

# FcγRIIIa-V/F 158 polymorphism in Turkish children with asthma bronchiale and allergic rhinitis

Zeyrek D, Tanac R, Altinoz S, Berdeli A, Gulen F, Koksoy H, Demir E. FcγRIIIa-V/F 158 polymorphism in Turkish children with asthma bronchiale and allergic rhinitis.

*Pediatr Allergy Immunol* 2008; 19: 20–24.

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Fc receptors (FcR) play an important role in immune regulation. This might be linked to the variability in immune response, therefore relating to the pathogenesis of atopic diseases. The aim of the present study was to evaluate the FcγRIIIa gene polymorphism in Turkish children with asthma and allergic rhinitis. The study included 364 atopic children (184 bronchial asthma, 180 allergic rhinitis) and 234 healthy subjects as the control group, aged between 5 to 16 years. Patients were recruited from outpatient clinics of allergy and general pediatric care. Plasma IgE concentrations were measured by immunoassays and skin prick test was done in children with atopic diseases. The FcγRIIIa gene polymorphism was determined using the polymerase chain reaction method. Distribution of V158V genotype was significantly different among patient groups compared to controls (for asthmatic children OR: 5.33, 95% CI: 2.80–10.23,  $p < 0.001$ ; for allergic rhinitis OR: 3.25, 95% CI: 1.75–6.07,  $p = 0.001$ ). Distribution of 158 V allele was significantly different among asthmatic children (OR: 2.20, 95% CI: 1.65–2.92,  $p < 0.001$ ) and allergic rhinitis patients (OR: 1.77, 95% CI: 1.32–2.35,  $p < 0.001$ ) compared to healthy controls. Our study shows that the V158V genotype in FcγRIIIa gene polymorphism may be a genetic risk factor for the development of atopic diseases.

**Dost Zeyrek<sup>1</sup>, Remziye Tanac<sup>1</sup>, Serdar Altinoz<sup>1</sup>, Afif Berdeli<sup>2</sup>, Figen Gulen<sup>1</sup>, Huseyin Koksoy<sup>1</sup> and Esen Demir<sup>1</sup>**

<sup>1</sup>Department of Pediatric Allergy and Pulmonology, Aegean University, <sup>2</sup>Molecular Research Laboratory, Aegean University, Izmir, Turkey

**Key words:** FcγRIIIa gene polymorphism; bronchial asthma; allergic rhinitis; children

Dost Zeyrek, Department of Pediatric Allergy and Pulmonology, Aegean University, Cocuk Klinigi Izmir, Bornova 35100, Izmir, Turkey  
Tel.: +903903500

E-mail: dost.zeyrek@ege.edu.tr

Accepted 13 January 2007

Allergic diseases are regarded as polygenic complex diseases and collective effects of multiple genetic and environmental factors are suggested to have a role in their development (1). Recent data on the genetics of atopic patients suggest a possible association of phenotype and treatment response variability with genetic polymorphism (2). Receptors on the Fc site of immunoglobulins maintain the information flow between cellular and humoral components of the immune system via different effector cells, thus playing an important role. Fc receptors of immunoglobulin G (IgG) (FcγR) initiate effector functions like phagocytosis, antibody-dependent cellular toxicity, superoxide production, degranulation, cytokine production and regulation of antibody synthesis, all of which

are essential for immune function (3). Fc gamma receptors are among the super family of immunoglobulins and they are divided into three groups: FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) (4). Related genes are located on chromosome 1 (5). Two isoforms of FcγRIII (CD16) have been described: FcγRIIIa (CD16a) and FcγRIIIb (CD16b) (6). FcγRIIIa is expressed in monocytes, macrophages, natural killer cells, and  $\gamma/\delta$  T cells (7). Two polymorphisms have been described for FcγRIIIa: first is the replacement of L with R or H at position 48, and the second is replacement of V with F at position 158. IgG1 and IgG3 binding affinity is higher for individuals with VV homozygous genotype compared with FF homozygous genotype (8).

In several studies, risk of infections and autoimmune diseases was found to be associated with FcγRIIIa gene polymorphism (9, 10); however, to the best of our knowledge, its relation with atopic disease has not been reported. This study aimed to investigate the relationship between FcγRIIIa gene polymorphism and atopic diseases.

### Materials and methods

This study included 364 children with IgE-mediated bronchial asthma and allergic rhinitis. The control group comprised 234 healthy subjects. The inclusion criteria were as follows:

- patients with allergic rhinitis or bronchial asthma;
- a positive specific IgE (spIgE) against respiratory allergens;
- a positive skin-prick test (SPT) against respiratory allergens.

### Diagnosis of allergy

*Evaluation of clinical status.* The asthmatic patients were evaluated according to Global Initiative for Asthma (GINA) criteria (11) and allergic rhinitis patients were evaluated according to symptom scoring system (12, 13). Evaluation of subjects included routine serum biochemical analysis and allergic workup based on the following laboratory determinants: eosinophilic granulocyte count in the peripheral blood, serum total IgE level, specific IgE levels against some inhaled allergens, and SPT. 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 using the enzyme immune assay method by using a 3M Diagnostic System Kit (Pharmacia, Uppsala, Sweden). Total IgE levels were related to the age-specific limits provided by the manufacturer. Values that exceeded 100 kU/l were considered to be high. The presence of allergen-specific IgE antibodies was estimated with selected aeroallergens, i.e. house dust, ascarids (*Dermatophagoides pteronyssinus* and *D. farinae*), feathers, cat and dog hair and pollens of Gramineae, weeds, and trees. Pharmacia CAP system was used; 0.35 kU/l was regarded as positive.

*Skin-prick test.* For all patients, SPT was performed by a pediatric allergy specialist. Standard respiratory allergen panel included tree pollen, grass pollen, dust mite, fungus,

animal dander, grains pollen, wild grass, flower pollen, and latex (Stallergenes S.A., Antony-France). Results were evaluated according to European Academy of Allergology and Clinical Immunology (EAACI) criteria (14). Control patients were evaluated with regard to chronic and/or severe infections, rheumatological and autoimmune disorders, and familial and personal history of atopy, and with laboratory tests. Children were included in the control group if they had no personal and familial history of atopy and no signs of atopic disorder, and if they were negative for spIgE.

### Exclusion criteria

Children with recurrent respiratory tract infections, bronchiolitis obliterans, and chronic rhinopharyngitis were excluded from the study. Patients with clinical and laboratory findings of other chronic systemic diseases or immune deficiency were also excluded.

### DNA purification

Laboratory personnel examining the blood samples were blinded to the study groups. Genomic DNA of patients and healthy controls was extracted from peripheral blood leukocytes with QIAamp DNA Blood Mini Kits (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions.

*FcγRIIIa genotyping.* FcγRIIIa genotyping was performed by using the method of van den Berg et al. (26). In this amplification refractory mutation system-polymerase chain reaction (PCR), the allele-specific primers [KIM-G (V): 5'-TCTCTGAAGACACATTCTACTCCCTA-C-3' and KIM-1 (F): 5'-TCTCTGAAGACACATTCTACTCCCTAA-3'] amplified a 160-bp fragment with their antisense downstream primer A013 (5'-ATATTTACAGAATGGCACAGG-3'). The control primers were Ctrl-1 and Ctrl-1, amplifying a 270-bp piece of DNA. Again, two PCR procedures were required for genotyping of a subject. Reactions were performed with 175 ng of genomic DNA in a 25-μl reaction volume, containing 2.5 μl of PCR Gold buffer 62.5 nmol MgCl<sub>2</sub>, 1.25 nmol each of the four dTNPs, 11.9 pmol each of the two control primers and 0.5 U Ampli Tag Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA). PCR conditions were denaturation for 10 min at 95°C; 32 cycles at 95°C for 30 s, 57°C for 20 s, 72°C for 25 s, and a final extension step at 72°C for 7 min.

To validate accuracy and reproducibility of the results obtained using the above-described techniques, we randomly ran 20% of samples from both groups (study and control groups) for FcγRIIIa genotypes by using direct DNA sequencing technique with ABI PRISM 310 Genetic Analyzer System (Applied Biosystems).

**Ethics.** Parents of all patients signed informed consent forms and the Ethics Committee of the Hospital approved the study.

#### Statistical analysis

Statistical analysis of the data was performed with SPSS 11.0 computer program. The mean age of both groups and total IgE levels between V158V and F158F genotypes were compared by Student's *t*-test. Genotypic distribution and allelic frequencies were statistically defined by the chi-squared test. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated

using the same software;  $p < 0.05$  is accepted as statistically significant.

#### Results

The characteristics of the study population are given in Table 1. Genotypic distributions of this case-control study did not deviate significantly from the Hardy-Weinberg equilibrium expectations in each group ( $p > 0.05$ ). Genotype frequency of FcγRIIIa in the asthmatic children and control group are presented in Table 2. V158V genotype (34.8%) was significantly more frequent in asthmatic children compared with the control group (17%) (OR 5.33, 95% CI 2.80–10.23,  $p < 0.001$ ).

Genotype frequency of FcγRIIIa in the allergic rhinitis group and the control group are presented in Table 3. V158V genotype was significantly more frequent in the allergic rhinitis group (29%) compared with the control group (17%) (OR 3.25, 95% CI 1.75–6.07,  $p < 0.001$ ).

The distribution of alleles were as follows: 158F allele 144 (39.1%), 158V allele 224 (60.9%)

Table 1. Characteristics of the study and control groups

|                                    | Study group<br>(n = 364) | Control group<br>(n = 234) | p-value |
|------------------------------------|--------------------------|----------------------------|---------|
| Diagnosis                          |                          |                            |         |
| Asthma (%)                         | 184 (50.5)               |                            |         |
| Allergic rhinitis (%)              | 180 (49.5)               |                            |         |
| Age, years ± SD (range)            | 11.4 ± 3.4 (5–16)        | 10.9 ± 2.6 (6–16)          | 0.06    |
| Gender                             |                          |                            |         |
| Female                             | 142                      | 100                        | 0.52    |
| Male                               | 222                      | 134                        |         |
| IgE (kU/l), mean ± SD              | 420.7 ± 443              | 33.6 ± 17.4                | <0.001  |
| Eosinophils (per mm <sup>3</sup> ) | 364 ± 213.4              | 138 ± 102.8                | 0.001   |

Table 4. The FcγRIIIa alleles in asthmatic and allergic rhinitis children and controls

| FcγRIIIa alleles | Asthma group, n (%)       | Control group, n (%) | OR (95% CI)      | p-value |
|------------------|---------------------------|----------------------|------------------|---------|
| F                | 144 (39.1)                | 274 (58.5)           | 2.20 (1.65–2.93) | <0.001  |
| V                | 224 (60.9)                | 194 (41.5)           |                  |         |
|                  | All rhinitis group, n (%) | Control group, n (%) |                  |         |
| F                | 160 (47)                  | 274 (58.5)           | 1.77 (1.32–2.35) | <0.001  |
| V                | 200 (53)                  | 194 (44.5)           |                  |         |

Table 2. The FcγRIIIa allotypes in asthmatic children and controls

| FcγRIIIa genotypes | Asthma group observed, n (%) | Asthma group expected, n* | Control group observed, n (%) | Control group expected, n* | OR   | 95% CI     | p-value |
|--------------------|------------------------------|---------------------------|-------------------------------|----------------------------|------|------------|---------|
| FF                 | 24 (13)                      | 28                        | 80 (34.3)                     | 77                         | 1    | 0.10–0.36  | 1       |
| FV                 | 96 (52.2)                    | 90                        | 114 (48.7)                    | 120                        | 2.81 | 1.60–4.95  | 0.001   |
| VV                 | 64 (34.8)                    | 66                        | 40 (17)                       | 37                         | 5.33 | 2.80–10.23 | <0.001  |

\*Hardy-Weinberg expectancy.

Table 3. The FcγRIIIa allotypes in children with allergic rhinitis and controls

| FcγRIIIa genotypes | All rhinitis group – observed, n (%) | All rhinitis group – expected, n* | Control group – observed, n (%) | Control group – expected n* | OR   | 95% CI    | p-value |
|--------------------|--------------------------------------|-----------------------------------|---------------------------------|-----------------------------|------|-----------|---------|
| FF                 | 32 (17.7)                            | 34                                | 80 (34.3)                       | 77                          | 1    | 0.16–0.56 | 1       |
| FV                 | 96 (53.3)                            | 92                                | 114 (48.7)                      | 120                         | 2.11 | 1.25–3.55 | 0.002   |
| VV                 | 52 (29)                              | 54                                | 40 (17)                         | 37                          | 3.25 | 1.75–6.07 | <0.001  |

\*Hardy-Weinberg expectancy.

Table 5. Atopy in the family, eosinophilia and total IgE levels in V158V and F158F genotypes

|                                 | V158V genotype (n = 156) | F158F genotype (n = 136) | OR (95% CI)      | p-value |
|---------------------------------|--------------------------|--------------------------|------------------|---------|
| Atopy in the family (%)         | 94 (60.2)                | 41 (30.1)                | 2.00 (1.27–3.16) | 0.001   |
| Total IgE (mg/dl),<br>mean ± SD | 384.7 ± 528.3            | 191.4 ± 259.7            |                  | 0.006   |
| Eosinophilia (%)                | 84 (53.8)                | 34 (25)                  | 2.15 (1.33–3.59) | 0.0009  |

in asthmatic children and 158F allele 160 (47%), 158V allele 180 (53%) in allergic rhinitis. 158V allele was more frequent among asthmatic children and children with allergic rhinitis, compared with the control group (OR 2.20, 95% CI 1.65–2.92,  $p < 0.001$  and OR 1.77, 95% CI 1.32–2.35,  $p < 0.001$  respectively) (Table 4). Total IgE levels were significantly higher, and eosinophilia and familial history of atopy were significantly more frequent among patients with V158V genotype (Table 5).

## Discussion

The Fcγ receptors for IgG constitute a family of hematopoietic cell-surface molecules that can stimulate cellular responses upon binding of antibody–antigen complexes and mediate antibody-dependent phagocytosis (16). Mast cells and basophils also express low-affinity and, under specific conditions, high-affinity IgG receptors. When co-engaging these receptors with FcεRI, IgG antibodies can amplify or dampen IgE-induced mast cell activation (17). The FcγR may also be involved in anaphylactic response. The ability of the immune system to respond appropriately to foreign antigen depends 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 (18). The important role of FcγR in anaphylaxis has been shown in mice; unfortunately, there are no available data showing the relevance of FcγR receptors in human anaphylaxis. 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 (FcγRI), causing the release of systemic mediators (19, 20). 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 com-

plexes triggers C57.1 mast cells to release serotonin, suggesting a greater potential complexity for the IgE-mediated anaphylactic response (18, 21). 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 (22, 23). In a small number of studies examining the association between atopic diseases and FcγR, FcγRIIB<sup>−/−</sup> mice were found to be more sensitive to allergic rhinitis (24), but no relation was found with FcγRIIA polymorphism in children with atopic disease (25).

This study examined the hypothesis that FcγRIIA polymorphism may be a heritable factor influencing susceptibility to atopic disease in Turkish children. V158V allele was significantly more frequent both in patients with allergic rhinitis and bronchial asthma compared with controls. In addition, higher frequency of familial atopy history and eosinophilia, and increased total IgE levels were found among patients having the V158V allele, supporting an increased tendency for this genotype to atopy. In conclusion, FcγRIIA gene polymorphism may be a genetic risk factor for the development of atopic diseases.

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