Genetic analysis of HLA-typing in Chinese patients with idiopathic thrombocytopenic purpura

We investigated the frequencies of HLA alleles in 40 Hong Kong Chinese with idiopathic thrombocytopenic purpura (ITP). The frequencies were similar to those in the local population and were not related to the response to steroids. ITP may be distinct from other autoimmune diseases which are associated with specific HLA phenotypes.

Adult chronic idiopathic thrombocytopenic purpura (ITP) is a common hematologic disease worldwide. The pathogenesis involves formation of auto-antibodies against platelet glycoproteins. The mechanism of autoimmunity is not fully understood but may involve binding of antigenic peptides to HLA antigens.[1] Therefore, specific HLA alleles may be associated with the occurrence of ITP. However, this association was not found in Caucasians.[2-4] Studies in Chinese are not available and it is important to see whether results from the West can be extrapolated to this population. We investigated the frequencies of HLA alleles in Hong Kong Chinese patients with ITP with reference to the database from volunteer bone marrow donors registered with the Hong Kong Marrow Match Foundation (HKMMF).[5] The latter reflects the local prevalence of HLA alleles in Hong Kong, in which over 98% of the population are ethnic Chinese and the majority have ancestral origins in the Guangdong Province in southern China. No donors were selected based on gender or the presence of specific HLA alleles.

Forty Chinese patients with ITP in a general hematology clinic were recruited. The diagnosis was based on thrombocytopenia with normal or increased megakaryocytopoiesis in the bone marrow. Patients with pre-existing systemic lupus erythematosus (SLE), concomitant chronic liver diseases with or without splenomegaly or intake of drugs known to be associated with thrombocytopenia were excluded from the analysis. Class I HLA typing (HLA-A and –B) was performed using standard serological techniques employing antisera standardized for use in a Chinese population. Class II typing was performed using polymerase chain reaction (PCR) with sequence specific primers as described previously.[5] Twenty-two primer mixes were used to identify 19 HLA-DR alleles. Based on the DR alleles identified by the first set of primers, further DRB1 allelic sub-typing was performed using different primer mixes for various alleles. The first-line treatment of ITP was prednisolone (1 mg/kg/day). Complete remission (CR) was defined as a rise of platelet count to greater than 100×10^9/L for at least 6 months (with or without treatment). Partial remission (PR) was defined as a platelet count of $50\text{-}100\times10^9$/L or platelet count greater than 100×10^9/L for less than 6 months. Refractory disease (non-remission, NR) was defined as the absence of a platelet count increase or a platelet count of less than $50\text{x}10^9$/L during treatment. The response to steroids was assessable in 31 patients. Comparisons of the frequencies of HLA alleles were performed using the Chi-squared test. A p value of less than 0.05 was considered statistically significant.

The characteristics of the patients are shown in Table 1. The female to male ratio was 3:1. The median age and platelet count at presentation were 40 (range: 20-82) years and 18×10^9/L (range: $1\text{-}78\times10^9$/L), respectively. The median duration of disease was 85 months (range: 12-469 months). Table 2a shows the frequencies of HLA-A, -B and -DR alleles. HLA-A11, A2, A24, B46, B13, B60, DRB1-15, DRB-0901, and DRB1-04 were the most prevalent alleles in patients with ITP. However, none of these alleles showed a significant difference in frequency when compared with controls (χ^2 test, $p > 0.05$, Table 2a). There was also no significant difference when the HLA types of patients with partial or no response to steroids were compared with those in patients who exhibited complete remission (Table 2b).

Table 1. The clinical characteristics and HLA typing of the patients.

Pt	Sex/Age	Plt*	A1	A2	B1	B2	DRB1a	DRB1b	Response°
1	F/41	24	2		35	38	08	11	-
2	M/29	35	2		60	62	12	08	-
3	F/20	15	29	33	7	44	16	10	PR
4	M/48	19	2	1101	62	54	15	14	PR
5	F/44	39	24	1101	13	61	15	0901	-
6	M/40	3	2	1101	55	46	15	04	CR
7	F/32	42	1101		75		04	12	CR
8	F/45	25	2	1101	13	61	15	04	PR
9	F/22	1	1101	31	60	61	04	0901	PR
10	F/31	6	1101		13	15	0901	51	CR
11	M/33	6	2	1102	13	46	12		PR
12	F/32	3	24	1101	13	38	15	12	CR
13	F/61	7	2	34	56	46	04	0901	PR
14	F/56	40	2	1101	46		0901		-
15	F/62	58	1101		38	60	15	0901	-
16	F/37	-	24	1102	48	46	0901		-
17	F/-	-	2		38	46	16	04	PR
18	F/82	54	2	1101	18	46	16	08	-
19	M/22	7	30	31	13	35	07	08	PR
20	M/38	3	24	33	5801	60	0901	13	NR
21	F/-	-	2	24	46		0901		PR
22	F/63	45	24		35	38	16	11	-
23	F/55	78	2	33	5801	60	15		CR
24	F/38	7	2	1101	13	46	15	16	PR
25	F/38	16	24		61	51	15	0901	PR
26	F/57	40	1101	30	13	71	04	0701	CR
27	M/63	13	2		13	46	15	0901	PR
28	F/51	62	24		13		15		NR
29	M/28	2	2	1101	60	46	08	0901	CR
30	F/25	8	33		5801		03	1302	PR
31	M/32	32	24	1101	75	60	15	04	PR
32	F/35	13	1101	33	75	44	16	11	CR
33	F/67	27	1101	1102	39	60	16	04	NR
34	M/48	14	1	24	35	27	15	14	CR
35	F/79	2	1101		13		15	14	CR
36	F/36	78	24	1101	60	46	04	0901	CR
37	F/27	6	2	29	7	46	0901	1001	CR
38	F/64	50	1101		13	38	15	0901	CR
39	F/66	-	2		38	39	15	08	-
40	F/81	51	1101	1102	60		15	11	NR

denotes the platelet count at presentation ($\times10^9$/L) and °denotes the response to steroids as defined in the text. – : clinical data not available. A blank in the HLA typing indicates homozygosities. NR: non-remission; PR: partial remission; CR: complete remission.

A recent study in Japanese patients showed that DRB1*0410 was more prevalent in ITP patients than in controls and was associated with a poor response to steroids, suggesting that genetic factors were involved in the clinical course of ITP.[6] DRB1*0410 is uncommon in Hong Kong Chinese with a phenotypic frequency of only 0.4%[5] and none of the 40 patients in this cohort possessed this allele. ITP in Hong Kong Chinese was not associated with specific HLA alleles and no specific alleles could predict the response to steroid therapy. In this respect, ITP may be pathogenetically distinct from other autoimmune diseases which are often associated with specific HLA phenotypes. Alternatively, HLA alleles may be associated with a common but currently unidentified factor that predisposes to ITP. This remains speculative and our results should be confirmed with a larger cohort of patients.

Table 2a. Prevalence of HLA alleles in 40 Hong Kong Chinese with ITP. There was no significant difference in HLA phenotype frequencies between patients with ITP and those in healthy volunteers (*) registered with the Hong Kong Marrow Match Foundation (HKMMF)5 (χ^2 test, $p > 0.05$). Only nine HLA-A, 20 HLA-B and 12 HLA-A alleles were shown and these are the commonest alleles reported in the Hong Kong population. Other HLA alleles were not present in this cohort of patients and they occur at low frequency in the local population.

HLA-allele	Phenotype frequency No.of patient (fraction)	Phenotype frequency* (N=18,774)
A11	23 (0.58)	0.56
A2	17 (0.43)	0.53
A24	11 (0.27)	0.27
A33	5 (0.13)	0.19
A31	2 (0.05)	0.03
A29	2 (0.05)	0.02
A30	2 (0.05)	0.03
A34	1 (0.03)	0.0012
A1	1 (0.03)	0.02
B46	13 (0.33)	0.27
B13	12 (0.30)	0.18
B60	10 (0.25)	0.28
B38	7 (0.18)	0.10
B61	4 (0.10)	0.05
B35	4 (0.10)	0.06
B75	3 (0.08)	0.19
B58	3 (0.08)	0.13
B7	2 (0.05)	0.03
B44	2 (0.05)	0.03
B62	2 (0.05)	0.09
B39	2 (0.05)	0.03
B51	1 (0.03)	0.09
B54	1 (0.03)	0.04
B56	1 (0.03)	0.02
B27	1 (0.03)	0.04
B55	1 (0.03)	0.04
B71	1 (0.03)	0
B18	1 (0.03)	0.08
B48	1 (0.03)	0.03 (N=250)
DRB15	18 (0.45)	0.24
DRB09	15 (0.38)	0.29
DRB04	10 (0.25)	0.16
DRB16	7 (0.18)	0.12
DRB08	6 (0.15)	0.09
DRB12	4 (0.10)	0.03
DRB14	4 (0.10)	0.14
DRB11	4 (0.10)	0.09
DRB07	1 (0.03)	0.10
DRB10	1 (0.03)	0.04
DRB13	1 (0.03)	0.02
DRB03	1 (0.03)	0.17

Table 2b. Prevalence of HLA alleles in 31 Hong Kong Chinese with ITP according to their responses to steroid therapy. There was no significant difference in HLA frequencies among patients who attained complete remission (CR) and those who had partial remission (PR) or non-remission (NR) (χ^2 test, $p > 0.05$).

HLA-typing	PR/NR to steroid No. of patients	CR to steroid No. of patients
A11	8	10
A2	8	4
A24	5	3
A33	3	2
B13	6	5
B46	6	4
B60	5	3
B75	1	2
B7	1	1
B35	1	1
B44	1	1
B58	2	1
B27	0	1
B38	1	2
B55	0	1
B71	0	1
B61	3	0
B51	1	0
B54	1	0
B56	1	0
B62	1	0
DRB15	8	7
DRB9	6	5
DRB04	6	4
DRB12	1	2
DRB14	1	2
DRB16	4	1
DRB07	1	0
DRB08	1	1
DRB10	1	1
DRB11	1	1
DRB03	1	0
DRB13	2	0

In conclusion, the present study showed that ITP in Hong Kong Chinese was not associated with specific HLA phenotypes and that there was no association between HLA-typing and the response to steroids in this population.

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