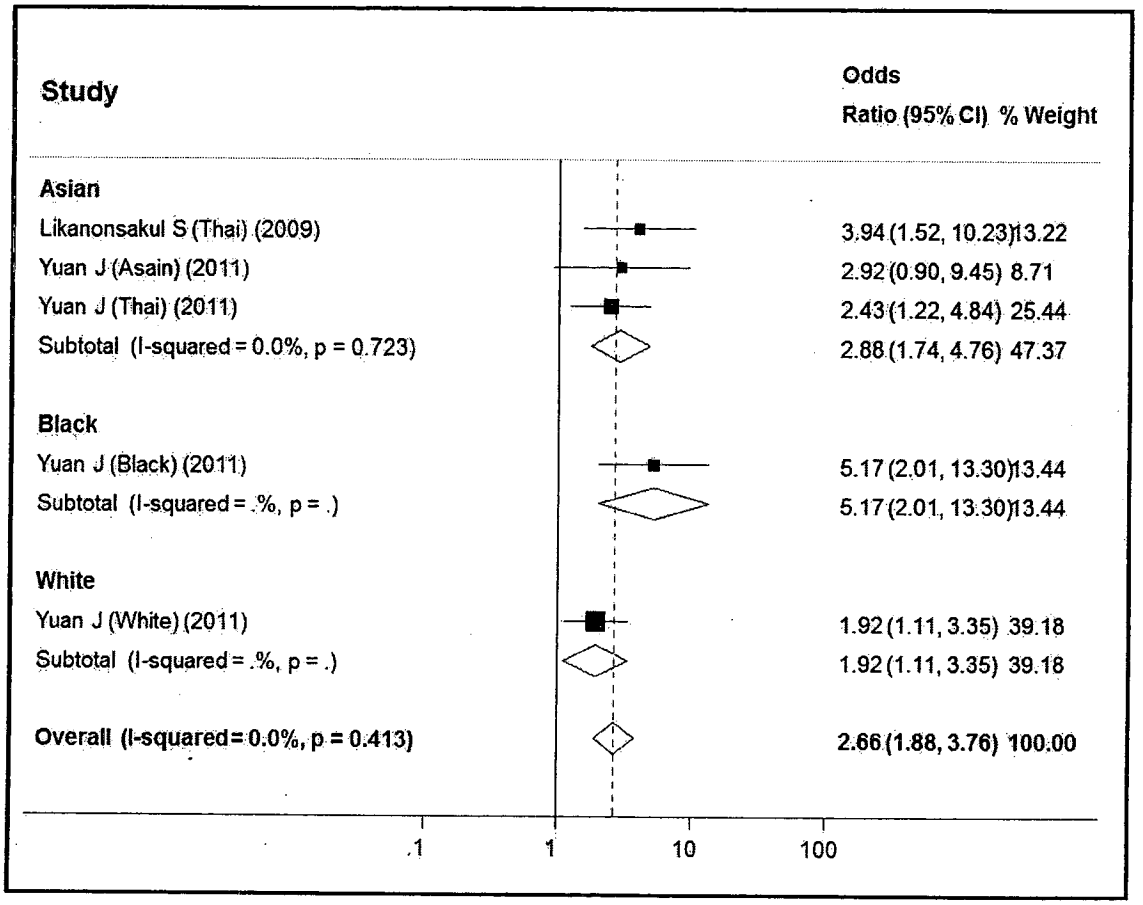

Figure 16 Random effects meta-analysis of nevirapine-induced skin reaction among HLA-Cw*04 positive

2. There were 3 studies that observed an association between HLA-Cw*08 and nevirapine-induced HSR [74,75]. In these 3 studies, the number of HLA-Cw*08 carriers was 11/57 in the cases and 10/136 in controls. However, the association between HLA-Cw*08 and nevirapine-induced HSR was not found (OR = 3.48; 95% CI = 0.88 – 13.70) (Figure 17).

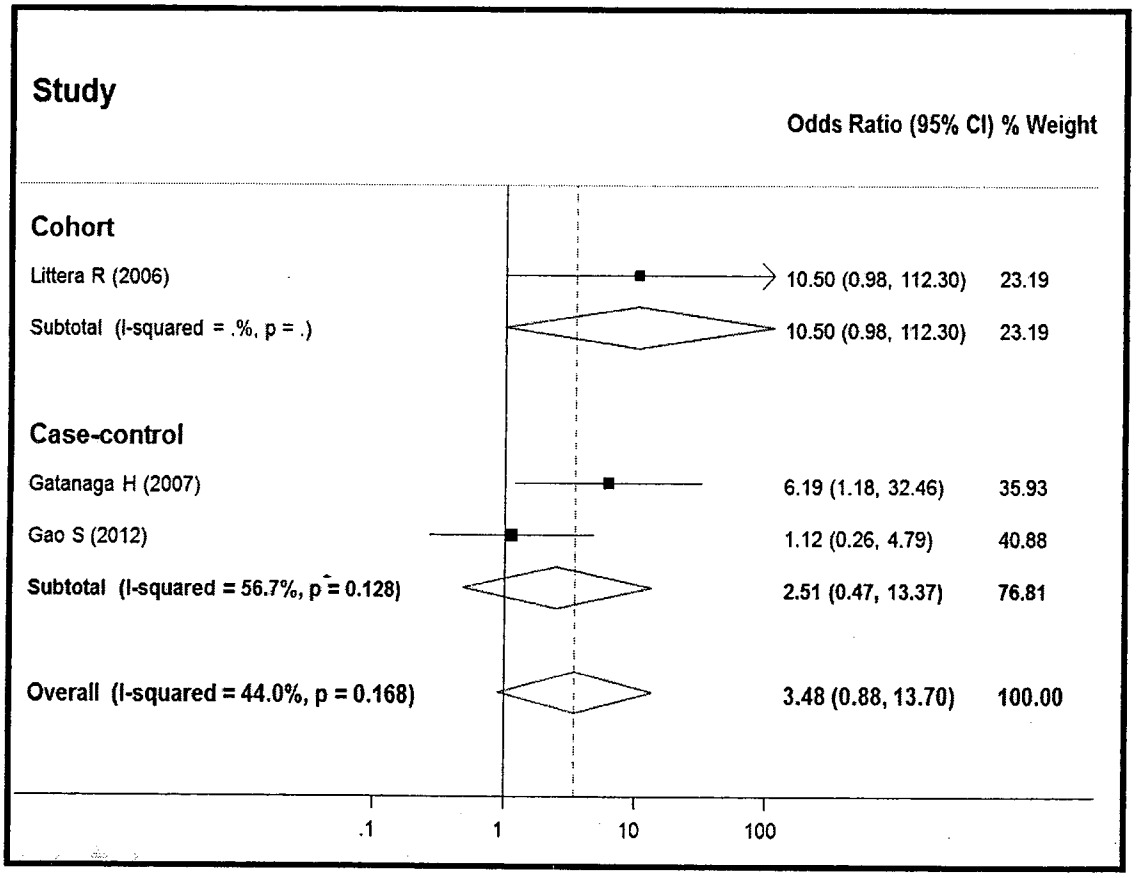

Figure 17 Random effects meta-analysis of nevirapine-induced HSR among HLA-Cw*08 positive

HLA class II and nevirapine-induced ADRs

There were 7 studies [24, 25, 43, 74, 76, 78, 79] studied HLA class II and nevirapine-induced ADRs. Six studies [24, 43, 74, 76, 78, 79] investigated HLA-DRB1 genotypes whereas Carr D, et al. study [25] investigated HLA-DQB1*05:01:01 (Table 7 and 8).

Vitezica Z, et al. [76] reported an association between HLA-DRB1*01 and nevirapine and efavarenz-induced HSR in French population. The number of HLA-DRB1*01 carriers was 5/6 in the cases and 1/15 in controls. After our calculation using the DerSimonian and Laird method under a random-effects model, the statistically significant was found (OR=70.00; 95% CI = 3.65 – 1342.66). Chantarangsu S, et al. [24] reported an association between HLA-DRB1*01202 and nevirapine-induced skin rash in Thai population. The number of HLA-DRB1*01202 carriers was 41/147 in the cases and 19/185 in controls. Thus, a significant association between HLA-DRB1*01202 and the nevirapine-induced skin rash in Thai population was identified. A reported OR using Haldane's modification method was 2.56 (95% CI 1.41-4.61). Nonetheless, our re-calculation using the DerSimonian and Laird method under a random-effects model was performed and the new OR was 3.38 (95% CI 1.86-6.13). In Yuan J, et al. [43] was observing an association between HLA-DRB1*01 and nevirapine-induced skin reaction in Asian, Black and White populations. In these studies, the statistically significant association between HLA-DRB1*01 and nevirapine-induced skin reaction in white population was found. The OR was 3.02 (95% CI = 1.66 – 5.49). The number of HLA-DRB1*01 carriers was 25/77 in the cases and 57/277 controls.

In Gao S, et al. [78] study an association between several HLA-DRB1 genotypes and nevirapine-induced HSR in Han-Chinese were investigated. Nonetheless only an association between HLA-DRB1*12 and nevirapine-induced HSR in Han-Chinese was statistically significant (OR= 13.01; 95% CI 5.62 -30.16).

In Carr D, et al. [25] study an association between several HLA-DQB1*05:01:01 and nevirapine-induced SJS/TEN was investigated. The number of HLA-DQB1*05:01:01 carriers was 3/35 in cases and 47/135 in controls. According to this study, a clear association between HLA-DQB1*05:01:01 and nevirapine-induced SJS/TEN was found. (OR= 0.18; 95% CI 0.05-0.60).

Phillips E, et al. [79], an association between HLA-DRB1*0101, HLA-DRB1*0102, HLA-DRB1*0301 and HLA-DRB1*1401 and nevirapine-induced hepatotoxicity in African population were observed. However, an association between HLA-DRB1*0102 and nevirapine-induced hepatotoxicity was identified (OR = 4.30; 95% CI = 1.23-15.03).

Table 8 Reported odds ratio of the nevirapine included studies categorized by HLA genotypes and ADRs

Author	HLA	ADR	Sample size (Positive/Total)		OR (95% CI)
			Case	Control	
HLA class I					
HLA-A genotypes					
Littera R, et al. [74]	HLA-A*33	HSR	1/13	1/36	2.92 (0.17 - 50.34)
Chantarangsu S, et al. [24]	HLA-A*2407	Skin	10/147	1/185	12.12 (1.96-74.24)
Phillips E, et al. [79]	HLA-A*6801	Hepatotoxicity	0/53	8/103	2.04 (0.125-33.27)
HLA-B genotypes					
Littera R, et al. [74]	HLA-B*14	HSR	3/13	1/36	10.50 (0.98-112.30)
Chantarangsu S, et al. [24]	HLA-B*3505	Skin rash	15/147	1/185	16.53 (2.74-98.98)
Yuan J, et al. [43]	HLA-B*35	Skin rash	10/52	7/173	5.65 (2.03-15.71)
	HLA-B*35 (Black)		3/27	10/77	0.84 (0.21-3.30)
	HLA-B*35 (White)		21/77	48/277	1.79 (0.99-3.23)
	HLA-B*35 (Asian)		4/19	8/54	1.53 (0.40-5.82)
Phillips E, et al. [79]	HLA-B*0705	Hepatotoxicity	3/53	1/106	6.30 (0.64-62.09)
	HLA-B*1510		4/53	20/106	0.35 (0.11-1.09)
	HLA-B*1801		4/53	2/106	4.25 (0.75-23.97)
	HLA-B*3501		1/53	4/106	0.49 (0.05-4.50)
	HLA-B*3505		1/53	1/106	2.02 (0.12- 32.93)
	HLA-B*4101		0/53	4/106	0.21 (0.01- 4.03)
	HLA-B*5801		12/53	9/106	3.15 (1.23-8.06)
	HLA-B*5802		6/53	25/106	0.41(0.16-1.08)

Table 8 (cont.)

Author	HLA	ADR	Sample size (Positive/Total)		OR (95% CI)
			Case	Control	
HLA-C genotypes					
Littera R, et al. [74]	HLA-Cw*08	HSR	3/13	1/36	10.50 (0.98-112.30)
Gatanaga H, et al. [75]	HLA-Cw*08	HSR	5/12	3/29	6.19 (1.18-32.46)
Chantarangsu S, et al. [24]	HLA-Cw*0401	Skin rash	17/147	4/185	5.92 (1.95-18.00)
	HLA-Cw*0702		14/147	34/185	0.47 (0.24-0.91)
Likanonsakul S, et al. [77]	HLA-Cw*01	Skin rash	12/39	12/60	1.07 (0.45-2.51)
	HLA-Cw*03		4/39	20/60	0.22 (0.07-0.71)
	HLA-Cw*04		16/39	9/60	3.94 (1.52-10.23)
	HLA-Cw*05		2/39	3/60	1.03 (0.16-6.44)
	HLA-Cw*06		8/39	9/60	1.46 (0.51-4.19)
	HLA-Cw*07		19/39	39/60	0.51 (0.23-1.16)
	HLA-Cw*08		9/39	15/60	0.90 (0.35-2.32)
	HLA-Cw*12		6/39	8/60	1.18 (0.38-3.71)
	HLA-Cw*14		0/39	3/60	0.21 (0.01- 4.14)
	HLA-Cw*15		2/39	2/60	1.57 (0.21-11.62)
Yuan J, et al. [43]	HLA-Cw*04 (Asian)	Skin rash	7/19	9/ 54	2.92 (0.90-9.45)
	HLA-Cw*04 [39]		18/52	32/179	2.43 (1.22-4.84)
	HLA-Cw*04 (Black)		15/27	15/77	5.17 (2.01-13.30)
	HLA-Cw*04 (White)		26/77	58/277	1.93 (1.11-3.35)
Gao S, et al. [78]	HLA-Cw*01	HSR	8/32	16/71	1.15 (0.43-3.04)
	HLA-Cw*02		0/32	1/71	0.73 (0.03-18.23)
	HLA-Cw*03		4/32	14/71	0.59 (0.18-1.93)
	HLA-Cw*04		4/32	3/71	3.24 (0.68-15.41)
	HLA-Cw*05		0/32	15/71	0.06 (0.003-0.97)
	HLA-Cw*06		2/32	4/71	1.12 (0.19-6.43)
	HLA-Cw*07		8/32	17/71	1.06 (0.40-2.79)
	HLA-Cw*08		3/32	6/71	1.12 (0.26-4.79)
	HLA-Cw*12		1/32	4/71	0.56 (0.06-5.21)
	HLA-Cw*14		4/32	5/71	1.91 (0.48-7.66)
	HLA-Cw*15		1/32	4/71	0.54 (0.06-5.04)
	HLA-Cw*16		1/32	0/71	6.81 (0.27-171.79)
Carr D, et al. [25]	HLA-Cw*0401	SJS/TEN	23/36	39/153	5.17 (2.39-11.18)
Phillips E, et al. [79]	HLA-Cw*0210	Hepatoto xicity	2/55	19/106	0.17 (0.04-0.77)
	HLA-Cw*0302		6/55	3/106	4.29 (1.03-17.89)
	HLA-Cw*0304		4/55	19/106	0.36 (0.12-1.11)
	HLA-Cw*0602		12/55	39/106	0.48 (0.23-1.02)
	HLA-Cw*1203		8/55	4/106	4.44 (1.27-15.47)

Table 8 (cont.)

Author	HLA	ADR	Sample size (Positive/Total)		OR (95% CI)
			Case	Control	
HLA class II					
Littera R, et al. [74]	HLA-DRB1*0101	HSR	0/13	2/36	0.54 (0.02-12.00)
	HLA-DRB1*0102		2/13	1/36	6.36 (0.53-77.08)
Vitezica Z, et al. [75]	HLA-DRB1*0101	HSR	5/6	1/15	70.00 (3.65-1342.66) Patients received NPVor efavirenz
Chantarangsu S, et al. [24]	HLA-DRB1*1202	Skin rash	41/147	19/185	3.38 (1.86- 6.13)
Yuan J, et al. [43]	HLA-DRB1*01 (Asian)	Skin rash	1/30	1/233	8.00 (0.49-131.37)
	HLA-DRB1*01 (Black)		2/14	8/77	1.44 (0.27- 7.60)
	HLA-DRB1*01 (White)		25/57	57/277	3.02 (1.66-5.49)
Gao S, et al. [78]	HLA-DRB1*01	HSR	0/32	2/71	0.43 (0.02-9.17)
	HLA-DRB1*03		1/32	4/71	0.54 (0.06-5.04)
	HLA-DRB1*04		4/32	5/71	1.89 (0.47- 7.55)
	HLA-DRB1*07		2/32	5/71	0.88 (0.16-4.80)
	HLA-DRB1*08		4/32	8/71	1.13 (0.31-4.05)
	HLA-DRB1*09		8/32	17/71	1.06 (0.40-2.79)
	HLA-DRB1*10		1/32	1/71	2.26 (0.14-37.28)
	HLA-DRB1*11		3/32	3/71	2.35 (0.45-12.31)
	HLA-DRB1*12		51/32	9/71	13.01 (5.62-30.16)
	HLA-DRB1*13		2/32	2/71	2.30 (0.31-17.10)
Gao S, et al. [78]	HLA-DRB1*14	HSR	2/32	1/71	4.67 (0.40- 53.45)
	HLA-DRB1*15		2/32	14/71	0.27 (0.06-1.27)
	HLA-DRB1*16		2/32	2/71	2.30 (0.31-17.10)
Carr D, et al. [25]	HLA-DQB1*05:01:01	SJS/TEN	3/35	47/135	0.18 (0.05-0.60)
Phillips E, et al. [79]	HLA-DRB1*0101	Hepatotoxicity	0/54	3/103	0.26 (0.02-5.19)
	HLA-DRB1*0102		8/54	4/103	4.30 (1.23-15.03)
	HLA-DRB1*0301		4/54	15/103	0.47 (0.15-1.49)
	HLA-DRB1*1401		0/54	4/103	0.20 (0.01-3.84)

Discussion of section II

In our study, we found several associations between HLA genotypes and nevirapine-induced adverse drug reaction. Those included: 1) HLA-DRB1*01 and nevirapine and efavarenz-induced HSR in French (OR = 70.00; 95% CI = 3.65–1342.66); 2) HLA-B*3505 and nevirapine-induced skin rash in Thai population (OR = 16.53; 95% CI = 2.74-98.98); 3) HLA-DRB1*12 and nevirapine-induced HSR (OR = 13.01; 95%CI = 5.62-30.16); 4) HLA-A*2407 and nevirapine-induced skin rash in Thai population (OR = 12.12; 95% CI = 1.96-74.24); 5) HLA-Cw*08 and nevirapine-induced HSR (OR = 6.19; 95% CI = 1.18-32.46); 6) HLA-Cw*0401 and nevirapine-induced HSR (OR = 5.95; 95% CI = 1.95-18.00); 7) HLA-B*35 and nevirapine-induced skin reaction in Thai populations (OR = 5.65; 95% CI = 2.03-15.71); 8) HLA-Cw*0401 and nevirapine-induced SJS/TEN (OR = 5.17; 95% CI = 2.39-11.18); 9) HLA-Cw*1203 and nevirapine-induced hepatotoxicity (OR = 4.44; 95% CI = 1.27-15.47); 10) HLA-DRB1*0102 and nevirapine-induced hepatotoxicity (OR = 4.30; 95% CI = 1.23-15.03); 11) HLA-Cw*0302 and nevirapine-induced hepatotoxicity (OR = 4.29; 95% CI = 1.03-17.89); 12) HLA-B*5801 and nevirapine-induced hepatotoxicity (OR = 3.15; 95% CI = 1.23-8.06); 13) HLA-DRB1*1202 and nevirapine-induced skin rash (OR = 3.38; 95% CI = 1.86-6.13); 14) HLA-DRB1*01 and nevirapine-induced skin rash (OR = 3.02; 95% CI = 1.66-5.49); 15) HLA-Cw*04 and nevirapine-induced skin reaction in pooling analysis (OR = 2.66; 95% CI = 1.88 – 3.76): 16) HLA-Cw*0702 and nevirapine-induced skin reaction (OR = 0.47; 95% CI = 0.24-0.91); 17) HLA-DQB1*05:01:01 and nevirapine-induced SJS/TEN (OR = 0.18; 95% CI =0.05-0.60) ,and; 18) HLA-Cw*05 and nevirapine-induced HSR (OR = 0.06; 95% CI = 0.003-0.97).

The strong association between HLA-DRB1*01 and nevirapine and efavarenz-induced HSR in French population (OR=70.00; 95% CI = 3.65 – 1342.66) was found by Vitezica Z, et al. [76]. However, patients receiving nevirapine and efavarenz were included in this study. Thus, these results may not be a direct effect of nevirapine. Also, the sample size of this study was small (6 cases and 15 controls). Taken together, a future prospective study with an appropriate study design (i.e. randomized control study, exposure to nevirapine only, a larger sample size, performing in other ethnicities) should be conducted.

The association between HLA-B*3505 and nevirapine-induced skin rash in Thai population was reported by Chantarangsu S, et al. [24], which is the only study available in the literature. In this study, OR was 16.53 (95% CI = 2.74-98.98). This study is only one available study that observed the association between HLA-B*3505 and nevirapine-induced skin rash in the databases. skin rash. Nontheless, the association between HLA-B*35 and nevirapine-induced skin reaction in Thai population was reported by Yuan J, et al., [43]. The OR was 5.65 (95% CI = 2.03-15.71) Thus, more epidemiologic studies are required to identify this plausible association between the gene and nevirapine-induced and a high resolution HLA genotyping should be performed to identify specific HLA genotypes.

Interestingly, our results demonstrated protective effects. These were the association between HLA-Cw*0702 and nevirapine-induced skin reaction (OR = 0.47; 95% CI = 0.24-0.91) [24] the association between HLA-DQB1*05:01:01 and nevirapine-induced SJS/TEN (OR = 0.18; 95% CI =0.05-0.60) [25] and the association between HLA-Cw*05 and nevirapine-induced HSR (OR = 0.06; 95% CI = 0.003-0.97) [78]. There are only three available studies demonstrating these protective effects. Therefore, a molecular mechanism of these protective effects has never been proposed, a study identifying a molecular protein-protein interaction is warranted.

Section III: Stavudine

Result of section I (Stavudine)

Study selection

Our systematic searching schematic is summarized as shown in Figure 18. A database search identified 1,955 articles. After following removal of duplicates, 1,681 abstracts were assessed and 220 articles fitted with our inclusion criteria for further review. Full-text articles of these 220 records were reviewed and 2 articles [23,80] met eligibility requirements for inclusion (Figure 18). We excluded 218 studies for the following reasons; (1) being a review article or case report (89 studies); (2) Not human study or validated method (30 studies); (3) Not receiving stavudine (15 studies), and; (4) Not reporting association between HLA genotype and/or ADRs (84 studies). No additional articles were identified in the bibliographies of the included studies.

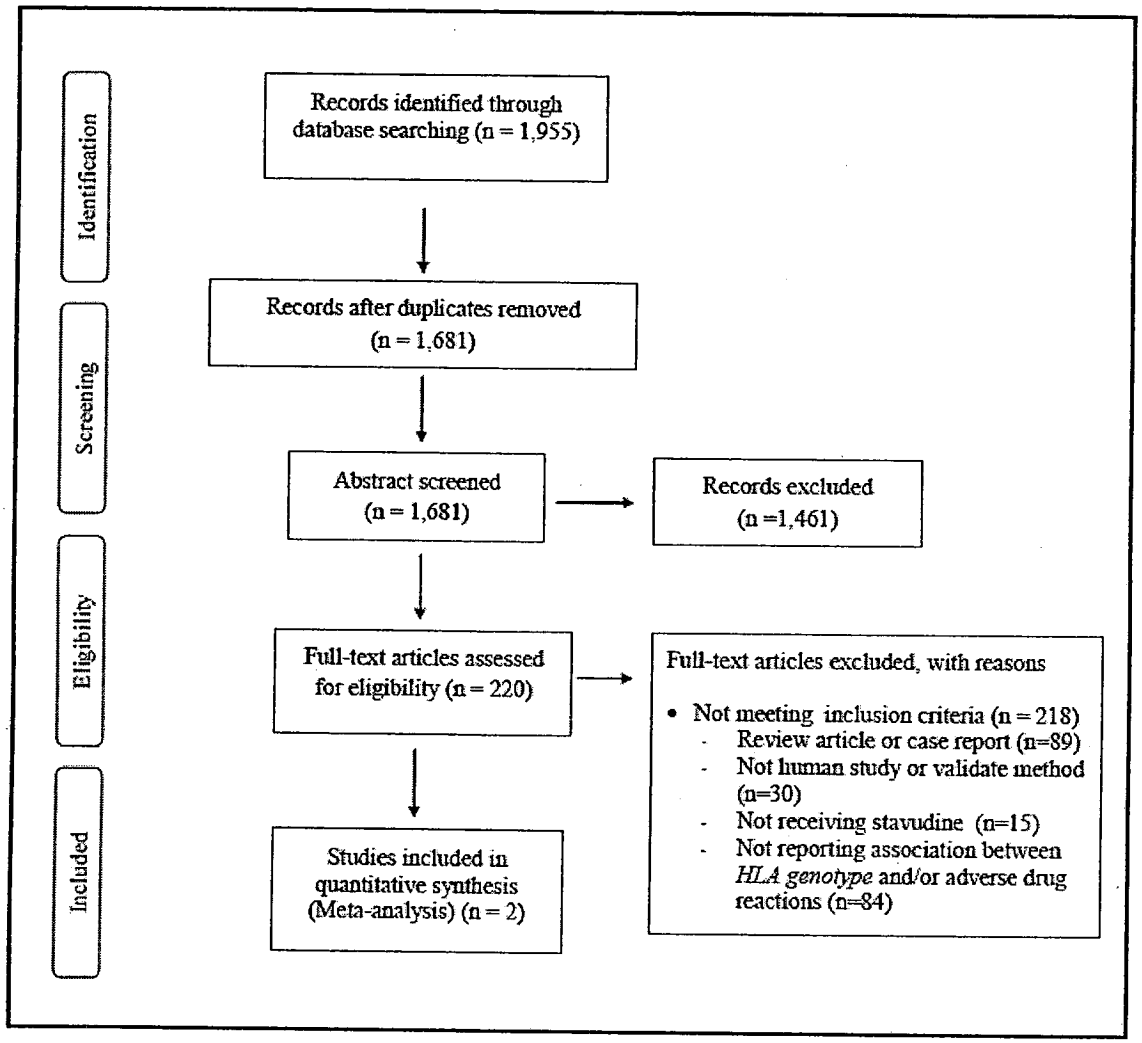

Figure 18 A flow diagram of our systematic searching procedure for the studies related to the association of stavudine-induced lipodystrophy and HLA-B*4001.

Study characteristics

Characteristics of the included studies are summarized in Table 9 and 10. The studies included 242 cases of stavudine-induced lipodystrophy and 197 control subjects [23, 80]. Wangsomboonsiri, et al. [23] was a cross-sectional study, whereas Domingo, et al.[80] and conducted in Thai and Spanish populations, respectively. The mean ages of the patients were 42.9 and 42.5 years in cases and controls, respectively. Males comprised 68.6% of the cases (166/242) and 70.0% of controls (138/197). The dose of stavudine in both studies was 30 mg or 40 mg twice daily based on the body weight [23, 80].

In Wangsomboonsiri, et al. [23] study all patients who had been exposed and continue to use stavudine at the time of lipodystrophy diagnosis were invited from the previous case-control studies [24, 81] to participate in this study. The median duration of stavudine treatment was 47.8 and 44.8 months in cases and controls, respectively. For Domingo, et al. [80] study the controls were matched by the duration of stavudine exposure (± 6 months). However, the duration of stavudine use and time after stopping stavudine were not reported. Only 75 out of 336 patients used stavudine at the time of study.

In both studies, lipodystrophy was independently assessed by patient self-evaluation and physical examination with a lipodystrophy severity grading scale [82, 83]. In brief, any lipoatrophy and diffused fat accumulation at each of the following region: face, neck, dorsocervical spine, arms, breasts, abdomen, buttocks, and legs, was recorded and rated as absent (score of 0), mild (noticeable on a close inspection; score of 1), moderate (readily noticeable by a patient and/ or a physician; score of 2) or severe (readily noticeable to a casual observer; score of 3) The diagnostic criteria for lipodystrophy in both studies were slightly different but still based on the severity, number and affected area of the body. Cases were defined as patients with "moderate to severe lipodystrophy" whereas the patients whose signs were too mild or no sign of lipodystophy were identified as controls.

HLA-B*4001 was the only genotype investigated in both studies. The polymerase chain reaction (PCR) technique and sequence-based typing were used to identify HLA-B*4001. Both studies did not report information on HWE.

The quality assessment of both studies by Downs and Black checklist [59] showed the same score (16/32) with the same answer for each item (Table 9). The items that were not reported and/or were incomplete involved selection process, follow-up process, blinding, and compliance.

Table 9 Characteristics of stavudine studies meeting the selection criteria.

No.	Study	Study design	Ethenicity	Case		Control		Downs and Black checklist
				Positive	Negative	Positive	Negative	
1	Wangsomboonsiri, et al. [23]	Cross-sectional	Thai	16	39	2	46	17
2	Domingo, et al. [80]	Cross-sectional	Spanish	9	178	6	143	16

Table 10 Summary patient demographic information of stavudine included studies for the meta-analysis.

Parameter	Wangsomboonsiri, et al. [23]			Domingo, et al. [80]		
	Case (n=55)	Control (n=48)	P	Case (n=187)	Control (n=149)	P
A. Clinical characteristics						
Male (%)	29 (52.7)	20 (41.7)	0.262	137 (73.3)	118 (79.1)	0.249
Mean age, year (range)	41.9	41.1	0.585	44 (40.0-51.7)	44 (41.0-49.0)	0.817
Start Dose, mg/day (range)	NR	NR	NR	NR	NR	NR
Mean Dose, mg/day (range)	NR	NR	NR	NR	NR	NR
Median Duration of d4T Exposure (months)	47.8 (31.0–67.0)	44.8 (25.9–58.4)	0.161	NR	NR	NR
B. Metabolic and fat characteristics						
Height [71]	162.8	161.4	0.343	NR	NR	NR
Body weight at ART initiation (kg)	53.2	56.8	0.94	66.5 (58.0-73.0)	70.0 (61.5-75.0)	0.229
BMI at ART initiation (kg/m^2)	20.2	23.1	0.16	23.4	23.4	0.9218
Waist circumference [71]	76.7	82.8	0.01	87.0	88.0	0.8412
Total fat (kg)	11.6	17.0	<0.001	12.0	14.1	<0.001
Total fat (%)	21.4	28.0	<0.001	8.1	22.5	<0.001
C. Laboratory characteristics						
FBS level (mg/dL)	95.0	95.0	0.992	97.2	93.6	0.022
Triglyceride level (mg/dL)	223.9	169.0	0.092	169.9	144.2	0.017
Total cholesterol level (mg/dL)	217.7	215.1	0.781	212.7	191.4	0.367
HDL cholesterol leve (mg/dL)	46.2	50.8	0.134	42.2	45.6	0.005
LDL cholesterol level (mg/dL)	122.1	132.1	0.247	120.6	107.1	<0.001

Note: NR = Not reported

Quantitative synthesis

Only one HLA genotype (HLA-B*4001) was extracted from the included studies to investigate its association with lipodystrophy induced by stavudine in HIV/AIDS patients. In the meta-analysis, overall frequencies of HLA-B*4001 allele were 10.3% (25 of 242) in the cases and 4.1% (8/197) in the control group. The overall OR for the association of HLA-B*4001 allele with the risk for stavudine-induced lipodystrophy was 3.13 (95% CI: 0.40-24.14) (Figure 19). The heterogeneity was substantial, indicating the significant variation between both studies (I^2 = 79.4%, p = 0.027). Therefore it cannot be concluded that the presence of HLA-B*4001 has effect on the risk of lipodystrophy in patients who received stavudine.

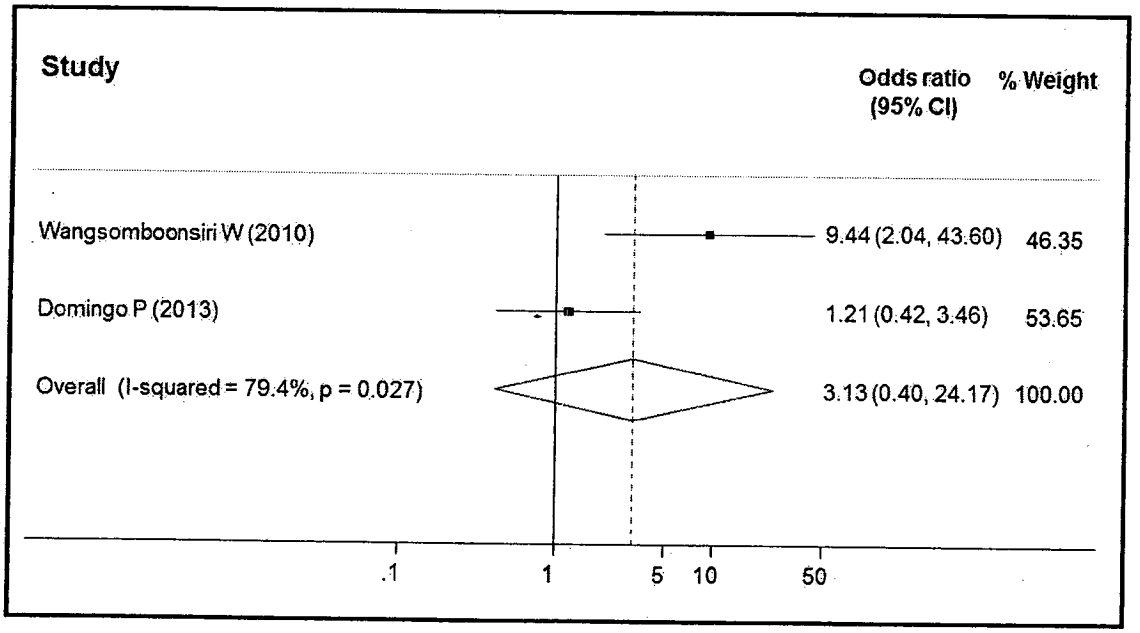

Figure 19 Random-effects meta-analyses of stavudine-induced lipodystrophy among HLA-B*4001 positive

Table 11 Reported odds ratio of the stavudine included studies and summary odds ratio

Study	Year	HLA-B*4001 Positive/Total		Odds Ratio (OR)	95% Confidence Interval
		case	control		
Wangsomboonsiri, et al. [23]	2010	16/55	2/48	9.44	2.04 – 43.60
Domingo, et al. [80]	2013	9/187	6/149	1.21	0.42 – 3.46
Overall OR (I-squared=79.4%, P=0.027)				**3.13**	**0.04 – 24.17**

Discussion of section III (Stavudine)

The objective of this study was to explore whether any specific HLA alleles were associated with stavudine-induced lipodystrophy. We analyzed two studies investigating the association between HLA-B*4001 alleles and lipodystrophy in patients who received stavudine. So far, HLA-B*4001 is the only HLA allele reported this association and only two studies provide sufficient data to conduct meta-analysis. The first study conducted in Thai population by Wangsomboonsiri, et al. [23] demonstrated a significant positive correlation between HLA-B*4001 allele and stavudine-induced lipodystrophy whereas no significant association was observed in another study conducted in Spanish population by Domingo, et al. [80]

The result from our meta-analysis cannot draw the conclusion due to substantial heterogeneity between both studies. To explore the potential sources of heterogeneity, we cannot conduct subgroup analysis due to a limited number of included studies. However, from our systematic review, the difference in results of Wangsomboonsiri, et al. [23] and Domingo, et al. [80] studies may be related to ethnicity, duration of stavudine exposure, and patient selection process.

Variability in HLA allele frequencies has been found in different populations. The frequency of HLA-B*4001 in Thai population is 0.055-0.085, which is much higher than that in Spanish population (0.025-0.05) [84]. Since the numbers of the available studies of HLA genotype and stavudine-induced lipodystrophy were limited

and the sample sizes were relatively small, the power might be too low to detect a difference in the distribution of HLA-B*4001 even though a true difference exists. More studies should be further conducted to confirm this association, particularly in Asian populations (e.g. Han-Chinese, Malaysian, Korean and Japanese).

Lipodystrophy is a delayed adverse effect induced by stavudine. Incidence rate lipodystrophy after one year on stavudine was 14.7% and Incidence rates per 100 person-years (95%-confidence interval) during the second year on stavudine was 11.4 [85]. Risk of lipodystrophy in HIV patients who received stavudine ≤ 2 years was about 26 fold-increased and about 15 fold-increased in ≥ 2 years user compare with those whose did not received [86]. Nevertheless, after switching stavudine to abacavir, patients with moderate-to-severe lipodystrophy significant improvements in subcutaneous fat continued over 104 weeks [87]. These factors should be appropriately controlled as well as other factors such as concurrent drug use. Previous studies also demonstrated the association between other genes and lipodystrophy in certain populations, for example, Fas - 670AA genotype and APOC3 [88, 89]. There might be a complex interplay between several genes that involves the development of stavudine-induced lipodystrophy rather than a single HLA allele.

The selection of patients is another contributing factor that may cause the heterogeneity. There is a selection bias in Wangsomboonsiri, et al. [23] study since the patients were selected from a group of patients in previous case-control studies. Only the patients who maintained stavudine use were eligible which, in fact, a number of individuals who had been exposed to stavudine and changed to other regimens may still have lipodystrophy. Moreover, HWE should be tested and reported to check whether or not the included individuals were in equilibrium for the frequencies of [90, 91]. Equilibrium suggested that the included individuals and, therefore, they are likely to be a representative of the population [92, 93].

Lipodystrophy can be mediated through tumor necrosis factor α (TNF-α) and its signal transduction [94, 95]. However, the mechanism by which HLA-B*4001 contributed to stavudine-induced lipodystrophy is not well understood. Nonetheless, elevated TNF-α level were observed in HIV positive patients with stavudine-induced lipodystrophy [96]. Thus, this cytokine might be responsible for lipodystrophy among these HIV patients.

Our meta-analysis has several limitations. Firstly, up to present, there are only two studies available in the literature, the association was observed in only two ethnicities (Thai and Spanish). Secondly, from these two studies, total sample size, was limited (242 cases and 197 controls). Thirdly, the diagnostic criteria for selected cases and control was different between the two studies. Thus, future prospective studies with a larger sample size in Thai population and different ethnic groups are needed to verify the association between HLA-B*4001 and stavudine-induced lipodystrophy.