

CHAPTER IV

RESULTS

4.1 Determination of the Quality of Complement

The quality of the lymphocytotoxicity test used in serological HLA typing depended on the specificity of the HLA anti-sera, quality of the cells and complement, and how well the technique was used. The potency and cytotoxic property of the complement was tested by using the lymphocytotoxic method. Eight non immunized rabbit sera were tested for cytotoxicity before using in HLA typing. All of them were of high quality in the lymphocytotoxicity test and strongly positive with the positive control. They did not have autolysis with the cell and negative serum control, as shown in Tables 4.1.1 and 4.1.8.

4.2 The Distribution of HLA-A2, A9, A11.1 and A11.2 Serological Typing in 3 Ethnic Groups of Northern Thai Populations

The distribution of HLA-A2, A9, A11.1 and A11.2 in 153 Khon Muang, 125 Khon Yawng and 113 Karen is given in Tables 4.2 to 4.4. Four serotypes: A2, A9, A11.1 and A11.2 were found in both Khon Muang and Khon Yawng, but A11.2 was completely absent in Karen, as seen in Table 4.5.

4.3 Comparison of HLA Gene Frequency within 3 Ethnic Groups of Northern Thai Populations

The distribution of gene frequency in HLA-A2 was highest in Khon Yawng (43.43%) compared with Khon Muang (33.83%) and Karen (12.76%). HLA-A9 was 20.18%, 15.62% and 12.55% in Karen, Khon Yawng and Khon Muang, respectively. HLA-A11.1 was very high in Karen (52.96%) when compared with Khon Muang (34.32%) and Khon Yawng (22.20%). The gene frequency of HLA-A11.2 was highest in Khon Yawng (7.05%) compared with Khon Muang (3.32%) and Karen (0%). HLA-A11.1 was significantly the highest in Karen ($P \leq 0.005$), whereas, HLA-A2 and A-11.2 were significantly higher in Khon Yawng ($P \leq 0.0001$) when compared with Karen. Also, HLA-A11.1 was significantly more common in Khon Muang than in Khon Yawng.

4.4 Comparison of HLA Gene Frequency between Khon Muang and Tai-speaking Groups

The distribution of HLA-A2, A9, A11.1 and A11.2 gene frequencies in Khon Muang was determined in Tai-speaking groups, namely present day Thais, Tai Lue and Tai Dam, as shown in Table 4.6. HLA-A2 was the most common gene frequency in Tai Dam (53.42 %), which was significantly different ($P < 0.001$) to Khon Muang. Comparing gene frequencies between the Khon Muang and present day Thais, HLA-A2, and A9 serotypes were significantly different ($P < 0.05$) in Khon Muang. In contrast, there was no significant difference ($P > 0.05$) in gene frequencies for 4 HLA-A serotypes in both Khon Muang and Tai Lue.

4.5 Comparison of HLA Gene Frequency between Khon Yawng and Tai-speaking Groups

HLA-A2, A9, A11.1 and A11.2 were determined in Khon Yawng and Tai speaking groups, as shown in Table 4.7. Overall, only HLA-A2 was significantly different ($P < 0.0001$) between Khon Yawng and present day Thais. HLA-A9, A11.1 and A-11.2 were not significantly different in Tai speaking groups.

4.6 Comparison of HLA Gene Frequency between Karen and Tai-speaking Groups

A comparison of 4 HLA serotypes in Karen and Tai-speaking groups is given in Table 4.8. The gene frequency of HLA-A2 was the highest in Tai Dam, while HLA-A11.1 was the highest in Karen by a significant difference ($P < 0.0001$). The gene frequencies of 4 HLA serotypes in Karen was significantly different ($P < 0.05$) to the Tai speaking groups, except for HLA-A9, where there was no significant difference ($P > 0.05$) to present day Thais.

4.7 Comparison of HLA Gene Frequency in 3 Ethnic Groups of Northern Thai Populations and non- Thai Mainland Southeast Asian Populations

Five non-Thai mainland southeast Asian populations in areas neighboring Thailand were southern Han (S. Han), Buyi, Miao (Hmong), Vietnamese (Viet), and Singapore Chinese (Sing. C). The gene frequencies of HLA-A2, A9, and A11.1 was determined during the 11HWS, which also included the comparison of Khon Muang, Khon Yawng and Karen, as shown in Tables 4.9 to 4.11.

4.8 Comparison of HLA Gene Frequency in Khon Muang and non- Thai Mainland Southeast Asian Populations

HLA-A2 and HLA-A11.1 gene frequencies in Khon Muang, as seen in Table 4.9, were significantly different ($P < 0.0001$) compared to Buyi and Miao, respectively. A slightly significant difference ($P < 0.05$) also occurred in the gene frequencies of HLA-A2 and A9 when compared to Singapore Chinese and southern Han.

4.9 Comparison of HLA Gene Frequency in Khon Yawng and non- Thai Mainland Southeast Asian Populations

When the gene frequency of HLA-A11.1 was compared between Khon Yawng and non-Thai, Southeast Asian populations, as shown in Table 4.10, there was a significant difference ($P < 0.0001$) to southern Han and Miao. In contrast, the gene frequency of HLA-A11.1 in Khon Yawng had a slightly significant difference ($P < 0.05$) compared to Buyi and Vietnamese.

4.10 Comparison of HLA Gene Frequency in Karen and non- Thai Mainland Southeast Asian Populations

Three prevalent blood types: HLA-A2, A9 and A11.1 were determined in Karen, as shown in Table 4.11. The gene frequency of HLA-A2 decreased significantly ($P < 0.0001$) in Karen, whereas, HLA-A11.1 had a slightly significant increase ($P < 0.05$) in that group compared with non-Thai, Southeast Asian populations. On the other hand, there was no significant difference ($P > 0.05$) in the HLA-A11.1 gene frequency between

Karen and Miao. The HLA-A9 gene frequency in Karen showed no significant difference to non-Thai, Southeast Asian groups.

4.11 Comparison of HLA Gene Frequencies in Khon Muang and Thais in Different Parts of Thailand

The distribution of HLA-A2, A9 and A11 gene frequencies in Khon Muang was compared with northern Thais, northeastern Thais, southern Thai-muslims and Thais, as shown in Table 4.12. HLA-A9 in Khon Muang was significantly lower compared with northeastern Thais ($P < 0.005$), southern Thai-muslims ($P < 0.001$) and Thais ($P < 0.05$). In contrast, HLA-A11 was significantly higher in Khon Muang when compared with northeastern Thais ($P < 0.01$), southern Thai-muslims ($P < 0.001$) and Thais ($P < 0.005$). The gene frequency of HLA-A2 was significantly higher in Khon Muang compared with southern Thai-muslims ($P < 0.05$) and Thais ($P < 0.005$). On the other hand, there was no significant difference ($P > 0.05$) in gene frequencies for 3 HLA-A serotypes in Khon Muang and previously studied northern Thais.

4.12 Comparison of HLA Gene Frequencies in Khon Yawng and Thais in Different Parts of Thailand

A comparison of 3 HLA serotypes in Khon Yawng and Thais in different parts of Thailand is given in Table 4.13. There was no significant difference ($P > 0.05$) in gene frequencies for 3 HLA-A serotypes in both Khon Yawng and previously studied northern Thais. HLA-A2 was the most common gene frequency in Khon Yawng (43.43%), which was significantly different ($P < 0.05$, $P < 0.0001$ and $P < 0.0001$) to northeastern Thais,

southern Thai-muslims and Thais, respectively. The gene frequency of HLA-A9 was significantly lower in Khon Yawng ($P < 0.05$) compared with northeastern Thais and southern Thai-muslims. In contrast, HLA-A11 in Khon Yawng was significantly higher ($P < 0.05$) compared with southern Thai-muslims.

4.13 Comparison of HLA Gene Frequencies in Karen and Thais in Different Parts of Thailand

HLA-A2, A9, and A11 were determined in Karen and Thais from various parts of Thailand, as shown in Table 4.14. The gene frequency of HLA-A2 in Karen was significantly different ($P < 0.0001$) compared with northern Thais and northeastern Thais. HLA-A11 was the most common gene frequency in Karen (52.96%) and was significantly different ($P < 0.05$) to northern Thais, while the most significant difference ($P < 0.0001$) occurred when compared with northeastern Thais, southern Thai-muslims and Thais.

4.14 The Distribution of HLA-A11 Allele Frequencies in 3 Ethnic Groups in Northern Thai Populations

Molecular typing of HLA-A11 allele frequencies in Khon Muang, Khon Yawng and Karen is given in Table 4.15. Only 3 of at least 5 known HLA-A11 alleles were identified in this study, as shown in Figure 4.1 to 4.3. In all populations, HLA-A*1101 was the most frequent allele. HLA-A*1101 and A*1102 were recognized in both Khon Muang and Khon Yawng, while Karen had HLA-A*1101, and A*1103. The distribution of the allele frequency of HLA-A*1101 was highest in Karen (38.05%) compared with Khon Muang (28.43%) and Khon Yawng (19.6%). The HLA-A*1102 allele frequency

was 6.80% in Khon Yawng and 3.27% in Khon Muang, while absolutely none was found in Karen (0%). Surprisingly, HLA-A*1103 was only identified in Karen with an allele frequency of 2.65%. HLA-A*1101 was significantly different ($P < 0.01$) in Karen compared with Khon Muang and there was a highly significant difference ($P < 0.0001$) when compared with Khon Yawng. HLA-A*1102 was only significantly different ($P \leq 0.0005$) in Karen when compared with Khon Yawng.

4.15 The Correlation of Serological Typing and Molecular Typing in HLA-A11 in 3 Ethnic Groups in Northern Thai Populations

The distribution of the serological typing of HLA-A11.1 and HLA-A11.2 correlated with the molecular typing (HLA-A*1101 and HLA-A*1102) in both Khon Muang and Khon Yawng, All of the HLA-A*1101 in Karen blood samples were also corrected with serologically define HLA-A11.1. Four out of six Karen samples, which were defined as HLA-A*1103, showed the positive reaction with the correspond anti-HLA-A11.1. The exceptional two samples, Karen 23 and Karen 62 scored as negative result.

4.16 HLA-A*1103 in the Karen Family

The family typing of Karen 23 and Karen 62 were selected to confirm the ambiguous result. Karen 23 family comprised of Karen 23, 140, 142 and 155 members, while as Karen 62 family consisted of Karen 62, 151 and 154 members. The HLA-serological typing using a special anti-HLA-A11 panel. The results (Table 4.16) showed that the HLA-A*1103 specificity could be discriminated from HLA-A*1101 by means of

negative result of anti-HLA-A11.1, 11.2 (serum no TP2456.35) and anti-HLA-A11 (serum no TP1433.42) specificity.

The HLA class I of the two family were also typed and showed in Table 4.17.

4.17 HLA-A*1103 Gene Sequencing

Finally, sequence based typing was selected to confirm HLA-A*1103 by direct detection of the nucleotide sequence of the HLA-A allele in exon 2 and 3. The samples Karen 23, 140, 142 and 155 of family Karen 23 and Karen 62, 151 and 154 of family Karen 62 were sequenced and all samples found the polymorphism at nucleotide 524 and 527 of the exon 3 were G and A, respectively.

Table 4.1 Determination of potency and toxicity of Rabbit complement**4.1.1 Rabbit No.1**

PC & NC dilution	Rabbit serum dilutions				
	Neat	1:2	1:4	1:8	1:16
PC (Neat)	8	8	6	1	1
PC (1:2)	8	8	6	1	1
PC (1:4)	8	8	6	1	1
PC (1:8)	8	8	4	1	1
NC (Neat)	1	1	1	1	1
NC (1:2)	1	1	1	1	1
NC (1:4)	1	1	1	1	1
NC (1:8)	1	1	1	1	1
Control*	1	1	1	1	1

PC = Positive serum control

NC = Negative serum control

* = Cells & complement control

Table 4.1 Determination of potency and toxicity of Rabbit complement (continued.)**4.1.2 Rabbit No.2**

PC & NC dilution	Rabbit serum dilutions				
	Neat	1:2	1:4	1:8	1:16
PC (Neat)	8	8	2	1	1
PC (1:2)	8	8	1	1	1
PC (1:4)	8	8	2	1	1
PC (1:8)	8	8	2	1	1
NC (Neat)	1	1	1	1	1
NC (1:2)	1	1	1	1	1
NC (1:4)	1	1	1	1	1
NC (1:8)	1	1	1	1	1
Control*	1	1	1	1	1

PC = Positive serum control

NC = Negative serum control

* = Cells & complement control

Table 4.1 Determination of potency and toxicity of Rabbit complement (continued.)

4.1.3 Rabbit No.3

PC & NC dilution	Rabbit serum dilutions				
	Neat	1:2	1:4	1:8	1:16
PC (Neat)	8	8	6	1	1
PC (1:2)	8	8	4	1	1
PC (1:4)	8	8	2	1	1
PC (1:8)	8	8	1	1	1
NC (Neat)	1	1	1	1	1
NC (1:2)	1	1	1	1	1
NC (1:4)	1	1	1	1	1
NC (1:8)	1	1	1	1	1
Control*	1	1	1	1	1

PC = Positive serum control

NC = Negative serum control

* = Cells & complement control

Table 4.1 Determination of potency and toxicity of Rabbit complement (continued.)**4.1.4 Rabbit No.4**

PC & NC dilution	Rabbit serum dilutions				
	Neat	1:2	1:4	1:8	1:16
PC (Neat)	8	8	6	1	1
PC (1:2)	8	8	4	1	1
PC (1:4)	8	8	4	1	1
PC (1:8)	8	8	2	1	1
NC (Neat)	1	1	1	1	1
NC (1:2)	1	1	1	1	1
NC (1:4)	1	1	1	1	1
NC (1:8)	1	1	1	1	1
Control*	1	1	1	1	1

PC = Positive serum control

NC = Negative serum control

* = Cells & complement control

Table 4.1 Determination of potency and toxicity of Rabbit complement (continued.)

4.1.5 Rabbit No.5

PC & NC dilution	Rabbit serum dilutions				
	Neat	1:2	1:4	1:8	1:16
PC (Neat)	8	8	6	1	1
PC (1:2)	8	8	4	1	1
PC (1:4)	8	8	2	1	1
PC (1:8)	8	8	2	1	1
NC (Neat)	1	1	1	1	1
NC (1:2)	1	1	1	1	1
NC (1:4)	1	1	1	1	1
NC (1:8)	1	1	1	1	1
Control*	1	1	1	1	1

PC = Positive serum control

NC = Negative serum control

* = Cells & complement control

Table 4.1 Determination of potency and toxicity of Rabbit complement (continued.)**4.1.6 Rabbit No.6**

PC & NC dilution	Rabbit serum dilutions				
	Neat	1:2	1:4	1:8	1:16
PC (Neat)	8	8	1	1	1
PC (1:2)	8	8	2	1	1
PC (1:4)	8	8	2	1	1
PC (1:8)	8	8	1	1	1
NC (Neat)	1	1	1	1	1
NC (1:2)	1	1	1	1	1
NC (1:4)	1	1	1	1	1
NC (1:8)	1	1	1	1	1
Control*	1	1	1	1	1

PC = Positive serum control

NC = Negative serum control

* = Cells & complement control

Table 4.1 Determination of potency and toxicity of Rabbit complement (continued.)**4.1.7 Rabbit No.7**

PC & NC dilution	Rabbit serum dilutions				
	Neat	1:2	1:4	1:8	1:16
PC (Neat)	8	8	8	1	1
PC (1:2)	8	8	6	1	1
PC (1:4)	8	8	1	1	1
PC (1:8)	8	8	1	1	1
NC (Neat)	1	1	1	1	1
NC (1:2)	1	1	1	1	1
NC (1:4)	1	1	1	1	1
NC (1:8)	1	1	1	1	1
Control*	1	1	1	1	1

PC = Positive serum control

NC = Negative serum control

* = Cells & complement control

Table 4.1 Determination of potency and toxicity of Rabbit complement (continued.)**4.1.8 Rabbit No.8**

PC & NC dilution	Rabbit serum dilutions				
	Neat	1:2	1:4	1:8	1:16
PC (Neat)	8	8	8	1	1
PC (1:2)	8	8	8	1	1
PC (1:4)	8	8	8	1	1
PC (1:8)	8	8	1	1	1
NC (Neat)	1	1	1	1	1
NC (1:2)	1	1	1	1	1
NC (1:4)	1	1	1	1	1
NC (1:8)	1	1	1	1	1
Control*	1	1	1	1	1

PC = Positive serum control

NC = Negative serum control

* = Cells & complement control

Table 4.2 HLA typing in Khon Muang

DNA number	HLA-A	HLA-A	A*1101	A*1102	A*1103
KM 001	2	9			
KM 002	2	9			
KM 003	2	9			
KM 004	2	9			
KM 005	2	9			
KM 006	2	9			
KM 007	2	9			
KM 008	2	9			
KM 009	2	9			
KM 010	2	9			
KM 011	2	9			
KM 012	2	11.1	1101		
KM 013	2	11.1	1101		
KM 014	2	11.1	1101		
KM 015	2	11.1	1101		
KM 016	2	11.1	1101		
KM 017	2	11.1	1101		
KM 018	2	11.1	1101		
KM 019	2	11.1	1101		
KM 020	2	11.1	1101		
KM 021	2	11.1	1101		
KM 022	2	11.1	1101		
KM 023	2	11.1	1101		
KM 024	2	11.1	1101		
KM 025	2	11.1	1101		
KM 026	2	11.1	1101		
KM 027	2	11.1	1101		
KM 028	2	11.1	1101		
KM 029	2	11.1	1101		
KM 030	2	11.1	1101		

Table 4.2 HLA typing in Khon Muang (continued.)

DNA number	HLA-A	HLA-A	A*1101	A*1102	A*1103
KM 031	2	11.1	1101		
KM 032	2	11.1	1101		
KM 033	2	11.1	1101		
KM 034	2	11.1	1101		
KM 035	2	11.1	1101		
KM 036	2	11.1	1101		
KM 037	2	11.1	1101		
KM 038	2	11.1	1101		
KM 039	2	11.1	1101		
KM 040	2	11.1	1101		
KM 041	2	11.1	1101		
KM 042	2	11.1	1101		
KM 043	2	11.1	1101		
KM 044	2	11.1	1101		
KM 045	2	11.1	1101		
KM 046	2	11.1	1101		
KM 047	2	11.1	1101		
KM 048	2	11.2		1102	
KM 049	2	11.2		1102	
KM 050	2	11.2		1102	
KM 051	2				
KM 052	2				
KM 053	2				
KM 054	2				
KM 055	2				
KM 056	2				
KM 057	2				
KM 058	2				
KM 059	2				
KM 060	2				

Table 4.2 HLA typing in Khon Muang (continued.)

DNA number	HLA-A	HLA-A	A*1101	A*1102	A*1103
KM 061	2				
KM 062	2				
KM 063	2				
KM 064	2				
KM 065	2				
KM 066	2				
KM 067	2				
KM 068	2				
KM 069	2				
KM 070	2				
KM 071	2				
KM 072	2				
KM 073	2				
KM 074	2				
KM 075	2				
KM 076	2				
KM 077	2				
KM 078	2				
KM 079	2				
KM 080	2				
KM 081	2				
KM 082	2				
KM 083	2				
KM 084	2				
KM 085	2				
KM 086	2				
KM 087	9	11.1	1101		
KM 088	9	11.1	1101		
KM 089	9	11.1	1101		
KM 090	9	11.1	1101		

Table 4.2 HLA typing in Khon Muang (continued.)

DNA number	HLA-A	HLA-A	A*1101	A*1102	A*1103
KM 091	9	11.1	1101		
KM 092	9	11.1	1101		
KM 093	9	11.1	1101		
KM 094	9	11.1	1101		
KM 095	9	11.1	1101		
KM 096	9	11.1	1101		
KM 097	9	11.1	1101		
KM 098	9	11.1	1101		
KM 099	9	11.1	1101		
KM 100	9	11.1	1101		
KM 101	9	11.2		1102	
KM 102	9				
KM 103	9				
KM 104	9				
KM 105	9				
KM 106	9				
KM 107	9				
KM 108	9				
KM 109	9				
KM 110	9				
KM 111	9				
KM 112	11.1	11.2	1101	1102	
KM 113	11.1		1101		
KM 114	11.1		1101		
KM 115	11.1		1101		
KM 116	11.1		1101		
KM 117	11.1		1101		
KM 118	11.1		1101		
KM 119	11.1		1101		
KM 120	11.1		1101		

Table 4.2 HLA typing in Khon Muang (continued.)

DNA number	HLA-A	HLA-A	A*1101	A*1102	A*1103
KM 121	11.1		1101		
KM 122	11.1		1101		
KM 123	11.1		1101		
KM 124	11.1		1101		
KM 125	11.1		1101		
KM 126	11.1		1101		
KM 127	11.1		1101		
KM 128	11.1		1101		
KM 129	11.1		1101		
KM 130	11.1		1101		
KM 131	11.1		1101		
KM 132	11.1		1101		
KM 133	11.1		1101		
KM 134	11.1		1101		
KM 135	11.1		1101		
KM 136	11.1		1101		
KM 137	11.1		1101		
KM 138	11.1		1101		
KM 139	11.1		1101		
KM 140	11.1		1101		
KM 141	11.1		1101		
KM 142	11.1		1101		
KM 143	11.1		1101		
KM 144	11.1		1101		
KM 145	11.1		1101		
KM 146	11.1		1101		
KM 147	11.1		1101		
KM 148	11.1		1101		
KM 149	11.2			1102	
KM 150	11.2			1102	

Table 4.2 HLA typing in Khon Muang (continued.)

DNA number	HLA-A	HLA-A	A*1101	A*1102	A*1103
KM 151	11.2			1102	
KM 152	11.2			1102	
KM 153	11.2			1102	

Table 4.3 HLA typing in Khon Yawng

DNA number	HLA-A	HLA-A	A*1101	A*1102	A*1103
Yawng 001	2	9			
Yawng 002	2	9			
Yawng 003	2	9			
Yawng 004	2	9			
Yawng 005	2	9			
Yawng 006	2	9			
Yawng 007	2	9			
Yawng 008	2	9			
Yawng 009	2	9			
Yawng 010	2	9			
Yawng 011	2	9			
Yawng 012	2	9			
Yawng 013	2	9			
Yawng 014	2	9			
Yawng 015	2	9			
Yawng 016	2	9			
Yawng 017	2	9			
Yawng 018	2	9			
Yawng 019	2	9			
Yawng 020	2	11.1	1101		
Yawng 021	2	11.1	1101		
Yawng 022	2	11.1	1101		
Yawng 023	2	11.1	1101		
Yawng 024	2	11.1	1101		
Yawng 025	2	11.1	1101		
Yawng 026	2	11.1	1101		
Yawng 027	2	11.1	1101		
Yawng 028	2	11.1	1101		
Yawng 029	2	11.1	1101		

Table 4.3 HLA typing in Khon Yawng (continued.)

DNA number	HLA-A	HLA-A	A*1101	A*1102	A*1103
Yawng 030	2	11.1	1101		
Yawng 031	2	11.1	1101		
Yawng 032	2	11.1	1101		
Yawng 033	2	11.1	1101		
Yawng 034	2	11.1	1101		
Yawng 035	2	11.1	1101		
Yawng 036	2	11.1	1101		
Yawng 037	2	11.1	1101		
Yawng 038	2	11.1	1101		
Yawng 039	2	11.1	1101		
Yawng 040	2	11.1	1101		
Yawng 041	2	11.1	1101		
Yawng 042	2	11.1	1101		
Yawng 043	2	11.1	1101		
Yawng 044	2	11.2		1102	
Yawng 045	2	11.2		1102	
Yawng 046	2	11.2		1102	
Yawng 047	2	11.2		1102	
Yawng 048	2	11.2		1102	
Yawng 049	2	11.2		1102	
Yawng 050	2	11.2		1102	
Yawng 051	2				
Yawng 052	2				
Yawng 053	2				
Yawng 054	2				
Yawng 055	2				
Yawng 056	2				
Yawng 057	2				
Yawng 058	2				

Table 4.3 HLA typing in Khon Yawng (continued.)

DNA number	HLA-A	HLA-A	A*1101	A*1102	A*1103
Yawng 059	2				
Yawng 060	2				
Yawng 061	2				
Yawng 062	2				
Yawng 063	2				
Yawng 064	2				
Yawng 065	2				
Yawng 066	2				
Yawng 067	2				
Yawng 068	2				
Yawng 069	2				
Yawng 070	2				
Yawng 071	2				
Yawng 072	2				
Yawng 073	2				
Yawng 074	2				
Yawng 075	2				
Yawng 076	2				
Yawng 077	2				
Yawng 078	2				
Yawng 079	2				
Yawng 080	2				
Yawng 081	2				
Yawng 082	2				
Yawng 083	2				
Yawng 084	2				
Yawng 085	2				
Yawng 086	9	11.1	1101		
Yawng 087	9	11.1	1101		

Table 4.3 HLA typing in Khon Yawng (continued.)

DNA number	HLA-A	HLA-A	A*1101	A*1102	A*1103
Yawng 088	9	11.1	1101		
Yawng 089	9	11.1	1101		
Yawng 090	9	11.1	1101		
Yawng 091	9	11.1	1101		
Yawng 092	9	11.1	1101		
Yawng 093	9	11.1	1101		
Yawng 094	9	11.2		1102	
Yawng 095	9	11.2		1102	
Yawng 096	9	11.2		1102	
Yawng 097	9	11.2		1102	
Yawng 098	9				
Yawng 099	9				
Yawng 100	9				
Yawng 101	9				
Yawng 102	9				
Yawng 103	11.1		1101		
Yawng 104	11.1		1101		
Yawng 105	11.1		1101		
Yawng 106	11.1		1101		
Yawng 107	11.1		1101		
Yawng 108	11.1		1101		
Yawng 109	11.1		1101		
Yawng 110	11.1		1101		
Yawng 111	11.1		1101		
Yawng 112	11.1		1101		
Yawng 113	11.1		1101		
Yawng 114	11.1		1101		
Yawng 115	11.1		1101		
Yawng 116	11.1		1101		

Table 4.3 HLA typing in Khon Yawng (continued.)

DNA number	HLA-A	HLA-A	A*1101	A*1102	A*1103
Yawng 117	11.1		1101		
Yawng 118	11.1		1101		
Yawng 119	11.1		1101		
Yawng 120	11.2			1102	
Yawng 121	11.2			1102	
Yawng 122	11.2			1102	
Yawng 123	11.2			1102	
Yawng 124	11.2			1102	
Yawng 125	11.2			1102	

Table 4.4 HLA typing in Karen

DNA number	HLA-A	HLA-A	HLA-A*1101	HLA-A*1102	HLA-A*1103
KAREN 001	2	9			
KAREN 002	2	9			
KAREN 003	2	9			
KAREN 004	2	9			
KAREN 005	2	9			
KAREN 006	2	9			
KAREN 007	2	9			
KAREN 008	2	9			
KAREN 009	2	9			
KAREN 010	2	9			
KAREN 011	2	11.1	1101		
KAREN 012	2	11.1	1101		
KAREN 013	2	11.1	1101		
KAREN 014	2	11.1	1101		
KAREN 015	2	11.1	1101		
KAREN 016	2	11.1	1101		
KAREN 017	2	11.1	1101		
KAREN 018	2	11.1	1101		
KAREN 019	2	11.1	1101		
KAREN 020	2	11.1	1101		
KAREN 021	2	11.1	1101		
KAREN 022	2	11.1	1101		
KAREN 023	2	11.1			1103
KAREN 024	2				
KAREN 025	2				
KAREN 026	2				
KAREN 027	2				
KAREN 028	9	11.1	1101		
KAREN 029	9	11.1	1101		
KAREN 030	9	11.1	1101		
KAREN 031	9	11.1	1101		

Table 4.4 HLA typing in Karen (continued.)

DNA number	HLA-A	HLA-A	HLA-A*1101	HLA-A*1102	HLA-A*1103
KAREN 032	9	11.1	1101		
KAREN 033	9	11.1	1101		
KAREN 034	9	11.1	1101		
KAREN 035	9	11.1	1101		
KAREN 036	9	11.1	1101		
KAREN 037	9	11.1	1101		
KAREN 038	9	11.1	1101		
KAREN 039	9	11.1	1101		
KAREN 040	9	11.1	1101		
KAREN 041	9	11.1	1101		
KAREN 042	9	11.1	1101		
KAREN 043	9	11.1	1101		
KAREN 044	9	11.1	1101		
KAREN 045	9	11.1	1101		
KAREN 046	9	11.1	1101		
KAREN 047	9	11.1	1101		
KAREN 048	9				
KAREN 049	9				
KAREN 050	9				
KAREN 051	9				
KAREN 052	9				
KAREN 053	9				
KAREN 054	9				
KAREN 055	9				
KAREN 056	9				
KAREN 057	9				
KAREN 058	9				
KAREN 059	11.1		1101		1103
KAREN 060	11.1		1101		1103
KAREN 061	11.1		1101		1103
KAREN 062	11.1		1101		1103

Table 4.4 HLA typing in Karen (continued.)

DNA number	HLA-A	HLA-A	HLA-A*1101	HLA-A*1102	HLA-A*1103
KAREN 063	11.1				1103
KAREN 064	11.1		1101		
KAREN 065	11.1		1101		
KAREN 066	11.1		1101		
KAREN 067	11.1		1101		
KAREN 068	11.1		1101		
KAREN 069	11.1		1101		
KAREN 070	11.1		1101		
KAREN 071	11.1		1101		
KAREN 072	11.1		1101		
KAREN 073	11.1		1101		
KAREN 074	11.1		1101		
KAREN 075	11.1		1101		
KAREN 076	11.1		1101		
KAREN 077	11.1		1101		
KAREN 078	11.1		1101		
KAREN 079	11.1		1101		
KAREN 080	11.1		1101		
KAREN 081	11.1		1101		
KAREN 082	11.1		1101		
KAREN 083	11.1		1101		
KAREN 084	11.1		1101		
KAREN 085	11.1		1101		
KAREN 086	11.1		1101		
KAREN 087	11.1		1101		
KAREN 088	11.1		1101		
KAREN 089	11.1		1101		
KAREN 090	11.1		1101		
KAREN 091	11.1		1101		
KAREN 092	11.1		1101		
KAREN 093	11.1		1101		

Table 4.4 HLA typing in Karen (continued.)

DNA number	HLA-A	HLA-A	HLA-A*1101	HLA-A*1102	HLA-A*1103
KAREN 094	11.1		1101		
KAREN 095	11.1		1101		
KAREN 096	11.1		1101		
KAREN 097	11.1		1101		
KAREN 098	11.1		1101		
KAREN 099	11.1		1101		
KAREN 100	11.1		1101		
KAREN 101	11.1		1101		
KAREN 102	11.1		1101		
KAREN 103	11.1		1101		
KAREN 104	11.1		1101		
KAREN 105	11.1		1101		
KAREN 106	11.1		1101		
KAREN 107	11.1		1101		
KAREN 108	11.1		1101		
KAREN 109	11.1		1101		
KAREN 110	11.1		1101		
KAREN 111	11.1		1101		
KAREN 112	11.1		1101		
KAREN 113	11.1		1101		

Table 4.5 HLA-A2, A9, A11.1 and A11.2 gene frequencies (%) in Khon Muang, Khon Yawng and Karen

HLA	Khon Muang (n=153)		Khon Yawng (n=125)		Karen (n=113)	
	Observed	GF (%)	Observed	GF (%)	Observed	GF (%)
A2	86	33.83 ^a	85	43.43 ^c	27	12.76 ^{a, c}
A9	36	12.55 ^a	36	15.62	41	20.18 ^a
A11.1	87	34.32 ^{a, b}	49	22.20 ^{b, c}	88	52.96 ^{a, c}
A11.2	10	3.32 ^a	17	7.05 ^c	0	0 ^{a, c}

Gene frequencies showed a significant ($P < 0.05$) difference between Khon Muang, Khon Yawng and Karen.

a = A2, A9, A11.1, A11.2 in Khon Muang vs Karen

b = A11.1 in Khon Muang vs Khon Yawng

c = A2, A11.1, A11.2 in Khon Yawng vs Karen

Table 4.6 HLA-A2, A9, A11.1 and A11.2 gene frequencies (%) in Khon Muang compared with present-day Thais (PDT), Tai Lue (TL) and Tai Dam (TD)

HLA	Khon Muang (n=153)	Tai-speaking group		
		PDT (n=140) ¹	TL (n=96) ²	TD (n=106) ³
A2	33.83 ^{a, b}	23.00 ^a	42.26	53.42 ^b
A9	12.55 ^a	20.27 ^a	10.44	12.05
A11.1	34.32	25.36	30.00	28.63
A11.2	3.32	3.64	7.58	5.33

Gene frequencies showed a significant ($P < 0.05$) difference between Khon Muang and Tai-speaking group.

a = A2, A9 in Khon Muang vs present day Thais

b = A2 in Khon Muang vs Tai Dam

1 = Data from Chandanayingyong et al., 1997

2 = Data from Chandanayingyong et al., 1992

3 = Data from Chandanayingyong et al., 1999

Table 4.7 HLA-A2, A9, A11.1 and A11.2 gene frequencies (%) in Khon Yawng compared with present-day Thais (PDT), Tai Lue (TL) and Tai Dam (TD)

HLA	Khon Yawng (n=125)	Tai-speaking group		
		PDT (n=140) ¹	TL (n=96) ²	TD (n=106) ³
A2	43.43 ^a	23.00 ^a	42.26	53.42
A9	15.62	20.27	10.44	12.05
A11.1	22.20	25.36	30.00	28.63
A11.2	7.05	3.64	7.58	5.33

Gene frequencies showed a significant ($P < 0.05$) difference between Khon Yawng and Tai-speaking group.

a = A2 in Khon Yawng vs PDT

¹ Chandanayingyong et al., 1997

² Chandanayingyong et al., 1992

³ Chandanayingyong et al., 1999

Table 4.8 HLA-A2, A9, A11.1 and A11.2 gene frequencies (%) in Karen compared with present-day Thais (PDT), Tai Lue (TL) and Tai Dam (TD)

HLA	Karen (n=113)	Tai-speaking group		
		PDT (n=140) ¹	TL (n=96) ²	TD (n=106) ³
A2	12.76 ^{a, b, c}	23.00 ^a	42.26 ^b	53.42 ^c
A9	20.18 ^{b, c}	20.27	10.44 ^b	12.05 ^c
A11.1	52.96 ^{a, b, c}	25.36 ^a	30.00 ^b	28.63 ^c
A11.2	0 ^{a, b, c}	3.64 ^a	7.58 ^b	5.33 ^c

Gene frequencies showed a significant ($P < 0.05$) difference between Karen and Tai-speaking group.

a = A2, A11.1, A11.2 in Karen vs PDT

b = A2, A9, A11.1, A11.2 in Karen vs TL

c = A2, A9, A11.1, A11.2 in Karen vs TD

¹ Chandanayingyong et al., 1997

² Chandanayingyong et al., 1992

³ Chandanayingyong et al., 1999

Table 4.9 HLA-A2, A9, and A11.1 gene frequencies (%) in Khon Muang compared with non-Thai SE Asian populations studied in 11HWS

HLA	Khon Muang (n=153)	Mainland SE Asian populations (11HWS) ¹				
		S.Han (n=138)	Buyi (n=69)	Miao (n=70)	Viet (n=149)	Sing.C (n=73)
A2	33.83 ^{b,d}	33.7	42.8 ^b	30.7	25.9	37.0 ^d
A9	12.55 ^a	19.9 ^a	16.7	16.4	13.5	16.4
A11.1	34.32 ^c	31.9	29.7	42.1 ^c	26.3	26.0
A11.2	3.32	ND	ND	ND	ND	ND

Gene frequencies showed a significant ($P < 0.05$) difference between Khon Muang and non-Thai mainland SE Asian populations.

a = A9 in Khon Muang vs S.Han

b = A2 in Khon Muang vs Buyi

c = A11.1 in Khon Muang vs Miao

d = A2 in Khon Muang vs Sing.C

¹ Non-Thai mainland SE Asian populations studied during the 11HWS were the southern Han (S.Han), Buyi, Miao (or Hmong), Vietnamese (Viet), and Singapore Chinese (Sing.C) (Imanishi et al., 1992).

Table 4.10 HLA-A2, A9, and A11.1 gene frequencies (%) in Khon Yawng compared with non-Thai SE Asian populations studied in 11HWS

HLA	Khon Yawng (n=125)	Mainland SE Asian populations (11HWS) ¹				
		S.Han (n=138)	Buyi (n=69)	Miao (n=70)	Viet (n=149)	Sing.C (n=73)
A2	43.43 ^{b,d}	33.7	42.8 ^b	30.7	25.9 ^d	37.0
A9	15.62	19.9	16.7	16.4	13.5	16.4
A11.1	22.20 ^{a,b,c,d}	31.9 ^a	29.7 ^b	42.1 ^c	26.3 ^d	26.0
A11.2	7.05	ND	ND	ND	ND	ND

Gene frequencies showed a significant ($P < 0.05$) difference between Khon Yawng and non-Thai mainland SE Asian populations.

A = A11.1 in Khon Yawng vs S.Han

B = A2, A11.1 in Khon Yawng vs Buyi

C = A11.1 in Khon Yawng vs Miao

D = A2, A9 in Khon Yawng vs Viet.

¹ Non-Thai mainland SE Asian populations studied during the 11HWS were the southern Han (S.Han), Buyi, Miao (or Hmong), Vietnamese (Viet), and Singapore Chinese (Sing.C) (Imanishi et al., 1992).

Table 4.11 HLA-A2, A9, and A11.1 gene frequencies (%) in Karen compared with non-Thai SE Asian populations studied in 11HWS

HLA	Karen (n=113)	Mainland SE Asian populations (11HWS) ¹				
		S.Han (n=138)	Buyi (n=69)	Miao (n=70)	Viet (n=149)	Sing.C (n=73)
A2	12.76 ^{a, b, c, d, e}	33.7 ^a	42.8 ^b	30.7 ^c	25.9 ^d	37.0 ^e
A9	20.18	19.9	16.7	16.4	13.5	16.4
A11.1	52.96 ^{a, b, d, e}	31.9 ^a	29.7 ^b	42.1	26.3 ^d	26.0 ^e
A11.2	0	ND	ND	ND	ND	ND

Gene frequencies showed a significant ($P < 0.05$) difference between Karen and mainland SE Asian populations.

a = A2, A9 in Karen vs S.Han

b = A2, A9 in Karen vs Buyi

c = A2 in Karen vs Miao

d = A2, A9 in Karen vs Viet

e = A2, A9 in Karen vs Sing.C

¹ Non-Thai mainland SE Asian populations studied during the 11HWS were the southern Han (S.Han), Buyi, Miao (or Hmong), Vietnamese (Viet), and Singapore Chinese (Sing.C) (Imanishi et al., 1992).

Table 4.12 HLA-A2, A9, and A11 gene frequencies (%) in Khon Muang compared with northern Thais (NT), northeastern Thais (NET), southern Thai-Muslims (STM), and Thais

HLA	Khon Muang (n=153)	Thais in different parts of Thailand			
		NT ¹ (n=146)	NET ² (n=100)	STM ³ (n=102)	Thais ⁴ (n=137)
A2	33.83 ^{b,c}	36.43	30.00	19.56 ^b	18.9 ^c
A9	12.55 ^{a,b,c}	15.60	24.50 ^a	26.57 ^b	19.4 ^c
A11	39.50 ^{a,b,c}	40.90	25.84 ^a	20.17 ^b	24.5 ^c

Gene frequencies showed a significance ($P < 0.05$) difference between Khon Muang and Thais in different parts of Thailand.

a = A9, A11 in Khon Muang vs NET

b = A2, A9, A11 in Khon Muang vs STM

c = A2, A9, A11 in Khon Muang vs Thais

¹ Fongsatikul et al., 1997

² Romphruk et al., 1997

³ Chiewsilp et al., 1997

⁴ Chandanayingyong et al., 1992

Table 4.13 HLA-A2, A9, and A11 gene frequencies (%) in Khon Yawng compared with northern Thais (NT), northeastern Thais (NET), southern Thai-Muslims (STM), and Thais

HLA	Khon Yawng (n=125)	Thais in different parts of Thailand			
		NT (n=146)	NET (n=100)	STM (n=102)	Thais (n=137)
A2	43.43 ^{a, b, c}	36.43	30.00 ^a	19.56 ^b	18.9 ^c
A9	15.62 ^{a, b}	15.60	24.50 ^a	26.57 ^b	19.4
A11	31.30 ^b	40.90	25.84	20.17 ^b	24.5

Gene frequencies showed a significant ($P < 0.05$) difference between Khon Yawng and Thais in different parts of Thailand.

a = A2, A9 in Khon Yawng vs NET

b = A2, A9, A11 in Khon Yawng vs STM

c = A2 in Khon Yawng vs Thais

¹ Fongsatikul et al., 1997

² Romphruk et al., 1997

³ Chiewsilp et al., 1997

⁴ Chandanayingyong et al., 1992

Table 4.14 HLA-A2, A9, and A11 gene frequencies (%) in Karen compared with northern Thais (NT), northeastern Thais (NET), southern Thai-Muslims (STM), and Thais

HLA	Karen (n=113)	Thais in different parts of Thailand			
		NT ¹ (n=146)	NET ² (n=100)	STM ³ (n=102)	Thais ⁴ (n=137)
A2	12.76 ^{a, b}	36.43 ^a	30.00 ^b	19.56	18.9
A9	20.18	15.60	24.50	26.57	19.4
A11	52.96 ^{a, b, c, d}	40.90 ^a	25.84 ^b	20.17 ^c	24.5 ^d

Gene frequencies showed a significant ($P < 0.05$) difference between Karen and Thais in different parts of Thailand.

a = A2, A11 in Karen vs NT

b = A2, A11 in Karen vs NET

c = A11 in Karen vs STM

d = A11 in Karen vs Thais

¹ Fongsatikul et al., 1997

² Romphruk et al., 1997

³ Chiewsilp et al., 1997

⁴ Chandanayingyong et al., 1992

Table 4.15 Allele frequencies (AF) of HLA-A11 subtypes in 3 ethnic groups of northern Thai population

Allele	Khon Muang (n=153)		Khon Yawng (n=125)		Karen (n=113)	
	Observed	AF (%)	Observed	AF (%)	Observed	AF (%)
A*1101	87	28.43 ^a	49	19.6 ^b	86	38.05 ^{a, b}
A*1102	10	3.27	17	6.80 ^c	0	0 ^c
A*1103	0	0 ^a	0	0 ^c	6	2.65 ^c
A*1104	0	0	0	0	0	0
A*1105	0	0	0	0	0	0

Allele frequencies showed a significant ($P < 0.05$) difference between Khon Muang, Khon Yawng and Karen.

a = A*1101, A*1103 in Khon Muang vs Karen

b = A*1101 in Khon Yawng vs Karen

c = A*1102, A*1103 in Khon Yawng vs Karen



Figure 4.1 The PCR product of HLA-A*1101 sample shows the specific band of 554 bp, 522 bp and 300 bp on lane 2, 3 and 7, respectively. The amplification control primers giving rise to a 796 bp fragment were included.

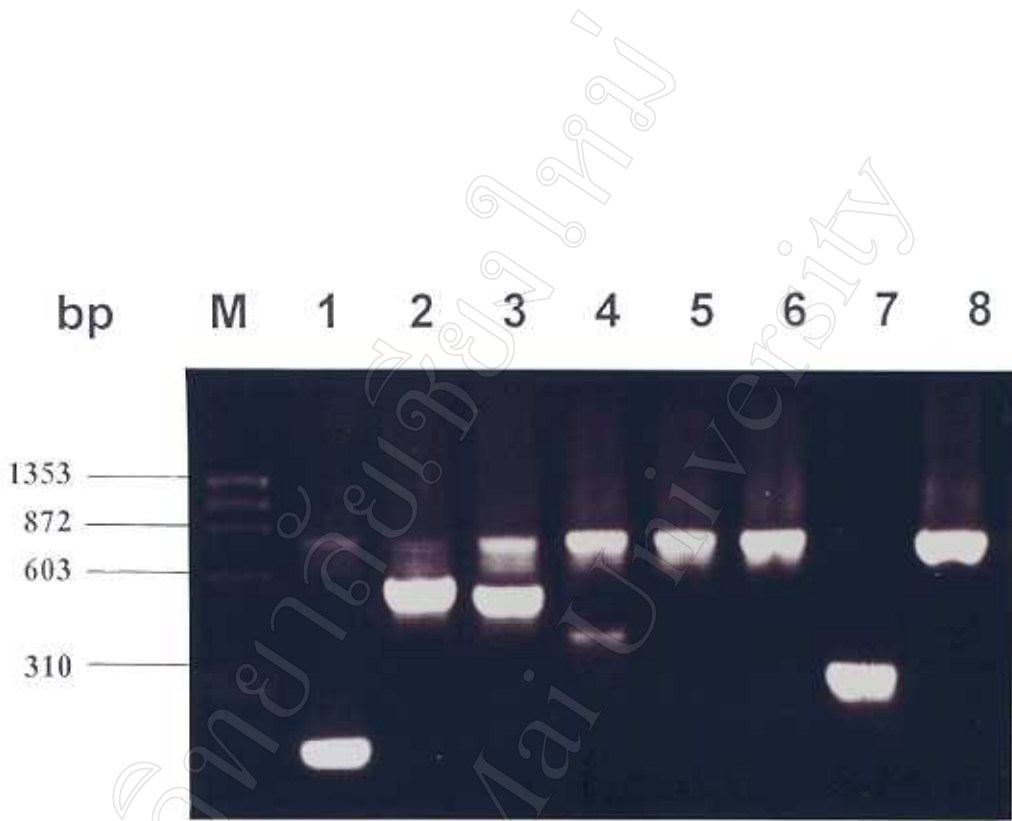


Figure 4.2 The PCR product of HLA-A*1102 sample shows the specific band of 170 bp, 554 bp, 522 bp and 300 bp on lane 1, 2, 3 and 7, respectively. The amplification control primers giving rise to a 796 bp fragment were included.

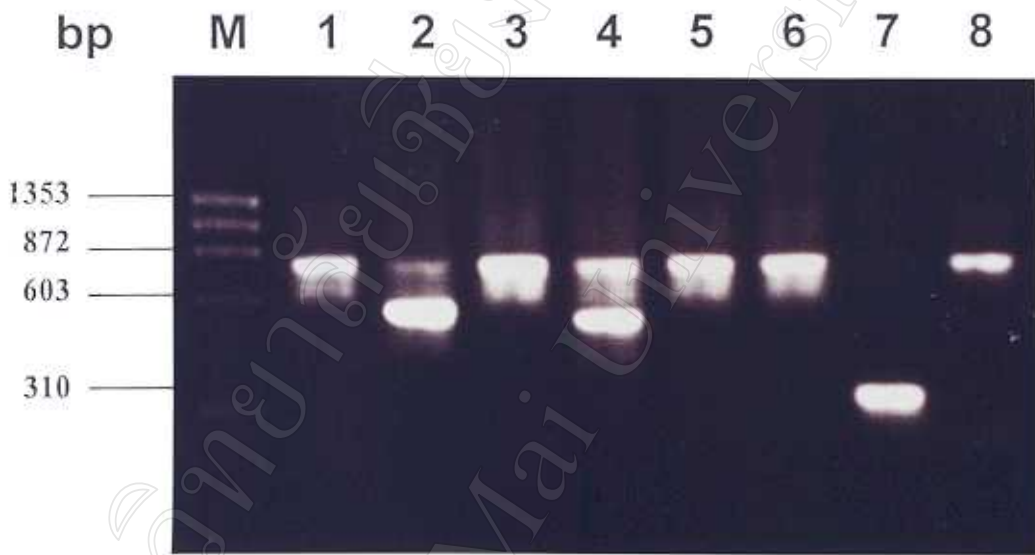


Figure 4.3 The PCR product of HLA-A*1103 sample shows the specific band of 554 bp, 522 bp and 300 bp on lane 2, 4 and 7, respectively. The amplification control primers giving rise to a 796 bp fragment were included.

Table 4.16 Serological reaction patterns of HLA-A*1101 and A*1103 in Karen family 1 and 2

Serum No.	Antibody specificity	Karen family 1*			Karen family 2*					
		K62 A*1101 A*1103	K151 A*1101 A*1103	K154 A*1103	K141 A*1101	K142 A*1101 A*1103	K155 A*1101 A*1103	K140 A*1103	K23 A*1103	
NC	Negative	1	1	1	1	1	1	1	1	
PC	Positive	8	8	8	8	8	8	8	8	
TP.4550.39	A11.1	8	8	8	8	8	8	6	6	
TP.3198.40	A11.1	8	8	8	8	8	8	8	8	
TP.45.43	A11.1	8	8	8	8	8	8	8	8	
S.1594.34	A11.2	1	1	1	1	1	1	1	1	
S.1759.3	A11.2	1	1	1	1	1	1	1	1	
TP11613.33	A11.2	1	1	1	1	1	1	1	1	
TP.7834.2	A11.1,11.2	8	8	8	8	8	8	8	8	
TP11287.33	A11.1,11.2	8	8	8	8	8	8	8	8	
TP2839.34	A11,10	8	8	8	8	8	8	8	8	
TP2456.35	A11.1,11.2	8	8	1	8	8	8	1	1	
TP1433.42	A11	8	8	1	8	8	8	2	2	

*These samples were typed by DNA typing and direct sequencing

Table 4.17 Molecular typing of HLA-A, -B in Karen family

Karen	Code	Status	A*	A*	B*	B*
Family 23	Karen 140	Grand father	0203	1103	1301	51021
	Karen 142	Daughter	11011	1103	1525	51021
	Karen 23	Niece	0203	1103	1301	51021
	Karen 155	Nephew	11011	1103	1525	51021
Family 62	Karen 62	Mother	11011	1103	52011	1502
	Karen 151	Daughter	11011	1103	52011	1502
	Karen 154	Daughter	0203	1103	52011	40011/40012