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# LACK OF AN ASSOCIATION OF THE GLU237GLY POLYMORPHISM IN THE GENE FOR THE FCε RECEPTOR β-SUBUNIT WITH ATOPIC DISEASES IN A CZECH POPULATION

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A b s t r a c t

The high-affinity IgE receptor (FcεRI) plays a central role in degranulation of mast cells and basophils. This case-control study investigated a possible association of the Glu237Gly polymorphism in exon 7 of the gene for the β-subunit of this receptor with a predisposition to atopy in the Czech population. It included 157 patients (75 men and 82 women, aged 31±15 years) with a history of one or more atopic conditions, i.e., asthma, allergic rhinitis or atopic dermatitis, and 77 healthy controls (40 men and 37 women, aged 40±15 years). The Glu237Gly polymorphism was detected by means of PCR with a subsequent restriction analysis using the XmnI enzyme. Out of 234 subjects examined, only one patient was found to display an amino acid substitution (glutamic acid replaced by glycine) at position 237, which gave rise to a heterozygous combination of Gly237/Glu237. All the other subjects were homozygotes with a Glu237/Glu237 combination. Therefore, there was no reason for evaluating the statistical significance of differences between the patient and the control group.

Our results clearly demonstrate that this mutation cannot be considered to be a polymorphism in our population. In some populations, this variant occurs more frequently and is associated with atopy, bronchial hyper-responsiveness, or a clinical manifestation of atopic asthma. However, the majority of recent studies, in agrreement with our results, have argued against an association of the Glu237Gly polymorphism with the development of atopy or asthma. These differences may be related to the effect of different environmental factor, particularly to the involvement of various allergens and parasitic infections which induce IgE-mediated immune reactions.

K e y  w o r d s

polymorphism, atopy, asthma, IgE, receptor

## INTRODUCTION

Atopy is defined as an inherited tendency towards an immune response characterised by a long-term production of IgE antibodies against numerous, commonly occurring allergens to which the organism is exposed *(1)*. Although the number of atopic patients in our population is estimated to be between 25 and 30 %, clinical manifestations of allergy become apparent only in some of them, most frequently as allergic rhinitis or bronchial asthma. Atopic dermatitis and allergic gastroenteropathy are less frequent. One or more clinical signs can be present during the course of or at different stages of an allergic disease in one patient.

Atopic diseases have a genetic background, with participation of several genes, combined with external environmental factors. The gene for the high-affinity IgE receptor β-subunit, which is localised on chromosome 11q13, can be regarded as one of the candidate genes *(2).* The high-affinity IgE receptor is present mainly on the surfaces of mast cells and basophils, but also on monocytes and Langerhans' cells. It consists of four subunits: α and β and two γ chains linked with disulphine bonds. About 20 % of the receptors are of FcεRIαβ$(\gamma)_2$ phenotype and the rest (about 80 %) are expressed as FcεRIαβ$(\gamma)_2$. The existence of a receptor lacking the β-subunit was described for Langerhans' cells. The β-subunit apparently takes part (by a mechanism which is not yet clear) in activation or release of some preformed mediators which are present in the cell granules of mast cells but not in Langerhans' cells *(3).* β and γ subunits contain the immunoreceptor tyrosine-based activation motif (ITAM), which is a tyrosine phosphorylation site essential for signal transduction and cell activation. The receptor activation eventually results in inducing degranulation of the cells. Considering the key role of this receptor in the degranulation of mast cells and basophils, it can be assumned that mutations in the genes coding for the IgE receptor influence the development of early hypersensitivity reactions in atopic persons.

Several polymorphic sites have so far been found within the gene region coding for the β-subunit: Rsa I and CA repetitions (in introns), Rsa I (in exon 7), Leu 181/183 (in exon 6) and Glu237Gly (in exon 7). However, only some of them are associated with atopic diseases or with some parameters of atopic predisposition *(4, 5, 6, 7).*

Our investigation was concerned with the Glu237Gly polymorphism. A single-nucleotide substitution of adenine for guanine at position 6843 causes

*Table 1*
Demographic data

| Groups | Age (years ±SD) | | |
|---|---|---|---|
| Controls (n = 77) | 40±15 | Men (n = 40) | 37±15 |
| | | Women (n = 37) | 42±15 |
| Atopic patients (n = 157) | 31±15 | Men (n = 75) | 26±13 |
| | | Women (n = 82) | 34±16 |

a replacement of glutamic acid (Glu) by glycine (Gly) at position 237 which is in the vicinity of ITAM. This substitution increases hydrophobic properties of the C-end of the amino acid chain and can thus influence signal transduction into the cell and lead to the development of early hypersensitivity in atopic persons.

The aim of our study was to test the hypothesis that atopic asthma, or other atopic diseases, can be associated with the Glu237Gly polymorphism in the β-subunit of the FcεRI gene.

## MATERIALS AND METHODS

*Subjects and diagnostic methods*

A group of 157 patients with symptoms and signs of atopic asthma, allergic rhinitis or atopic dermatitis (75 men and 82 women, aged 31±15 years) and 77 healthy controls (40 men and 37 women, aged 40±15 years) were included in the study *(Table 1)*.

In accordance with widely used clinical criteria *(8, 9)*, atopy was defined by the presence of at least one three criteria:

1) Positive skin prick test (diameter of 3 mm or larger than in a negative control) after administration of one or more of the following common aeroallergens: house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), common grass and tree pollens, animal fur extracts (cat and dog dandruff), and common moulds (*Alternaria*, *Cladosporium* and *Aspergillus*). Histamine was used as a positive control and physiological solution as a negative control.

2) Total serum IgE level above the normal values (more than 150 IU/ml in non-smoking adults). Total serum IgE levels were measured by an immunoturbidimetric test (Boehring).

3) Raised specific serum IgE levels >0.35 kU/l, ELISA, DPC Germany) produced in response to one or more of the common antigens.

Asthma was diagnosed according to the criteria of the American Thoracic Society *(10)* and atopy by the tests mentioned above. Atopic dermatitis was defined according to the major and minor criteria proposed by *Hanifin and Rajka* in *1980 (11)*. Subjects with allergic rhinitis had atopy, and the symptoms of either hay fever or the whole year's rhinitis, as confirmed by a specialist before they were included in the study.

All the subjects gave their written informed consent to participation in the study. The study was approved by the Committee for the Ethics of Medical Experiment on Human Subjects at the Faculty of Medicine, Masaryk University in Brno.

*Table 2*
Polymorphisms in exon 7 of the gene coding for the IgE receptor β-subunit and their distribution in our sample

| Genotypes | Healthy controls N = 77 | Atopic patients N = 157 |
|---|---|---|
| Glu237Glu | 77 | 156 |
| Glu237Gly | 0 | 1 |
| Gly237Gly | 0 | 0 |

*Polymorphism determination*

The Glu237Gly polymorphism was detected by means of the polymerase chain reaction (PCR) with a subsequent restriction analysis using the Xmnl enzyme. Genomic DNA was prepared from peripheral blood leukocytes and used as a template in PCR according to *Shirakawa et al., 1996 (6).* The reaction was performed in a final volume of 50l containing 0.5μmol of each primer, 3.2 mmol/l $MgCl_2$, 200μmol of each dNTP and 0.8 U Taq polymerase (Biogen). The DNA was amplified in 36 cycles with the initial denaturation at 97°C for 2 min, annealing at 50°C for 1 min and extension at 58°C for 1 min. This was followed by 35 cycles with denaturation at 94°C, 1 min, annealing 50°C, 1 min, extension at 58°C, 1 min, and a final extension at 58°C for 5 min. PCR products were cleaved with the Xmnl restriction enzyme at 37°C for 16 to 20 h. Fragments were separated by electrophoresis in 3 % agarose gel and visualised with ethidium bromide in UV light. Based on the detected lengths of the fragments, each sample was designated as one of the following three genotypes:

Gly237/Gly237 homozygote, a fragment of 103 bps (without the target sequence for Xmnl enzyme) which was found in neither the analysed sample nor the literature.

Gly237/Glu237 heterozygote, fragments of 103, 80 and 23 bps

Glu237/Glu237 homozygote, fragments of 80 and 23 bps.

## RESULTS

Although we examined 234 persons, we found only one patient to have an amino acid substitution (glutamic acid replaced by glycine) at position 237, which gave rise to a heterozygous combination of Gly237/Glu237. For this reason, we did not test the statistical significance of differences in allelic frequencies or genotype combinations between the patient and control groups. The distribution of genotypes is shown in *Table 2.*

## DISCUSSION

The Gly237 allele has been found in an Australian population at a frequency of 5.3 *% (5)* and in a Japanese population at a frequency of 6 *%. (6)* In both studies, the presence of Gly237 was strongly associated with bronchial asthma (P=0.005) and, in the Australian population, also with bronchial hyper-responsiveness (BHR), (P=0.009). The Gly237 allele also occurs in the population of Great Britain at a frequency of about 3.5 % and in the Italian population at 4 *% (12).* An analysis of the data on the British population has revealed only a weak association of this allele with BHR (P=0.02), but a significant association with the development of bronchial asthma (P=0.009) and the manifestation of atopic predisposition (P=0.0001). *Green et al., 1998 (13)* reported a significant difference in the frequency of the Glu237Gly polymorphism between black and white populations in the Republic of South Africa. This variant prevailed in the black population. In view of the statistically significant association of the Gly237Glu with BHR (even in the absence of atopy), other authors have speculated on its possible influence on the development of more severe stages of asthma or a higher mortality rate in the black than the white populations *(14).*

In light of these inconsistent reports, we designed a study to investigate whether a similar distribution of the Glu237Gly polymorphism can also be found in other populations and whether a similar association between this mutation and a predisposition to atopic diseases exists in the Czech population. In a group of 157 patients with different manifestations of atopic disease (bronchial asthma, allergic rhinitis or atopic dermatitis) and in 77 healthy controls, we examined samples of their DNA for the presence of the Glu237Gly polymorphism. However, this genotype was identified only in one patient with allergic rhinitis. He was a man with a family history of atopic disease and a personal medical history of allergic rhinoconjunctivitis of seasonal nature, without other associated diseases. The laboratory findings revealed only moderately increased levels of total IgE (between 300 and 400 IU/ml) and positive skin prick tests for grasses and spring pollen.

The data so far published on the occurrence of this variant in different populations *(5,7,14)* suggest a significant influence of the geographic location, with an apparent trend for this variant to decline from south to north and, perhaps, from east to west. The highest frequency is recorded in populations with a high incidence of parasitic diseases (Africa, Australia), while a substantially lower occurrence is in European countries (Great Britain, Italy). The polymorphism discussed here also appears to be race-related. In our population, at least on the basis of the results presented here, this phenomenon cannot be regarded as a polymorphism (i.e., mutation in which the frequency of the less frequent allele is lower than 1 %). This can be due to partly the geographic location (Central Europe *vs.*maritime countries such as Great Britain and Italy) and partly the stronger homogeneity of the Czech population. Our results allow us to draw a conclusion that the Glu237Gly polymorphism does not contribute significantly to the genetic risk of developing a predisposition to atopy in the Czech population. Positive associations observed in different geographic regions can simply express the genetic heterogeneity of atopy and asthma, and may relate to differences in the effects of various environmental factors. However, the recent studies from countries representing a variety of nationalities, which are in agreement with our results, have not confirm any association of the Glu237Gly polymorphism with the development of atopy or asthma *(7, 15, 16, 17).*

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## CHYBĚJÍCÍ ASOCIACE GLU237GLY POLYMORFISMU GENU PRO β-PODJEDNOTKU FCε RECEPTORU S ATOPICKÝMI NEMOCEMI V ČESKÉ POPULACI

S o u h r n

Vysokoafinitní receptor pro IgE (FcεRI) hraje centrální roli v degranulaci žírných buněk a bazofilů. Práce studuje možnou asociaci polymorfismu Glu237Gly v 7. exonu genu pro β-podjednotku tohoto receptoru s atopickou predispozicí v české populaci. Použili jsme metodu „case-control". Studovali jsme 157 pacientů s anamnézou atopického asthmatu, alergické rhinitidy, atopické dermatitidy nebo jejich kombinací (75 mužů a 82 žen, věk 31±15 let) spolu se 77 zdravými kontrolami (40 mužů a 37 žen, věk 40±15 let). K detekci Glu237Gly polymorfismu jsme použili PCR metodu s následnou restrikční analýzou enzymem XmnI. Přestože jsme vyšetřili 234 osob, pouze u jednoho pacienta jsme prokázali záměnu aminokyseliny (glutamové kyseliny za glycin) v pozici 237, což vedlo k heterozygotní kombinaci Gly237/Glu237, ostatní osoby byly homozygoti s kombinací Glu237/Glu237. Statistickou vžznamnost rozdílu mezi pacienty a kontrolní skupinou nemělo proto smysl testovat.

Ze získaného výsledku je patrné, že v naší populaci lze stěží pokládat tuto mutaci za mutaci vytvářející polymorfismus. V některých populacích se tato varianta vyskytuje častěji a byla asociována s atopií, případně bronchiální hyperreaktivitou nebo klinickou manifestací atopického asthmatu. Většina prací z poslední doby však v souhlasu s našimi výsledky vztah polymorfismu Glu237Gly k rozvoji atopie či asthmatu popírá. Rozdíly mohou souviset s variabilitou zevního prostředí, zvláštně co se týká zastoupení různých alergenů a parazitárních infekcí, u nichž se uplatňují imunitní reakce zprostředkované IgE.

REFERENCES

1. *Stites DP, Terr AI.* Základní a klinická imunologie /Basic and Clinical Immunology/. 1991, Victoria Publishing: 49–72.
2. *Sandford AJ, Shirakawa T, Moffatt MF, Daniels SE, Ra C, Faux JA, Young RP, Nakamura Y, Lathrop GM, Cookson WOCM, Hopkin JM.* Localisation of atopy and β subunit of high-affinity IgE receptor (FcεRI) on chromosome 11q. Lancet 1993;341: 332–334.
3. *Jürgens M, Wollenberg A, Hanau D, de la Salle H, Bieber T.* Activation of human epidermal Langerhans cells by engagement of the high affinity receptor for IgE, Fc epsilon RI. J Immunol 1995;155: 5184–9.
4. *Shirakawa T, Mao XQ, Sasaki S, Enomoto T, Kawai M, Morimoto K, Hopkin J.* Association between atopic asthma and coding variant of FcεRIβ in a Japanese population. Hum Mol Genet 1996;5: 1129–1130.
5. *Hill MR, Cookson WOCM.* A new variant of the subunit of the high-affinity receptor for Immunoglobulin E (FcεRIβ- E237G): association with measures of atopy and bronchial hyper-responsiveness. Hum Mol Genet 1996;5: 959–962.
6. *Shirakawa T, Mao XQ, Sasaki S, Kawai M, Morimoto K, Hopkin JM.* Association between Fc epsilon RI beta and atopic disorder in a Japanese population. Lancet 1996;347: 394–395.
7. *Rohrbach M, Kraemer R, Liechti-Gallati S.* Screening of the Fc epsilon RI-beta gene in a Swiss population of asthmatic children: no association with E237G and identification of new sequence variations. Dis Markers 1998;14: 177–86.
8. *Holla L, Vasku A, Znojil V, Siskova L, Vacha J.* Association of 3 gene polymorphisms with atopic diseases. J Allergy Clin Immunology 1999;103 (4): 702–708.
9. *Holla L, Vasku A, Jurajda M, Znojil V.* Polymorphism of the endothelin-1 and angiotensinogen genes in atopic diseases. Scripta Medica 1998 71: 341–350.
10. American Thoracic Society. Standards for the *diagnosis* and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Am Rev Respir Dis 1988 136: 225–244.
11. *Hanifin JM, Rajka G.* Diagnostic features of atopic dermatitis. Acta Derm Venereol (Stockh) 1980 92: 44–47.

12. *Trabetti E, Cusin V, Malerba G, Martinati LC, Casartelli A, Boner AL, Pignatti PF.* Association of the FcepsilonRIbeta gene with bronchial hyper-responsiveness in an Italian population. J Med Genet1998;35: 680–1.
13. *Green SL, Gaillard MC, Song E, Dewar JB, Halkas A.* Polymorphisms of the beta chain of the high-affinity immunoglobulin E receptor (Fcepsilon RI-beta) in South African black and white asthmatic and nonasthmatic individuals. Am J Respir Crit Care Med 1998;158: 1487–92.
14. *Walls RS, Ordman LJ.* Presentation of patients with asthma at an allergy clinic in Cape Town. S.A.M.J 1983;64: 942–945.
15. *Wilkinson J, Thomas NS, Morton A, Holgate ST.* Candidate gene and mutational analysis in asthma and atopy. Int Arch Allergy Immunol 1999; 118: 265–7.
16. *Ishizawa M, Shibasaki M, Yokouchi Y, Noguchi E, Arinami T, Yamakawa-Komayashi K, Matsui A, Hamaguchi H.* No Association between atopic asthma and coding variant of Fc epsilon R1 beta in a Japanese population. J Hum Genet 1999; 44: 308–11.
17. *Dickson PW, Wong ZY, Harrap SB, Abramson MJ, Walters EH.* Mutational analysis of the high affinity immunoglobulin E receptor beta subunit gene in asthma. Thorax 1999; 54: 409–12.