

The FcγRIIa Polymorphism in Children with Atopic Diseases

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Key Words

FcγRIIa · Polymorphism · Atopic · Children

Abstract

Background: Fcγ receptor II (FcγRIIa) is the most widely distributed of the classes of FcR and is expressed in polymorphic forms on most types of hematopoietic cells. Recent data suggest that this polymorphism may be relevant to FcγRIIa function. This might be linked to variability in immune response and therefore related to the pathogenesis of atopic diseases. The aim of the study was to evaluate the FcγRIIa polymorphism in children with atopic diseases. **Methods:** In the study were included 140 atopic children, 77 with food allergy and 126 healthy subjects as the control group. The FcγRIIa polymorphism was determined using the polymerase chain reaction method. **Results:** The distribution of FcγRIIa genotypes in atopic children did not differ from that of healthy controls. Moreover, there was no association between the FcγRIIa genotypes and atopic diseases. **Conclusion:** It seems that the FcγRIIa polymorphism does not represent an important genetic risk factor for atopic diseases susceptibility.

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Introduction

Fc receptors (FcR) are glycoproteins found on the surface of hematopoietic cells that bind the Fc portion of immunoglobulin and provide a link between the humoral and cellular immune systems. Interaction of FcR with immunoglobulin provides the host with protection against infection by means of phagocytosis, antibody-dependent cellular cytotoxicity, release of inflammatory mediators, and enhancement of antigen presentation [1]. In addition to host defense, FcR play a role in autoimmune diseases and immunohematologic disorders [2]. FcγRII, which binds IgG in its oligomeric form or when it is bound to cells, is found on the surface of monocytes, macrophages, neutrophils, platelets, basophils, eosinophils, and other cells [3]. The FcγRIIa gene is polymorphically expressed, resulting in an amino acid change (arginine or histidine) in the second immunoglobulin domain of the extracellular region, containing the ligand-binding pocket at position 131 in the FcγRIIa protein [4]. This polymorphism alters ligand-binding properties. Histidine/histidine (H/H131) cells have markedly higher binding affinity for human IgG2 than arginine/arginine (R/R131) cells, whereas heterozygotes (H/R131) have intermediate affinity. There are also major differences in binding of murine IgG1 (R/R131>H/H131) and minor differences with human IgG3 (H/H131>R/R131) [5]. Differ-

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1018–2438/04/1333–0233\$21.00/0

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ences in binding affinity have been demonstrated in a variety of assays measuring phagocytosis, monocyte-dependent T cell proliferation, rosetting of opsonized erythrocytes, antibody-mediated platelet activation, and anti-FcγRII monoclonal antibody reactivity [6]. The variation in ligand specificity of FcγRIIa polymorphic forms has clinical relevance. Human IgG2 targets are bacteria with a polysaccharide capsule and FcγRII is the only FcR with binding affinity for human IgG2. Especially, only the H/H131 genotype can effectively bind human IgG2. The FcγRIIa genotype might influence the ability to clear encapsulated organisms [7]. The FcγRIIa genotype is also related to a risk of other immune-mediated diseases, including heparin-associated thrombocytopenia [8, 9] and immune complex clearance in systemic lupus erythematosus [10]. The FcγRIIa genotypes vary widely among different ethnic groups. The Japanese as an example have a low frequency of infections by encapsulated organisms and a high frequency of the H/H(131) phenotype [11, 12]. The aim of the study was to evaluate the FcγRIIa polymorphism in children with atopic diseases.

Material and Methods

This study included 217 children (109 girls and 98 boys; age range 1.5–17 years) with IgE-mediated allergic diseases. The children either stayed at the hospital (Pediatric Department of the University Hospital in Szczecin, Poland) or were seen in the Pediatric Outpatient Department of the Division of Allergology. The control group comprised 124 healthy subjects (69 girls and 56 boys; age range 5–17 years). Subjects with histories and familial histories that suggested an allergic background and those with some abnormalities shown by the routine biochemical tests and IgE level were excluded from the control group. All subjects were Caucasians and came from the Pomeranian region of Poland. The study was approved by the local ethics committee on human research, and informed consent was obtained from all parents of the children.

Children with recurring respiratory tract infections, bronchitis obliterans, pneumonia, and chronic rhinopharyngitis were excluded from the study. Moreover, we excluded any subjects whose history suggested more than an allergic background, as well as those with some abnormalities shown by routine biochemical tests and immunoglobulin levels.

Diagnostics

The evaluation of subjects included routine biochemical blood analysis and allergic workup based on the following laboratory determinants: eosinophilic granulocyte count in the peripheral blood, total IgE serum level, specific IgE levels against some inhaled and alimentary allergens, and skin prick test. A history of an atopic disease in first- or second-degree relatives and physical signs (recurring cough on exertion or at night and wheezy breathing, sometimes associated

with a cutaneous or digestive form of allergy or seasonal rhinitis) were considered for classification of bronchial asthma.

The eosinophilic granulocyte count was considered to be elevated when it exceeded 4% of total white blood cell count. Total IgE and specific IgE levels were estimated with an immunoenzymatic method by use of a 3M diagnostic System Kit. Total IgE levels were related to the age-specific limits given by the manufacturer. Values that exceeded 15 IU/ml were considered to be elevated. The presence of allergen-specific IgE antibodies was estimated with selected aeroallergens, i.e. house dust, ascarids (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), feathers, cat and dog hair, food allergens (protein of cow's milk, egg yolk, and egg white), and pollens of Gramineae, weeds, and trees. The level of allergen-specific IgE was classified from 0 to 4; a score of 1 or more accounted for an elevated level. All skin tests were done with a point method by use of a Bencard Beechem Kit. The same allergens were used for skin tests and in specific IgE assays. The results were obtained with the two following tests: a negative control of allergens and a positive control with a histamine solution of 1 mg/ml. The result of the control test was considered to be positive if the diameter of the blister was 3 mm larger than that of a negative control or if some reaction occurred that was at least half the size of that with histamine. No skin diagnostic tests were performed on children with advanced skin symptoms and signs or those younger than 4 years old. The stool of each child was examined 3 times for parasites to exclude an infection.

The food allergy had been diagnosed when the children were 1–2 years old on the basis of positive (to 2 h) open controlled food challenge tests (performed 2–3 times), positive skin prick tests and increased blood serum level of specific IgE (>0.7 kIU/l). The presence of allergen-specific IgE antibodies was estimated with selected food allergens (e.g. proteins of cows milk, egg white, wheat flour, rye flour, soybean, codfish, pork, beef, chicken, chocolate, tomato, nuts including peanuts, celery, orange, kiwi) and aeroallergens (e.g. house dust mite *D. pteronyssinus* and *D. farinae*), molds and yeast, feathers, cat and dog epithelium, and pollens of Gramineae weeds and trees. All skin tests were made as prick tests by use of the commercial allergen solution of Allergopharma (3-ml solution: 5% glycerol, 0.9% sodium chloride, and 0.2% phenol; Joachim Ganzer KG, Reinbeck, Germany). The results were compared with negative controls (solvent of allergens only) and positive controls (1 mg/l histamine solution). The results of the controls were considered to be positive if the diameter of a blister was >3 mm larger than that of a negative control or if some reaction occurred that was at least half the size of that with histamine. On the basis of performed tests and studies, the children received diagnoses of both food allergy and other allergic diseases, such as atopic asthma, atopic dermatitis, allergic seasonal rhinitis and conjunctivitis.

Atopic asthma + atopic rhinitis and conjunctivitis + atopic dermatitis + food allergy were diagnosed in 11 cases, atopic asthma + atopic rhinitis and conjunctivitis + food allergy in 5 children, atopic asthma + atopic dermatitis + food allergy in 2 patients, atopic asthma + food allergy in 19, atopic rhinitis and conjunctivitis + food allergy in 25, atopic asthma + atopic rhinitis and conjunctivitis in 8, atopic asthma + atopic dermatitis in 2, atopic rhinitis and conjunctivitis + atopic dermatitis in 9, atopic asthma + atopic rhinitis and conjunctivitis + food allergy in 1, atopic asthma only in 30, atopic rhinitis and conjunctivitis only in 28, food allergy only in 77.

The children were classified into three groups as follows: children with atopic diseases (140 subjects); children with food allergy only (77 subjects); healthy children as control group (124 subjects).

Table 1. The distribution of FcγRIIa allotypes in control subjects and children with atopic diseases

FcγRIIa allotypes	Food allergy only			Atopic diseases			Controls		
	n	%	95% CI	n	%	95% CI	n	%	95% CI
RR131	13	16.9	9.3–27.1	28	20.0	13.7–27.6	27	21.8	15.4–29.8
RH131	37	48.1	36.5–59.7	71	50.7	42.1–59.3	59	47.6	39.0–56.3
HH131	27	35.0	24.5–46.8	41	29.3	21.9–37.6	38	30.6	23.2–39.2

Methods

FcγRIIa Genotyping

Genomic DNA was extracted manually (precipitation with trimethylammonium bromide salts) from leukocytes contained in 450 µl of venous blood with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant [13]. We performed 25-µl PCRs containing 2.5 µl of genomic DNA (approx. 100 ng), 2.5 µl of 10 × PCR buffer (contains 15 mM MgCl₂, Gibco BRL), 200 µM of dNTP (Gibco BRL), and 0.5 U of Taq polymerase (Gibco BRL). We used 0.5 µM H131-specific sense primer (5'-ATCCCAGAAATTCTCCCA-3') from the second extracellular domain or 0.5 µM R131-specific sense primers (5'-ATCCCAGAAATTCTCCCG-3') (all primers were synthesized by Gibco BRL) and 0.5 µM common antisense primer from an area of the downstream intron where the sequences for FcγRIIa, FcγRIIb, and FcγRIIc diverge (5'-CAATTTTGCTGCTATGGGC-3'). The resulting fragment was 253 bp in length. As internal control, we used 0.125 µM human growth hormone (HGH)-I primer (5'-CAGTG-CCTTCCCAACCATTCCCTTA-3') and 0.125 µM HGH-II primer (5'-ATCCAACGATTTCTGTTGTGTTTC-3'), which resulted in a 439-bp fragment. We used a thermal cycler (Mastercycler Eppendorf) to perform a hot-start PCR followed by 5 min at 95°C, 10 cycles of 1 min at 95°C, 2 min at 57°C, and 1 min at 72°C. Thereafter, to enhance the sensitivity, we used 22 cycles of 1 min at 95°C, 2 min at 54°C, and 1 min at 72°C and a final extension step for 5 min at 72°C. Each PCR analysis was performed with control samples for the R/R, H/R and H/H genotypes. The PCR amplification products were separated on 1.5% agarose and visualized by using ethidium bromide [9].

Statistical Analysis

Frequencies of FcγRIIa alleles were given with 95% confidence intervals (95% CI). The distribution of FcγRIIa alleles in patients with RA was compared with healthy subjects and statistically evaluated by use of the χ^2 test with Yates correction for small groups.

Results

The distribution of FcγRIIa genotypes in 124 Polish healthy subjects did not differ from that of Caucasian populations (table 1). The genotypes R/R131, H/R131 and H/H131 were found in 27 (21.8%), 59 (47.8%) and 38 (30.6%) subjects, respectively. The distribution of al-

leles were as follows: R allele 113 (45.6%), H allele 135 (54.4%).

For the group of 140 atopic children, the distribution was: R/R131 – 28 subjects (20.0%), H/R131 – 71 (50.7%) and H/H131 – 41 (29.3%); R allele 127 (45.4%), H allele 153 (54.6%) and there were not significant differences in comparison with the control group (table 1).

In the group of 77 children with food allergy only, there were 13 (16.9%) subjects with R/R131 genotype, 37 (48.1%) with H/R131 and 27 (35%) with H/H131. The distribution of alleles were as follows: R allele 63 (40.9%), H allele 91 (59.1%). There was no statistically significant difference in the distribution of FcγRIIa genotypes and alleles between atopic children, children with food allergy and the control group. With the sample size analyzed in the present study, the 90% probability to detect significant genotype distribution differences between atopic children and the control group occurs when there is at least 16.3% difference in the distribution of these genotypes in the general population.

To establish the homogeneity of the studied children, the Hardy-Weinberg equilibrium was calculated. In the control group (124 subjects) as well as in children with atopic diseases (140 cases) and in children with food allergy only (77 subjects), the FcγRIIa genotypes were normally distributed: $p = 0.95$ for the control group, $p = 0.99$ for atopic children, $p = 0.99$ for children with food allergy only.

In relation to FcγRIIa genotypes, atopic asthma was diagnosed in 13 (46.4%) children with the R/R131 genotype, in 41 (57.7%) with H/R131 and in 24 (58.5%) with genotype H/H131. The odds ratio for the risk of developing atopic asthma among individuals with the H/H131 genotype was 1.63 times greater than among subjects with genotype R/R131. The differences are not statistically significant (table 2). In relation to FcγRIIa alleles, atopic asthma was diagnosed in 52.8% of children with the R allele and 58.2% with the H allele. The odds ratio for the

Table 2. Diagnosed atopic diseases in relation to FcγRIIa allotypes

Children with diagnosed diseases	FcγRIIa allotypes						OR*	95% CI
	RR131 (n = 28)		RH131 (n = 71)		HH131 (n = 41)			
	n	%	n	%	n	%		
Atopic asthma	13	46.4	41	57.7	24	58.5	1.63	0.62–4.29
Rhinitis and conjunctivitis	12	42.9	53	74.6	22	53.7	1.54	0.59–4.06
Atopic dermatitis	4	14.3	10	14.1	10	24.4	1.94	0.54–6.93

OR = Odds ratio, i.e. the risk of developing disease in children with HH131 allotype vs. children with RR131 allotype.

H allele was 1.25 times greater than for the R allele. The differences are not statistically significant.

Rhinitis and conjunctivitis were diagnosed in 12 children (42.9%) with the R/R131 genotype, in 53 (74.6%) with genotype H/R131 and in 22 (53.7%) with genotype H/H131. The risk of rhinitis and conjunctivitis was 1.54 times greater for HH131 genotype in comparison with RR131 genotype (odds ratio 1.54) but not statistically significant (table 2). In relation to FcγRIIa alleles, atopic rhinitis and conjunctivitis were diagnosed in 60.6% of children with the R allele and in 63.4% with allele H. The odds ratio for the H allele is 1.12 times greater than for the R allele. The differences are nonsignificant.

Atopic dermatitis was diagnosed in 4 (14.3%), 10 (14.1%) and 10 (24.4%) children with genotypes R/R131, H/R131 and H/H131, respectively. The odds ratio for the risk of atopic dermatitis development in individuals with H/H131 genotype is 1.93 times greater than in subjects with genotype R/R131. The differences are not statistically significant (table 2). In relation to the FcγRIIa alleles, atopic dermatitis was diagnosed in 14.2% of children with the R allele and 19.6% with allele H. The odds ratio for the H allele was 1.48 times greater than for the R allele. The differences are not statistically significant.

With the sample size analyzed in the present study, there is a 90% probability to detect significant differences in atopic asthma, atopic rhinitis, conjunctivitis, and dermatitis diagnosis in relation to FcγRIIa genotypes when there is a difference of at least 31.5, 34.0 and 43.6% in the distribution of these genotypes in the general population, respectively; as concerns the FcγRIIa alleles, the differences in the general population should be at least 16.1, 15.7 and 24.5%, respectively.

Discussion

This study examined the hypothesis that FcγRIIa polymorphism may be a heritable factor influencing susceptibility to atopic disease. The distribution of FcγRIIa allotypes in the control population was similar to that obtained in other European populations. Comparing the distribution of FcγRIIa allotypes among children with atopic diseases and controls, there was no significant difference between these two groups. Also the frequency of diagnosed atopic diseases in FcγRIIa allotypes was similar. The study was carried out in a group of 140 atopic children comparing it with healthy children and children with food allergy only. In most cases, 2 or 3 atopic diseases were diagnosed; in some of cases the diagnosis of atopic disease was accompanied by a diagnosis of food allergy. The aforementioned group of atopic children seems to be sufficiently numerous and homogeneous to establish differences in FcγRIIa allotype distribution. With this sample size, the detection of differences in FcγRIIa allotype distribution with 90% probability between atopic and healthy children is possible if these differences in the general population are at least 16.3%. Moreover, the correlation between different atopic diseases and FcγRIIa allotypes was calculated. Although these groups were smaller, it does not seem that only some atopic diseases correlate with FcγRIIa allotypes. The immunologic background of different atopic diseases is similar and it is unlikely that FcγRIIa receptors play a role only in some atopic diseases.

The Fcγ receptors for IgG constitute a family of hemopoietic cell surface molecules that can stimulate cellular responses upon binding of antibody-antigen complexes and mediate antibody-dependent phagocytosis. Impaired

handling and removal of immune complexes by the mononuclear phagocyte system results in its deposition and in the occurrence of inflammatory processes in organs and tissues [6]. However, the underlying mechanism of such defective immune complex clearance and its relation to the observed clinical manifestation remain to be defined. Downregulation of FcγRIIa expression and functional effects on signal transduction and anti-Fcγ antibodies have been implicated as factors in aberrant FcγR functioning [5]. Indeed, there is increasing evidence that the FcγRIIa polymorphism is a factor for the manifestation of immune-complex-mediated diseases. The impaired phagocytosis of immune complexes seen in patients with systemic SLE can be correlated to the presence of the R/R 131 allele, but the H/H 131 allele was significantly more frequent in patients with heparin-induced thrombocytopenia [9, 10]. Several authors have evaluated the association between FcγRIIa polymorphism and susceptibility to infections, and the R/R131 allotype has been found to be a risk factor for recurrent bacterial respiratory infections [7].

The FcγR may also be involved in anaphylactic response. The ability of the immune system to respond appropriately to foreign antigen is dependent on a balance of activating and inhibitory signals. Recently, the role of cell surface inhibitory receptors in attenuating immune responses, thereby preventing pathologic conditions including autoimmunity and atopy, has been recognized

[14]. Unfortunately, the important role of FcγR in anaphylaxis has been shown in mice, but there are no available data showing the relevance of FcγRIIa receptors in anaphylaxis in humans. The anaphylaxis reaction in mice has been considered to be a typical immediate hypersensitivity response determined by the activation of mast cells via antigen-induced aggregation of an IgE-sensitized high-affinity receptor for IgE (FcεRI) causing the release of systemic mediators [15, 16]. Earlier observations indicated that the low-affinity FcR for IgG (FcγRIIb and FcγRIII) on mouse mast cells, macrophages, and rat mucosal type mast cell RBL 2H3 can bind IgE immune complexes in vitro and the engagement of FcγRII with IgE immune complexes triggers C57.1 mast cells to release serotonin, suggesting a greater potential complexity to the IgE-mediated anaphylactic response [14, 17]. In addition to modulating IgG-triggered hypersensitivity responses, FcγRII and III on mast cells are potent regulators of IgE-mediated responses and reveal the existence of a regulatory pathway for IgE triggering of effector cells through IgG FcR that could contribute to the etiology of the atopic response [18–21]. Although the evidence supporting a direct role for IgG and FcγR in the anaphylaxis reaction in mice is compelling, the contribution of the receptors to the IgE-mediated response in humans is generally believed to be minimal. It seems that FcγRIIa polymorphism does not represent an important genetic risk of developing atopic diseases.

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