FcγRIIa and FcγRIIIa polymorphisms in childhood primary immune thrombocytopenia: implications for disease pathogenesis and outcome

Andromachi Papagianni[a], Marina Economou[a], Athanasios Tragiannidis[b], Eliza Karatza[c], Fekri Samarah[d], Nikolaos Gombakis[a], Fani Athanassiadou-Piperopoulou[b], Norma Vavatsi–Christaki[d] and Miranda Athanassiou-Metaxa[a]

Primary immune thrombocytopenia (ITP) is the commonest acquired cause of bleeding in childhood. The aim of the present study was to evaluate the role of FcγRIIa and FcγRIIIa polymorphisms in the pathogenesis and therapeutic result of childhood ITP. The genotypic frequencies for two Fcγ receptor single-nucleotide polymorphisms, FcγRIIa-131 arginine (R) versus histidine (H) and FcγRIIIa-158 valine (V) versus phenylalanine (F) were examined in 53 children diagnosed with ITP. The genotype frequencies were compared with those of 45 healthy controls. The association between the above frequencies and disease natural course as well as therapeutic result following intravenous immunoglobulin (IVIG) administration was investigated. FcγRIIIa-158V was significantly overrepresented in children with ITP versus controls ($P = 0.029$), whereas no statistically significant difference was noted in FcγRIIa polymorphism distribution. No statistically significant difference was noted in the above genotype frequencies' distribution between children with newly diagnosed and chronic ITP, as well as with regards to the therapeutic result following IVIG administration. High-affinity FcγRIIIa variant (158 V) is possibly implicated in disease susceptibility, but neither of the two Fcγ receptor single-nucleotide polymorphisms seem to have any impact on chronicity or therapeutic effect of IVIG. *Blood Coagul Fibrinolysis* 24:35–39 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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[a]1st Paediatric Department, Ippokration General Hospital, [b]2nd Paediatric Department, AXEPA General Hospital, [c]3rd Paediatric Department, Ippokration General Hospital and [d]Biochemistry Laboratory Department, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

Correspondence to Andromachi Papagianni, MD, Megalou Alexandrou 25b, T.K. 54641, Thessaloniki, Greece
Tel: +003 023 1083 2566/003 069 4919 0537;
e-mail: andromachipap@hotmail.com

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Introduction

Primary immune thrombocytopenia (ITP) is an autoimmune disorder characterized by isolated thrombocytopenia in the absence of any other underlying cause [1]. Fcγ receptors (FcγRs) are the main mediators of the immunoglobulin G (IgG) autoantibody-coated platelets' destruction by the macrophages of the reticuloendothelial system, especially in the spleen and liver [2].

FcγRs are proteins, belonging to the immunoglobulin superfamily and are divided in three main classes: FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16), each with specific genetic, functional and structural properties [3,4]. Data from clinical trials [5,6], as well as experimental data from animal models [7–9], suggest that the low-affinity receptors FcγRIIa and FcγRIIIa are primarily responsible for the removal of opsonized platelets in ITP [2].

Several polymorphisms exist for both FcγRII and FcγRIII in humans. Their importance as biological markers stands in the fact that these polymorphisms exhibit altered affinities for IgG, possibly leading to different clearance rates of immune complexes in patients who express these variants [3]. This in turn might contribute to altered susceptibility of these patients to certain immune disorders [3]. Two of these polymorphisms have been related to ITP, namely a single amino acid substitution in FcγRIIa [histidine (H) instead of arginine (R) at position 131] and a single amino acid substitution in FcγRIIIa [valine (V) instead of phenylalanine (F) at position 158], which can both significantly affect antibody-binding capacity [10–12].

There is conflicting data regarding the exact significance of the above-named polymorphisms in childhood ITP. In view of the former considerations, we conducted a prospective multicenter study in order to further investigate the role of FcγRIIa and FcγRIIIa polymorphisms in the pathogenesis and therapeutic result of childhood ITP.

Patients and methods

Patients and controls

We evaluated consecutive children between the ages of 6 months and 15 years with newly diagnosed or chronic primary ITP, who were diagnosed in the three (first,

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second and third) Paediatric Departments of Aristotle University of Thessaloniki, from March 2008 to June 2009. Chronicity was defined as persistence of thrombocytopenia for more than 12 months from diagnosis [1]. All children were followed up until August 2010. Patient information obtained via history taking included patient age at diagnosis, patient's sex, initial platelet-enhancing therapy (if any), bleeding stage at diagnosis according to the grading by Buchanan and Adix [13] and revised according to recent guidelines [14]. All patients had their immunoglobulin levels checked before any therapeutic intervention and they were all screened for HIV and hepatitis C virus infection. A direct Coombs test was also performed in all patients. The above tests were run in order to exclude cases of secondary thrombocytopenia.

The control population consisted of adult healthy blood donor volunteers, with no history of thrombocytopenia. A peripheral blood sample (approximately 2 ml in EDTA) was obtained from each patient via venepuncture. For controls, the sample was in addition to their blood donation. The study was approved by the Ethics Review Board of Aristotle University of Thessaloniki.

In order to investigate the association of FcγR polymorphisms with the therapeutic result of IVIG in newly diagnosed childhood ITP, we used the outcome criteria defined by the International Working Group on ITP in 2009 [1]. According to those, complete response (CR) was defined as an increase of platelet count $>100 \times 10^9$/l. Response was defined as an increase of platelet count >30 and less than 100×10^9/l and at least a two-fold increase of baseline count and as no response was defined the failure to increase the platelet count more than 30×10^9/l. The time of response was set at 48 h after the IVIG infusion.

Laboratory analysis

All samples were analyzed in the laboratory of the Biochemistry Department of Aristotle University of Thessaloniki. DNA was extracted from peripheral blood (buffy coat) using QIAmp DNA Mini Kit according to the manufacturer's instructions.

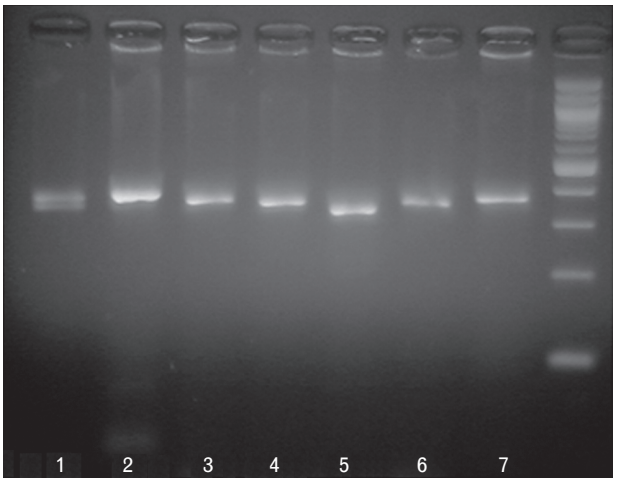
FcγRIIa genotyping

This was performed using PCR-amplified genomic DNA and allele-specific restriction enzyme digestion as described by Jiang *et al.* [15] Fig. 1.

FcγRIIIa genotyping

The latter was performed using PCR-amplified genomic DNA and a modification of the FcγRIIIa restriction fragment length polymorphism analysis as described by Koene *et al.* [12]. For this purpose, the primers and PCR conditions were essentially as described, with the exception of the antisense primer in the nested PCR which was replaced by the FcγR3A intron 4 primer, 5′-ATCACCAGGAGGGAACCACATA-3′ (invitrogen accession number: D5346E10), as described by Carcao *et al.* [16]. This primer leads to a larger (207 bp) PCR-amplified fragment that includes an internal control restriction site for NlaIII digestion and can be detected on agarose gel (Fig. 2).

Fig. 1



Allele-specific restriction enzyme digestion for detection of *FCGR2A* genotypes by PCR of genomic DNA. Shown is the agarose gel electrophoresis result for analysis of six patients. Lanes 1, 3–7: *Bst*UI-digested genomic DNA, Lane 2: uncut PCR product. Lanes 3, 4, 6, 7: *FCGR2A*-A/A [FcγRIIa-131H/H, one fragment 343 base pairs (bp)]. Lane 1: *FCGR2A*-A/G (FcγRIIa-131H/R, two fragments 343 and 322 bp) and Lane 5: *FCGR2A*-G/G (FcγRIIa-131R/R, one fragment 322 bp). Lane 2: uncut PCR (366 bp). F, phenylalanine; H, histidine; R, arginine.

Statistical analysis

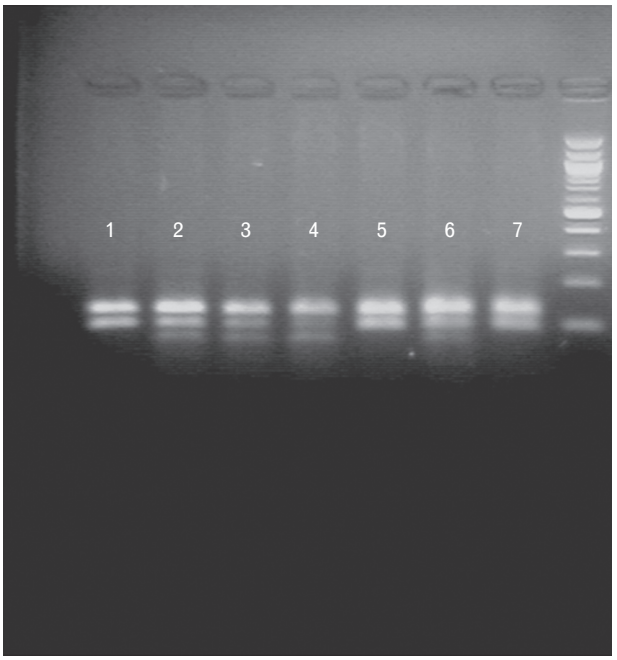
Genotypic distribution and allelic frequencies between children with ITP and controls were compared using 2×3 and 2×2 contingency tables accordingly and data were analysed for significant differences using χ^2 analysis [and Fisher's exact test with small numbers of frequencies (<5)]. Analysis of variance was used to compare genotype frequencies with the course of ITP (newly diagnosed versus chronic). The association of genotype frequencies with the therapeutic response to IVIG was analysed using 2×7 contingency tables and χ^2 analysis. A two-tailed *P*-value of less than 0.05 was considered as statistically significant. The Statistical Package for Social Science (SPSS Inc, version 13.0 for Windows) was used for statistical analyses.

Results

Baseline characteristics

A total of 53 patients and 45 controls were evaluated during the study period. Baseline characteristics are presented in Table 1. Mean and median age at presentation of ITP was 5.9 and 4.7 years, respectively (range 0.5–14.83 years). A total of 30 patients (56.6%) had newly diagnosed ITP and went into remission in less than 3 months from diagnosis; two patients (3.8%) had

Fig. 2



*Nla*III restriction fragment length polymorphism detection of *FCGR3A* genotypes by PCR of genomic DNA. Shown is the agarose gel electrophoresis result for analysis of seven patients. Lanes 1, 5, 7: *FCGR3A*-T/T (FcγRIIIa-158F/F, two fragments 123 and 84 bp), Lanes 2, 3, 6: *FCGR3A*-G/T (FcγRIIIa-158 V/F, three fragments 123, 84 and 61 bp) and Lane 4: *FCGR3A*-G/G (FcγRIIIa-158 V/V, two fragments 123 and 61). F, phenylalanine; H, histidine; R, arginine; V, valine.

persistent ITP, and both recovered within 12 months from diagnosis. The remaining 21 patients (39.6%) had chronic ITP. The relatively high percentage of children with chronic ITP was due to the fact that all paediatric departments that participated in the study are reference centres and follow-up most of the chronic haematology cases.

Genotype distribution and allelic frequencies of FcγRIIa and FcγRIIIa polymorphisms between children with immune thrombocytopenia and healthy controls

FcγRIIa

Allelic gene (FcγRIIa-131H and FcγRIIa-131R) frequencies between controls and ITP patients were essentially similar. No statistically significant difference was noted between the two groups in the genotype distribution of FcγRIIa polymorphism ($P = 0.886$).

Table 1 Patient demographics and course of disease

Patient demographics	
Patient age at diagnosis (years)	Mean age: 5.9 ± 3.9, Median age: 4.7, Range: 0.5–14.83 years
Sex	26 boys, 27 girls, M : F = 1: 1.03
Newly diagnosed ITP (≤3months)	$n = 30$ (56.6%)
Persistent ITP (3–12 months)	$n = 2$ (3.8%)
Chronic ITP (>12 months)	$n = 21$ (39.6%)

ITP, immune thrombocytopenia.

Table 2 FcγRIIIa genotype frequency distribution and allele frequencies for controls and immune thrombocytopenia patients

	ITP patients ($n = 53$)	Controls ($n = 45$)	*P*
FcγRIIIa			0.029
158F/F	$n = 6$ (11%)	$n = 15$ (33%)	0.008
158V/F	$n = 46$ (87%)	$n = 29$ (64%)	0.009
158V/V	$n = 1$ (2%)	$n = 1$ (2%)	0.999
Allele frequencies			0.019
158F	0.55	0.66	
158V	0.45	0.34	

F, phenylalanine; H, histidine; ITP, immune thrombocytopenia; R, arginine; V, valine.

FcγRIIIa

Allelic gene frequencies of FcγRIIIa-158F and FcγRIIIa-158V in the healthy control group were 0.655 and 0.344, respectively, whereas these frequencies in the ITP patients were 0.547 and 0.453, respectively ($P = 0.019$ between patients and controls – Table 2). Genotype distribution had statistically significant difference between patients and controls ($P = 0.029$).

We also studied the distribution between different FcγRIIa/IIIa genotype combinations in the patient and control group, as there are data regarding their nonrandom distribution [17], but we found no statistically significant difference. When comparing children with newly diagnosed and chronic ITP with regards to FcγRs' polymorphisms distribution (Table 3), no statistically significant difference was detected between the two groups ($P = 0.873$ for FcγRIIa-131H/R and $P = 0.661$ for FcγRIIIa-158V/F). Children with persistent ITP were included in the newly diagnosed group of patients.

Association between FcγRIIa/FcγRIIIa polymorphisms and therapeutic result

The association between the FcγR polymorphisms and the therapeutic result following IVIG infusion was investigated by comparing the increase in platelet count following the intravenous infusion of γ-immunoglobulin (1–2 g/kg) shortly after diagnosis, but no later than 3 months from diagnosis, and with a sufficient time

Table 3 FcγRs single-nucleotide polymorphisms distribution and course of disease

	Chronic ($n = 21$)	Newly diagnosed/ persistent ($n = 32$)	*P*
FcγRIIa			0.873
131H/H	35% (7/21)	36% (12/32)	
131H/R	55% (12/21)	46% (14/32)	
131R/R	10% (2/21)	18% (6/32)	
FcγRIIIa			0.661
158F/F	10% (2/21)	12% (4/32)	
158F/V	90% (19/21)	85% (27/32)	
158V/V	0% (0/20)	3% (1/32)	

F, phenylalanine; H, histidine; R, arginine; V, valine.

Table 4 Distribution of FcγRIIa and FcγRIIIa genotypes in children with newly diagnosed immune thrombocytopenia receiving intravenous immunoglobulin subdivided by clinical response (no response/response/complete response)

	No response ($n=5$)	Response ($n=21$)	Complete response ($n=13$)	P
FcγRIIa				0.228
H/H	40% (2/5)	24% (5/21)	54% (7/13)	
H/R	20% (1/5)	57% (12/21)	39% (5/13)	
R/R	40% (2/5)	19% (4/21)	8% (1/13)	
FcγRIIIa				0.432
F/F	40% (2/5)	14% (3/21)	8% (1/13)	
F/V	60% (3/5)	81% (17/21)	92% (12/13)	
V/V	0% (0/5)	5% (1/21)	0% (0/13)	

F, phenylalanine; H, histidine; R, arginine; V, valine.

interval from other therapeutic means that could influence platelet count. Thirty-nine out of 53 patients received IVIG, whereas response to therapy (CR/R/NR, as explained earlier) was evaluated at 48 h following infusion. No statistically significant difference was found in either of the FcγR receptors with regards to response to therapy (Table 4), using 3×3 tables and χ^2 test ($P=0.228$ for FcγRIIa-131H/R and $P=0.432$ for FcγRIIIa-158V/F).

Discussion

Our findings suggest that genotype FcγRIIIa-158VF and high-affinity allele V respectively present with a statistically significant higher frequency in children with ITP, whereas FcγRIIa polymorphic alleles do not seem to play an important role in childhood ITP. Variant allele V has a higher affinity for subclasses IgG1, IgG3 και IgG4 [12,18]. Neither of the two Fcγ receptors' polymorphisms appears to have any prognostic value for chronicity in childhood ITP. Moreover, no association was documented between the polymorphic variants' distribution and response following IVIG infusion in children with newly diagnosed ITP.

Our study did not document any difference in the FcγRIIa polymorphism distribution between children with ITP and controls in contrast to a previous study [16] in which FcγRIIa-131H was significantly overrepresented in children with ITP versus controls. Our results come in agreement with a former pilot study in childhood chronic ITP [19], as well as another in adult patients with chronic ITP [20]. On the contrary, our results indicate FcγRIIIa as a major participant in disease pathogenesis, as the high-affinity FcγRIIIA-158V allele is overrepresented in patients with ITP ($P=0.019$) in contrast to the low-affinity FcγRIIIA-158F allele, an observation also highlighted by previous studies [16,19,20]. FcγRIIIA-158V allele has a higher binding capacity for γ-immunoglobulin, a property that may contribute to a higher destruction rate of IgG-autoantibody-coated platelets, thus leading to a higher susceptibility for development of ITP. It needs here to be mentioned that a main limitation of our study is the relatively constrained number of case controls. A greater number would certainly allow a better interpretation of our study results.

Neither of the two FcγRIIa or FcγRIIIa single-nucleotide polymorphism genotype distributions seems to correlate with disease progression to chronicity, a finding also supported by Carcao *et al.* [16]. No association was found between the two Fcγ receptors' polymorphisms and the therapeutic response to IVIG. This finding may be explained by the fact that Fcγ receptor blockade is only one of the different mechanisms by which IVIG acts. Therefore, other mechanisms, such as complement pathway, soluble Fas or activation of the inhibitory FcγRIIb receptor, may play a more significant role in the response to IVIG administration [21]. One of the limitations of our study is the relatively small number of children who received IVIG, and more patients would be necessary in order to reach safer conclusions.

Overall, our study supports the importance of 158V allele and FcγRIIIa in predisposing patients to present with immune thrombocytopenia, suggesting that it may be a genetic marker for disease susceptibility. On the contrary, neither FcγRIIa nor FcγRIIIa constitute prognostic factors for chronicity in childhood ITP. Neither of the two receptors seems to influence the therapeutic result of IVIG administration in newly diagnosed childhood ITP.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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