

## mRNA Expression and RNA Editing (2451 C-to-U) of IL-12 Receptor $\beta_2$ in Adult Atopic Patients

Interleukin (IL)-12 activates T helper (Th) 1 cells to produce interferon (IFN)- $\gamma$  which inhibits atopic inflammation. IL-12 acts through interaction with its receptor, especially  $\beta_2$  subunit. In several studies, the low production of IFN- $\gamma$  in peripheral mononuclear cells of atopic patients on response to IL-12 stimulation has been reported. Therefore we investigated the IL-12 receptor  $\beta_2$  (IL-12R $\beta_2$ ) mRNA expression and RNA editing, nucleotide 2451 C-to-U conversion, to find the cause of low responsiveness to IL-12 in atopy. Quantitative real time PCR for mRNA expression and sequence analysis for RNA editing were performed in 80 atopic patients and 54 healthy controls. The expression of IL-12R $\beta_2$  mRNA was significantly lower in atopic patients than healthy controls ( $p < 0.05$ ). In sequence analysis, RNA editing on nucleotide 2451 was not found from either atopic patients or healthy controls. In additional evaluation, there was no relationship between expression of IL-12R $\beta_2$  mRNA and serum total IgE or blood eosinophil count. Reduced IL-12R $\beta_2$  mRNA expression in atopic patients indicate the reduced capacity to respond to IL-12 which induce IFN- $\gamma$  production and this may contribute to Th2-skewed immune response in atopy.

**Key Words :** interleukin-12 receptor; IL-12R $\beta_2$ ; Gene Expression Profiling; Atopy; Asthma; Dermatitis; Atopic; RNA Editing

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## INTRODUCTION

Atopy is characterized by enhanced immunoglobulin E (IgE) response to common environmental antigens and leads to clinical disorders such as asthma, eczema and rhinitis (1). Allergic inflammation and IgE synthesis are known to be a T helper (Th) 2 phenomenon promoted by a distinctive allergen-specific Th2 cell subset (2).

Th cells can be divided into two subpopulations, Th1 and Th2, based on the cytokines they produce. Th2 cells produce interleukin (IL)-4, IL-5 and IL-13, which cause eosinophilia and promote immunoglobulin class switching to IgE (3). Th1 cells produce interferon (IFN)- $\gamma$ , which inhibit Th cells to differentiate to Th2 cells and down-regulates the production of IgE.

IL-12 is the single most powerful factor inducing the synthesis of IFN- $\gamma$  and acts by binding to its specific receptor. The receptor of IL-12 (IL-12R) is composed of two subunits,  $\beta_1$  and  $\beta_2$ , that assemble to form a high affinity IL-12 receptor complex (4). While the  $\beta_1$  subunit is relatively constantly expressed, the  $\beta_2$  subunit expression is restricted to Th1 cells and highly regulated by several cytokines.

Several investigators have shown that peripheral blood mononuclear cells (PBMCs) from atopic patients produced less IFN- $\gamma$  than those of healthy individuals when they were stimulated with IL-12 (5-9). The underlying mechanisms of

the low responsiveness to IL-12 in atopic patients have not been known. In one study, reduced expression of IL-12R $\beta_2$  mRNA was found to have relation with low production of IFN- $\gamma$  in atopic patients (13). They explained that the decreased capacity of up-regulation of IL-12R $\beta_2$  might decrease the responsiveness of Th1 cells to IL-12. In another study, RNA editing, especially, nucleotide 2451 C-to-U conversion of IL-12R $\beta_2$ , was reported in atopic patients (1). They suggested that above RNA editing might give rise to a conformational change in IL-12R $\beta_2$  and cause impairment of Th1 cells to respond to IL-12.

The aim of this study was to investigate mRNA expression of IL-12R $\beta_2$  as well as the RNA editing, nucleotide 2451 C-to-U conversion, of IL-12R $\beta_2$  as a possible cause of low responsiveness to IL-12 in atopic patients. In additional evaluation, the correlation of the IL-12R $\beta_2$  mRNA expression with serum total IgE concentration and blood eosinophil count were evaluated in atopic patients.

## MATERIALS AND METHODS

### Subjects

Eighty atopic patients (52 male, 28 female,  $34.2 \pm 18.9$  yr) with major allergic diseases such as bronchial asthma (10 male,

4 female,  $30.7 \pm 22.1$  yr), atopic dermatitis (26 male, 15 female,  $36.5 \pm 18.5$  yr) or allergic rhinitis (17 male, 8 female,  $31.6 \pm 18.1$  yr) were studied. The diagnosis of allergic diseases was established by specific allergic symptoms, positive results of allergen specific IgE test and/or increased serum total IgE concentration. Fifty four healthy controls (23 male, 31 female,  $47.1 \pm 14.3$  yr) who had no history of atopic disease and had normal serum total IgE concentration were studied.

## Methods

Venous blood was drawn from atopic patients and healthy controls. PBMCs were isolated by gradient centrifugation method using Ficoll-Paque™plus (Amersham Biosciences, Uppsala, Sweden) and were suspended in the RPMI 1640 (Invitrogen, Grand Island, NY, U.S.A.) culture medium in  $1 \times 10^6$  density. Then the cells were cultured in the presence of  $10 \mu\text{g}/\text{mL}$  PHA for 24 hr at  $37^\circ\text{C}$  in a humidified atmosphere containing 5%  $\text{CO}_2$  (Forma  $\text{CO}_2$  incubator model

**Table 1.** Primers for quantitative real time PCR and sequencing analysis

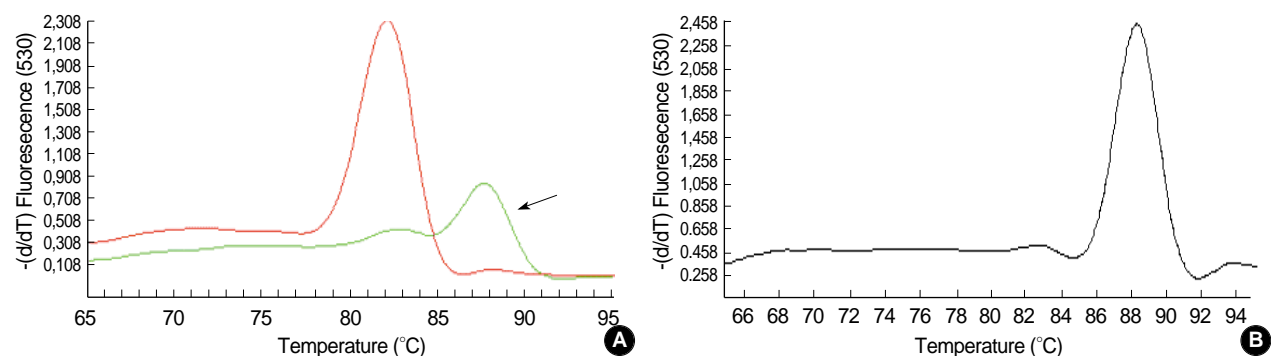
Uses	Target	Primer sequences
Quantitative real time PCR	IL-12R $\beta_2$	S 5' -ACATTCTTGGACATAGTG-AGGCC-3'
		AS 5' -GTACATCTGCTACGGAA-GCC-3'
	GAPDH	S 5' -CGTCTTACCACCATGGA-GA-3'
		AS 5' -CGGCCATCAGCCACAG-TTT-3'
Sequencing analysis	IL-12R $\beta_2$ cDNA	S 5' -GATGACAGCTCTGACAG-CTG-3'
		AS 5' -GGCCTGATGACCTTGGA-IT-3'
	IL-12R $\beta_2$ genomic DNA	S 5' -GATGACAGCTCTGACAGC-TG-3'
		AS 5' -CATTGTCTCCAGGAAGA-TAG-3'

S, sense; AS, antisense.

3039, Formal Scientific Inc, Ohio, U.S.A.).

Total cellular RNA was extracted from cultured PBMCs using High Pure RNA isolation kit (Roche, Penzberg, Germany) and  $5 \mu\text{g}$  of RNA was converted to complementary DNA (cDNA) by reverse transcription (RT) PCR in a total reaction mixture volume of  $20 \mu\text{L}$ . The real time PCR reactions for quantification of IL-12R $\beta_2$  cDNA and GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) were performed, respectively. GAPDH was analyzed as an internal control. The master PCR reaction mixture was contained  $2 \mu\text{L}$  of LightCycler® FastStart DNA master SYBR green I (Roche Molecular Biochemicals, Mannheim, Germany),  $2.5 \mu\text{L}$  of  $\text{MgCl}_2$  in final concentration of  $4 \text{ mM}$ , each  $10 \text{ pM}$  of sense and antisense primers,  $2 \mu\text{L}$  of cDNA and distilled water in final volume of  $20 \mu\text{L}$ . The LightCycler® FastStart DNA master SYBR green I was composed of *Taq* DNA polymerase, SYBR green I dye,  $10 \text{ mM}$   $\text{MgCl}_2$  and dNTPs (dATP, dCTP, dGTP, dUTP) in the reaction buffer. The PCR thermal conditions were as follows: 10 min at  $95^\circ\text{C}$  and 40 cycles of 10 sec at  $95^\circ\text{C}$ , 5 sec at  $57^\circ\text{C}$  and 4 sec at  $72^\circ\text{C}$  for IL-12R $\beta_2$  and 10 min at  $95^\circ\text{C}$  and 40 cycles of 10 sec at  $95^\circ\text{C}$ , 5 sec at  $55^\circ\text{C}$  and 15 sec at  $72^\circ\text{C}$  for GAPDH. The primer sequences were summarized in Table 1. The quantitative real time PCR was performed using LightCycler® system (Roche Molecular Biochemical, Mannheim, Germany). During each cycle on the real time of reaction and after completion of primer extension at  $72^\circ\text{C}$ , fluorescence at  $530 \text{ nm}$  (F1 channel) was recorded. The amount of IL-12R $\beta_2$  mRNA was calculated relative to the amount of GAPDH present in each sample and described as IL-12R $\beta_2$ /GAPDH ratio. All the analysis was performed in duplicate. Melting curve analysis was used to detect non-specific amplification which could be occurred during PCR procedures using SYBR green I (Fig. 1).

Fragment of exon 13 including nucleotide 2451 of IL-12R $\beta_2$  was amplified from mRNA and genomic DNA, respectively. The PCR thermal conditions were as follows: 5 min at  $94^\circ\text{C}$  and 40 cycles of 20 sec at  $94^\circ\text{C}$ , 20 sec at  $65^\circ\text{C}$  and 45 sec at  $72^\circ\text{C}$  for mRNA and 10 min at  $94^\circ\text{C}$  and 40 cycles of 1 min at  $94^\circ\text{C}$ , 1 min at  $55^\circ\text{C}$  and 1 min at  $72^\circ\text{C}$



**Fig. 1.** Melting curves of amplification products of IL-12R $\beta_2$  mRNA (A) and GAPDH (B). Solid arrow indicates non-specific primer-dimers product.

for genomic DNA. The primer sequences were summarized in Table 1. The amplified products were purified and sequenced in automatic sequencer ABI prism® 3730xI (Applied Biosystems, Foster City, CA, U.S.A.).

Serum total IgE concentration was measured using RIDA AllergyScreen test kit (R-biopharm AG, Darmstadt, Germany). Peripheral blood eosinophil count was measured using ADVIA120 (Bayer, Dublin, Ireland).

**Statistical analysis**

The comparison of IL-12Rβ<sub>2</sub> mRNA expression between atopic patients including 3 disease groups and healthy controls was analyzed using independent t test. The comparison of IL-12Rβ<sub>2</sub> mRNA expression among 3 disease groups was analyzed using one way ANOVA. The relationship between IL-12Rβ<sub>2</sub> mRNA expression and serum total IgE were analyzed using one way ANOVA. The relationship between IL-12Rβ<sub>2</sub> mRNA expression and blood eosinophil count was analyzed using Spearman’s correlation test. All the statistical analysis was performed with SPSS program (version 12.0, SPSS Inc, Chicago, IL, U.S.A.) and the *p* value of <0.05 was

considered as significant.

**RESULTS**

The PHA stimulated PBMCs from atopic patients had significantly lower expression of IL-12Rβ<sub>2</sub> mRNA than the cells from healthy controls (*p*<0.05) (Fig. 2). The average IL-12Rβ<sub>2</sub>/GAPDH ratio was 0.035 ± 0.02 in healthy controls and 0.018 ± 0.047 in atopic patients. When patients were divided into 3 disease groups (asthma, atopic dermatitis, allergic rhinitis), each disease group showed lower IL-12Rβ<sub>2</sub> mRNA expression than controls (*p*<0.05, respectively), also. But there was no significant difference of IL-12Rβ<sub>2</sub> mRNA expression between 3 groups (*p*>0.05) (Table 2).

In the results of sequencing analysis, all the nucleotide 2451 was cytidine (C) in cDNA as well as genomic DNA from atopic patients and healthy controls (Fig. 3). Therefore, the RNA editing phenomenon, C-to-U conversion at nucleotide 2451 of IL-12Rβ<sub>2</sub> was not found in present study.

The atopic patients were divided into 3 groups according to the semiquantitative results of serum total IgE (<100 IU/mL, 100-200 mIU/mL and >200 IU/mL) and the average IL-12Rβ<sub>2</sub>/GAPDH ratio was 0.008 ± 0.007, 0.008 ± 0.007 and 0.028 ± 0.064, respectively. When the expression of IL-12Rβ<sub>2</sub> mRNA was compared, there was no significant differences among groups (*p*>0.05) (Fig. 4). The correlation between IL-12Rβ<sub>2</sub> mRNA expression and blood eosinophil count was not found, either (*p*<0.05) (Fig. 5).

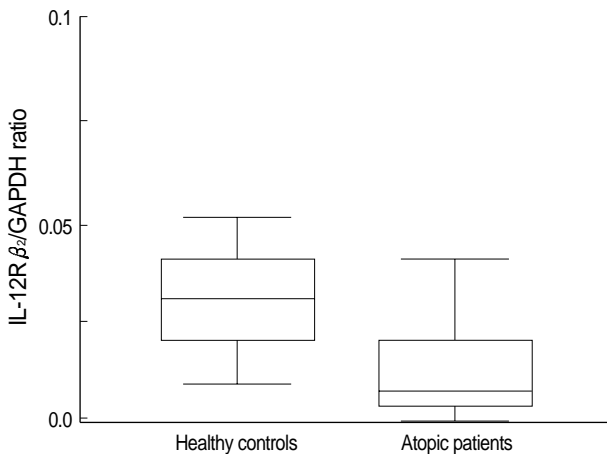


Fig. 2. Comparison of IL-12Rβ<sub>2</sub>/GAPDH ratio between healthy controls (n=54) and atopic patients (n=80). The mRNA expression of IL-12Rβ<sub>2</sub> is significantly lower in atopic patients than healthy controls (*p*<0.05).

Table 2. Results of IL-12Rβ<sub>2</sub> mRNA expression manifested by IL-12Rβ<sub>2</sub>/GAPDH ratio

	IL-12Rβ <sub>2</sub> /GAPDH ratio
Atopic patients (n=80)	0.0177 ± 0.0465*
Asthma (n=14)	0.0102 ± 0.0072 <sup>†</sup>
Allergic rhinitis (n=42)	0.0232 ± 0.063 <sup>†</sup>
Atopic dermatitis (n=26)	0.0128 ± 0.0191 <sup>†</sup>
Healthy controls (n=54)	0.0345 ± 0.0193

\**p*<0.05 by independent t test compared with healthy controls. <sup>†</sup>*p* >0.05 by one way ANOVA compared among asthma, allergic rhinitis and atopic dermatitis. Values are mean ± SD.

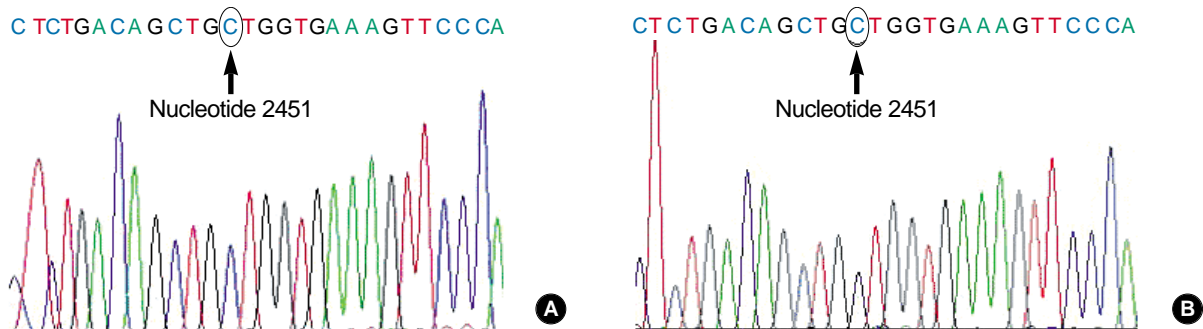


Fig. 3. Results of sequencing analysis of IL-12Rβ<sub>2</sub> cDNA (A) and genomic DNA (B). Both bases of nucleotide 2451 of cDNA and genomic DNA are cytosines in atopic patients and healthy controls. Arrows indicate the base composition of nucleotide 2451 on exon 13.

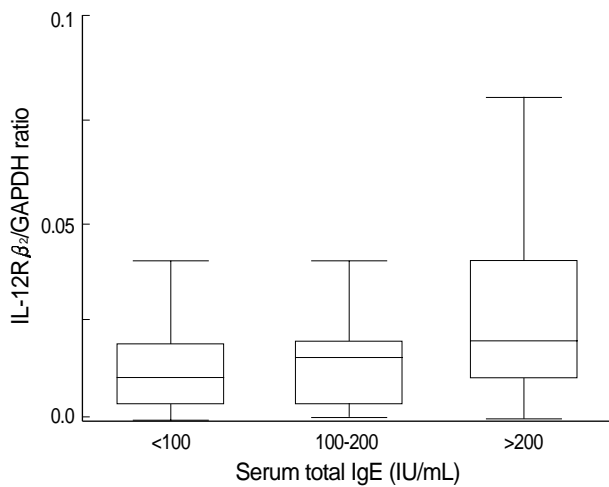


Fig. 4. Comparison of IL-12R $\beta_2$ /GAPDH ratio among 3 groups divided by serum total IgE concentration. Significant difference is not found between 3 groups (one way ANOVA,  $p>0.05$ ).

## DISCUSSION

IL-12 is the single most powerful factor to induce Th1 immune response. It is produced by antigen present cells (APCs), including monocytes and dendritic cells. IL-12 is a heterodimeric cytokine composed of 35 and 40 kDa peptides and the genes are located on chromosome 3 and 5, respectively (11). By binding to its specific receptor complex,  $\beta_1$  and  $\beta_2$ , IL-12 promotes the development of Th1 responses and induces production of IFN- $\gamma$ . The  $\beta_2$  subunit is the signal transducing component of the receptor and highly regulated in contrast to the  $\beta_1$  subunit. IFN- $\gamma$  inhibits Th cells to differentiate to Th2 cells and down-regulates the production of IgE in B lymphocytes. IFN- $\gamma$  is also able to augment IL-12. This IL-12/IFN- $\gamma$  loop helps the predominant Th1 cell differentiation and inhibits Th2 cell differentiation (12).

Since it was reported that PBMCs from atopic patients produced less IFN- $\gamma$  than those of healthy individuals when they were stimulated with IL-12 (5-9), several investigations to find genetic mechanisms from interaction between IL-12 and IL-12R have been reported. Several IL-12 polymorphism or IL-12R $\beta_2$  mutations such as truncated (2496 del 91) or missense (1577 A to G and 2799 A to G) mutation has been reported (13). Although these mutations were thought to lead to change in the conformational structure of  $\beta_2$  receptor, these are controversial, yet. In a recent study, the relationship between RNA editing of IL-12R $\beta_2$  and atopy was reported by Kondo et al. (1). RNA editing is defined when there is any specific alteration in primary sequence of a RNA molecule. Such changes have marked effects on gene expression. They found that the patients who had nucleotide 2451 C-to-U conversion showed lower IFN- $\gamma$  production and faint tyrosine phosphorylation of STAT-4 than those who did not have editing. This C-to-U conversion cause substitution of amino

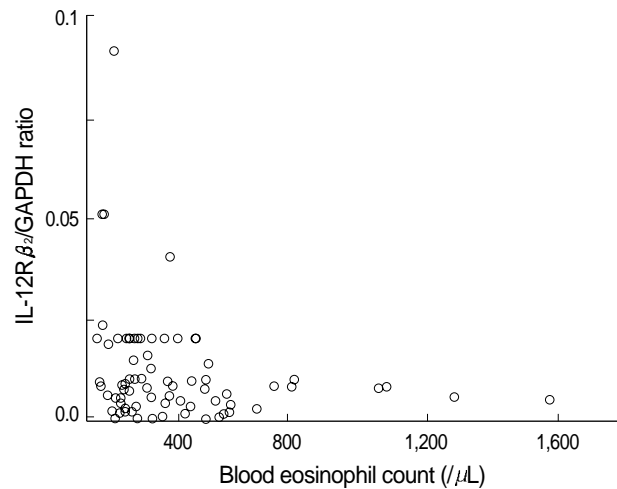


Fig. 5. Relationship between IL-12R $\beta_2$ /GAPDH ratio and blood eosinophil count in atopic patients. Significant relationship between IL-12R $\beta_2$  mRNA expression and blood eosinophil count is not found (Spearman's correlation test,  $p>0.05$ ).

acid alanine to valine. Therefore, they proposed that the RNA editing might cause impairment of the IL-12 signal cascade and the subsequent reduction in IFN- $\gamma$  production by disturbing conformational binding of IL-12 to IL-12R. Unfortunately, we could not find the C-to-U conversion at nucleotide 2451 from either atopic patients or healthy controls.

In other studies, the expression of IL-12R $\beta_2$  mRNA was studied in atopy (10, 14). PBMCs from children with atopic airway symptoms showed lower mRNA expression of IL-12R $\beta_2$  than non-atopic children and the production of IFN- $\gamma$  after stimulation with IL-12 was also lower in atopic children. In present study, we also found that PBMCs from atopic patients expressed lower IL-12R $\beta_2$  mRNA than those of healthy controls. This low expression findings of IL-12R $\beta_2$  mRNA was found in all 3 atopic disease groups, but significant differences was not found between groups. These results correspond with previous studies and support their hypothesis that reduced capacity of up-regulation of IL-12R $\beta_2$  may contribute to the Th2-skewed immune response in atopic patients.

The underlying mechanism of low mRNA expression of IL-12R $\beta_2$  in atopic patients cannot be known yet. But there was a suggestion to introduce association of CD2 defects and low responsiveness to IL-12. In previous and present studies, the PBMCs were stimulated with phytohaemagglutinin, which activates lymphocytes via CD2. CD2 is an accessory molecule of T cell and has been thought to synergize the IL-12 action by up-regulation of the  $\beta_2$  chain (15, 16). Besides, the lower proportion of CD2+ lymphocytes was detected in atopic children (17). But further studies will be required whether there is connection between CD2 and atopy.

In additional evaluation, we also analyzed the relationship between IL-12R $\beta_2$  mRNA expression and serum total IgE concentration or eosinophil count. There were not any significant associations between them. This result indicates that

the IL-12R $\beta_2$  mRNA expression would not affect production of IgE or eosinophils, directly. However, we thought that this analysis would be more reliable if we analyzed serum IgE concentration with quantitative method. In one study, a negative correlation between IL-2-induced IL-12R $\beta_2$  mRNA expression and serum IgE concentration had been reported (10).

In conclusion, RNA editing which might cause the conformational changes in IL-12R $\beta_2$  was not found, but less IL-12R $\beta_2$  mRNA on response to PHA stimulation was found in atopic patients. This low expression of IL-12R $\beta_2$  did not have relationship with atopic disease category, serum IgE concentration or blood eosinophil count. We guess low expression of IL-12R $\beta_2$  may contribute to low responsiveness to IL-12, low production of IFN- $\gamma$  and Th2-skewed immunity in atopic patients, subsequently.

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