FcγRIIIa polymorphism in pediatric immune thrombocytopenia: impact on clinical course and outcome

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Abstract

Fcγ receptors (FcγRs) is an important family of receptors involved in the recognition of IgG- coated particles and complexes. Engagement of activating FcγRs initiates phagocytosis, antibody- dependent cellular cytotoxicity, and the release of inflammatory mediators. Many systemic autoimmune diseases are under FcγR control. Immune thrombocytopenic purpura (ITP) is the most common cause of thrombocytopenia in children. Multiple pathophysiologic mechanisms contribute to thrombocytopenia in ITP, including phagocytosis and destruction of autoantibody-coated platelets. **Aim** To study the impact of FcγRIIIa polymorphism on development of ITP in Egyptian children, and its impact on bleeding severity, response to treatment and disease chronicity. **Subjects and methods:** This is a case-control study including 40 patients with ITP (25 newly diagnosed, 15 chronic ITP) and 20 normal controls. Medical history and physical examination were performed. Laboratory investigations included CBC, Coombs test, serum complement and antinuclear antibody, platelet- associated IgG, ELISA test for H. pylori antigen in stools, and FcγRIIIa genotyping by PCR. Patients were followed up for one year to assess severity of the disease and response to treatment. **Results**: The high affinity FcγRIIIa genotype (158 V/V) and the heterozygous genotype (V/F) were significantly overrepresented in ITP patients compared to the control group. There was no significant difference among ITP patients carrying different FcγRIIIa genotypes regarding response to therapy with corticosteroids or IVIg, and FcγRIIIa genotypes were similar in acute and chronic ITP. **Conclusion**: FcγRIIIa polymorphism might confer susceptibility to ITP, however, different FcγRIIIa genotypes did not affect response to therapy or development of chronic ITP.

ΦcγPIIIα πολψμορπησμ ιν πεδιατρις ιμμυνε τηρομβοcψτοπενια: ιμπαcτ ον clinical course and outcome

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Abbreviated title : FcγRIIIa genotyping in children with ITP

Key words : ITP, FcγRIIIa polymorphism, children, Egypt

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Abbreviations :

FcγRs	Fc gamma receptors
ITP	Immune thrombocytopenic purpura
CBC	Complete blood count
PCR	Polymerase chain reaction
	antibody- dependent cellular cytotoxicity
SLE	Systemic lupus erythematosus
IgG	Immunoglobulin G
C3, C4	Complement 3, complement 4
ANA	Antinuclear antibodies
MHC	major histocompatibility complex
IVIg	Intravenous immunoglobulin

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Aim

To study the impact of FcγRIIIa polymorphism on development of ITP in Egyptian children, and its impact on bleeding severity, response to treatment and disease chronicity.

Subjects and methods:

This is a case-control study including 40 patients with ITP (25 newly diagnosed, 15 chronic ITP) and 20 normal controls. Medical history and physical examination were performed. Laboratory investigations included CBC, Coombs test, serum complement and antinuclear antibody, platelet- associated IgG, ELISA test for H. pylori antigen in stools, and FcγRIIIa genotyping by PCR. Patients were followed up for one year to assess severity of the disease and response to treatment.

Results :

The high affinity FcγRIIIa genotype (158 V/V) and the heterozygous genotype (V/F) were significantly overrepresented in ITP patients compared to the control group. There was no significant difference among ITP patients carrying different FcγRIIIa genotypes regarding response to therapy with corticosteroids or IVIg, and FcγRIIIa genotypes were similar in acute and chronic ITP.

Conclusion :

FcγRIIIa polymorphism might confer susceptibility to , however, different FcγRIIIa genotypes did not affect response to therapy or development of chronic ITP.

Introduction:

Childhood immune thrombocytopenic purpura () is an acquired autoimmune disease characterized by low platelet count that can be primary or secondary to other conditions. Primary ITP is more common in children than adults, and the incidence peaks in winter and spring following the incidence of viral infection. The condition is classified as newly diagnosed ITP (ITP duration of <3 months), persistent ITP (ITP duration of 3-12 months) and chronic ITP (ITP duration of >12 months). [1]

Multiple pathophysiologic mechanisms appear to be responsible for thrombocytopenia in . Phagocytosis and destruction of autoantibody-coated platelets is an important mechanism of thrombocytopenia in ITP. Other possible mechanisms include complement- mediated platelet lysis, T cell- dependent cytotoxicity of target platelets, abnormal dendritic cells expressing self-antigens, and impaired megakaryopoiesis. Some studies suggested that H. pylori could initiate or perpetuate by different mechanisms including molecular mimicry, expression of Lewis antigen, association with Th-1 immune response and stimulation of B-1 cells to produce self- reactive antibodies. [2]

Fcγ receptors found primarily on neutrophils, macrophages, and monocytes, are involved in the recognition of IgG- coated particles and complexes. Fcγ receptors are divided into three classes: FcγRI, FcγRII, and FcγRIII. Within each class, isoforms have been identified that vary in molecular weight, binding affinity to different subclasses of human IgG, and in distribution on the surface of hematopoietic cells. These isoforms have different biologic roles. FcγRI, FcγRIIa, FcγRIIc, and FcγRIIIa are stimulatory receptors are found on the platelets and most leukocytes including monocytes, granulocytes, macrophages, and natural killer cells. Engagement of activating FcγRs initiates phagocytosis, antibody- dependent cellular cytotoxicity (), and the release of inflammatory mediators by phagocytes. By contrast, FcγRIIb is an inhibitory receptor expressed on B lymphocytes (FcγRIIb1) and on myeloid cells (FcγRIIb2). Inhibitory FcγRIIb can abolish cellular signaling. In cells that express both stimulatory and inhibitory FcγRs, the relative level of these two types of receptors may determine the state of activation after interaction with immune complexes.[34]

Many systemic autoimmune diseases are under FcγR control. Activating FcγRs control autoimmune reactions by increasing the uptake of immune complexes and by triggering effector macrophages, whereas the inhibitory receptors control the activation of autoreactive B lymphocytes and thus maintain peripheral tolerance.[5] Allelic variants of FcγRs are common within the population. Functionally relevant FcγR polymorphisms that have a profound influence on IgG binding capacity might explain heritable predisposition to immune complex disease. Linkages between such FcγR polymorphisms and autoimmune diseases such as SLE, rheumatoid arthritis, Guillian Barre syndrome and multiple sclerosis have been reported. [4,6,7]

The human low affinity activating FcγRs (FcγRIIa and FcγRIIIa) might play important roles in platelet destruction in . The role of polymorphism of the low- affinity activating FcγRs in has been studied in both adults and children. In most studies, no difference was found in FcγRIIa genotypes 131 H/R between patients and controls. Similarly, response to treatment was not influenced by FcγRIIa genotypes. These data might reflect that most of the antiplatelet antibodies in ITP are IgG1 or IgG3; while it is the affinity for IgG2 that is most affected by FcγRIIa genotype.[8–10]

For FcγRIIIa polymorphism, conflicting results were obtained regarding its relationship to disease incidence and response to medication/ splenectomy in patients. Also, little data are present correlating FcγRIIIa genotypes to disease chronicity especially in childhood . The present study aimed at studying FcγRIIIa polymorphism in children with in relation to disease severity, response to treatment and development of chronic disease.[11]

Subjects and methods:

This is a case-control study that was conducted on 40 pediatric patients with , and 20 age and sex- matched normal children. Informed consents from patients' legal guardians were obtained, and the study was approved

by the ethical committee of Alexandria University. Twenty-five of the patients were enrolled at diagnosis and followed up for one year, whereas the other 15 were chronic patients followed up in the hematology outpatient clinic at Alexandria University Children Hospital, Alexandria, Egypt. Seventeen of the newly diagnosed cases achieved normal platelet count within 3 months after diagnosis, fulfilling the definition of acute ITP (Group 1, acute ITP=17 patients). The other 8 patients turned to suffer from the chronic form of , and they were grouped with the 15 chronic patients collected from the clinic (Group 2, chronic ITP= 23 patients).

For all patients included in the study, full medical history was obtained regarding the onset of symptoms, the duration before presentation to hospital, and preceding viral infection. Complete physical examination was performed. Investigations included CBC, Coombs' test, serum level of C3, C4, and antinuclear antibody, flowcytometic detection of platelet- associated IgG, ELISA test for detection of H. pylori Ag in stool samples. Genotyping of FCGR3A-158V/F polymorphism was performed as described by Koene et al using a nested PCR followed by allele-specific restriction enzyme digestion (figure 1).[12]

The patients were carefully followed up for one year to assess the course and severity of the disease and response to different modalities of treatment. Severe bleeding was defined as episodes requiring hospital admission and/or blood transfusions or symptoms interfering seriously with quality of life.

Results:

The high affinity FcγRIIIa genotype (158 V/V) and the heterozygous genotype (V/F) were significantly overrepresented in ITP patients compared to the control group, who had higher prevalence of the low affinity genotype (158 F/F). (p= 0.001) (Table 1). However, there was no significant difference between acute and chronic cases as regards the prevalence of different FcγRIIIa genotypes. (Table 2)

No significant impact of FcγRIIIa polymorphism on the course or severity of bleeding manifestations in patients was noted, despite that it was apparent that patients with the high affinity genotype (V/V), tended to frequently suffer a more severe course. In addition, there was no significant correlation between FcγRIIIa genotypes and platelet count or antiplatelet antibodies. (Table 3)

FcγRIIIa polymorphism did not influence the response of patients to therapy with corticosteroids, however, although not significantly different, patients with the high affinity genotype V/V tended to respond to IVIg more frequently than patients with the other genotypes. The number of patients receiving anti- D, vincristine, azathioprine, danazol, rituximab, or who underwent splenectomy was too small to be statistically sound for analysis. None of the patients included in the current report received thrombopoietic agents, as they were not commercially available at the time of conducting the study (Table 4)

Patients with chronic ITP tended to have longer duration of symptoms before presenting to hospital, responded less frequently to steroid therapy, and had higher prevalence of H pylori antigen in their stools. There was no statistically significant difference between acute and chronic cases regarding other epidemiological, clinical or laboratory parameters. (Table 5).

Discussion:

Childhood ITP probably results from a spectrum of polymorphic susceptibility factors, including FcγR functional variants.[13] In the present study, the distribution of FcγRIIIa genotypes in Egyptian children with ITP was significantly different from the control populations. The high affinity FcγRIIIa-158V genotype (V/V) and the heterozygous genotype (V/F) were significantly overrepresented in ITP patients compared to the control group, while the low affinity genotype (F/F) was overrepresented in the control subjects. Similar results were reported by Foster et al [8] and Carcao et al who reported a genotypic distribution of FcγRIIIa subtypes comparable to our results.[8,13] FcγR variants with the higher affinities for IgG were detected in a much larger number of children with ITP, lending further strength to the argument that FcγRIIIa polymorphisms contribute to the pathogenesis of childhood ITP.[13]

Two possible mechanisms may explain the over-representation of FcγRIIIa-158V in childhood ITP as seen in

the present study. An over-representation of the high IgG binding alleles might result in increased clearance of IgG-sensitized platelets. The more efficient clearance of cross-reactive antibody-sensitized platelets by the higher binding FcγRs may lead to enhanced platelet antigen presentation by major histocompatibility complex (MHC) class II cells. In addition, viral immune complex binding to the higher affinity FcγRs on platelets can increase platelet consumption leading to increased self-antigen presentation by phagocytic cells bearing MHC class II.

Fcγ receptors also regulate antibody production indirectly through phagocytosis followed by antigen presentation. However, in the present study, no significant correlation was observed between the presence of platelet antibodies or their titer and the polymorphisms of the Fcγ receptors. Also, there was no significant correlation between Fcγ receptor IIIa genotypes and the initial platelet count. These results are in agreement to those of Fujimoto et al and might indicate that the polymorphisms of the Fcγ receptors did not regulate the production of autoantibody against platelets at least in some population.[9]

Although not significantly different, it was noted that patients with the high affinity genotype (V/V) tended to suffer a more severe course of thrombocytopenia with frequent bleeding episodes and recurrent hospitalization. (71.4% of patients with V/V genotype compared to 33.3%, 40% of patients with V/F, F/F genotypes respectively). This was also reported by Binstadt BA et al who attributed these data to the better ability of the high affinity genotype to phagocytose IgG1- and IgG3-autoantibody-coated platelets, resulting in increased disease severity. More efficient phagocytosis results in lower platelet counts, putting the patient at a higher risk for bleeding.[14]

Regarding the response to therapy, the present work did not show a significant correlation between Fcγ receptor IIIa genotypes and response to corticosteroids. The response rate was high (>65%) in all three genotypes. On the other hand, although not significant, it was observed that patients with the high affinity genotype (V/V) responded more frequently to IVIg (80%) than patients with the lower affinity genotypes. As the major mechanism of IVIg action in ITP is Fcγ receptor blockade, it seems reasonable that the higher the affinity of the receptor, the more it is liable to be blocked by IVIg, and hence, the higher the response rate.

In the same context, the influence of Fcγ receptor IIIa polymorphism on the response to treatment in ITP was studied by Fujimoto and colleagues.[9] They found that patients with FcγRIIIA158 V/V genotype trended to respond to medications more effectively than to splenectomy compared with other genotypes, while patients with 158 F/F and V/F genotypes did not response to medication, but better controlled by splenectomy. One possible explanation is that in the V/V type, which has a higher affinity for antibody-sensitized platelets, medications such as prednisolone are more effective because of suppression of the function of phagocytes carrying this receptor. In contrast, splenectomy is less effective because the other reticuloendothelial organs such as the liver could still destroy platelets with high affinity even after the spleen was removed. In the F/F or the V/F type, however, the effectiveness of the elimination of the main platelet destruction site (the spleen) is more obvious because phagocytes of the other organs have only lower affinity for the platelets, which may induce the compensatory state for the platelet destruction by platelet production.[9]

In the current study three children with chronic ITP have undergone splenectomy (V/V, V/F and F/F genotypes; one patient each), none of them showed response to splenectomy. Three patients received anti-D immunoglobulin, two of them responded to such a treatment, one carrying the V/V genotype and the other was V/F. The one who was refractory to anti- D had the low affinity genotype F/F. One patient -carrying the V/V genotype- with severe refractory chronic ITP received rituximab with no sustainable response. The small number of patients treated with these modalities did not allow drawing conclusion regarding the impact of FcγRIIIA polymorphism in such response.

None of the patients included in this report received thrombopoietic agents, as they were not available commercially at the time of conducting this study. Further studies are needed to assess whether this polymorphism can predict response to thrombopoietic agonist, thus helping decision making by clinicians taking care of children with ITP.

Declarations of interest: The authors have no conflict of interests to disclose

Conclusion:

FcγRIIIa polymorphism is a potential genetic factor that confers susceptibility to development of and may have an impact on the response to some modalities of therapy. FcγRIIIa polymorphism did not affect the probability to progress to the chronic in our patients. Factors such as type of onset of symptoms, duration before presentation, infection with H pylori, and pattern of response to corticosteroid therapy may be risk factors that predict the liability to development of chronic ITP.

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Figure Legend:

FIGURE 1 Gel electrophoresis showing Restriction Fragment Length Polymorphism (RFLP) analysis of FCGR3A gene polymorphisms (NlaIII restriction analysis of the 94- bp)

TABLE 1 Distribution of different FcγRIIIa genotypes in ITP cases and control group.

Group		ITP	Control	Total	Pearson Chi-Square
Genotype V/V	Count	7	0	7	χ^2= 15.082 P value 0.001
	% within genotype	100.0%	0.0%	100.0%	
	% within group	17.5%	0.0%	11.7%	
V/F	Count	18	2	20	
	% within genotype	90.0%	10.0%	100.0%	
	% within group	45.0%	10.0%	33.3%	
F/F	Count	15	18	33	
	% within genotype	45.5%	54.5%	100.0%	
	% within group	37.5%	90.0%	55.0%	
Total	Count	40	20	60	

* Statistically significant (p< 0.05)

TABLE 2 Distribution of different FcγRIIIa genotypes in acute and chronic ITP cases.

Group		Acute ITP	Chronic ITP	Total	Pearson Chi-Square
Genotype V/V	Count	3	4	7	χ2= 0.067 P value 0.967
	% within genotype	42.9%	57.1%	100.0%	
	% within group	17.6%	17.4%	17.5%	
V/F	Count	8	10	18	
	% within genotype	44.4%	55.6%	100.0%	
	% within group	47.1%	43.5%	45.0%	
F/F	Count	6	9	15	
	% within genotype	40.0%	60.0%	100.0%	
	% within group	35.3%	39.1%	37.5%	
Total	Count	17	23	40	

TABLE 3 Antiplatelet antibodies, platelet count, and bleeding severity in ITP cases according to different FcγRIIIa genotypes.

		ΦςγPIIIα γε-νοτψπε	**ΦςγPIIIα γε-νοτψπε**	**ΦςγPIIIα γε-νοτψπε**	**ΦςγPIIIα γε-νοτψπε**	**ΦςγPIIIα γε-νοτψπε**	**ΦςγPIIIα γε-νοτψπε**	**Total** n= 40	**Total** n= 40
		V/V n= 7	V/V n= 7	V/F n= 18	V/F n= 18	F/F n= 15	F/F n= 15		
Anti-platelet Ab	**Anti-platelet Ab**	2 28.6%	2 28.6%	8 44.4%	8 44.4%	10 66.7%	10 66.7%	20 50%	20 50%
Positive	Positive								
Negative	Negative	5 71.4%	5 71.4%	10 55.6%	10 55.6%	5 33.3%	5 33.3%	20 50%	20 50%
Platelet count	5 - 35	5 - 35	5 - 85	5 - 85	4 - 67	4 - 67	4 - 85	4 - 85	F= 0.379 P= 0.687
min- max									
mean± SD	19 ± 11.1	19 ± 11.1	22.4± 19.8	22.4± 19.8	26.1± 19.7	26.1± 19.7	23.2± 18.3	23.2± 18.3	
Bleeding severity	2 28.6%	2 28.6%	12 66.7%	12 66.7%	9 60%	9 60%	23 57.5%	23 57.5%	χ^2= 3.054 P = 0.217
Mild									
Severe	5 71.4%	5 71.4%	6 33.3%	6 33.3%	6 40%	6 40%	17 42.5%	17 42.5%	

TABLE 4 Relationship between FcγRIIIa genotype and response to corticosteroids and IVIg

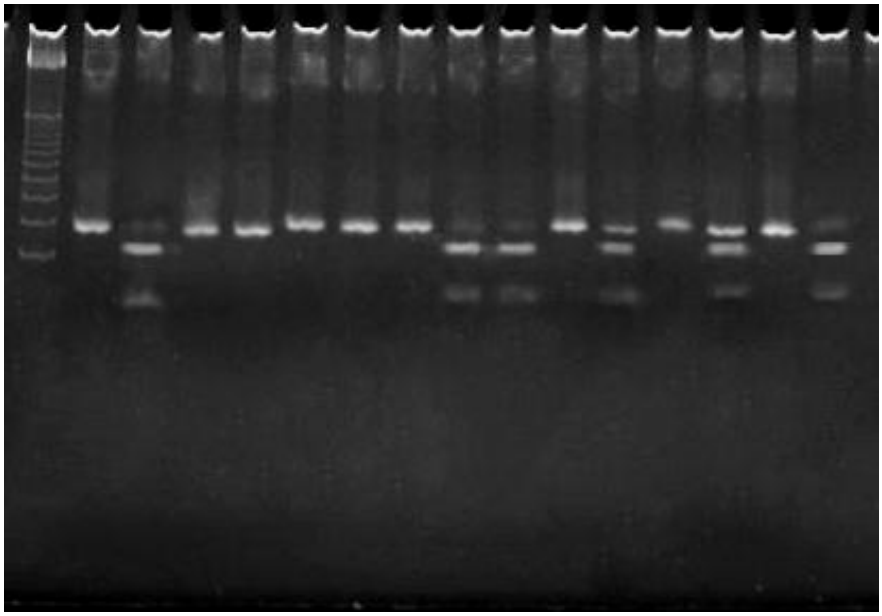
Steroids	**Steroids**	**Genotype**	**Genotype**	**Genotype**	**Genotype**	**Genotype**	**Genotype**	**Total** n= 40	Te sig
		V/V n= 7	V/F n= 18	V/F n= 18	V/F n= 18	F/F n= 15	F/F n= 15		
Response	No %	5 71.4%	12 66.7%	12 66.7%	12 66.7%	12 80%	12 80%	29 72.5%	χ^2= P =
No response	No %	2 28.6%	6 33.3%	6 33.3%	6 33.3%	3 20%	3 20%	11 27.5%	
IVIg	**IVIg**	**Genotype**	**Genotype**	**Genotype**	**Genotype**	**Genotype**	**Total** n= 13	**Total** n= 13	
		V/V n= 5	V/V n= 5	V/F n= 6	F/F n=2	F/F n=2			
Response	No %	4 80%	4 80%	2 33.3%	1 50%	1 50%	7 53.8%	7 53.8%	χ^2= P =
No response	No %	1 20%	1 20%	4 66.7%	1 50%	1 50%	6 46.2%	6 46.2%	

TABLE 5 Comparison between acute and chronic ITP patients: epidemiology, clinical/ laboratory parameters, and response to treatment.

	Acute ITP (n= 17)	Chronic ITP (n=23 (8+15)	Test of significance
Sex Male Female	9 (52.9%) 8 (47.1%)	13 (56.5%) 10 (43.5%)	χ^2= 0.051 p = 0.822
Age (in months) min-max X± SD	9 - 120 45.7 ±30.2	9 - 124 45.5 ± 29.	t = 0.024 p = 0.0981
Onset Acute Gradual	9 (52.9%) 8 (47.1%)	10 (43.5%) 13 (56.5%)	χ^2= 1.319 p = 0.251
Duration before presentation (days) min- max X± SD	1 - 45 8.6 ±11.6	1 - 90 22.4 ± 21.7	t = -2.598 p = 0.014*
History of viral infection Negative positive	9 (52.9%) 8 (47.1%)	14 (60.9%) 9 (39.1%)	χ^2= 0.251 p = 0.616
H pylori Ag in stools Positive Negative	4 (23.5 %) 13 (76.5 %)	10 (66.7 %) 5 (33.3 %)	χ2= 6.026 P = 0.014*
C3 (mg/dl) min- max X± SD	86.2 – 216.4 137.9± 34.0	97.3 – 207.4 136.9 ± 34.4	Z = -0.151 P = 0.880
C4 (mg/dl) min- max X± SD	10.5 – 52.7 27.1 ± 11.3	13.2 – 55.1 31.1 ± 17.8	Z = -1.058 P = 0.290
ANA (µ/ ml) min- max X± SD	4.2 – 12.6 6.4 ± 1.9	3.9 – 13.3 6.5 ± 2.9	Z = -0.907 P = 0.364
Antiplatelet Ab Positive Negative	7 (41.2 %) 10 (58.8 %)	8 (58.3 %) 7 (46.7 %)	χ2= 0.473 P = 0.492
Platelet count (x103/cmm) min- max X± SD	5 - 85 22.2 ± 19.8	4- 67 24.0 ± 17.6	t = -0.307 p = 0.760
Course: Mild Severe	10 (58.8%) 7 (41.2%)	13 (56.5%) 10 (43.5%)	χ^2= 0.021 P = 0.884
Response to steroids: Response No response	16 (94.1%) 1 (5.9%)	13 (56.5%) 10 (43.5%)	χ^2= 6.930 p = 0.008*
Response to IVIg: Response No response	1 (100%) 0 (0%)	6 (50 %) 6 (50%)	χ^2= 0.929 P = 0.335

* Statistically significant (p< 0.05)

FIGURE 1 Gel electrophoresis showing Restriction Fragment Length Polymorphism (RFLP) analysis of FCGR3A gene polymorphisms (NlaIII restriction analysis of the 94- bp)



Lanes 1,3,4,5,6,7,10,12, and 14 show homozygous FCGR3A genotype F/F (94 bp).

Lanes 2,8,9, and 15 show homozygous FCGR3A genotype V/V (61 and 33 bp).

Lanes 11, and 13 show heterozygous FCGR3A genotype V/F (94, 61 and 33 bp).