Review Article

Genetic defects in downregulation of IgE production and a new genetic classification of atopy

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ABSTRACT

Atopic disorders, such as asthma, eczema and rhinitis, develop due to the interactions between genetic and environmental factors. Atopy is characterized by enhanced IgE responses to environmental antigens. The production of IgE is upregulated by Th2 cytokines, in particular interleukin (IL)-4, and downregulated by Th1 cytokines, in particular interferon (IFN)-γ. In the present review, we present the genetic factors responsible for IgE production and genetic defects in the downregulation (brake) of IgE production, especially in terms of IL-12 and IL-18 signaling, mutations of the IL-12 receptor β2 chain gene and mutations of the IL-18 receptor α chain gene in atopy. Moreover, we newly present a genetic classification of atopy. There are four categories of genes that control the expression of allergic disorders, which include: (i) antigen recognition; (ii) IgE production (downregulation = brake; and upregulation); (iii) the production and release of mediators; and (iv) events on target organs. In the near future, this genetic classification will facilitate the development of tailor-made treatment.

Key words: atopy, IgE production downregulation, interferon-γ, interleukin-12 receptor β2, interleukin-18 receptor α.

INTRODUCTION

Atopic disorders, such as asthma, eczema and rhinitis, develop due to the interactions between genetic and environmental factors. Atopy is characterized by enhanced IgE responses to environmental antigens. The production of IgE is upregulated by Th2 cytokines, in particular interleukin (IL)-4, and is downregulated by Th1 cytokines, in particular interferon (IFN)-γ.1 Interleukin-12, which is a cytokine that promotes cell-mediated Th1 responses and the production of IFN-γ, is one of the important cytokines that downregulates IgE production. Interleukin-18, originally known as an IFN-γ-inducing factor, is a recently cloned cytokine of approximately 18 kDa secreted by Kupffer cells of the liver and activated macrophages.2 Interleukin-18 strongly augments IFN-γ production by T lymphocytes, natural killer (NK) cell cytotoxicity and T lymphocyte proliferation.

In the present review, we discuss the genetic factors responsible for IgE production and the genetic defects in the downregulation (brake) of IgE production in atopy. Moreover, we newly present a genetic classification of atopy.

DEVELOPMENT OF ALLERGIC DISEASES

A questionnaire was distributed in March 1991 to children under 16 years of age who were attending kindergarten or elementary or junior high school in two Japanese cities, namely Gifu, with a temperate climate, and Itoman, with a subtropical climate. The number of subjects analyzed was 1243 in Gifu and 1953 in Itoman. Multiple logistic regression analysis was performed using SAS (SAS Institute, Cary, NC, USA).
Multiple logistic regression analysis showed that, in both cities, children of families with a history of allergy have a significantly higher risk (relative risk 3.58 and 4.22 for Gifu and Itoman, respectively) of contracting an allergic disease (Table 1). These results show that there is a genetic accumulation in the development of allergic disorders. Therefore, the development of allergic disorders is correlated with some genes. We think that multiple causative genes, but not a single gene, are correlated, because there are multiple pathogeneses of allergic reactions.

**GENETIC FACTORS OF ENHANCED IgE PRODUCTION AND ATOPY**

Serum IgE levels of atopic children were plotted against serum IgE levels of their parents (Fig. 1) and a good correlation was found ($P < 0.016$). Therefore, this indicates that IgE production shows genetic accumulation. Several linkage analyses and mutations for candidate genes of atopy (i.e. enhanced IgE production) have been reported. In 1989, Cookson et al. reported a linkage between IgE responses underlying asthma and rhinitis and chromosome 11q. Moreover, Shirakawa et al. 

![Figure 1](image.png)

**Fig. 1** Relationship between serum total IgE levels of atopic children and the IgE levels of their parents (the highest IgE level of two spouses was used). Children older than 6 years were selected. $y = 1.38 + 0.3461x; P < 0.016$.

### Table 1  Genetic and environmental factors in relation to any allergic diseases as analyzed by multiple logistic regression

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Relative risk (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gifu ($n = 1243$)</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3.58 (2.17–5.91)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>0.93 (0.69–1.27)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>0–3</td>
<td>1.72 (0.87–3.40)</td>
</tr>
<tr>
<td>4–6</td>
<td>1.47 (0.93–2.31)</td>
</tr>
<tr>
<td>7–9</td>
<td>1.30 (0.81–2.07)</td>
</tr>
<tr>
<td>10–12</td>
<td>1.15 (0.71–1.85)</td>
</tr>
<tr>
<td>13–15</td>
<td>1</td>
</tr>
<tr>
<td>Structure of house</td>
<td></td>
</tr>
<tr>
<td>Made of wood</td>
<td>1</td>
</tr>
<tr>
<td>Made of reinforced concrete</td>
<td>1.22 (0.87–1.72)</td>
</tr>
<tr>
<td>Apartment house</td>
<td>1.27 (0.66–2.42)</td>
</tr>
<tr>
<td>Flooring</td>
<td></td>
</tr>
<tr>
<td>Wooden floor</td>
<td>1</td>
</tr>
<tr>
<td>Tatami</td>
<td>0.98 (0.64–1.49)</td>
</tr>
<tr>
<td>Carpet on tatami</td>
<td>1.17 (0.79–1.72)</td>
</tr>
<tr>
<td>Carpet on wooden floor</td>
<td>2.00 (1.17–3.42)</td>
</tr>
<tr>
<td>Pets</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>0.88 (0.62–1.23)</td>
</tr>
</tbody>
</table>
reported that a common variant of the β-subunit of the high-affinity IgE receptor (FcεRIβ) on chromosome 11, Ile181Leu within the 4th transmembrane domain, shows significant association with positive IgE responses. Several associations have been noted between atopy and genes on the chromosome 5 cytokine cluster, including IL-4. In 1998, Mitsuyasu et al. reported that the Ile50Val variant of the IL-4 receptor α (IL-4Rα) chain upregulates IgE synthesis and is associated with atopic asthma. Moreover, Shirakawa et al. noted genetic variants of IL-13. Very recently, we found that reduced IFN-γ production by peripheral blood mononuclear cells (PBMC) following stimulation with IL-12 or IL-18 is associated with heterozygous IL-12 receptor β2 (IL-12Rβ2) chain gene or IL-18 receptor α (IL-18Rα) chain gene mutations in atopic subjects.

**GENETIC DEFECTS IN THE DOWNREGULATION OF IgE PRODUCTION IN ATOPY**

The production of IgE is upregulated by Th2 cytokines, in particular IL-4, and is downregulated by Th1 cytokines, in particular IFN-γ. Interleukin-12 and IL-18 are the important cytokines that induce IFN-γ and downregulate IgE production (Fig. 2).

In this section, the genetic defects in the downregulation (brake) of IgE production, especially, in terms of IL-12 and IL-18 signaling, are discussed.

**Interleukin-12 and IL-12R**

Interleukin-12, which is produced by activated antigen-presenting cells, is a cytokine that consists of two disulfide-linked subunits, p35 and p40. Interleukin-12

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**Fig. 2** The Th1 and Th2 lymphocyte balance and upregulation and downregulation of IgE production. IL, interleukin; DH, delayed-type hypersensitivity; IFN, interferon; APC, antigen-presenting cell; HLA, human leukocyte antigen; TCR, T cell receptor; Baso, basophils; Mast, mast cells; Eo, eosinophils.
plays a central role in promoting Th1-type immune responses and, thus, cell-mediated immunity.\textsuperscript{12–15} Interleukin-12 also induces IFN-γ production by T lymphocytes and NK cells.\textsuperscript{16–18} The receptor for IL-12 (IL-12R) is composed of two distinct subunits, β1 and β2\textsuperscript{19} (Fig. 3). Although the β2 chain of the IL-12R is expressed only in Th1 lymphocytes, the β1 chain is expressed in both Th1 and Th2 lymphocytes.\textsuperscript{20} The IL-12Rβ1 chain does not contain any cytoplasmic tyrosine residues, whereas the cytoplasmic region of the IL-12Rβ2 chain contains three tyrosine residues. This suggests that the β2 subunit plays an important role in IL-12 signal transduction. Interleukin-12 induces activation of specific members of the signal transducers and activators of transcription (Stat) family of transcription factors and it has been shown that Stat4-deficient mice manifest impaired production of IFN-γ\textsuperscript{21} and the phenotype of the IL-12-p40-deficient mouse is similar to that of the Stat4-deficient mouse.\textsuperscript{15} Therefore, Stat4 is particularly important. Interleukin-12 induces rapid tyrosine phosphorylation of Stat4 and the formation of nuclear complexes capable of binding to DNA sequences, such as the Stat4-binding site.\textsuperscript{21,22}

**Interleukin-18 and IL-18R**

A variety of biological functions have been associated with human IL-18, including the induction of the proliferation of activated T lymphocytes, enhancement of NK cytotoxicity, induction of the production of IFN-γ and granulocyte–macrophage colony stimulating factor (GM-CSF), and promotion of a Th1 response.\textsuperscript{2,23–25} The activity of IL-18 is via an IL-18R complex. This IL-18R complex is composed of a binding chain termed IL-18Rα, a member of the IL-1R family previously identified as the IL-1R-related proteins, and a signaling chain, also a member of the IL-1R family. The IL-18R complex recruits the IL-1R-activating kinase and tumor necrosis factor (TNF)-associated factor 6, which phosphorylates nuclear factor (NF)-κB-inducing kinase, with subsequent activation of NF-κB\textsuperscript{26–28} (Fig. 4).

![Fig. 3](image-url)  
**Fig. 3** Interleukin (IL)-12 signaling. TYK2, tyrosine kinase 2; JAK2, Janus kinase 2; STAT4, signal transducers and activators of transcription 4; IFN, interferon.

![Fig. 4](image-url)  
**Fig. 4** Interleukin (IL)-18 signaling. IL-18Rα, IL-18Rβ, IL-18 receptor α and β chains, respectively; IKK-1, Ikk-2, IκBα kinases 1 and 2, respectively; NF-κB, nuclear factor-κB; NIK, NF-κB-inducing kinase; TRAF-6, tumor necrosis factor receptor-associated factor 6; IRAK, IL-1 receptor-associated kinase.
Interferon-γ production by IL-12 or IL-18 in atopy

We examined the production of IFN-γ in PBMC of atopic patients and healthy controls following stimulation with IL-12 or IL-18.\textsuperscript{10,11} The PBMC of non-atopic healthy controls showed adequate IFN-γ production following stimulation with either IL-12 or IL-18. Although the concentrations of IFN-γ in IL-18-stimulated PBMC were correlated with those of IL-12-stimulated PBMC in atopic patients, there were cases showing different responses to IL-12 and IL-18, as shown in Fig. 5. The production of IFN-γ following stimulation with IL-12 (or IL-18) was poor, but IL-18 (or IL-12) stimulation elicited detectable IFN-γ production in some atopic patients. The discrepancy in IFN-γ production following stimulation with IL-12 or IL-18 suggests a disturbance in the IL-12 or IL-18 signal cascade in these patients.

Role of mutations of the IL-12Rβ2 chain gene in atopy

Recently, it was shown that homozygous nonsense mutation of the IL-12Rβ1 chain gene resulted in impairment of immunity against Salmonella and mycobacteria.\textsuperscript{29} Moreover, IL-12Rβ1-knock out mice showed impaired development of Th1.\textsuperscript{30} In a previous study,\textsuperscript{10} sequence analysis of the cDNA of IL-12Rβ2 revealed three types of distinct genetic mutations (2496del91, 1577 A to G (R313G), 2799 A to G (H720R)) in some atopic patients (Fig. 6). Reduced production of IFN-γ by PBMC following stimulation with IL-12, but not IL-18, is associated with heterozygous IL-12Rβ2 chain cDNA mutations in atopic subjects. In these atopic patients, a heterozygous IL-12Rβ2 chain cDNA mutation results in decreased tyrosine phosphorylation of Stat4 and subsequently reduced production of IFN-γ following stimulation with IL-12. Such reduced production of IFN-γ could cause insufficient suppression of accelerated IgE production in B lymphocytes by IL-4, resulting in the elevation of serum IgE.
IgE levels (Fig. 7). The 2496del91 mutation of IL-12Rβ2, which is found all over the transmembrane portion, causes premature termination. The heterozygous missense mutations, 1577 A to G (R313G) and 2799 A to G (H720R), may lead to changes in the conformational structure. Moreover, these heterozygous mutations may play a role via a dominant negative effect. At least, these patients with heterozygous mutations of IL-12Rβ2 chain cDNA have not exhibited impairment of immunity against Salmonella and mycobacteria.

The balance between IFN-γ-producing Th1 lymphocytes and proallergic Th2 lymphocytes is important. Heterozygous mutations of IL-12Rβ1 or β2 may result in impairment of the downregulation (brake) of IgE production, whereas homozygous mutations of IL-12Rβ1 or β2 may lead to an obvious impairment of Th1-type cell-mediated immunity in addition to impairment of the downregulation of IgE production. The results of our study indicate that atopic diseases are caused, in part, by impairment of the IL-12 signal cascade, which downregulates IgE production, and that the mutation of...
the IL-12β2 chain gene is one of the causative genes for atopy.

Role of mutation of the IL-18Rα chain gene in atopy

The IL-18Rα chain cDNA of atopic patients was sequenced. We identified a three-base deletion of the IL-18Rα chain cDNA (950delCAG), which was generated by alternative splicing, as determined on the basis of genomic sequence data for the IL-18Rα chain gene (Fig. 8). Peripheral blood mononuclear cells with the predominant expression of 950delCAG significantly showed reduced IFN-γ production after IL-18 stimulation. There was a significant difference in the expression pattern of the IL-18Rα chain transcript between atopic patients and non-atopic controls. According to these results, the dominant expression of the 950delCAG transcript of IL-18Rα chain cDNA, which was associated with reduced IFN-γ production following IL-18 stimulation and high serum IgE levels, predisposes to some atopic diseases.

Role of mutation of the IFN-γR1 chain gene in atopy

We identified a novel heterozygous single-nucleotide substitution 1400 T to C (Leu467Pro) in the seventh exon of the IFN-γR1 chain gene. This substitution was detected in six of 89 allergic patients, but not in 72 non-allergic subjects. There was a difference in the Leu467Pro frequency between allergic and non-allergic subjects (P < 0.05). Serum IgE levels of allergic patients with Leu467Pro were higher than those of non-allergic
subjects (P < 0.001). These results suggest that Leu467Pro in the IFN-γR1 chain gene is one of the candidate susceptibility genes for atopic diseases.

**GENETIC CLASSIFICATION OF ATOPY**

Recently, mutations or genetic polymorphisms of several genes, such as those encoding the FcεRIβ, IL-4Ra subunit, and IL-13, have been reported as the probable causative genes of atopy, which is characterized by enhanced IgE production. Based on these reports and our results, we present a new genetic classification of atopy in Fig. 9. There are four categories of genes that control the expression of allergic disorders, which include: (i) antigen recognition; (ii) IgE production (downregulation = brake; and upregulation); (iii) the production and release of mediators; and (iv) events on target organs. In the near future, this genetic classification will facilitate the development of tailor-made treatment.

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