DNA Typing of the HLA-A, -B and -C Genes : Possible MHC Class I Haplotypes in the Northeastern-Thais

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Abstract

The phenotype and gene frequencies of HLA class I were studied in the Northeastern Thai population. Blood samples were collected from 100 unrelated healthy northeastern-Thais. HLA-A, -B and -Cw alleles were determined using the polymerase chain reaction- amplification refractory mutation system (PCR-ARMS). 12 HLA-A, 20 HLA-B and 14 HLA-Cw alleles were found. Linkage disequilibrium analysis indicated the existence of 7 HLA-A-B and 19 HLA-B-Cw haplotypes. A*0207-Cw*01-B*4601 was the most common possible haplotype in this population. These results provide regional basic information for further studies in anthropology, organ transplantation and MHC disease associations in the northeastern-Thais.

Human major histocompatibility complex (MHC) is located on the short arm of the 6th chromosome. The MHC consists of polymorphic gene clusters, including HLA class I and class II, occupying at least 4 Mb[1]. The MHC class I and II genes encode cell surface antigens that play a role in self/nonself recognition. The HLA class I genes comprise of three classical (HLA -A, -B, -C) and seven non-classical class I genes (HLA -E, -F, -G, -H, -J, -K and -L). Of these non-classical class I genes, HLA -E, -F and -G are probably functional genes[2]. Indeed, HLA-G was found to be one of the inhibitory ligands for cytotoxicity induced by natural killer cells[3]. To date, over 83 HLA -A, 186-B, 44-Cw, 5-E and 7-G allelic sequences have been reported[4].

Typing for HLA class II genes by molecular methods is well established and has been incorporated in the routine[5-11]. Recently, many investigators have developed several molecular typing methods for HLA class I genes. The methods include DNA amplification and hybridization with sequence-specific oligonucleotide probes (SSOP) [12,13], amplification with sequence-specific oli-

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gonucleotide primers (SSP or ARMS)[14-16], with single-stranded conformation polymorphism (SSCP)[17,18] analysis and sequence-based typing (SBT)[19,20].

Identifying alleles of MHC class I and II genes are of great importance in many medical areas, including tissue and bone marrow transplantation, assessment of disease susceptibility, the development of synthetic vaccines and anthropology study. There are some reports on the study of HLA class I in the Thai population by serological technique including Northeastern-Thai population (NE-Thais)[21-24], but there is no information on the distribution of HLA class I alleles at molecular level. The purpose of this study was to determine the gene frequencies of HLA class I (HLA -A, -B, -Cw) in the Northeastern-Thai population using the polymerase chain reaction amplification refractory mutation system (PCR- ARMS) technique.

MATERIAL AND METHOD

Subjects

One hundred unrelated healthy NE-Thais were studied. They came from 18 provinces in the Northeast of Thailand. All of them were interviewed for their family histories. They were Thais and have lived in the Northeast of Thailand for at least two generations. All of samples were typed for HLA-A and -B by microlymphocytotoxicity test and the results had been previously reported[24].

DNA preparation

Genomic DNA was extracted from buffy coat by proteinase K digestion and salting out technique[5].

HLA-class I DNA typing

HLA-A, -B and -C were studied by PCR-ARMS techniques, according to the 12th International Histocompatibility Workshop (IHW) protocol. The oligonucleotide primers were from 12th IHW including 32 primer mixes for HLA-A, 27 primer mixes for HLA-B and 23 primer mixes for HLA-C. Briefly, the PCR reactions were carried out in 13 ml volumes containing 100 ng of genomic DNA and PCR buffer (67 mM Tris HCl *p*H 8.8, 17 mM ammonium sulfate, 0.1% Tween 20, 200 mM each of dNTP, 2 mM $MgCl_2$). Each PCR reaction contains between 0.1 mM-0.8 mM of the control primers, 1 mM of the allele specific primers and 0.27 units of Taq DNA polymerase (Promega, Medison, WI, U.S.A.).

PCR amplifications were carried out in a 480 DNA thermal cycler (Perkin-Elmer Instrument, Cetus Corp., Norwalk CT.). The PCR cycling conditions were as follows: 1 cycle denaturation at 96°C for 2 min, followed by denaturation at 96°C for 30 sec, annealing at 68°C for 60 sec, extension at 72°C for 40 sec for 5 cycles, followed by 21 cycles of denaturation at 96°C for 30 sec, annealing at 65°C for 60 sec, extension at 72°C for 40 sec, followed by 4 cycles of denaturation at 96°C for 30 sec, annealing at 55°C for 75 sec and extension at 72°C for 120 sec with a final extension at 72°C for 10 min.

PCR products were electrophoresed through 1.0 per cent agarose gel containing 0.5 mg/ml ethidium bromide. The gels were run for 30 min at 15 v/cm in 0.5X TBE (89 mM Tris-base, 89 mM boric acid, 2 mM EDTA *p*H 8.0) and visualized by UV transilluminator.

Statistical analysis

Phenotype frequencies (PF) were calculated using the formular as follows:

PF = sum of particular antigen/N

when N = total number of individual tested

Gene frequencies (GF) were estimated from the formular:

$$GF = 1 - (1 - PF)^{1/2}$$

The linkage disequilibrium was calculated from a 2 x 2 table. The significant association between the two loci was calculated by the chi-square test.

RESULTS

The phenotype and gene frequencies of HLA-A, in 100 Northeastern-Thais are shown in Table 1. Twelve alleles of HLA-A were recognized in this group. The common HLA-A alleles were A*11 at GF of 0.2584, A*24 at GF of 0.2450, A*0207 at GF of 0.1398, A*0203 at GF of 0.1282. The frequencies of A*0205, A*3401 and A*3101 were very low. In A*02 alleles, A*0207 is the most common subtype.

Table 2 shows the phenotype and gene frequencies of HLA-B alleles. Twenty alleles of HLA-B* were determined. B*4601 at GF of 0.1398, B*15 (02,08,11,15) at GF of 0.0890, B*35 and B*55/56 at GF of 0.0780 are commonly found. Although HLA-B*4601 cannot be definitly assigned

Table 1. HLA-A phenotype and gene frequencies in 100 Northeastern-Thais.

HLA-A*	number positive	Phenotype frequencies	Gene frequencies
0203	24	0.24	0.1282
0205	1	0.01	0.0050
0207	26	0.26	0.1398
11(01,02)	45	0.45	0.2584
24(02,03)	43	0.43	0.2450
26(01,02,04)	4	0.04	0.0202
29(01,02)	3	0.03	0.0151
30(01-03)	6	0.06	0.0305
3101	2	0.02	0.0101
33(01,02)	13	0.13	0.0673
3401	1	0.01	0.0050
7401	3	0.03	0.0151
blank	29	0.29	0.1574

Table 2. HLA-B phenotype and gene frequencies in 100 Northeastern-Thais.

HLA-B*	number positive	Phenotype frequencies	Gene frequencies
07(02-05)	9	0.09	0.0461
13(01,02)	14	0.14	0.0726
15(02,08,11,15)	17	0.17	0.0890
15(01,04-07,12,19,20)	8	0.08	0.0408
18(01,02)	12	0.12	0.0619
27(02-05,06,07,09)	9	0.09	0.0461
35(01-08)	15	0.15	0.0780
38(01,02)	8	0.08	0.0408
39/6701	7	0.07	0.0356
4001	11	0.11	0.0566
40(02,04-06)	5	0.05	0.0253
44(02-04)	7	0.07	0.0356
4601	26	0.26	0.1398
4801	3	0.03	0.0151
5001	1	0.01	0.0050
51/5201	8	0.08	0.0408
5401	4	0.04	0.0202
57(01,02)	1	0.01	0.0050
5801	10	0.10	0.0513
55/56	15	0.15	0.0780
blank	10	0.10	0.0513

Table 3. HLA-C phenotype and gene frequencies in 100 Northeastern-Thais.

HLA-Cw*	number positive	Phenotype frequencies	Gene frequencies
01(01-02)	34	0.34	0.1876
0302	10	0.10	0.0513
0303	4	0.04	0.0202
0304	18	0.18	0.0945
04(01,02)	23	0.23	0.1225
0602	6	0.06	0.0305
07(01-03)	36	0.36	0.2000
0704	11	0.11	0.0566
08(01-03)	18	0.18	0.0945
12(01-02)	5	0.05	0.0253
1203	7	0.07	0.0356
14(01-03)	5	0.05	0.0253
15(01-03,05)	7	0.07	0.0356
1504	2	0.02	0.0101
blank	14	0.14	0.0726

by PCR-ARMS, it was defined as HLA-B*4601 from HLA-B15* (02,08,11,15) by serology[24]. B*57, B*5001, B*4801 are rare in this population.

Fourteen HLA-Cw alleles are shown in Table 3, Cw*07 at GF of 0.2, Cw*01 at GF of 0.1876, Cw*04 at GF of 0.1225 and Cw*0304 at GF of 0.0945 were common. Cw*1504 and Cw*0303 represent at low frequencies.

The linkage disequilibrium (LD) of 12 HLA-A and 20 HLA-B alleles was analysed. Seven HLA-A and -B haplotypes with significant associations are shown in Table 4 (only delta value more than 100). The most common combination of alleles was HLA-A*0207-B*4601. Table 5 shows nineteen HLA-B and C haplotypes with significant association. HLA-B*4601-Cw*01 was the most common haplotype. Possible HLA-A, -B and C haplotypes were also analysed. Table 6 shows 22 possible HLA class I haplotypes in this population. The most common haplotype was A*0207-Cw*01-B*4601.

DISCUSSION

HLA class I polymorphisms have conventionally been detected at the cell surface by serology using alloantisera or monoclonal antibodies. Serology is a quick and convenient method for

Table 4. Singnificant HLA-A and -B association in 100 Northeastern-Thais.

HLA-A*	HLA-B*	n	Delta@
0207	4601	19	796
24	27	7	214
33	5801	7	316
30	13	4	174
33	4801	3	141
33	44	3	115
29	07(02-05)	3	144

@ = delta x 10000

Table 5. Significant HLA-B and -C association in 100 Northeastern-Thais.

HLA-B*	HLA-Cw*	n	Delta@
07(02-05)	07(01-03)	8	305
13	0304	8	320
13	0602	4	174
15(02,08,11,15)	08	14	645
15(01,04-07,12,19,20)	0304	4	137
15(01,04-07,12,19,20)	04	6	232
18	0704	8	370
27	0304	5	193
35	04	11	453
38	07(01-03)	7	264
39/6701	07(01-03)	7	285
4001	0304	6	232
40(02,04-06)	15(01-03)	4	192
44	07(01-03)	7	285
4601	01	26	1135
51/5201	14	6	292
5401	01	4	164
55/56	1203	7	328
5801	0302	10	487

@ = delta x 10000

Table 6. Possible MHC class I haplotypes in 100 Northeastern-Thais.

HLA-A*	HLA-Cw*	HLA-B*	n
29	07(01-03)	07(02-05)	2
11	07(01-03)	07(02-05)	3
30	0602	13	4
11	0304	13	7
11	08	15(02,08,11,15)	8
24	04	15(01,04-07,12,19,20)	5
24	0704	18	7
0203	07(01-03)	18	2
24	0304	27	5
24	04	35	6
11	07(01-03)	38	3
24	07(01-03)	38	5
0203	07(01-03)	39/6701	6
11	0304	4001	5
24	04	4001	3
11	15(01-03,05)	40(02,04-06)	2
33	07(01-03)	44	3
0207	01	4601	19
11	01	4601	6
11	14	51/5201	3
24	01	5401	2
33	0302	5801	7

HLA class I detection but it is not clear in many cases by serological cross reactivities and a lack of high quality reagents. The DNA typing of the HLA class II genes has become popular and has been efficiently used in the investigation of HLA-matching in tissue transplantation and disease associations. Recently, HLA class I DNA typing has been developed[12-20] and PCR-ARMS was introduced in the 12th International Histocompatibility Workshop for HLA class I typing, using 32 primer mixes for HLA-A[14], 27 pimer mixes for HLA-B [15] and 23 primer mixes for HLA-Cw[16]. The resolution offered by this PCR-ARMS is low to medium level. Definite designations of particular alleles will need further characterization.

To date, 83 HLA-A, 186 HLA-B and 44 HLA-Cw have been defined[4]. In this study 12 HLA-A*, 20 HLA-B* and 14 HLA-Cw* alleles were demonstrated. We found only one allele of HLA-A*, -B* and -Cw* in 29, 10 and 14 samples,

respectively. The individuals may present homozygotes at these loci. Alternatively, these individuals may possess unidentified alleles undetectable by the method used in this study. This phenomenon has often been seen in A*11, A*24, B*4601, Cw*07(01-03) and Cw*01. Interestingly, these are the most common alleles found in each locus. In our previous study[24], one antigen of HLA-A or -B expressed in 36 and 13 samples, respectively. This is the result of high sensitivity of the molecular typing method compared to the serological method. The discrepancy of the results between PCR-ARMS and serology found in 7 samples of HLA-A (7%) and 20 samples of HLA-B (20%). Bozon et al and Bunce et al[25,26] reported the discrepancy rates between serology and SSP was 7.1 per cent in HLA-A and 7.5 per cent in HLA-B. Our result showing high discrepancy in HLA-B may be due to the specificity of antisera used in serological typing.

It has been established that there is a strong association between HLA-A-HLA-B and HLA-B-HLA-Cw. The HLA-A-B and HLA-B-Cw associations in NE-Thais are quite similar to those found in Southern Han, Thais, Thai-Chinese, Vietnamese and Singapore Chinese[27]. The possible HLA-A*-Cw*-B* haplotypes in this study were also analysed. A*0207-Cw*01-B*4601 is the most common in NE-Thais which is similar to that found in Southern Han, Thais, Vietnamese, Singapore Chinese, while A33-Cw10-B58 was commonly found in Thai-Chinese[27].

High heterogeneity of HLA-B15 has been described in the Southeast Asian population[18]. 25 alleles of HLA-B15 have been reported[3]. B*15 haplotypes in this study can be devided into two groups : B*15(02,08,11,15) -Cw*08-A*11 and B*15(01, 04-07, 12, 19, 20)- Cw*04-A*24. Further characterization of these HLA-B15 alleles will be needed to elucidate and define the HLA-B15 haplotypes in this population.

In conclusion, this study revealed the distributions of HLA-A, -B and -Cw alleles in the NE-Thais. The results provide the basic data of HLA class I genes at the DNA level which will be useful for further studies in anthropology, organ transplantation and MHC disease associations in the Northeastern-Thais.

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การศึกษายีน HLA-A, -B และ -C ด้วยเทคนิค DNA : MHC class I haplotypes ในชาวไทยภาคตะวันออกเฉียงเหนือ

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ได้ทำการศึกษาความถี่ของยีนที่ควบคุมการสร้างแอนติเจน human leukocyte antigen (HLA) class I ในประชากรไทยภาคตะวันออกเฉียงเหนือด้วย เทคนิค polymerase chain reaction-amplification refractory mutation system (PCR-ARMS) โดยเก็บตัวอย่างทดสอบจากประชากรเชื้อชาติไทย ที่มีภูมิลำเนาในภาคตะวันออกเฉียงเหนือและไม่เป็นเครือญาติกันจำนวน 100 ราย พบยีน HLA-A, B และ Cw จำนวน 12, 20 และ 14 ชนิด ตามลำดับ เมื่อวิเคราะห์ลักษณะ linkage disequilibrium ใน class I พบ HLA-A-B จำนวน 7 ชนิด HLA-B-CW จำนวน 19 ชนิด นอกจากนี้ลักษณะ possible HLA class I haplotype ที่พบได้บ่อยที่สุด คือ A*0207-Cw*01-B*4601 จากผลการศึกษาที่ได้ สามารถนำไปใช้เป็นข้อมูลพื้นฐาน ซึ่งมีประโยชน์ทางด้านมนุษยวิทยา การปลูกถ่ายอวัยวะ และศึกษาความสัมพันธ์ต่อการเกิดโรคบางชนิดในประชากรไทยภาคตะวันออกเฉียงเหนือต่อไป

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