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HLA-KIR-associated sites in Gag and their effects on clinical outcome in Thailand.

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Abstract

Background:
Viral peptides (epitope) are presented by class I HLA molecules to both cytotoxic T lymphocyte (CTL) through T cell receptor (TCR), and natural killer (NK) cell through killer immunoglobulin-like receptor (KIR). Compared to the studies about anti-HIV immune pressure by CTL and its related viral adaptation, studies about NK cell remain sparse.

Methodology:
208 HIV-1 CRF01_AE-infected treatment naïve Thais were recruited. Their clade I HLA allele, KIR, viral load, and Gag sequence data were collected, and
1) HLA-KIR-associated Gag mutation or preservation sites by Fisher's exact test with 95% confidential interval,
2) effects of HLA-KIR-associated sites on viral load by Mann-Whitney U-test,
3) correlation between the number of associated sites and viral load by Spearman's correlation test, and
4) the number of associated sites and survival rate by log rank test and Cox hazard model,
were analyzed.

Results:
1) 52 HLA-KIR-associated sites (20 sites at p17, 15 sites at p24, and 17 sites at p15) were identified,
2) 6 out of 52 sites also scored viral load difference between association positives and negatives with significance; 4 out of 6 sites scored lower viral load among association positives.
3) Negative correlation between the number of association at above 4 protective sites and viral load was identified (r=-0.18, p=0.002). Among 4 sites, 3 sites were HLA-Bw4-KIR3DL1-associated sites (I104I, N252N, and T454T).
4) Finally in longitudinal analysis, among HLA-Bw4-KIR3DL1 positives, higher number of HLA-Bw4-KIR3DL1-associated mutations also scored higher survival rate (p=0.009 by log rank test, and 0.63 (0.47-0.84) of hazard ratio (95% CI), p=0.002 by Cox hazard model).

Conclusions:
KIR-HLA-associated sites in Gag were identified, and several sites were strongly associated with clinical outcomes by cross sectional and longitudinal analysis. These findings imply the existence of immune pressure by NK cell and consequent viral adaptation. These findings will contribute to further development of epitope-based vaccine study.

Background

- Genome wide association study (GWAS) reported class I HLA allele as the best contribution factor for HIV viral control, and that interacts with both cytotoxic T lymphocytes (CTLs) through their T cell receptors (TCR), and natural killer (NK) cells through their killer immunoglobulin-like receptor (KIR).

- Related with KIR and HIV control,
1. Advantege of KIR3DL1-HLA-Bw4 for clinical outcome (Martin et al, *Nature Genetics 2007*)
2. Existence of HIV-1 daptation to NK cell-mediated immune pressure (Alter et al. *Nature 2011*)
were previously reported.

- However, compared to CTLs, the contribution of NK cells to HIV viral control in vivo is less clear.

Our objective is to identify KIR-HLA-associated Gag sites and evaluate their effects on viral control among CRF01_AE-infected South-east Asians.

Methodology

Subjects and data:
208 treatment naïve HIV-1 CRF01_AE-infected patients from Lampang hospital, Thailand.
- 105 female and 103 male
- Med. age 32 years old (IQR 29-36)
- CD4+ T cell count at enrollement: Med. 161 /ul (IQR 23-370)
- Viral load at enrollment: Med. 5.2 log copies/ml (IQR 4.2-5.6)
- KIR2DL1+ 201 (97%)
- KIR2DL2+ 63 (30%)
- KIR2DL3+ 205 (99%)
- KIR2DS1+ 89 (43%)
- KIR2DS2+ 74 (36%)
- KIR3DL1+ 202 (97%)
- KIR3DS1+ 88 (42%)



Statistical analysis:
- Analysis of 1) - 4) enlisted at Methodology in Abstract was performed.

Results

HLA-KIR	Site	Num.	Predominant seq.	Odds ratio	95% CI	p (Fisher)
HLA-C1+KIR2DL2+	p17	93	E	2.4	1.2-4.8	0.022
	p17	115	T	4.4	1.02-19	0.046
	p17	122	T	2.6	1.1-6.1	0.033
	p24	135	V	6.6	1.3-35	0.022
HLA-C1+KIR2DL3+	p17	15	A	0.1	0.03-0.6	0.019
	p17	46	L	0.2	0.07-0.8	0.018
	p17	112	Q	0.1	0.03-0.6	0.024
	p17	115	T	0.1	0.02-0.4	0.007
	p15	425	T	0.1	0.03-0.6	0.024
	p15	467	I	4.4	1.2-17	0.031
HLA-C1+KIR2DS2+	p17	18	K	2.4	1.1-5.1	0.039
	p17	31	M	0.4	0.2-0.8	0.014
	p17	83	V	0.5	0.3-0.9	0.019
	p17	94	V	2.2	1.03-4.7	0.045
	p15	434	K	0.1	0.01-0.8	0.008
HLA-C2+KIR2DL1+	p17	26	K	5.1	1.8-15	0.002
	p17	28	K			0.020
	p17	46	L	2.2	1.1-4.2	0.023
	p17	127	K	2.8	1.1-6.7	0.026
	p24	147	L	2.9	1.5-5.5	0.002
	p24	247	I			0.020
	p24	281	S	0.2	0.06-0.7	0.006
	p24	310	T	0.3	0.09-0.8	0.014
	p15	372	H	2.7	1.4-5.0	0.002
	p15	377	M	18	2.1-150	0.002
	p15	425	T	6.0	1.7-21	0.004
	p15	464	G	0.3	0.1-0.9	0.034
	p15	473	Q			0.037
	p15	477	D	3.0	1.1-7.9	0.048
HLA-C2+KIR2DS1+	p17	7	V			0.017
	p17	53	T			0.049
	p17	108	Q			0.015
	p24	186	M			0.015
	p24	247	I			0.002
	p24	256	I	15	1.3-173	0.042
	p15	374	N	3.2	1.2-8.5	0.027
	p15	377	M	5.8	1.2-28	0.043
	p15	401	R	5.8	1.2-28	0.043
	p15	471	L	0.3	0.1-0.9	0.045
	p15	498	Q			0.015
HLA-Bw4+KIR3DL1+	p17	34	L	0.3	0.2-0.8	0.010
	p17	104	I	0.4	0.2-0.8	0.008
	p24	147	L	0.4	0.2-0.7	0.004
	p24	242	T	19	2.6-144	<0.001
	p24	252	N	0.5	0.3-0.8	0.013
	p24	271	N			0.040
	p24	280	V	0.3	0.1-0.7	0.008
	p24	310	T	2.6	1.1-6.3	0.035
	p15	377	M	0.1	0.01-0.7	0.008
	p15	454	T	0.5	0.2-0.9	0.037
HLA-Bw4+KIR3DS1+	p24	252	N	0.5	0.3-0.9	0.034
	p15	465	E	0.1	0.02-0.9	0.027

Table 1. HLA-KIR-associated sites in Gag. HLA-KIR-associated sites in Gag identified by Fisher's exact test with p<0.05 and 95% CI,

Associated vs non-associated	VL (median (IQR) log copies/ml) (n) Associated	Non-associated	p (U-test)
HLA-C1+KIR2DL2+ associated			
E93X vs E93E	**5.5 (5.3-5.8) (19)**	**5.1 (4.5-5.6) (165)**	**0.009**
T115X vs T115T	5.6 (4.5-5.8) (5)	5.2 (4.6-5.6) (200)	0.50
T122X vs T122T	4.8 (4.5-5.1) (12)	5.2 (4.5-5.6) (183)	0.061
V135X vs V135V	5.5 (5.2-5.8) (5)	5.2 (4.5-5.6) (201)	0.31
HLA-C1+KIR2DL3+ associated			
A15A vs A15X	5.2 (4.6-5.6) (188)	5.4 (4.6-5.79 (11)	0.66
L46L vs L46X	5.2 (4.5-5.6) (147)	5.4 (4.8-5.6) (56)	0.35
Q112Q vs Q112X	5.2 (4.6-5.6) (187)	5.2 (4.7-5.6) (12)	0.94
T115T vs T115X	5.2 (4.6-5.6) (191)	5.0 (4.2-5.7) (8)	0.79
T425X vs T425T	5.2 (4.5-5.6) (187)	5.3 (4.8-5.6) (12)	0.41
I467X vs I467I	5.2 (4.7-5.6) (117)	5.0 (4.5-5.5) (88)	0.14
HLA-C1+KIR2DS2+ associated			
K18X vs K18K	5.6 (4.9-5.8) (16)	5.2 (4.5-5.6) (177)	0.10
M31M vs M31X	5.3 (4.6-5.7) (54)	5.2 (4.5-5.6) (74)	0.22
V83V vs V83X	5.2 (4.6-5.6) (47)	5.2 (4.4-5.7) (95)	0.63
V94X vs V94V	4.7 (4.5-5.4) (16)	5.2 (4.6-5.6) (176)	0.26
K434K vs K434X	**5.2 (4.5-5.6) (70)**	**5.6 (5.1-5.9) (18)**	**0.017**
HLA-C2+KIR2DL1+ associated			
K26X vs K26K	5.6 (5.2-5.7) (10)	5.2 (4.6-5.6) (192)	0.26
K28X vs K28K	5.7 (5.3-5.7) (3)	5.2 (4.5-5.6) (205)	0.33
L46X vs L46L	5.2 (4.1-5.6) (22)	5.1 (4.5-5.6) (152)	0.80
K127X vs K127K	5.4 (5.0-5.8) (11)	5.2 (4.5-5.6) (185)	0.21
L147X vs L147L	5.4 (4.8-5.7) (26)	5.2 (4.4-5.6) (148)	0.33
I247X vs I247I	5.4 (5.4-5.5) (3)	5.2 (4.5-5.6) (205)	0.38
S281S vs S281X	5.2 (4.5-5.6) (54)	5.2 (4.4-5.6) (34)	0.94
T310T vs T310X	5.2 (4.6-5.6) (53)	5.5 (4.6-5.9) (37)	0.077
H372X vs H372H	5.4 (4.7-5.7) (33)	5.0 (4.5-5.6) (124)	0.10
M377X vs M377M	5.6 (4.7-5.9) (6)	5.2 (4.5-5.6) (201)	0.33
T425X vs T425T	5.5 (5.2-5.6) (8)	5.2 (4.5-5.6) (196)	0.25
G464G vs G464X	5.2 (4.5-5.6) (53)	5.2 (4.6-5.6) (33)	0.72
Q473Q vs Q473X	5.2 (4.5-5.6) (57)	5.1 (4.6-5.7) (11)	0.85
D477X vs D477X	5.0 (4.2-5.8) (9)	5.2 (4.6-5.6) (190)	0.89
HLA-C2+KIR2DS1+ associated			
V7V vs V7X	5.2 (4.3-5.6) (26)	5.3 (4.9-5.6) (32)	0.39
T53T vs T53X	5.2 (4.3-5.6) (26)	5.4 (4.8-5.7) (25)	0.41
Q108X vs Q108Q	4.3 (4.2-4.4) (2)	5.2 (4.6-5.6) (206)	0.12
M186X vs M186M	**5.9 (5.9-6.0) (2)**	**5.2 (4.5-5.6) (206)**	**0.042**
I247X vs I247I	5.4 (5.4-5.5) (3)	5.2 (4.5-5.6) (205)	0.38
I256X vs I256I	4.9 (4.4-5.3) (2)	5.2 (4.5-5.6) (205)	0.88
N374X vs N374N	4.7 (4.1-5.4) (7)	5.2 (4.6-5.6) (182)	0.34
M377X vs M377M	5.5 (4.7-5.8) (3)	5.2 (4.5-5.6) (201)	0.64
R401X vs R401R	5.2 (4.8-5.6) (3)	5.2 (4.5-5.6) (201)	0.73
L471L vs L471X	5.1 (4.3-5.5) (22)	5.1 (4.5-5.6) (71)	0.67
Q498X vs Q498Q	5.4 (5.3-5.5) (2)	5.2 (4.5-5.6) (206)	0.64
HLA-Bw4+KIR3DL1+ associated			
L34L vs L34X	5.2 (4.4-5.6) (124)	4.9 (4.5-5.3) (28)	0.22
I104I vs I104X	**4.7 (4.2-5.5) (66)**	**5.3 (4.7-5.6) (121)**	**<0.001**
L147L vs L147X	5.1 (4.4-5.7) (106)	5.3 (4.7-5.6) (60)	0.15
T242X vs T242T	4.6 (4.3-5.6) (29)	5.2 (4.6-5.6) (178)	0.15
N252N vs N252X	**5.0 (4.4-5.5) (80)**	**5.4 (4.7-5.7) (99)**	**0.022**
N271N vs N271X	5.1 (4.4-5.6) (136)	5.8 (5.2-5.8) (3)	0.27
V280V vs V280X	5.1 (4.4-5.6) (128)	5.2 (4.5-5.8) (21)	0.40
T310X vs T310T	5.5 (4.5-5.9) (30)	5.2 (4.5-5.5) (171)	0.088
M377M vs M377X	5.2 (4.4-5.6) (135)	5.8 (4.9-5.9) (7)	0.13
T454T vs T454X	**5.0 (4.4-5.6) (112)**	**5.3 (4.9-5.8) (46)**	**0.012**
HLA-Bw4+KIR3DS1+ associated			
N252N vs N252X	5.2 (4.6-5.5) (39)	5.4 (4.7-5.7) (99)	0.41
E465E vs E465X	5.3 (4.5-5.6) (60)	5.2 (4.2-5.6) (18)	0.65

Table 2. HLA-KIR-associated sites and viral load. Viral load differences between HLA-KIR-associated site positives and negatives by Mann-Whitney U-test are shown. In total, 6 sites scored significant VL difference between them; 4 sites with lower viral load, and 2 sites with higher viral load.

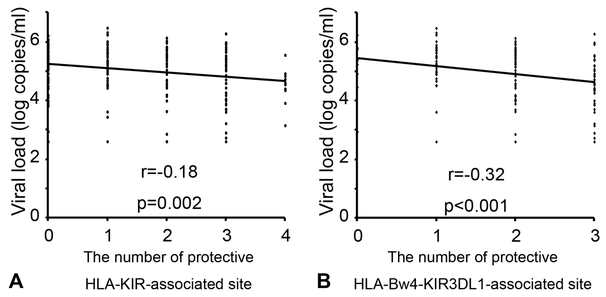

Figure 1. Correlation between the number of protective HLA-KIR-associated site and viral load. Correlation between the number of all protective HLA-KIR-associated site and viral load among n=208 (Figure 1A), and the number of protective HLA-Bw4-KIR3DL1-associated site and viral load among n=136 with HLA-Bw4+KIR3DL1+ (Figure 1B) by Spearman's correlation analysis, are shown.

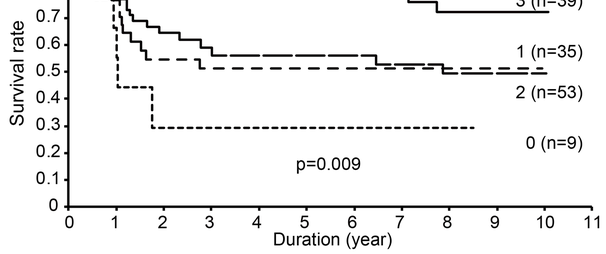

Figure 2. The number of protective HLA-Bw4-KIR3DL1-associated site and survival rate. Survival rate differences among the number of protective HLA-Bw4-KIR3DL1-associated site (0-3), n=136 with HLA-Bw4+KIR3DL1+ by log rank test is shown. There was no significant differece in the analysis among the number of all protective HLA-KIR-associated site (p=0.21).

	HR (95% CI)	p
The num. of protective HLA-Bw4-KIR3DL1-associated site	0.63 (0.47-0.84)	0.002
HLA-Bw4-KIR3DL1-associated I104I	0.46 (0.26-0.81)	0.007
HLA-Bw4-KIR3DL1-associated N252N	0.56 (0.33-0.96)	0.036
HLA-Bw4-KIR3DL1-associated T454T	0.74 (0.38-1.4)	0.36

Table 3. Cox hazard model analysis about protective HLA-Bw4-KIR3DL1-associated site and survival rate.

Conclusions

In conclusion, we ideitified several HLA-KIR-associated Gag sites and their effects on clinical outcomes, especially the contributon of HLA-Bw4-KIR3DL1-associated sites to viral control and survival rate.
These findigs implies the existence of NK cell-induced anti-HIV immune pressure and consequent viral adaptation.

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