

HLA in Southern Thai-Muslims

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Abstract

One hundred and two Southern Thai-Muslims (STM) from Nakhon Si Thammarat province were studied for HLA class I and II by SSP ARMS-PCR and PCR-SSO, respectively. The allele frequencies, haplotype frequencies, delta value and linkage disequilibrium between alleles were expressed. The most frequent alleles for HLA-A, HLA-B and HLA-C were A*24(02,03), A*11 (01,02), A*02(01,03,05-07,11); B*15(01,04-07,12,19,20), B*07(02-05), B*51(01-05)/B*52 (011,012); and Cw*07(01-03), Cw*04(01,02), Cw*08(01-03), respectively. The HLA class II alleles frequently found were DRB1*1202, DRB1*15021, DRB1*0701; DRB3*0301; DRB5*0101; DQA1*0101, DQA1*0103, DQA1*0601; DQB1*0301, DQB1*0501, DQB1*0201; and DPB1*1301, DPB1*2301 and DPB1*0501. Two common HLA class I and II haplotypes with significant linkage disequilibrium were A*24 (02,03)-Cw*08 (01-03)-B*15 (01,04-07,12,19,20) -DRB1*1202 and A*33 (01,02)-Cw*0302-B*5801-DQB1*0201. The absence of B*27 and DRB1*1401, the presence of A*2301 and high frequency of A*68 were observed in STM. Conclusion : Certain level of genetically distinction among STM, CT and NET existed. However, the genetic diversity of STM was relatively closer to CT than NET.

Southern Thai-Muslims (STM) constitute more than half of all Thai-Muslims which is approximately 10 per cent of the country's population. Their ancestors were mostly from Malaysia⁽¹⁾. The STM in this study were from Nakhon

Si Thammarat province located at Lat. 8° 24' 58" N, and Long. 99° 57' 59" E. The approximate geographic size of the studied area was 1,084,047 sq km, whereas, approximate size of the population from which samples were drawn was 127,567 sq km.

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Table 1. HLA class I allele frequencies in Southern Thai-Muslim (STM) by SSP ARMS-PCR.

HLA-A	N (n=102)	Allele frequency (%)	HLA-B	N (n=102)	Allele frequency (%)	HLA-Cw	N (n=102)	Allele frequency (%)
A*01(01,02)	6	3.2	B*51(01,05)/B*52(01,01,012)	14	8.4 c	Cw*01(01,02)	11	6.0
A*0201	8	3.9	B*07(02,05)	17	8.7 b	Cw*0302	14	7.0
A*0203	15	8.0	B*0801	3	1.5	Cw*0303	1	0.5
A*0205	3	1.5	B*44(02,04)	4	2.0	Cw*0304	2	1.0
A*0206	1	0.5	B*13(01,02)	7	3.5	Cw*04(01,02)	34	17.8 b
A*0207	7	3.4	B*15(01,04-07,12,19,20)	23	12.2 a	Cw*0602	13	6.5
A*0211	1	0.5	B*15(02,08,11,15)/B*4601	7	5.9	Cw*07(01-03)	39	23.1 a
A*02	1	0.5	B*1503/B*4802	13	6.8	Cw*08(01-03)	31	17.6 c
A*03(01,02)	8	3.9	B*15(09,10,18)	1	0.5	Cw*12(01,02)	8	3.9
A*11(01,02)	37	19.8 b	B*15(13,16,17)	11	5.1	Cw*1203	3	1.5
A*2301	2	1.0	B*1521	1	0.5	Cw*14(01-03)	4	2.0
A*24(02,03)	45	22.5 a	B*38(01,02)	4	2.0	Cw*15(01-03,05)	9	4.5
A*26(01,02,04)	3	1.5	B*39(01,013,021,03-07)/B*6701	2	0.5	Cw*1504	1	0.5
A*2603	1	0.5	B*57(01,02)	5	3.0	Cw*1601	1	0.5
A*3401/A*66(01,02)	6	3.4	B*5801	11	5.8	Cw*1602	1	0.5
A*29(01,02)	3	1.5	B*18(01,02)	8	4.0	Cw*blank		7.1
A*30(01-03)	1	0.5	B*5401	2	1.0			
A*3101	7	3.4	B*55(01,02)/B*56(01,02)	10	5.5			
A*3201	1	0.5	B*35(01-08)	8	4.1			
A*33(01,02)	21	11.0	B*3701	1	0.5			
A*7401	1	0.5	B*40(01,012)	4	2.0			
A*3601	1	0.5	B*40(02,04-06)	4	2.0			
A*68	9	4.4	B*4007	1	0.5			
A*6801	1	0.5	B*4701	2	1.0			
A*6901	2	1.0	B*4801	1	0.5			
A*blank		2.1	B*5301	2	1.0			
			B*5901	5	2.5			
			B*7801	3	1.5			
			B*blank		7.8			

Only present alleles were expressed.
a, b and c were the most three frequent
alleles found in STM.

Table 2. HLA Class II allele frequencies in Southern Thai-Muslim (STM) by PCR-SSO.

DRB1 Allele	N (n=93)	Allele frequency(%)	DRB3,DRB5 Allele	N (n=87)	Allele frequency(%)	DQA1 Allele	N (n=90)	Allele frequency(%)	DQB1 Allele	N (n=79)	Allele frequency(%)	DPB1 Allele	N (n=91)	Allele frequency(%)
DRB1*1501	9	4.8	DRB3*0101	6	3.3 c	DQA1*0101	40	25.5 a	DQB1*0201	18	12.3 c	DPB1*01011	10	5.8
DRB1*15021	22	12.4 b	DRB3*0202	23	14.7 b	DQA1*0102	9	5.2	DQB1*0301	29	20.6 a	DPB1*02011	2	1.1
DRB1*1504	5	2.7	DRB3*0301	34	22.0 a	DQA1*0103	28	16.6 b	DQB1*0302	1	0.6	DPB1*02012	6	3.4
DRB1*1602	5	2.7	DRB3*blank		60.0	DQA1*0201	10	6.0	DQB1*03032	7	4.5	DPB1*0301	9	5.0
DRB1*1604	1	0.5				DQA1*03011	15	8.7	DQB1*0401	4	2.6	DPB1*0401	5	2.8
DRB1*02	1	0.5				DQA1*03012	2	1.1	DQB1*0402	5	3.2	DPB1*0402	7	3.9
DRB1*03011	6	3.3	DRB5*0101	17	10.2 a	DQA1*0401	4	2.2	DQB1*0501	21	14.2 b	DPB1*0501	11	6.2 c
DRB1*0304	3	1.7	DRB5*0102	11	6.5 b	DQA1*0501	15	8.7	DQB1*0502	7	4.5	DPB1*0801	2	1.1
DRB1*0403	2	1.1	DRB5*blank		83.3	DQA1*0601	26	15.8 c	DQB1*05031	11	7.2	DPB1*1301	21	12.1 a
DRB1*0405	7	3.8				DQA1*blank		10.0	DQB1*0504	1	0.6	DPB1*1401	4	2.2
DRB1*0406	1	0.5							DQB1*0601	14	9.4	DPB1*1901	1	0.5
DRB1*1101	2	1.1							DQB1*0602	3	1.9	DPB1*20011	2	1.1
DRB1*1106	2	1.1							DQB1*0604	1	0.6	DPB1*2101	2	1.1
DRB1*1111	1	0.5							DQB1*06	6	3.9	DPB1*2301	17	10.0 b
DRB1*1113	2	1.1							DQB1*blank		13.9	DPB1*2501	8	4.4
DRB1*1202	28	16.4 a										DPB1*26011	1	0.5
DRB1*1203	1	0.5										DPB1*26012	2	1.1
DRB1*1301	4	2.2										DPB1*2701	3	1.6
DRB1*1302	5	2.7										DPB1*2801	7	3.9
DRB1*1303	1	0.5										DPB1*3101	3	1.6
DRB1*1307	4	2.2										DPB1*3201	1	0.5
DRB1*1311	1	0.5										DPB1*3301	1	0.5
DRB1*1312	7	3.8										DPB1*3601	4	2.2
DRB1*1404	2	1.1										DPB1*3701	1	0.5
DRB1*1405	2	1.1										DPB1*3801	5	2.8
DRB1*1408	1	0.5										DPB1*3901	1	0.5
DRB1*1410	4	2.2										DPB1*4001	1	0.5
DRB1*14	1	0.5										DPB1*4101	3	1.6
DRB1*0701	15	8.6 c										DPB1*4501	4	2.2
DRB1*0803	1	0.5										DPB1*4601	1	0.5
DRB1*0805	1	0.5										DPB1*4701	1	0.5
DRB1*08	1	0.5										DPB1*4801	1	0.5
DRB1*0901	5	2.7										DPB1*5001	1	0.5
DRB1*1001	11	6.0										DPB1*5201	6	3.4
DRB1*blank		9.2										DPB1*5401	1	0.5
												DPB1*blank		13.4

Only present alleles were expressed.
a, b and c were the most three frequent alleles found in STM

The HLA class I and class II have been studied in Central Thais (CT), Northeastern Thais (NET) and Northern Thais (NT)(2-5). Variation in HLA frequencies was observed in different parts of Thailand. However, the HLA typing in the Southern part of Thailand has not yet been studied. Therefore, Thai-Muslims, a group of people living in a unique area of the Southern part of Thailand with their own specific religion, culture and way of living, was studied for HLA class I and class II. The document of variations in HLA typings in various parts of Thailand will be useful for further application of HLA in many fields including anthropology study, organ transplantation, disease associations, etc.

MATERIAL AND METHOD

One hundred and two STM were studied for HLA class I and II by amplification refractory mutation system (SSP ARMS-PCR) and sequence specific oligonucleotide probe hybridization (PCR-SSO), respectively. The HLA class I and HLA-A*02 SSP ARMS-PCR typing kits were purchased from 12th International Histocompatibility Workshop (IHW) which included 32, 27 and 23 primers mixes for HLA-A, -B and -C typings, respectively. The primers and oligonucleotide probes for HLA class II (DRB1, DRB3, DRB5, DQA1, DQB1 and DPB1) were purchased from DNAgency, U.S.A. Both SSP ARMS-PCR and PCR-SSO typings were performed according to the 12IHW protocols(6-8).

Table 3. The rarest and absent alleles in Southern Thai-Muslim (STM).

The rarest alleles :		AF		AF	
A*3601	0.5 %	DRB1*0803	0.5 %	DPB1*1901	0.5 %
A*7401	0.5 %	DRB1*0805	0.5 %	DPB1*3201	0.5 %
				DPB1*3301	0.5 %
B*3701	0.5 %	DRB3*0101	3.3 %	DPB1*3701	0.5 %
B*4801	0.5 %			DPB1*3901	0.5 %
		DRB5*0102	6.5 %	DPB1*4001	0.5 %
Cw*1601	0.5 %			DPB1*4601	0.5 %
Cw*1602	0.5 %	DQA1*0401	2.2 %	DPB1*4701	0.5 %
				DPB1*4801	0.5 %
		DQB1*0401	2.6 %	DPB1*5001	0.5 %
		DQB1*0402	3.2 %	DPB1*5401	0.5 %
The alleles were not found :					
A*02(02,04,08-10,12-17), A*2501,		B*0802, B*14(01,02), B*15(14,22-25),		Cw*02(01,02), Cw*0301, Cw*0501	
A*3402, A*4301, A*6802, A*8001		B*27(02-07,09), B*4003, B*41(01,02),		Cw*0601, Cw*1701	
		B*4201, B*4501, B*8101			
DRB3*0201				DQB1*03(031,04,05), DQB1*05032,	
		DQA*0104, DQA*0302, DQA1*0502		DQB1*06(03,051,052,06-09)	
DRB5*02(01-03)					
				DPB1*01012, DPB1*0202, DPB1*0601, DPB1*0901,	
				DPB1*1001, DPB1*11(011,012), DPB1*1501,	
DRB1*01(01,02), DRB1*15(022,03), DRB1*1601,				DPB1*1601, DPB1*1701, DPB1*1801, DPB1*20012,	
DRB1*03(012,02,03), DRB1*04(01,02,04),				DPB1*2201, DPB1*2401, DPB1*2901, DPB1*3001,	
DRB1*1102, DRB1*1201, DRB1*13(04-06,08-10),				DPB1*3401, DPB1*3501, DPB1*4401, DPB1*4901,	
DRB1*14(01-03,06,07,09,11), DRB1*08(01,02,04)				DPB1*5101, DPB1*5301, DPB1*5501	

AF = allele frequency

Table 4. Most frequent haplotypes significantly found in Southern Thai-Muslim (STM).

Haplotype	HF (%)	LD x 100	Haplotype	HF (%)	LD x 100
A*24(02,03) - Cw*08(01-03)	9.5	5.5	A*24(02,03) - B*15(01,04-07,12,19,20)	8.8	6.0
Cw*08(01-03) - B*15(01,04-07,12,19,20)	7.7	5.6	A*24(02-03)-Cw*08(01-03)-B*15(01,04-07,12,19,20)	6.8	6.3
A*24(02,03)-B*15(01,04-07,12,19,20)-DRB1*1202	3.2	2.8	A*33(01,02)-B*5801-DQB1*0201	2.3	2.2
Cw*08(01-03)-B*15(01,04-07,12,19,20)-DRB1*1202	2.6	2.3	Cw*0302-B*5801-DQB1*0201	2.9	2.9
A*24(02,03)-Cw*08(01-03)-B*15(01,04-07,12,19,20)-DRB1*1202	2.7	2.6	A*33(01,02)-Cw*0302-B*5801-DQB1*0201	2.3	2.3
DRB1*1202-DRB3*0301	15.0	11.4	DRB1*15021-DRB5*0102	5.6	4.9
DRB1*1202-DQA1*0601	11.2	8.7	DRB1*1202-DQB1*0301	10.4	7.2
DQA1*0601-DQB1*0301	11.9	8.9	DQA1*0101-DQB1*0501	11.7	8.4
DRB1*1202-DPB1*1301	4.6	2.6	DRB3*0301-DQA1*0601	12.7	9.3
DRB3*0301-DQB1*0301	12.3	8.1	DRB3*0301-DPB1*1301	4.8	2.2
DRB5*0102-DQA1*0101	4.2	2.6	DRB5*0101-DQB1*0601	4.5	3.7
DRB5*0102-DPB1*1301	2.1	1.4	DQA1*0101-DPB1*0301	3.0	1.7
DQB1*0201-DPB1*2301	5.1	4.0			
DRB1*1202-DRB3*0301-DQA1*0601	10.4	9.8	DRB1*15021-DRB5*0102-DQA1*0101	4.1	3.9
DRB1*1202-DRB3*0301-DQB1*0301	9.6	8.9	DRB1*15021-DRB5*0102-DQB1*0501	3.9	3.8
DRB1*1202-DQA1*0601-DQB1*0301	9.4	8.9	DRB1*1202-DQA1*0601-DPB1*1301	4.3	4.0
DRB1*0701-DQB1*0201-DPB1*2301	3.8	3.7	DQA1*0601-DQB1*0301-DPB1*2301	3.8	3.5
DRB3*0301-DQA1*0601-DQB1*0301	9.8	9.2	DRB5*0101-DQA1*0103-DQB1*0601	2.7	2.6
DRB1*1202-DRB3*0301-DPB1*1301	4.5	4.0	DRB3*0301-DQA1*0601-DPB1*2301	3.0	2.7
DRB1*0701-DQA1*0201-DQB1*0201-DPB1*2301	2.7	2.7	DRB1*15021-DRB5*0102-DQA1*0101-DQB1*0501	3.0	3.0
DRB1*1202-DQA1*0601-DQB1*0301-DPB1*0301	2.7	2.7	DRB1*1202-DRB3*0301-DQA1*0601-DQB1*0301-DPB1*1301	2.3	2.2

Allelic frequencies were obtained by direct counting. The haplotype frequencies and delta value were analysed by computer programme kindly provided by Prof. T. Juji, The Japanese Red Cross Central Blood Center, Tokyo, Japan. The significance of the linkage disequilibrium between allele was tested using Chi-square analysis.

RESULTS

HLA-ABC allele frequencies of 102 STM are shown in Table 1. The most frequent alleles for HLA-A, HLA-B and HLA-C were A*24 (02,03), A*11 (01,02), A*02(01,03,05-07,11); B*15 (01,04-07,12,19,20), B*07(02-05), B*51(01-05)/B*52 (011, 012); and Cw*07(01-03), Cw*04(01,02), Cw*08 (01-03), respectively.

HLA class II allele frequencies of STM are shown in Table 2. The most frequent alleles for DRB1, DRB3, DRB5, DQA1, DQB1 and DPB1 were DRB1*1202, DRB1*15021, DRB1*0701; DRB3*0301, DRB3*0202, DRB3*0101; DRB5*

0101, DRB5*0102; DQA1*0101, DQA1*0103, DQA1*0601; DQB1*0301, DQB1*0501, DQB1*0201; and DPB1*1301, DPB1*2301, DPB1*0501, respectively. The rarest alleles and alleles which were not found in STM for HLA class I and class II are shown in Table 3.

The most frequent haplotypes, haplotype frequencies (HF) and linkage disequilibrium (LD) significantly found in STM are shown in Table 4. A*24(02,03)-Cw*08(01-03)-B*15(01,04-07,12,19,20) was the most common HLA class I haplotype found in STM with HF 6.8 per cent and LD 6.3 whereas DRB1*1202-DRB3*0301-DQA1*0601-DQB1*0301-DPB1*1301 was the most common HLA class II haplotype found in STM with HF 2.3 per cent and LD 2.2. Two HLA class I and II haplotypes commonly found in STM were A*24(02,03)-Cw*08(01-03)-B*15(01,04-07,12,19,20)-DRB1*1202 and A*33(01,02)-Cw*0302-B*5801-DQB1*0201 with HF 2.7 per cent and 2.3 per cent and LD 2.6 and 2.3, respectively.

Table 5. Comparison of molecular HLA class I and II in various parts of Thailand.

HLA Allele	STM	CT	NET	NT
A*01(01,02)	Present	Present*	Absent	Rare*
A*0201	Present	Present	Absent	ND
A*03(01,02)	Present	Present*	Absent	Rare*
A*2301	Present	Absent*	Absent	Absent*
A*68	Present	Rare*	Absent	Absent*
B*15(13,16,17)	Present	ND	Absent	ND
B*27	Absent	Present*	Present	Present*
B*7801	Present	ND	Absent	ND
DRB1*1312	Present	ND	Absent	Absent
DRB1*1401	Absent	Present	Present	Present
DQB1*0402	Present	Present	Absent	Present

STM = Southern Thai-Muslim

CT = Central Thais (A*02 from Chiewsilp P, et al. while DRB1 and DQB1 from Sujirachato K, et al.)

NET = Northeastern Thais (data from Romphruk A, et al.)

NT = Northern Thais (HLA class I from Fongsatitkul L, et al. while HLA class II from Mancernaroj R, et al.)

ND = Molecular HLA typing was not done.

* = HLA typing was performed by lymphocytotoxicity test.

DISCUSSION

The molecular HLA class I and II were compared among STM, CT NET and NT (Table 5) (9-13). The interesting findings were B*27 and DRB1*1401 which were present in CT, NET and NT (B27-serology) were absent in STM. The absence of HLA-B27 in Blacks (South Africans, Bushmen, Zimbabweans), Tribal Indians, and Amerinds⁽⁴⁾ and the absence of DRB1*1401 were also observed in Huilliches, Guarani, Kaingang and Ticuna of Amerindian and Colombian of Mestizo

(14). In addition, A*2301 was present in STM but absent in CT and NET. Furthermore, A*68 was present with higher frequency in STM but rare in CT and absent in NET.

It was noticed that there is a certain level of genetically distinction among STM, CT and NET, though sharing of most frequent alleles in class I, II and even four-loci linkage disequilibrium among STM, CT and NET were also observed⁽¹⁰⁻¹³⁾. Intermarriage among CT and NET may account for these findings.

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การศึกษาหมู่เลือดเมตโลhitชาว HLA ในคนไทยมุสลิมภาคใต้

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จากการศึกษา HLA class I (ABC) และ II (DRB1, DRB3, DRB5, DQA1, DQB1, DPB1) ในคนไทยมุสลิม (STM) ที่ นครศรีธรรมราช จำนวน 102 ราย โดยวิธี SSP ARMS-PCR และ PCR-SSO พบว่า หมู่เลือดเมตโลhitชาว ที่พบบ่อยคือ A*24 (02,03), A*11 (01,02), A*02 (01,03,05-07,11); B*15 (01,04-07,12,19,20), B*07 (02-05), B*51 (01-05)/ B*52 (011,012); Cw*07 (01-03), Cw*04 (01,02), Cw*08 (01-03); DRB1* 1202, DRB1* 15021, DRB1* 0701; DRB3* 0301; DRB5* 0101; DQA1* 0101, DQA1* 0103, DQA1* 0601; DQB1* 0301, DQB1* 0501, DQB1* 0201; DPB1* 1301, DPB1* 2301 และ DPB1* 0501

HLA class I และ II haplotype ที่พบใน STM ที่มี significant linkage disequilibrium คือ A* 24 (02,03)-Cw* 08 (01-03)- B* 15 (01,04-07,12,19,20)- DRB1* 1202 และ A* 33 (01,02)- Cw* 0302-B* 5801-DQB1* 0201 ความหลากหลายทางพันธุกรรมของคนไทยมุสลิมภาคใต้ใกล้เคียงกับคนไทยในภาคกลาง (CT) มากกว่าคนไทยอีสาน (NET) ความแตกต่างที่น่าสนใจใน STM คือไม่พบ B*27, DRB1*1401 แต่พบ A*2301 และมี A*68 สูง ซึ่งเป็นข้อสังเกตที่ไม่พบในคนไทยอื่นๆที่เคยศึกษามา สรุป มีความแตกต่างทางพันธุกรรมในระดับหนึ่ง ในกลุ่ม STM, CT และ NET แต่ในเชิงเปรียบเทียบความหลากหลายทางพันธุกรรมของ STM ใกล้เคียงกับ CT มากกว่า NET

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