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ORIGINAL ARTICLE

Clinical features and major histocompatibility complex genes as potential susceptibility factors in pediatric immune thrombocytopenia

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Background/Purpose: Immune thrombocytopenia (ITP) is a heterogeneous autoimmune disorder with diverse response rates to treatments that include corticosteroids, intravenous immunoglobulins (IVIG), and splenectomy. The predisposing causes of this autoimmune disorder, one of which is immunogenetic susceptibility, have not been fully determined. We investigated whether clinical features and human leukocyte antigen (HLA) genotypes influence the occurrence, treatment response, and disease duration of childhood ITP in Taiwan.
Methods: We performed HLA genotyping of 70 Taiwanese children with ITP and of 70 healthy controls and compared the data. Demographic data were also collected and evaluated.
Results: The frequencies of heterozygous HLA-A11 and the HLA-Cw1 allele were both significantly decreased in the ITP group ($p = 0.0160$ and $p = 0.0089$, respectively), whereas the frequency of heterozygous HLA-DQ5 was significantly increased in the ITP group ($p = 0.0057$). Patients with HLA-DRB1*11 or -DRB1*15 were more likely to respond poorly to corticosteroids than IVIG ($p = 0.0446$ and $p = 0.0008$, respectively). In addition, we observed a positive association between HLA-A11 homozygosity and the development of persistent or chronic ITP [odds ratio (OR) = 6.3165, $p = 0.0479$]. The presence of HLA-DRB1*08 was, however, negatively correlated with the development of persistent or chronic ITP

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(OR = 0.1729, $p = 0.0657$). Children with antecedent of preceding illness (API) and with a younger age of onset were more likely to experience a better treatment response and shorter course of ITP.
Conclusion: We suggest that API, age of onset, and particular HLA class I and class II alleles, may be involved in and influence the occurrence and disease duration of childhood ITP, as well as responses to different therapeutic approaches.

Introduction

Immune thrombocytopenia (ITP) is a heterogeneous disease characterized by increased platelet destruction and thrombocytopenia. Most researchers believe that this platelet destruction is immune mediated and may also involve the inhibition of platelet release by megakaryocytes. Autoantibodies against platelet antigens are the diagnostic hallmark of ITP; however, this mechanism may not account for all cases, as antiplatelet autoantibodies are detected in only 50%–70% of patients with ITP.[1–4] Failure to detect autoantibodies could be due to limited test sensitivity, undetected antigens, or additional mechanisms of platelet loss. Other contributing mechanisms for ITP have been proposed, such as complement-mediated lysis,[5] tolerance checkpoint defects, ineffective thrombopoiesis, direct T-cell cytotoxicity, and a combination of genetic susceptibility and environmental factors.[3]

The functional role of human leukocyte antigen (HLA), which is the human form of the major histocompatibility complex (MHC), is to present antigens to the immune system, and the genetic diversity of the HLA is postulated to have arisen as a host strategy to counter the antigenic diversity of infectious organisms. To identify potential susceptibility factors for ITP, several groups have focused on the identification of MHC genes; however, findings from these HLA association studies in ITP have produced inconsistent results. Nomura and colleagues[6] examined the clinical significance of HLA class II genes in more than 100 Japanese patients with ITP and showed that the frequency of the DRB1*0410 allele was significantly increased in patients with ITP compared with controls. Moreover, this allele was found significantly less frequently in patients who showed a good response to prednisolone. Stanworth and others[7] reported an association between the presence of HLA-A2 and ITP, particularly in female patients, with HLA-A2 also present at increased frequency in patients with chronic ITP that progressed to requiring splenectomy. Another study revealed strong associations between anti-glycoprotein autoantibodies and HLA class II alleles: antibodies against GPIIb-IIIa were associated with DRB1*0405 and DQB1*0401, and antibodies against GPIb-IX were associated with DRB1*0803 and DQB1*0601.[8] When factors influencing therapeutic responses to splenectomy were examined, a poor response was correlated with the presence of DRB1*0405, DQB1*0401, and anti–GPIIb-IIIa. Furthermore, some groups have reported weak associations, such as an increased frequency of HLA-Bw56 and HLA-DR2, in patients with ITP.[9,10]

Based on these previous studies and two additional aspects of ITP, we hypothesized that the HLA class I genes (in addition to the HLA class II genes) may play critical roles in childhood ITP. First, the platelet surface expresses HLA class I molecules (rather than HLA class II molecules), but platelet interactions with cytotoxic T cells, natural killer (NK) cells, and killer cell immunoglobulin-like receptors (KIRs) in patients with ITP are not well understood. Second, it is commonly accepted that childhood ITP is associated with infection, either viral or bacterial.[11] Thus, to investigate whether HLA classes I and II genotypes play a critical role in childhood ITP in Taiwan, we first identified demographic factors, laboratory parameters, and other clinical characteristics that may be linked to the course and treatment responses of childhood ITP. We performed HLA class I (HLA-A, -B, and -C) and class II (HLA-DQ and -DR) genotyping to explore the relationship between HLA susceptibility genes and the occurrence, treatment responses, and disease duration of childhood ITP in Taiwan.

Materials and methods

Participants

Patients were identified following a retrospective review of medical records at National Taiwan University Hospital and Shin Kong Wu Ho-Su Memorial Hospital from January 2007 to December 2009. For this study, 70 patients with ITP were enrolled who had attended pediatric hematology clinics, visited emergency departments, or been referred from other hospitals. For the control group, blood was collected from January 2007 to December 2009 from 70 healthy children (37 boys and 33 girls; mean age, 4.3 ± 3.8 years) who were in our vaccine study or were brought to our pediatric hematology clinic for blood typing. These children had no underlying diseases, and a hemogram showed no evidence of thrombocytopenia.

The diagnosis of primary ITP was established on the basis of history, physical examination, and a laboratory result showing isolated thrombocytopenia (peripheral blood platelet count $<100{,}000/mm^3$) in the absence of other disorders that may be associated with thrombocytopenia.[12] If a patient had atypical features or was not responding to therapy, a bone marrow aspirate was performed to exclude other diseases that result in thrombocytopenia. The age and season of onset, sex, antecedent of preceding illness (API), initial platelet count at diagnosis, treatment choice and response, and disease duration of the patients were also evaluated. API was defined as the occurrence of viral infection or immunization within 4 weeks prior to presenting with bleeding manifestations.[13] This study was approved by the Institutional Review Board of National Taiwan University Hospital and Shin Kong Wu Ho-Su Memorial Hospital. Informed written consent was obtained from the parents of each participant.

Treatments, treatment responses, and disease duration

Corticosteroids (predominantly prednisolone) and intravenous immunoglobulins (IVIGs) were the major medical treatments in our study patients. Splenectomy was indicated for severe acute ITP with acute life-threatening hemorrhage or for older children ($\geq$ 4 years) with severe ITP that had lasted $>$1 year (chronic ITP) and whose symptoms were not easily controlled with medical therapy. Prednisolone was administered at a dose of 2 mg/kg/day (maximum 60 mg/day) for 21–28 days or until an increase in the platelet count to $>$20,000/mm^3 was achieved. IVIG was administered at a dose of 1 g/kg/day for 2 days. Each patient was assessed for his or her response to treatment. The timing of monitoring of platelet counts and response assessment was determined by the expected kinetics of platelet increase after each treatment.[12] Therefore, treatment response was monitored on Day 28 of corticosteroid treatment and on Day 3 of IVIG administration. The duration of follow-up was $\geq$ 12 months from diagnosis. A good response was defined as an increase in the platelet count of $\geq$50,000/mm^3. A poor response was defined as an increase in the platelet count of $<$50,000/mm^3. Based on the International Working Group consensus definitions for disease duration, acute ITP (i.e., newly diagnosed ITP) indicates that the platelet count returned to normal ($>$150,000/mm^3) within 3 months from diagnosis. Persistent ITP refers to patients who did not achieve spontaneous remission or did not maintain their response after stopping treatment between 3 and 12 months from diagnosis. Chronic ITP is reserved for patients with ITP lasting for more than 12 months.[12]

HLA DNA typing with PCR and sequence-specific oligonucleotides

HLA DNA typing was performed with Lifecodes HLA-SSO (sequence-specific oligonucleotides) typing kits (Tepnel, Stamford, CT, USA). Genomic DNA from patients and controls was isolated at a concentration of 10–200 ng/μl. DNA was amplified with Taq polymerase and biotinylated primers supplied with the kit. The reaction mixture was subjected to eight cycles of denaturation for 30 seconds at 95°C, annealing for 45 seconds at 60°C, and extension for 45 seconds at 72°C. Another 32 cycles were performed that consisted of denaturation for 30 seconds at 95°C, annealing for 45 seconds at 63°C, and extension for 45 seconds at 72°C. After amplification, the amplified DNAs were subjected to the following incubation conditions for hybridization: 97°C for 5 minutes, 47°C for 30 minutes, and 56°C for 10 minutes. During hybridization, streptavidin-phycoerythrin was added for labeling. The HLA DNA was then typed and analyzed with a Luminex instrument (Luminex Corporation, Austin, TX, USA).

Statistical analysis

Statistical analyses were performed using SAS software, version 9.1.3 (SAS Institute Inc., Cary, NC, USA). Associations between categorical variables were analyzed with a chi-square test or Fisher's exact test, whereas the means of continuous variables were compared with a two-sample *t*-test or a Wilcoxon rank-sum test.

To analysis the HLA genotype distributions in control and ITP groups and to investigate the predictive values of age of onset, gender, season of onset, API, the initial platelet count, and HLA genotype on the treatment responses and disease duration for patients with ITP, we conducted regression analyses. First, we carried out a logistic regression analysis with only the first episode for each patient. Then we fitted a marginal logistic regression model using the generalized estimating equations (GEE) method for all the observed episodes for each patient. The GEE method helped us obtain consistent estimates of the standard errors for the estimated regression coefficients to make consistent inferences about the regression coefficients from the correlated data based on the correct *p* values. In the GEE analysis, if the first-order autocorrelation structure fits the repeated measures data well, the model-based estimates of the standard errors for the estimated regression coefficients are used. Otherwise, the empirical standard error estimates are reported.

The goal of regression analysis is to find one or a few parsimonious regression models that fit the observed data well. To ensure the quality of the analysis results, basic model-fitting techniques for (1) variable selection, (2) goodness-of-fit (GOF) assessment, and (3) regression diagnostics were used in our regression analyses. Specifically, the stepwise variable selection procedure (with iterations between the forward and backward steps) was applied to obtain a candidate final regression model. All the relevant univariate co-variates (both significant and nonsignificant ones) were initially included on the variable list, and the significance levels for entry and for stay were set to 0.15 or larger. Then, with the aid of substantive knowledge, the best final regression model was identified manually by reducing the significance levels to 0.05. Any discrepancy between the results of the univariate analysis and multivariate analysis was likely due to confounding effects of uncontrolled co-variates in the univariate analysis. Both the GOF measures (including the percentage of concordant pairs, estimated area under the receiver operating characteristic curve, and adjusted generalized R^2) and the GOF tests (including the deviance GOF test, Pearson chi-square GOF test, and Hosmer-Lemeshow GOF test) were examined to assess the GOF of the fitted logistic regression model. Because the value of the adjusted generalized R^2 for the logistic regression model is usually low, the larger *p* values of the deviance GOF test, Pearson chi-square GOF test, and Hosmer-Lemeshow GOF test indicate better fits. In addition, the statistical tools for regression diagnostics such as residual analysis, detection of influential cases, and a check for multicollinearity were used to discover model or data problems.

Results

Demographic and clinical characteristics of pediatric patients with ITP

Among the 70 patients with ITP who were included in this study, 33 were males and 37 were females. The mean age at onset was 6.0 $\pm$ 3.9 years, with 23 patients (32.9%) who

were ≥ 8 years of age at the onset of ITP. There was no apparent proclivity of season of onset for the occurrence of ITP (December–February, 25.7%; March–May, 22.9%; June–August, 24.3%; and September–November, 27.1%). A total of 42 patients (60%) had a history of API. The mean platelet count at diagnosis was 14,266 ± 16,156/mm^3, with 54 patients (77%) whose initial platelet count was < 20,000/mm^3. A total of 67 patients (95.7%) had received various treatments, predominantly corticosteroids (47 cases, 67.1%) or IVIG (43 cases, 61.4%). Thirty patients (42.3%) had received two or more types of treatment (data not shown). Four patients (5.7%) had undergone a splenectomy for the treatment of ITP (Table 1). In addition, 18 of the 47 patients (38%) who had ever received corticosteroids showed a good response, and 20 of the 43 patients (47%) who had ever received IVIG showed a good response. After a follow-up period of at least 12 months from diagnosis, 32 patients had acute ITP (45.7%), eight patients had persistent ITP (11.4%), and 30 patients had chronic ITP (42.9%).

Analysis of HLA genotype distributions in control and ITP groups

We analyzed the differential distribution of HLA class I and II genotypes between the ITP patient population and the control population (Table 2). Multivariate analysis showed that the frequency of heterozygous HLA-A11 was significantly decreased in the ITP group [odds ratio (OR) = 0.396, $p = 0.0160$] compared with the control group (Table 3). The presence of the HLA-Cw1 allele was also decreased in the ITP group (OR = 0.348, $p = 0.0089$). By contrast, the frequency of heterozygous HLA-DQ5 was significantly increased in the ITP group (OR = 3.838, $p = 0.0057$). None of the other alleles were differentially distributed between the control and ITP groups.

Analysis of demographic and clinical parameters and HLA genotypes with respect to ITP treatment responses

When analyzing demographic and clinical parameters among patients with ITP, we found that individuals with an older age of onset were at higher risk for a poor response to either or both treatments ($p < 0.001$). These results are shown in Table 4. When choosing the cut-off age at 8 years, we found that patients whose age of onset was ≥ 8 years were at higher risk for a poor response to either or both treatments ($p = 0.012$). Patients with API were more likely to show a good response to either or both treatments ($p = 0.004$). When analyzing demographic and clinical

Table 1 Demographic and clinical characteristics of the control individuals and patients with ITP.

Characteristics	Control	ITP	*p* value[a]
Number of patients	70	70	
Sex ratio (male:female)	37:33	33:37	0.612
Age at onset, y			
Mean ± SD	4.3 ± 3.8[b]	6.0 ± 3.9	0.018
<8 y	55 (78.6%)	47 (67.1%)	0.183
≥8 y	15 (21.4%)	23 (32.9%)	
Season of onset			
December–February		18 (25.7%)	
March–May		16 (22.9%)	
June–August		17 (24.3%)	
September–November		19 (27.1%)	
API		42 (60%)	
Initial platelet count			
Mean ± SD (per mm^3)		14,266 ± 16,156	
<20,000/mm^3		54 (77%)	
≥20,000/mm^3		16 (23%)	
Medical treatment			
Any		67 (95.7%)	
Corticosteroids (predominantly prednisolone)		47 (67.1%)	
IVIG		43 (61.4%)	
Danazol		14 (20%)	
Azathioprine		3 (4.3%)	
Quinidine		2 (2.9%)	
Vincristine		1 (1.4%)	
Mycophenolate mofetil		1 (1.4%)	
Patients progressing to requiring splenectomy		4 (5.7%)	

API = antecedent of preceding illness; HLA = human leukocyte antigen; ITP = immune thrombocytopenia; IVIG = intravenous immunoglobulin; SD = standard deviation.

[a] Continuous variables were tested by the Wilcoxon rank-sum test, whereas categorical variables were tested by the Fisher's exact test.

[b] The value here indicates the mean age of the control participants.

Table 2 HLA classes I and II genotypes of the control and ITP groups.

Genotype	Gene dose	*n* (%)		*p* value[a]
		Control group (*n* = 70)	ITP group (*n* = 70)	
HLA-A				
A2	Heterozygous	32 (45.7)	30 (42.9)	0.86
	Homozygous	6 (8.6)	8 (11.4)	
A11	Heterozygous	34 (48.6)	23 (32.9)	0.05
	Homozygous	5 (7.1)	13 (18.6)	
A33	Heterozygous	9 (12.9)	6 (8.6)	0.79
	Homozygous	1 (1.4)	1 (1.4)	
A24	Heterozygous	25 (35.7)	14 (20)	0.06
	Homozygous	0 (0)	1 (1.4)	
A26	Heterozygous	4 (5.7)	4 (5.7)	>0.99
	Homozygous	0 (0)	0 (0)	
A29	Heterozygous	0 (0)	4 (5.7)	0.12
	Homozygous	0 (0)	0 (0)	
HLA-B				
B13	Heterozygous	15 (21.4)	13 (18.6)	0.68
	Homozygous	1 (1.4)	0 (0)	
B15	Heterozygous	11 (15.7)	12 (17.1)	>0.99
	Homozygous	1 (1.4)	1 (1.4)	
B38	Heterozygous	7 (10)	8 (11.4)	>0.99
	Homozygous	1 (1.4)	1 (1.4)	
B40	Heterozygous	20 (28.6)	26 (37.1)	0.64
	Homozygous	4 (5.7)	3 (4.3)	
B51	Heterozygous	8 (11.4)	9 (12.9)	0.80
	Homozygous	0 (0)	1 (1.4)	
B58	Heterozygous	10 (14.3)	12 (17.1)	0.82
	Homozygous	1 (1.4)	0 (0)	
B46	Heterozygous	16 (22.9)	8 (11.4)	0.20
	Homozygous	4 (5.7)	3 (4.3)	
B27	Heterozygous	4 (5.7)	9 (12.9)	0.24
	Homozygous	0 (0)	0 (0)	
B55	Heterozygous	6 (8.6)	4 (5.7)	0.74
	Homozygous	0 (0)	0 (0)	
B39	Heterozygous	4 (5.7)	6 (8.6)	0.74
	Homozygous	0 (0)	0 (0)	
B52	Heterozygous	0 (0)	2 (2.9)	0.50
	Homozygous	0 (0)	0 (0)	
B7	Heterozygous	1 (1.4)	4 (5.7)	0.37
	Homozygous	0 (0)	0 (0)	
HLA-C				
Cw3	Heterozygous	24 (34.3)	24 (34.3)	>0.99
	Homozygous	8 (11.4)	7 (10)	
Cw7	Heterozygous	29 (41.4)	25 (35.7)	0.70
	Homozygous	1 (1.4)	2 (2.9)	
Cw8	Heterozygous	11 (15.7)	8 (11.4)	0.38
	Homozygous	0 (0)	2 (2.9)	
Cw12	Heterozygous	9 (12.9)	11 (15.7)	0.64
	Homozygous	0 (0)	1 (1.4)	
Cw15	Heterozygous	4 (5.7)	11 (15.7)	0.10
	Homozygous	0 (0)	0 (0)	
Cw4	Heterozygous	5 (7.1)	5 (7.1)	>0.99
	Homozygous	0 (0)	0 (0)	
Cw1	Heterozygous	23 (32.9)	15 (21.4)	0.15
	Homozygous	6 (8.6)	3 (4.3)	
Cw14	Heterozygous	2 (2.9)	6 (8.6)	0.27
	Homozygous	0 (0)	0 (0)	
HLA-DQ				
DQ2	Heterozygous	12 (17.1)	8 (11.4)	0.28
	Homozygous	0 (0)	2 (2.9)	
DQ3	Heterozygous	35 (50)	35 (50)	0.49
	Homozygous	15 (21.4)	20 (28.6)	
DQ5	Heterozygous	12 (17.1)	22 (31.4)	0.02
	Homozygous	4 (5.7)	0 (0)	
DQ6	Heterozygous	27 (38.6)	24 (34.3)	0.38
	Homozygous	2 (2.9)	0 (0)	
DQ4	Heterozygous	10 (14.3)	7 (10)	0.61
	Homozygous	0 (0)	0 (0)	
HLA-DRB1				
DRB1*03	Heterozygous	8 (11.4)	8 (11.4)	>0.99
	Homozygous	0 (0)	0 (0)	
DRB1*04	Heterozygous	18 (25.7)	19 (27.1)	>0.99
	Homozygous	0 (0)	0 (0)	
DRB1*09	Heterozygous	19 (27.1)	15 (21.4)	0.52
	Homozygous	1 (1.4)	3 (4.3)	
DRB1*11	Heterozygous	10 (14.3)	13 (18.6)	0.62
	Homozygous	1 (1.4)	2 (2.9)	
DRB1*12	Heterozygous	14 (20)	15 (21.4)	0.90
	Homozygous	2 (2.9)	3 (4.3)	
DRB1*15	Heterozygous	16 (22.9)	16 (22.9)	>0.99
	Homozygous	1 (1.4)	1 (1.4)	
DRB1*16	Heterozygous	9 (12.9)	10 (14.3)	>0.99
	Homozygous	1 (1.4)	0 (0)	
DRB1*08	Heterozygous	14 (20)	10 (14.3)	0.75
	Homozygous	1 (1.4)	1 (1.4)	
DRB1*07	Heterozygous	4 (5.7)	2 (2.9)	0.68
	Homozygous	0 (0)	0 (0)	
DRB1*13	Heterozygous	4 (5.7)	5 (7.1)	>0.99
	Homozygous	0 (0)	0 (0)	
HLA-DRB3	Heterozygous	39 (55.7)	38 (54.3)	>0.99
	Homozygous	0 (0)	0 (0)	
HLA-DRB4	Heterozygous	31 (44.3)	31 (44.3)	>0.99
	Homozygous	0 (0)	0 (0)	
HLA-DRB5	Heterozygous	25 (35.7)	21 (30)	0.59
	Homozygous	0 (0)	0 (0)	

HLA = human leukocyte antigen; ITP = immune thrombocytopenia.
[a] Fisher's exact test.

parameters and HLA classes I and II alleles among patients with ITP by multivariate analysis, we found that an older age of onset was a predictor for poor response to either or both treatments (OR = 1.3476, $p = 0.0010$). These results are shown in Table 5. The presence of API was a predictor for good response to treatment (OR = 0.1594, $p = 0.0380$). In addition, patients with ITP who responded poorly to either or both treatments revealed an increase in the frequency of HLA-DQ3 (OR = 15.5428, $p = 0.0024$) and

Table 3 Multivariate analysis of demographic parameters and HLA alleles for the occurrence of ITP.[a]

	Regression coefficient	Standard error	Wald	*p* value	Odds ratio	95% confidence interval
(Intercept)	−0.3740	0.3416	1.1984	0.2736		
HLA-A11 (heterozygous)	−0.9251	0.3839	5.8077	0.0160	0.396	0.187—0.841
HLA-Cw1	−1.0547	0.4031	6.8446	0.0089	0.348	0.158—0.768
HLA-DQ5 (heterozygous)	1.3448	0.4861	7.6549	0.0057	3.838	1.480—9.949

HLA = human leukocyte antigen; ITP = immune thrombocytopenia.
[a] Multiple logistic regression model: $n = 140$, adjusted generalized $R^2 = 0.161$, estimated area under the receiver operating characteristic curve = 0.698, and the Hosmer and Lemeshow goodness-of-fit Chi-square test $p = 0.564 > 0.05$ (df = 6).

a decrease in the frequency HLA-DRB1*08 (OR = 0.0529, $p = 0.0095$). Furthermore, patients with ITP with HLA-DRB1*11 or -DRB1*15 were more likely to respond poorly to corticosteroids compared with their response to IVIG (OR = 56.1135, $p = 0.0446$; OR = 144.4028, $p = 0.0008$, respectively). By contrast, patients who were heterozygous for HLA-A11 were more likely to have a better response to corticosteroids than to IVIG (OR = 0.0711, $p = 0.0338$), as were patients who harbored the HLA-A24 allele (OR = 0.0009, $p = 0.0014$). Therefore, API, age of onset, and particular HLA alleles exhibited strong correlations with treatment responses in this population.

Analysis of demographic and clinical parameters and HLA genotypes in patients with persistent or chronic ITP

When analyzing demographic and clinical parameters among patients with persistent or chronic ITP, we found that patients with an older age of onset and absence of API were more likely to develop persistent or chronic ITP ($p = 0.004$ and $p = 0.001$, respectively). See Table 4 for more detail. When analyzing demographic and clinical parameters and HLA classes I and II alleles among patients with persistent or chronic ITP by multivariate analysis, we

Table 4 Demographic and clinical characteristics in relation to treatment response and disease duration for patients with ITP.

Characteristics	Treatment response		*p* value[a]	Disease duration		*p* value[b]
	Good	Poor		Acute ITP	Persistent or chronic ITP	
Number	38[c]	52[c]		32	38	
Sex ratio (male/female)	18:20	27:25	0.760	16:16	17:21	0.810
Age at onset, y						
Mean ± SD	4.0 ± 3.3	7.7 ± 5.3	<0.001	4.2 ± 3.6	7.5 ± 5.1	0.004
<8 y	31 (81.6%)	29 (55.8%)	0.012	25 (78.1%)	22 (57.9%)	0.727
≥8 y	7 (18.4%)	23 (44.2%)		7 (21.9%)	16 (42.1%)	
Season of onset						
December—February	11 (28.9%)	12 (23.1%)	0.612	9 (28.1%)	9 (23.7%)	0.323
March—May	7 (18.4%)	12 (23.1%)	0.978	6 (18.7%)	11 (28.9%)	0.719
June—August	9 (23.7%)	14 (26.9%)	0.571	7 (21.9%)	10 (26.3%)	0.295
September—November	11 (28.9%)	14 (26.9%)	0.488	10 (31.3%)	8 (21.1%)	0.375
API	29 (76.3%)	25 (48.1%)	0.004	26 (81.3%)	16 (42.1%)	0.001
Initial platelet count						
Mean ± SD (per mm^3)	12,710 ± 6,258	14,690 ± 14,924	0.516	12,840 ± 17,688	14,870 ± 13,826	0.125
<20,000/mm^3	31 (81.6%)	42 (80.8%)	0.855	26 (81.3%)	29 (76.3%)	0.552
≥20,000/mm^3	7 (18.4%)	10 (19.2%)		6 (18.7%)	9 (23.7%)	
Medical treatment			0.938			
Corticosteroids	18 (47.4%)	29 (55.8%)		11 (34.4%)[d]	36 (94.7%)[d]	<0.001
IVIG	20 (52.6%)	23 (44.2%)		22 (68.8%)[e]	21 (55.3%)[e]	0.317

API = antecedent of preceding illness; GEE = generalized estimating equation; ITP = immune thrombocytopenia; IVIG = intravenous immunoglobulin; SD = standard deviation.
[a] Both continuous and categorical variables were tested with a logistic regression model using the GEE method. An exchangeable correlation structure was specified, and a robust variance estimate was used in the GEE analysis.
[b] Continuous variables were tested by the Wilcoxon rank-sum test, whereas categorical variables were tested by the Fisher's exact test.
[c] These figures are the number of treatments. Thirty patients received two treatments because one treatment had failed.
[d] These figures are the number of patients who had ever received corticosteroids treatment.
[e] These figures are the number of patients who had ever received IVIG treatment.

Table 5 Multivariate analysis of the predictors of a poor response.[a]

Covariate	Regression coefficient	Standard error	Wald	*p* value	Odds ratio	95% confidence interval
(Intercept)	−4.3793	1.5171	8.3324	0.0039		
Poor response to either or both treatments						
Age of onset	0.2983	0.0905	10.8759	0.0010	1.3476	1.1286−1.6090
API	−1.8366	0.8854	4.3030	0.0380	0.1594	0.0281−0.9037
HLA-DRB1*08	−2.9394	1.1330	6.7302	0.0095	0.0529	0.0057−0.4874
HLA-DQ3	2.7436	0.9044	9.2036	0.0024	15.5428	2.6406−91.7431
Likelihood of responding more poorly to corticosteroids than IVIG						
HLA-A11 (heterozygous)	−2.6433	1.2454	4.5047	0.0338	0.0711	0.0062−0.8169
HLA-A24	−7.0298	2.2007	10.2037	0.0014	0.0009	0.0001−0.0661
HLA-DRB1*11	4.0274	2.0056	4.0323	0.0446	56.1135	1.1012−2859.3960
HLA-DRB1*15	4.9726	1.4881	11.1665	0.0008	144.4028	7.8144−2668.4180

API = antecedent of preceding illness; GEE = generalized estimating equation; HLA = human leukocyte antigen; IVIG = intravenous immunoglobulin.

[a] Multiple logistic regression model using the GEE method: $n = 67$ (90 observations), adjusted generalized $R^2 = 0.523 > 0.3$, estimated area under the receiver operating characteristic curve = 0.9236 > 0.7, and the Hosmer and Lemeshow goodness-of-fit F test $p = 0.7487 > 0.05$ (df = 9,80).

found a positive association between HLA-A11 homozygosity and the development of persistent or chronic ITP (OR = 6.3165, $p = 0.0479$). These results are shown in Table 6. The presence of HLA-DRB1*08 was, however, negatively correlated with the development of persistent or chronic ITP (OR = 0.1729, $p = 0.0657$). In addition, patients with API were less likely to develop persistent or chronic ITP (OR = 0.2493, $p = 0.0241$), whereas patients with an older age of onset were more likely to develop persistent or chronic ITP (OR =1.1664, $p = 0.0379$).

Discussion

We characterized children with ITP who were seen at two major referral centers in Taiwan. Our goal was to delineate demographic factors, laboratory parameters, and HLA genotypes to better understand the characteristics of childhood ITP and to explore the relationships between these factors and the natural history and treatment response of this disorder. We observed that there was no predisposition to the occurrence of childhood ITP based on gender or season of onset. There were, however, twice as many patients whose age of onset was < 8 years as compared with those whose age of onset was ≥ 8 years, suggesting an age predisposition for this disorder. These findings are similar to those in previous studies.[14,15]

Various autoimmune diseases are associated with particular HLA class I alleles, class II alleles, or both. Therefore, several groups previously investigated the role of HLA class I and HLA-DR in ITP, albeit with somewhat inconsistent results.[9,10,16−18] Few pediatric studies on this issue have been performed. Intriguingly, Olsson and colleagues[19] found increased expression of several cytotoxic genes in patients with ITP as compared with controls. As apparent compensation for this increased cytotoxicity, they also found increased expression of the KIR family of receptors on $CD3^+$ T cells, including KIR3DL1, KIR3DL2, and KIR2DL3, in patients with ITP in remission (i.e., acute ITP) as compared with controls and with patients with active ITP. Later, they reported[20,21] that apoptotic resistance of activated T lymphocytes in patients with active ITP may lead to defective clearance of autoreactive T lymphocytes through activation-induced cell death, which might cause

Table 6 Multivariate analysis of the predictors for persistent or chronic ITP.[a]

	Regression coefficient	Standard error	Wald	*p* value	Odds ratio	95% confidence interval
(Intercept)	−1.5992	1.1657	−1.3718	0.1701		
Age of onset	0.1539	0.0741	2.0755	0.0379	1.1664	1.018−1.368
API	−1.3890	0.6158	−2.2556	0.0241	0.2493	0.070−0.810
HLA-A11 (homozygous)	1.8432	0.9318	1.9781	0.0479	6.3165	1.207−53.662
HLA-DRB1*08	−1.7552	0.9535	1.8407	0.0657	0.1729	0.020−0.9653

API = antecedent of preceding illness; HLA = human leukocyte antigen; ITP = immune thrombocytopenia.

[a] Multiple logistic regression model with the stepwise variable selection method: $n = 70$, adjusted generalized $R^2 = 0.373$, estimated area under the receiver operating characteristic curve = 0.809, and the Hosmer and Lemeshow goodness-of-fit F test $p = 0.171 > 0.05$ (df = 9,60).

a continued destruction of platelets (i.e., persistent or chronic ITP). HLA-A11 and HLA-Cw1, which are HLA class I molecules, have long been known to be associated with specific biological functions. HLA-A11 is the natural ligand for the inhibitory KIR3DL2 receptor,[22] whereas HLA-Cw1, a group 1 HLA-C molecule, is the natural ligand for KIR2DL2 and KIR2DL3 receptors on NK cells and T cells.[23] KIRs downregulate cytotoxic T-lymphocyte and NK-cell responses by binding to MHC class I molecules, thereby preventing lysis of target cells. Several epitopes recognized by cytotoxic T lymphocytes are HLA-A11 restricted, including peptides derived from human papillomavirus,[24] hepatitis C virus,[25,26] human immunodeficiency virus,[27–29] and Epstein-Barr virus.[30–32] HLA-DQ5 is associated with a number of autoimmune disorders, such as myasthenia gravis,[33] juvenile rheumatoid arthritis,[34–36] and other diseases.[37,38]

Our study results showed that HLA-A11 heterozygosity and the presence of the HLA-Cw1 allele seemed to be protective factors for the development of childhood ITP, whereas HLA-DQ5 heterozygosity seemed to be a risk factor for the development of this disorder. Moreover, in contrast to the apparent protective effects and the tendency of a better response to corticosteroids that are conferred by HLA-A11 heterozygosity, in the subgroup analysis of HLA genotypes for patients progressing to persistent or chronic ITP, we found a positive association between HLA-A11 homozygosity and the development of persistent or chronic ITP (Table 6). Whether the balance (or ratio) between the persistent existence of autoreactive T lymphocytes that is due to apoptotic resistance and the inhibitory effects of KIRs on NK cells and T cells contributed to the opposing results of HLA-A11 homozygosity and heterozygosity needs further investigation. These findings do, however, suggest a possible mechanism for the HLA bias we found in this population of patients with ITP. Thus, the relationship between particular HLA class I alleles and the mechanism of cytotoxicity and childhood ITP warrants further study in the near future.

In contrast to outcomes associated with two copies of HLA-A11, the presence of HLA-DRB1*08 was less likely to show a poor response to treatment (Table 5) and to develop persistent or chronic ITP (Table 6). HLA-DRB1*08, an HLA class II molecule, is associated with several diseases in particular populations, including primary biliary cirrhosis,[39–42] ulcerative colitis,[43] and ankylosing spondylitis.[44,45] Additional investigation is needed to determine the role that HLA-DRB1*08 plays in the pathogenesis of pediatric ITP in Taiwan.

In this study, we found that particular HLA class I allele patterns were associated with a better response to corticosteroids than IVIG, including heterozygosity for HLA-A11 and harboring the HLA-A24 allele. Particularly, HLA class II alleles, such as HLA-DRB1*11 or HLA-DRB1*15, were more likely to be related to a poor response to corticosteroids than to IVIG. IVIG may exert its main action by blocking Fcγ receptors or neutralizing autoantibodies by idiotype–anti-idiotype interactions.[46] We suggest that patients with ITP who harbor the particular HLA class II alleles described above are more likely to experience a disease course with a greater likelihood of Th2 bias or autoantibody production. The utility of corticosteroids does, however, suggest that disruption of T-cell regulation[47] and inhibition of phagocytosis, which increases the lifespan of platelets,[6] are critical for ITP. It remains to be determined whether patients with ITP who harbor the particular HLA class I alleles described above are more likely to experience a disease course with a greater likelihood of Th1 bias or cell-mediated cytotoxicity.

Childhood ITP is most commonly associated with a preceding viral illness, and the mechanism of "molecular mimicry" along with an inflammatory state may play a critical role. ITP may also occur after vaccination against MMR,[48] pneumococcus, *Haemophilus influenzae* B, diphtheria-tetanus-pertussis (DTP), hepatitis B virus, or varicella-zoster virus (VZV).[49] In this study, we found that patients with API and with a younger age of onset were more likely to experience a better treatment response and shorter course of ITP, which is in agreement with the results reported by Donato and colleagues.[13] and France and others.[48]

Analysis of the relationship between the HLA-A11 allele and the duration of ITP revealed that two copies of A11 predisposed patients to persistent and chronic ITP. HLA-A11 heterozygosity, in contrast, was a protective factor for the development of childhood ITP and also predisposed patients to respond better to corticosteroids than to IVIG. The Taiwanese population is highly positive for HLA-A11[50]; whether this allele is a susceptibility gene for a particular virus or other pathogens in the pediatric population, as well as the identification of its role in childhood ITP, warrants further investigation.

There are several caveats intrinsic to small-group population studies that require acknowledgment when attempting to apply findings to the population as a whole. First, it is possible that a population of children with ITP who were referred to a tertiary university hospital referral center is inherently more likely to respond poorly to therapies and to have a persistent or chronic form of the disease. In addition, the response rate to various therapies may be affected by the chosen cut-off point for the change in platelet counts following cessation of therapy (in our study, it was set at 50,000/mm^3). Although some HLA class I and class II alleles were significantly associated with childhood ITP in our study, additional combinations of predisposing gene variants and non-HLA genetic factors are likely to contribute to the development and disease course of ITP and to the treatment response of individual patients with ITP.

Furthermore, our findings are statistically significant within the population tested, but the power of an association study depends critically on the sample size. This case-control study made several important discoveries and advances, but its retrospective and nonrandomized nature inevitably limited the strength of the conclusions. Yet, based on these findings, prospective randomized controlled trials can be conducted in larger groups of patients to provide further insight into the immunogenetic susceptibility and clinical correlations of childhood ITP.

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