Genetic Variability of the High-affinity IgE Receptor \( \alpha \) Subunit (Fc\( \varepsilon \)RI\( \alpha \)) is Related to Total Serum IgE levels in Allergic Subjects

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**ABSTRACT**
Known susceptibility genes to atopy and asthma have been identified by linkage or associations with clinical phenotypes, including total serum IgE levels. IgE-mediated sensitivity reactions require a high-affinity IgE receptor (Fc\( \varepsilon \)RI), which immobilizes the immunoglobulin on the surface of the effector cells, mostly mast cells and basophils. In this mini-review, recent findings are presented on genetic variation of this receptor, as related to atopy. Transcription of FCER1A gene encoding the receptor \( \alpha \) subunit can be initiated from two separate promoters, the proximal one and the distal one, which results in a transcript containing two novel untranslated exons (1A, 2A). Our knowledge on the role of this mechanism in allergic diseases is still at an infancy stage. Within regulatory elements of FCER1A some common single nucleotide polymorphisms have functional associations, which were recently reported and replicated in different ethnical groups. Interestingly, these associations do not confer susceptibility to allergic diseases, but rather modulate serum concentrations of IgE. Similarly to the previously investigated \( \beta \) subunit of the receptor, FCER1A is a good candidate for a quantitative trait locus (QTL) in allergic diseases, and appears to participate in the systemic regulation of IgE levels.

**KEY WORDS**
allergy, FCER1A, Fc\( \varepsilon \)RI\( \alpha \), genetic polymorphism, IgE, omalizumab

**INTRODUCTION**
Allergic diseases, such as asthma or atopic dermatitis, run in families suggesting the presence of a common genetic predisposition. Genomic studies are frequently conducted in asthma and other allergy-associated diseases, with the aim of developing a better understanding of underlying processes.\(^{1,2}\) Many genetic loci and defined genes have been associated with the etiology of allergic disorders.\(^1\) One of the pioneering discoveries in allergy-related genetics was the association between atopy and the 11q13 genetic locus.\(^{1,3,5}\) Subsequent research revealed that the 11q13 genetic locus contains among others the FCER1B gene, encoding for the high-affinity IgE receptor (Fc\( \varepsilon \)RI) \( \beta \) chain (Fc\( \varepsilon \)RI\( \beta \)).\(^{4,5}\)

**HIGHER-ORDER IgE RECEPTOR STRUCTURE AND FUNCTION**
FceRI is expressed mainly on the surface of mast cells, basophils, dendritic cells, Langerhans cells and monocytes.\(^{5,9}\) Binding of the polyvalent antigens to specific IgE molecules occupying FceRI on basophils and mast cell membranes is an initial step in the sequence of the atopic reaction.\(^{7,13}\) On these cells, FceRI has a heterotetrameric form and consists of an FceRI\( \beta \) and \( \alpha \) subunit (FceRI\( \alpha \)), and two \( \gamma \) chains (FceRI\( \gamma \)), that build the \( \alpha \beta \gamma \) complex (Fig. 1).\(^{7,8,11,14}\) In humans but not in mice, the trimeric molecule of the receptor also exists, with one FceRI\( \alpha \) and two FceRI\( \gamma \) chains comprising the \( \alpha \gamma \) complex.\(^{7,8,14}\) FceRI\( \alpha \) is the receptor subunit responsible for the IgE binding. It has two extracellular immunoglobulin-like domains, a transmembrane hydrophobic region,
and a positively-charged short cytoplasmic tail. (Fig. 1).7,10-12,14 The remaining FcεRI subunits, arranged in a βγ complex, are responsible for signal transduction across the cell membrane. FcεRIβ has four transmembrane domains and two cytoplasmic tails (Fig. 1). FcεRIγ has a single transmembrane domain and a cytoplasmatic tail. Intracellular components of FcεRI undergo protein phosphorylation upon signaling at the characteristic immunoreceptor tyrosine-based activation motifs (ITAM) (Fig. 1).7,8,13,14 IgE is produced by B cells in the spleen, lymph nodes, and locally. Recently demonstrated local tissues, e.g., sinus mucosa, may be populated by IgE producing cells at a much higher ratio following allergen exposure.15 Despite common beliefs, stability of circulating IgE is similar to IgG. There is only binding to FceRI, which increases the half-life of this immunoglobulin, due to a high avidity and low dissociation constant of such a complex.16

**GENETICS OF HIGH-AFFINITY IgE RECEPTOR SUBUNITS**

Genetic association studies on FCER1B variability were animated by the cloning of the gene at locus 11q13,3,5 formerly linked with allergy-related disorders or atopy measures, such as bronchial hyperreactivity. Many reports published during the last decade dealt with the association between FCER1B gene polymorphic variants and allergic disorders. These findings were in general replicated within several ethnic groups, encompassing Caucasians, Japanese, Chinese and South Africa Blacks. Variants of the FCER1B gene have been associated with asthma,17,18 atopy,19,20 bronchial hyperreactivity,19 serum IgE levels17 and atopic dermatitis.5

Likewise, the FcεRIγ encoding gene (FCER1G) was screened for the presence of genetic variants. In the study on systemic lupus erythematosus, the gene turned out to be conservative in its coding sequence both within patients and healthy controls.21

Genetic variability of the gene encoding for FcεRIα (FCER1A) was previously out of the focus of studies and until quite recently only two relevant papers were published.8,22,23 This is surprisingly in contrast with many investigations on structural and functional properties of the FCER1A gene.24-33

**STRUCTURE AND FUNCTION OF THE GENE ENCODING FOR HIGH-AFFINITY IgE RECEPTOR α SUBUNIT (FCER1A)**

FCER1A gene is localized on chromosome 1q23. Originally, FCER1A gene had been described as consisting on five exons (Ex1–5), and its expression being regulated by a single promoter (Fig. 2).24-26 By FCER1A transcripts studies, presence of two additional FCER1A exons remotely localized at 12,000 bps (Ex2A) and 18,000 bps (Ex1A) upstream to the coding sequence was discovered (Fig. 2).28,32 Initially, the Ex1A sequence of 9 nucleotides was determined, however, it was still expected to extend further upstream.28 Indeed, subsequent studies revealed the Ex1A to be much longer (335 bp).32 Two alternative FCER1A transcription start sites were also identified, both within the Ex1A neighbourhood.32 This was accompanied by a discovery of a supplementary (‘distal’) promoter region.32 Due to this alternative FCER1A gene promoter, the one formerly known and localized just upstream of translated Ex1 was renamed the ‘proximal’ one (Fig. 2). Thus, FCER1A gene expression is controlled by two different promoter regions.29,31-33 In mast cells, under normal conditions, FCER1A gene transcription is regulated by the proximal promoter29,31-33 this requires some transcription factors, namely: GATA-1 and PU.1, as well as YY1 and Elf-1.27,29,31,33 In mast cells, eosinophils and monocytes FcεRI expression is enhanced by interleukin-4 (IL-4). Effects of this cytokine on FCER1A gene transcription seem I, however, not to be mediated by the proximal promoter but rather by the human specific distal one.32,33 The distal promoter is negatively regulated by a limited set of transcription factors, such as Elf-1, PU.1 and YY1.32,33 Therefore, both promoters vary not only in their response to IL-4, but also their regulatory mechanisms are different.27,29-33 The increased FcεRIα expression in aller-
In the study by Shikanai et al.,\textsuperscript{23} mutational screening of the proximal promoter and five protein coding \textit{FCER1A} gene exons (Ex1–5) was performed on Caucasian and Afro-American asthmatics and healthy controls. No mutations causing an amino acid change were detected in Ex1–5.\textsuperscript{23} However, in the regulatory region 5’ to the Ex1 (proximal promoter) three polymorphisms were found, i.e. −770 A > C, −664 G > A and −335 C > T, the last one being the most frequent.\textsuperscript{23} No significant differences in genotype distributions and allelic frequencies were found between asthmatics and healthy controls.\textsuperscript{21} Some interracial differences were detected in allelic frequencies. The frequency of the −770 A > C \textit{FCER1A} polymorphism varied between Caucasian and Afro-American asthmatics: −335 C > T allelic frequencies showed interracial differences both in patients with asthma and in healthy controls.\textsuperscript{23} Shikanai et al. found also a greater proportion of CC homozygotes of −335 C > T polymorphism in Caucasian asthmatic patients with total serum IgE levels in the lower quartile, contrasted to those with higher IgE concentrations.\textsuperscript{23}

In the Japanese study Hasegawa et al.\textsuperscript{22} screened for \textit{FCER1A} variability in patients with atopic dermatitis and in a healthy control group. They found another polymorphism of the region, named −66 T > C within the \textit{FCER1A} regulatory sequence of the proximal promoter.\textsuperscript{22} A difference in of −66 T > C \textit{FCER1A} genotypes frequencies between healthy non-allergic subjects and patients with atopic dermatitis suggested a possible association of the gene with predisposition to this allergic condition. Further molecular experiments showed that −66 T > C \textit{FCER1A} gene polymorphism affects binding of transcriptional factor GATA-1 to the proximal promoter and, thus, a transcriptional activity of the gene.\textsuperscript{22}

In a study recently completed in Poland, we used a similar mutational screening strategy on the proximal promoter region of \textit{FCER1A}, examining the allergic subjects with asthma or urticaria, and Polish population-based age- and sex-matched controls.\textsuperscript{35} We confirmed the presence of the two common polymorphisms, the one previously reported by Shikanai et al.\textsuperscript{23} (−335 C > T) and the other reported by Hasegawa et al.\textsuperscript{22} (−66 T > C). In order to unify the nomenclature, these polymorphisms are currently referred to as −344 C > T and −95 T > C, using a translation start nucleotide as the first one.\textsuperscript{22,23,35} Both genetic variations were in complete linkage disequilibrium. No differences were found in genotypes distributions between the groups of patients and healthy controls. Similarly, a haplotype analysis did not reveal any genetic associations with allergic diseases.\textsuperscript{35} Interestingly, −344 TT genotype was associated with higher total serum IgE levels in allergic patients but not in controls.\textsuperscript{35} This observation indirectly corroborated with previous results; e.g. Shikanai et al. described a more frequent −344 CC genotype in asthmatic patients with lower serum IgE levels.\textsuperscript{23,35} This genetic association between −344 C > T polymorphism and total serum IgE levels was replicated by Bae et al.\textsuperscript{36} In a study on the association between −344 C > T or −95 T > C polymorphisms and aspirin-induced urticaria, they found the carriers of the −344 T allele to have higher total serum IgE concentrations than subjects with the CC genotype.\textsuperscript{36} Moreover, functional studies of the −344 C > T polymorphism suggested an altered proximal promoter transcriptional activity possibly related to binding of the transcription factor MAZ.\textsuperscript{26}

In an extended study, in which we continued mutational screening of distal \textit{FCER1A} gene regions, including Ex1A and the distal promoter no variants were encountered in the distal promoter.\textsuperscript{37} However, two novel common polymorphisms were found in Ex1A (−18483 A > C, −18674/−18675 delAA). All the four \textit{FCER1A} polymorphisms (−344 C > T, −95 T > C, −18483A > C and −18674/−18675delAA) were in tight linkage disequilibrium. Again, no genotypes or reconstructed haplotypes frequencies differed between the subject groups of allergic patients and healthy controls.\textsuperscript{37} The association between the −344 TT genotype and higher serum IgE levels was confirmed in a group composed of allergic patients suffering from asthma, urticaria, persistent allergic rhinitis or simple pollinosis.\textsuperscript{37} This relationship remained significant using QTL analysis contrasting −344C > T genotypes against the other three polymorphisms. Interestingly enough, −18483 CC homozygotes also showed a statistical tendency toward higher total serum IgE levels in allergic patients.\textsuperscript{37} However, in a subgroup of asthmatics only, both −18483 CC and −
344 TT genotypes were highly significantly associated with higher total serum IgE levels.37

No common mutations in FcεRIa gene Ex2A were found, however, a rare single nucleotide polymorphism (−12663 A > G) was located several nucleotides upstream.38

**PERSPECTIVES FOR FcεRIa GENE VARIABILITY STUDIES: FceRI-TARGETTED ANTIALLERGIC DRUGS**

IgE increases FcεRI expression on the cell surface by preventing its degradation8,39,40 and serum IgE levels correlate with FcεRI expression on different cell types.39,41 Therefore, the presence of a hypothetic positive feedback loop involving IgE upregulation of FcεRI on basophils and mast cells, greater IL-4 secretion induced by upregulated FcεRI, and higher IgE production resulting from IL-4-dependent B-cell switch for IgE synthesis was proposed.8 FcεRI present on basophils and mastocytes might also affect IgE levels by increasing the plasma half-life of the immunoglobulin.16

Our knowledge on FcεRI function and its relation with IgE levels is in a large part derived from omalizumab studies. Anti-IgE therapy effectively downregulates FcεRI expression on mast cells,42 basophils,43,44 dendritic cells45 and monocytes.46 This further mediates cellular and clinical omalizumab effects. A similar response, though tested only in animals, was obtained by a vaccination against an IgE specific CH3 domain, which is responsible for the immunoglobulin binding to FcεRI.16 It might be also speculated, that targeting the regulatory regions of FcεRIa gene using recently discovered strategies, e.g. interfering RNA molecules, could bring similar effects.33,34 FcεRIa gene variants, by their altered transcriptional activities, offer a natural model for studying relations between serum IgE levels and clinical course of the disease. The role of FcεRIa variants in FcεRI cellular expression seems especially important on antigen presenting cells, because they lack expression of the FcεRIβ subunit in humans.7,47,48

**ACKNOWLEDGEMENTS**

We would like to express our gratitude to Mrs. Agata Pletnia-Potaczek for drawing the figures for the present work.

D. P. P. was awarded a stipend from the Foundation of Polish Science START 2007.

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