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Genetic studies in pediatric ITP: outlook, feasibility and requirements

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Abstract

The genomic revolution in medicine has not escaped attention of clinicians and scientists involved in medical management and research studies of immune thrombocytopenic purpura (ITP). In principle, ITP biology and care will benefit greatly from modern methods to understand the patterns of gene expression and genetic markers associated with fundamental parameters of the disease including predictors of remission; risk factors for severity; determinants of response to various therapies; and possibly biological sub-types. However, applying modern genetics to ITP carries severe challenges: (i) achieving adequate sample sizes is a fundamental problem because ITP is rare (and in pediatric ITP, chronic cases constitute only about 1/4 of the total); (ii) familial transmission of childhood ITP is so rare that a convincing pedigree requires consideration of other immunologic or hematologic disorders; (iii) ITP is probably biologically heterogeneous, based on clinical observations, immunological studies and animal models. Here we review the advantages and disadvantages of potential genetic approaches. Sufficient information is available to set reasonable bounds on which genetic analyses of ITP are feasible, and how they are most likely to be accomplished. The highest priority is for accurate *phenotypes* to compare to genetic analyses. Several registries worldwide hold promise for accomplishing this goal.

Keywords

GWAS; immune thrombocytopenic purpura; registries; cohort studies

INTRODUCTION

Immune thrombocytopenic purpura (ITP) is an acquired autoimmune disorder, and the most common cause of isolated thrombocytopenia in children. ITP is diagnosed clinically, based upon onset of thrombocytopenia in the absence of other hematologic abnormalities, or other causes of low platelets, and with a characteristic blood smear. Degree of thrombocytopenia is highly variable, and while bleeding manifestations also vary, severe bleeding is rare. Around 50% of typical childhood acute ITP is preceded by a viral or bacterial infection and commonly resolves within weeks to months without treatment. The incidence of pediatric ITP is on the order of 4–6 cases/100,000 population annually. About 1/4 of these patients go on to chronic disease (which depends in part on the definition of “chronic” as discussed below). For the US pediatric population (~70 million under age 18), this corresponds to

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~3000–4000 total cases annually, and around 1,000 new patients with chronic ITP each year. The *prevalence* of chronic ITP is somewhat higher, as patients continue to manifest their illness. Similar calculations can be done for any country or region, and an upper bound on number of cases can be set. These numbers inform the analyses that are possible.

While ITP in adults is defined as it is in children, fewer adult cases are acute and self-resolving. In young adults, particularly females, cases are more likely than in children to represent an initial manifestation of lupus, and in older adults, many cases are related to lymphoproliferative disorders. Overall though, typical adult primary ITP is very similar clinically to chronic pediatric ITP. Splenectomy remains a more common treatment in adult than pediatric disease by a wide margin. Whether *all* adult and pediatric chronic ITP cases are safely “lumped” together for the purpose of genetic analyses remains a matter of conjecture, but we and most treaters believe that the condition in older teens and young adults is indistinguishable clinically and biologically. Genes and pathways identified as important modifiers of lupus and lupus-like states are likely to be important candidate genes for ITP evaluation as well. Based on the lack of familial cases in ordinary ITP, it is fair to ask, “Why is genetic analysis important at all?” In our view, a search for genes and pathways which modify ITP will improve research and future clinical practice in the field in resourceful/multi-sided ways:

Novel genes and loci identified in unbiased genetic screens (genetic association studies or “transcriptome” analysis) may help elucidate biology of ITP, and suggest the possibility of novel testing for biomarkers of disease, or targets for therapy. These may be true even if the relative contribution of a novel gene to biological variability in ITP is small.

Candidate genes based on our current understanding of pathogenesis and pharmacologic responses can be evaluated to confirm or potentially to rule out postulated contributions.

Pharmacogenomic correlates of response to treatments may have direct clinical relevance in choosing therapies for patients.

DEFINITIONS

The temporal cutoff between “acute” and chronic ITP in childhood is arbitrary, and opinion about the best time-point is evolving. While chronic ITP was traditionally defined by a platelet count of $\leq 150 \times 10^9/L$ at 6 month following diagnosis, remissions after 6 months are common. The Intercontinental Childhood ITP Study Group (ICIS) has used a 12 month cutoff to define chronicity, and a recent international consensus group proposed nine months (1). It remains to be proven how best to apply these newer proposed criteria to pediatric ITP. Various defining criteria have been published. For further clinical review we refer to the guidelines of The American Society of Hematology, the UK guidelines, the AIEO ITP guidelines, the references cited therein, and the recently established criteria by Rodeghiero *et al* (1–4).

Differences in clinical ITP management approaches are substantial (5). “Which drug treatment should be chosen in refractory patients?” and “How can we predict disease progression and treatment response?” are examples of unanswered questions.

Therapeutics in ITP vary broadly, including immunomodulatory agents, immunosuppression, and more recently thrombopoietin-receptor agonists. A detailed discussion is beyond the scope of this paper, except to say that responses to individual therapies among patients also vary widely. Biological heterogeneity in ITP course, duration, and/or in drug responsiveness are crucial “phenotypes” and may prove to have genetic bases. Although several lines of investigation have focused on the pathophysiology of this

immune-mediated disease, little is understood about the underlying cause(s) of ITP (reviewed by 6–8).

IMPORTANCE OF REGISTRIES

As is true for many rare disorders, we believe that carefully designed multicenter registries are the only means to achieve sufficient level of detail in *salient outcomes* of ITP for comparison to genotype or gene expression results. While the extant registries (Table I) vary by methodology, they have in common the ability to discern various patient characteristics including demographics, duration of disease, and response to treatment(s). The largest registries are on the scale of a few hundred to 1–2 thousand patients. None have more than a few hundred chronic ITP patients. This is an important number in considering applicability of genome-wide approaches, discussed below.

In ITP, several key features of phenotype can only be determined by observation over time:

Differentiation of “chronic” from “acute” disease (by any of the definitions above) necessitates EITHER a retrospective diagnosis after the diagnostic time interval (e.g. 6, 9 or 12 months), OR prospective analysis with this length of follow up. *As a practical matter for the current state of ITP registry research, it would be helpful if all registries attempt to ascertain the platelet count status at 6, 9 AND 12 months from diagnosis to facilitate comparisons at a later time.* Longitudinal follow up at intervals after 12 months can also define a “**remission**” status in chronic ITP. For the purpose of genetic analysis, we propose that very long-term follow up will lead to a problem of diminishing returns. The fraction of patients achieving remission after more than two years is certainly too small for any primary genetic analyses, but would be amenable to analysis of novel candidate genes once they are discovered. The same can be said for patients with “**relapse**” of ITP months or years after achieving a remission off all therapies. While overt relapse is an important phenotypic characteristic, it occurs in only a small fraction of ITP patients (perhaps 5%), and insufficient power would be available for primary analysis of this outcome in all of the world’s registries combined.

Response to therapies is a key component of the ITP phenotype, but this outcome is highly variable among ITP patients, and there is no clinical consensus for a particular treatment at a particular time in the disease course for various ITP clinical situations. Therefore, even in the largest registries, only a minority of patients will have received any particular therapy. Less-severely affected patients will receive fewer pharmacologic treatments, and even severely affected, chronic ITP patients achieve experience with various therapies only over months or years. Furthermore, in clinical practice and most registries, (as opposed to prospective research studies), pre-and post-therapy platelet counts will not be reliably available for each treatment in all circumstances.

TYPES OF GENETIC ANALYSIS

Two ITP multicenter registries already include the collection of DNA samples and others aim to extend their registries in similar way. Advantages and disadvantages of different genetic approaches impact the possibilities of genetic studies in ITP.

Family-based-Linkage-Studies

Family-based-linkage-studies will be limited since familial cases of ordinary ITP are very rare. It is unlikely that such cases will be found in significant numbers in registries. Our center’s experience is not more than a handful of convincing pedigrees of “familial” ITP in more than one thousand consecutive probands. Other genetic disorders with

thrombocytopenia which may be (mis)diagnosed as ITP in the proband include myosin heavy chain disorders (MYH9 gene on chromosome 22q11.2, Online Mendelian Inheritance in Man (OMIM) #605249), Autoimmune Lymphoproliferative Syndrome (ALPS), related to defects in FAS pathway (OMIM 601859), and Wiscott-Aldrich Syndrome/X-linked thrombocytopenia (OMIM 301000). (<http://www.ncbi.nlm.nih.gov/sites/omim> queried 7/31/09)

Genome-Wide Association Studies (GWAS)

In these times of ready single nucleotide polymorphism (SNP) chip analysis and whole genome sequencing, GWAS (genome wide association studies) are technically feasible and could reveal novel insights especially in the pathophysiology and disease susceptibility. However, the most challenging requirement is to achieve adequate sample size to conduct association studies with reliable and conclusive results. Three major factors affect the sample size, “n” required to have significant results. First, the smaller the effect of any given gene, the larger a population will be required to see the effect. GWAS studies on populations of many thousand individuals can detect relative risks much less than 1.5-fold, but these will be impossible in chronic ITP. Second, rare but important genes responsible for “familial” ITP would be very difficult to ascertain in population studies. For all practical purposes, alleles with less than 5 or 10% prevalence in the population would need enormous “risk” to be discovered by unbiased approaches. Third, when the number of loci tested becomes very large (Affymetrix 6.0 SNP-chips allow testing of nearly 10^6 loci, and up to 2.5 million SNPs can be inferred), an enormous statistical price is paid for multiple comparisons. For the traditional accepted type I error of 5%, to test 1 million loci means that P values need to be less than 5×10^{-8} to be statistically significant. This fact alone drives sample sizes into the thousands for unbiased analyses. Table III demonstrates how effect size and allele frequency drive sample size requirements for recessive, co-dominant or dominant inheritance

Candidate gene approach

Candidate gene studies do not carry the same severe statistical penalty as GWAS techniques, although corrections for multiple comparisons are often not mentioned at all in published reports. A few association studies in ITP have been published (table IV). Results of these studies have to be confirmed in larger cohorts to make conclusions about pathogenetic implications. These reports include diverse groups of patients and populations which can make the analyses difficult to generalize. Several are of borderline statistical significance (p values not much less than 0.05), perhaps because of small sample size, chance associations, or biological heterogeneity. Only by replication in additional populations will true significance become clear. Investigators will achieve most meaningful results if they are able to assign patients definitively to “acute” and “chronic” ITP phenotypes (in case these are biologically distinct). It is imperative to have controls which match the ethnic and racial background of the case group: Frequencies of polymorphisms differ among groups, so that overrepresentation of different ethnic groups between cases and controls by ethnicity complicates analysis and accurate interpretation of genetic data (9).

Comparative genomics

Convincing familial or breed-specific ITP is common in some dog groups, including old English sheep dogs, poodles and American cocker spaniels. The fact that ITP is more common in some breeds (33% of all canine cases are seen in Cocker Spaniels) supports the hypothesis that genetic variation is a key factor in the development of ITP. Comparative studies of identified candidate genes in canine models is feasible due to the availability of the complete dog genome genomic sequences and the fact that linkage studies based on affected and unaffected littermates and pedigrees could be generated.

Pharmacogenomics

Hematologists routinely practice pharmacogenetics with any ITP patient under consideration for Anti-D therapy. The Rh blood group locus on chromosome 1 determines RhD expression and d/d individuals don't respond to this drug because they don't express the D antigen on erythrocytes. Although treaters do not routinely think of blood type as a genetic analysis, we see this as a nontrivial example; on the contrary, the Rh gene (which has nothing to do with ITP pathogenesis) demonstrates how specific genomic loci may have *powerful genetic effects* on environmental (drug treatment) responses to ITP. It is controversial if a gene which may be a key receptor protein for the chimeric anti-CD20 antibody, rituximab, namely the FCGR3A V/V allele, influences the response to Rituximab (10,11). The identification of further candidates in the metabolism of Rituximab would make pharmacogenetic studies in ITP feasible.

6-Mercaptopurine (6MP) and the related azathioprine, must be converted to active 6-thioguanine, for biological activity; these compounds are then cleared in part by thiopurine-methyltransferase (TPMT). A common allelic variant of the TPMT gene (approximately 10% in Caucasians) causes slow elimination of the active drug, and homozygotes for this variant (about 1% in the population) have high risk of severe myelosuppression and other toxicities (12).

CONCLUSION

Logistic and statistical considerations set strong boundaries on how genetic methods may be used for analysis of pediatric ITP pathophysiology and phenotypic expression. To the extent that ITP is biologically heterogeneous (T vs. B cell origin, for example), these restrictions will be even more severe. However, the several ITP registries world-wide will play an important role in defining phenotype and identifying subjects for feasible genetic studies. We propose that if the registries can have common phenotypic data elements, they will be much more powerful tools to assist basic scientists studying mechanisms of disease. If registries and samples were pooled collaboratively, larger samples sizes would be possible but replication of analyses would be hard. If registries are kept distinct, replication data sets will be easy to come by, but sample sizes will be small. Pharmacogenetics and comparative genomics have not yet been optimally tested. Unbiased GWAS studies will be particularly challenging due to sample size problems.

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Table I

ITP registries

Registry	Inclusion criteria	Locale	Enrolled patients (as of July 2009)	DNA collection	Web page
North American Chronic ITP Registry (NACIR), since 2002	<ul style="list-style-type: none"> - 6 m–18 y - Clinical diagnosis of ITP (<i>including primary ITP (isolated immune thrombocytopenia), secondary ITP and Evans Syndrome</i>) - Chronic ITP (duration > 6 m) - enrollment 6 mo after ITP diagnosis 	North America	439	Yes	www.itpkids.org
Intercontinental Childhood ITP Study Group (ICIS) and Pediatric and Adult Intercontinental Registry on Chronic ITP (PARC), since 2004	<ul style="list-style-type: none"> - Children >2m and adults - Clinical diagnosis of ITP (acute and chronic) 	International (30 countries)	1886	Yes	http://pages.unibas.ch/itpbase/
UK Pediatric ITP registry, since 2006 (data are transferred to ICIS)	<ul style="list-style-type: none"> - 3 m–16 y - Clinical diagnosis of ITP (acute and chronic) - enrollment with in 12 month of ITP diagnosis 	UK	240	Anticipated 2009	www.uk-ipt.org
Nordic Society of Pediatric Haematology and Oncology (NOPHO) ITP working group, 1998–2000	<ul style="list-style-type: none"> - 0–14 years - Clinical diagnosis of ITP (acute and chronic p.l.c. below $30 \times 10^9/L$) - enrollment at ITP diagnosis 	Scandinavia	506	No	closed (plan to continue in 2010/2011)
Associazione Italiana Ematologia ed Oncologia Pediatrica (AIEOP) ITP study group, 1999–2002	<ul style="list-style-type: none"> - 6 m–18 y - Clinical diagnosis of ITP (acute and chronic) - enrollment at ITP diagnosis 	Italy	609	No	closed

m= months, y= years

Relevant genetic studies in pediatric ITP

Table II

Type of study	Requirements (in addition to well described phenotypes)	Advantages	Disadvantages
Linkage Studies (Family based)	Must choose statistical approaches and models of inheritance. Examples: autosomal recessive: 10–15 affected individuals (unaffected family members uninformative except with consanguinity). vs. dominant: ~5 affected individuals (unaffected family members are informative)	Potential for adequate statistical power with a relatively small number of kindreds.	Familial cases of ordinary chronic ITP are extremely rare.
Genome-wide-Association Studies (Population based)	Power calculation of sample size based on expected effect size of a given gene.	Unbiased, powerful approach for multifactorial disorders in which single genes contribute only a fraction of the variance in phenotype. Genomic DNA is not affected by current therapy or current disease status; controls may be population based from public databases.	Requires at least hundreds of patients even for relatively strong effect sizes; thousands for modest effects; ethnic diversity may be an impediment; cost
Comparative genomics	Adequate animal models	Family studies are much easier in domestic animals. Informative pedigrees may be easy to obtain. Genomes available for many species (to compare syntenic regions of human chromosomes)	Not all animal models are generalizable to human disease. Focus on conserved genetic regions can be biased.
Candidate gene approach	Pathophysiology needs to be partially understood.	Several kinds of candidate gene analyses are possible – Gene expression (“transcriptome” analysis or proteomics may help define candidate genes); allows many kinds of polymorphisms (not only SNPs); allele transmission studies (if parental DNA has been isolated as well)	Biased: only known genes can be analyzed; genes of interest to investigators may or may not be the right genes. Generally, every study will require controls. In ITP, not clear which cells to study for gene expression or proteomics. (e.g. subsets of lymphocytes)
Pharmacogenetic Studies	Candidates in drug metabolism need to be identified, e.g. “DMET chip” analysis	Inexpensive, may account for variable response to medications, which is a key feature in ITP	Potentially biased: only known genes can be analyzed; genes of interest to investigators may or may not be the right genes.

Table III

Sample Size Estimates for 80% Power

Mode of Inheritance	Relative Risk	Allele Frequency 0.05	Allele Frequency 0.1	Allele Frequency 0.3
Dominant	1.3	2,500	1,250	750
	2	300	150	110
Recessive	1.3	8*10 ⁴	2*10 ⁴	2,500
	2	1*10 ⁴	2,500	300
Co-Dominant (2df) ^b	1.3	3,000	1,500	1,000
	2	350	200	150

Number of cases calculated with the "Power for Genetic Association analyses 1" program (Ref. 13).
Parameters used: Incidence 5/100,000 (0.00005), Type I error (alpha) = 0.05, Effective degree of freedom = 1, Control to Case ratio = 1
^b Co-Dominant (2 degree of freedom): This model test the effect of each genotype independently, therefore it is assumption-free.
Only **bold** numbers are sample sizes likely to be achieved in the pediatric ITP population.

Table IV

reported DNA variations in ITP

Gene/gene product	SNP	Outcome	Number and characteristics of patients ^b	Ethnicity	Reference
B-cell associated					
<i>TNFSF13B</i> (<i>BAFF</i> / <i>g</i> / <i>TNFI3B</i>)	-871 T/Ce	No significant association (no p value, chi-square calculated for this review not significant)	53 adult cITP (serum) 17 adult cITP (genetics) 10 controls	Caucasian (German)	(14)
<i>Ig</i> variable (<i>v</i>) gene (<i>h</i> v3005)	deletion	Deletion in 14/44 cITP patients, compared to 7/88 in controls (p=0.002)	44 adult cITP 88 controls	Caucasian	(15)
T-cell associated					
<i>IL-4</i> , <i>IL-6</i> , <i>IL-10</i>	IL-4: 70-bp VNTR in Intron 3 IL-6: -572 G/C IL-10: -627 C/A	IL-4 (RP1/RP2) genotype (p=0.04), IL-4 (RP2) allele (p=0.03), and IL-10 (A/C) genotype (p=0.01) were less frequently detected in children with chronic ITP than in controls	50 pediatric aITP 30 pediatric cITP 100 controls	Asian (Chinese)	(16)
<i>Interleukin(IL)-1β</i> and <i>IL-1</i> receptor antagonist (<i>IL-1 Ra</i>)	IL-1β: +3953 IL-1Ra: 86-bp VNTR in Intron 2 (I=410 bp; II=240 bp)	IL-1 <i>Ra</i> (I/II) genotype is underrepresented in patients with aITP (p=0.02)	50 pediatric aITP 30 pediatric cITP 100 controls	Asian (Chinese)	(17)
<i>TNF-α</i> <i>TNF-β</i> <i>IL-1β</i>	<i>TNF-α</i> : 238 G/A, -308 G/A <i>TNF-β</i> : +252 G/A IL-1β: -511 C/T, +3953 T/C	<i>TNF-β</i> +252 (G/G) genotype significantly higher in patients with ITP (p=0.04)	84 adult cITP 56 controls	Asian (Japanese)	(18)
<i>CTLA-4</i>	49 A/G (exon 1)	No significant association	60 ITP patients ^c 100 controls	Albanian (Macedonian)	(19)
<i>T-cell immunoglobulin- and mucin-domain-containing molecule-3</i> (<i>TIM-3</i>)	-1516 G/T, -574 T/G, 4259 G/T	No significant association	187 adult/pediatric, a/cITP ^c	Asian (Chinese)	(20)
<i>PTPN22</i>	1858 C/T	10 patients (22%) were heterozygous and 2 (4.4%) homozygous for this SNP compared to frequency of 8.6% observed in a published population study of 960 controls (p<0.0001)	45 adult a/cITP	Caucasian	(21)
<i>SOX13</i>	SOX13 coding region	Two sequence variations 1603C/T- <i>Pro</i> 534Ser and 1836C/T-synonymous were detected in 4 patients. The rare heterozygous genotypes are 3.5 (1603C>T) respectively 2.3 times (1836C>T) more common than in published non-ITP controls.	34 patients		(22)
B-/T-cell associated					
<i>CD72</i>	VNTR in Intron 8	No significant association	206 adult/pediatric, a/cITP ^c	Asian (Chinese)	(23)

Gene/gene product	SNP	Outcome	Number and characteristics of patients ^b	Ethnicity	Reference
Fcγ receptor associated					
<i>TNF</i> , <i>LTA</i> (<i>lymphotoxin</i> = <i>TNF</i> superfamily member-1), <i>IL-1</i> RN, <i>IL-1A</i> , <i>IL-1B</i> , <i>IL-4</i> , <i>IL-6</i> , <i>IL-10</i> , <i>FcγR2A</i> , <i>FcγR3A</i> , <i>FcγR3B</i>	TNF: -308 1/2, LTA: NcoI 1/2; IL-1 RN: VNTR in Intron 2 (2-5) IL-1A: -889 C/T, IL-1B: 3955 C/T, IL-4: -590 G/T, IL-6: -174 C/G, IL-10: -1082 A/G, <i>FcγR2A</i> : 131 H/R, <i>FcγR3A</i> : 158 V/F, <i>FcγR3B</i> : NAI/NA2	<i>FcγR3A</i> 158 (V/F) increased in cITP patients (p=0.017) <i>FcγR3B</i> (NAI/NA1) genotype overrepresented in cITP patients (p=0.0022) LTA NcoI (2/2) genotype overrepresented in cITP patients (p=0.0070) TNF -308 (1/2) and (2/2) genotypes were observed less in cITP patients (p=0.027)	37 pediatric cITP 218 controls	Caucasian	(24)
<i>FcγR2A</i> , <i>FcγR3A</i>	<i>FcγR2A</i> : 131 H/R, <i>FcγR3A</i> : 158 V/F,	<i>FcγR3A</i> 158 (F/F) is less frequently in ITP (p<0.05); in patients with <i>FcγR3A</i> 158 (V/V) was the remission rate with medications higher (p<0.05)	104 adult cITP 59 controls	Asian (Japanese)	(25)
<i>FcγR2A</i> , <i>FcγR3A</i>	<i>FcγR2A</i> : 131 H/R <i>FcγR3A</i> : 158 V/F	<i>FcγR2A</i> 131 H and <i>FcγR3A</i> 158 V allelic frequencies are associated with childhood ITP (p= 0.03)	101 pediatric a/c ITP 130 controls	Unknown	(26)
<i>FcγR2C</i> , <i>FcγR3A</i> , <i>FcγR3B</i>	CNV <i>FcγR2A</i> : 131 H/R <i>FcγR2B</i> : 232 I/T <i>FcγR3A</i> : 158 V/F <i>FcγR3B</i> : HNA1a/b/c <i>FcγR2C</i> : Stop (in Exon 3) <i>FcγR2B/C</i> : -386 G/C	Overrepresentation of <i>FcγR3A</i> 158V allele in pediatric ITP (p<0.001); <i>FCGR2B/C</i> : -386 C/C rare in cITP patients (p=0.03 in adult and 0.05 in pediatric ITP patients) Overrepresentation of ORF <i>FcγR2C</i> gene in ITP patients (p<0.009)	116 adult/pediatric, a/cITP ^c 100 controls	Caucasian	(27)
<i>FcγR2A</i>	131 H/R	No significant association	29 post splenectomy patients, refractory ITP 61 controls	Caucasian (Irish)	(28)
Cytokine					
<i>Transforming growth factor-β1</i> (<i>TGF β1</i>)	509 C/T Codon 10 (L/P) Codon 25 (R/P)	No significant association	35 pediatric aITP 40 pediatric cITP 97 controls	Turkish	(29)
DNA methylation					
<i>DNMT3B</i>	-149 C/T	No significant association	201 adult/pediatric, a/cITP ^c 136 controls	Asian (Chinese)	(30)
Antigens					
<i>Human Platelet antigen 1,2,3 and 5 systems</i>	HPA 1a-+/+1b-/+ HPA 2a-+/+2b-/+ HPA 3a-+/+3b-/+ HPA 4a-+/+4b-/+	Only HPA2-a was detected in ITP patients not HPA-2b (p=0.017)	33 adult chronic refractory ITP patients 80 controls	Caucasian	(31)
<i>dHLA-A, -B and -C antigen and -DR DNA typing</i>	HLA-A, -B and -C antigen and -DR DNA typing	DRB1*0410 was significantly increased in ITP patients (p < 0.05)	111 adult aITP 71 controls	Asian (Japanese)	(32)

other HLA typing studies (33–36) have discrepant results and used the non-genetic two-stage microlymphocytotoxicity test according to the NIH Tissue Typing Manual. Therefore these reports are not reported in this table.

^a synonymous: *BLYS*, *CD257*, *TALL-1*, *TALL1*, *THANK*, *TNFSF20*
^b aITP= acute ITP, cITP= chronic ITP
^c not reported in more detail