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Functional analysis of a polymorphism in the promoter region of the IL-12/23p40 gene

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Summary

Background Human *IL-12B* gene on chromosome 5q31 encodes the common p40 subunit of IL-12 and IL-23. IL-12 is known to play critical roles in the generation of T-helper type 1 (TH₁) cells, whereas IL-23 is involved in maintenance and/or population expansion of TH₁₇ cells. Although several reports suggested an association between a polymorphism (–6415CTCTAA/GC) in *IL-12B* and asthma, the molecular mechanism how this polymorphism is involved in allergic inflammation is still unclear.

Methods The transcription activity was analysed by reporter assay. A transcription factor binding to –6415 polymorphic site was identified by electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) assay. The amount of cytokines produced from peripheral monocytes were determined by ELISA.

Results Reporter assay showed that the transcription activity of the GC allele was higher than that of the CTCTAA allele. A transcription factor Sp1 bound to the region including the GC allele with a higher affinity than that of the CTCTAA allele in EMSA. *In vivo* binding of Sp1 to *IL-12B* gene carrying –6415GC was confirmed by ChIP assay. Overexpression of Sp1 up-regulated transcription activity of promoter carrying GC allele sequence, whereas the CTCTAA promoter was not affected by Sp1. We examined the correlation between –6415CTCTA/GC polymorphism and production of cytokine IL-12/23p40, IL-12p70, and IL-23 on peripheral blood monocytes, and monocytes with the GC/GC allele exhibited significantly higher expression of IL-12p70 protein than those with the CTCTAA/CTCTAA allele ($P = 0.009$).

Conclusions The –6415 polymorphism is involved in cytokine production potential by affecting Sp1-mediated transcription activity.

Keywords IL-12/23p40, polymorphism, promoter, Sp1

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Introduction

Human chromosome 5q31 contains numerous immunoregulatory genes that are linked to the phenotypes of asthma and atopy in many genetic studies [1–4]. *IL-12B* is located in this genomic region and encodes the 40 kDa subunit (termed p40) of cytokine, IL-12 [5]. In brief, functional IL-12 is a heterodimer of IL-12p40 and IL-12p35 encoded by *IL-12A* [6]. IL-12 is produced by antigen-presenting cell and plays a central role in the differentiation of T-helper type 1 (TH₁) cells [7]. IL-12p40 also forms a heterodimer with a p35-related protein p19, which is known as IL-23, a cytokine and member of the IL-

12 family. Several studies have shown significant roles of IL-12 and IL-23 in various immunorelated diseases. IL-12/23p40^{–/–} mice have reduced NK cell responses and delayed-type hypersensitivity responses, and lacked TH₁ development [8]. In addition, IL-23 is important for the survival and population expansion of TH₁₇ cells and mediates autoimmune pathologies, in the brain, gut, joints [9–13] as well as initiates inflammation in the skin [14].

A –6415 promoter polymorphism of *IL-12B* has been reported to be associated with atopic asthma in previous reports [15–17]. However, allelic effects of this polymorphism on asthma susceptibility and asthma-related phenotypes are controversial. The first report suggested

that heterozygous genotype was more frequently detected in severe childhood asthmatics [15]. In contrast, the promoter polymorphism was not associated with asthma susceptibility, severity, or atopy at ages 7 and 42 years in an Australian population. However, total serum IgE levels of adult men (age 42 years) with at least one CTCTAA allele were higher than those homozygous for the GC allele, whereas no difference was observed for adult women [16]. In addition, a Japanese population, GC allele was significantly frequent in childhood asthmatic patients with higher serum IgE but not associated with severity [17].

To test whether the genetic variation in the promoter of *IL-12B* displays functional activity, we investigated the effects of the polymorphism on gene expression mechanism and cytokine production capacity.

Material and methods

Cell culture

Human monocyte cell lines, THP-1 and U937, were cultured in RPMI 1640 (Sigma-Aldrich, St. Louis MO, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich), 100 U/mL penicillin, and 100 µg/mL streptomycin.

Transfection and luciferase assay

THP-1 or U937 cells (4×10^6) were transfected with 10 µg reporter plasmid carrying -6415CTCTAA or -6415GC sequence [17], and 25 ng pRL-CMV (Promega, Madison, WI, USA), which was used as the internal control of transfection efficiency, by electroporation using a Bio-Rad Gene Pulsar II (Bio-Rad, Hercules, CA, USA), as described previously [18]. For co-expression analysis, 2 µg expression plasmid, pCR-3.1 (mock), or pCR-Sp1 [19] was introduced into the cells in addition to each reporter plasmid and pRL-CMV. After 20–24 h of culture, luciferase activity of each whole-cell lysate was measured using a Dual-luciferase assay kit (Promega) and a luminescence detector, Micro Lumat Plus (Berthold, Germany), as previously described [20, 21].

Electrophoretic mobility shift assay

Probes for electrophoretic mobility shift assay (EMSA) were prepared by annealing 5'-FITC-labelled synthesized oligonucleotides of complementary sequences. The sequences of sense strand of probes were as follows: probe for -6415CTCTAA (-6389/-6439 with CTCTAA); 5'-ACAGCCTGTCTCCGAGAGAGGCTCTAATGTGGCCCCACATTAGAACAAC-3', and probe for -6415GC (-6389/-6435 with GC); 5'-ACAGCCTGTCTCCGAGAGAGGGCTGTGGCCCCACATTAGAACAAC-3' (Invitrogen, Invitrogen Life Technologies, Leek, The Netherlands). The non-labelled double-stranded oligonucleotides were prepared in a

similar manner and used as competitor. Nuclear proteins were extracted from THP-1 cells by a previously described method [18, 21]. Anti-Sp1 antibody (Ab) (clone #1C6, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or Sp3 Ab (Santa Cruz Biotechnology) was used in a supershift assay. Ten picomole probes were mixed with the nuclear extract and subjected to 4% PAGE as described in our previous report [20, 21]. Fluorescent images were analysed on a FluorImager 595 (Molecular Dynamics, Sunnyvale, CA, USA).

Western blot analysis

Whole cell lysate of each sample (1×10^6 cells transfected with 20 µg expression plasmid) was subjected to Western blot analysis. Western blot analysis was performed as described previously [22]. The same anti-Sp1 Ab, as that of EMSA, or anti-β-actin Ab (Santa Cruz Biotechnology) was used as a primary Ab. Alexa Fluor 680 goat anti-mouse IgG (Molecular Probes, Eugene, OR, USA) was used as a secondary Ab to detect Sp1 and β-actin. Infrared fluorescence on membranes was detected by Odyssey infrared imaging system (model ODY-9201-SC; LI-COR, Lincoln, NE, USA).

Chromatin immunoprecipitation assay

The chromatin immunoprecipitation (ChIP) assay was performed as described in our previous report [23, 24]. Anti-Sp1 mouse IgG1 Abs, clone #1C6 and #E-3 (Santa Cruz Biotechnology) and mouse control IgG1 (Invitrogen) were used. Quantitative PCRs were performed using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and a 7500 Real-Time PCR System (Applied Biosystems). The following primers and TaqMan probe sequences were used for this analysis: for the promoter region of the human *IL-12B* gene, -6452/-6387, forward primer -6452/6431 (5'-CTGGCCATGTA CAGCCTGTCT-3'), reverse primer -6395/-6387 (5'-TGGTCATTGGCAGGTTGTTCTA-3'), and TaqMan probe -6425/-6410 (5'-CCACAGCCCTCTCT-3'). The ratio of the amount of a specific DNA fragment in each immunoprecipitate to the amount of that fragment in the DNA in whole cell lysate (input DNA) was calculated from each the cycle threshold (C_t) value, which was determined using 7500 SDS software (Applied Biosystems).

Genotyping analysis

Genomic DNA was prepared from peripheral blood using DNA Quick II (Dainippon Pharmaceutical, Osaka, Japan). The promoter polymorphisms -6415 CTCTAA/GC were genotyped by use of the TaqMan system (Applied Biosystems) using the primers 5'-GGCCATGTACAGCCTGTCT-3' and 5'-TGGTCATTGGCAGGTTGTTGTTCTAAT-3', and two probes: 5'-VIC-CCACATTAGAGCCTCTCT-3' for CTCTAA allele, and 5'-FAM-CCACAGCCCTCTCT-3' for GC allele.

Monocyte isolation from human peripheral blood

The human peripheral blood mononuclear cells (PBMC) were enriched from 20 mL peripheral blood of healthy volunteers by means of Separate-L (Muto Pure Chemicals Co Ltd, Tokyo, Japan) density gradient centrifugation. The PBMC layer was washed with sterile PBS and each monocyte subpopulation was purified from PBMC by positive-selection program of AutoMACS (Miltenyi Biotech) after labelling with CD14 MicroBeads (Miltenyi Biotech). All of PBMCs isolated from 18 subjects, in brief, homozygous for CTCTAA/CTCTAA ($n=6$), heterozygous for CTCTAA/GC ($n=7$), and homozygous for GC/GC ($n=5$) were used for following cytokine measurement experiment without any omit.

Measurement of cytokine concentration

Monocyte cells at a concentration of 2×10^5 cells in 250 μ L complete medium were incubated in the presence of 100 U/250 μ L human IFN- γ (Roche Diagnostics, Indianapolis, IN, USA) for 16 h, and then stimulated with 1 μ g/mL lipopolysaccharide (LPS) (*Escherichia coli* serotype O111: B4; Sigma-Aldrich) for an additional 24 h. The amounts of IL-12/23 p40, IL-12 p70, and IL-23 in the culture supernatants were determined using a corresponding ELISA kit (R&D systems, Bender MedSystems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Statistical analysis of cytokine production

Non-parametric Mann-Whitney *U*-tests were applied using SPSS software (version 11.0; SPSS, Chicago, IL, USA) to assess differences in the amount of cytokines between each genotypes. *P*-values < 0.05 were considered to be statistically significant.

Results

The -6415CTCTAA/GC polymorphism in human IL-12B affects the binding affinity of a transcription factor Sp1

The CTCTAA/GC polymorphism at -6415 of human *IL-12B* was shown to be associated with atopic asthma in previous studies [15, 17]. Although the effect of this polymorphism on promoter activity was observed [17], molecular mechanism of this finding remains unclear. To assess the functional role of the nucleotide in the *IL-12B* promoter region, we performed EMSA using nuclear proteins extracted from a human monocyte cell line THP-1 with double-stranded oligonucleotides including the polymorphic site at -6415CTCTAA/GC. Specific bands with the same mobility indicated with an arrow were detected in EMSAs using a probe for -6415/GC and -6415/CTCTAA; and the band intensity with -6415/GC was stronger than that of -6415CTCTAA (Fig. 1a, lanes 2

and 8). When non-labelled oligonucleotides -6415/CTCTAA or -6415/GC were added as the competitor, 50-fold excess of the competitor GC was sufficient for eliminating the GC-specific band (Fig. 1a, lane 1). In contrast, the band with GC probe was still detected even in the presence of 100-fold excess of the competitor CTCTAA (Fig. 1a, lane 9). These results suggested that a certain protein binds around -6415 of *IL-12B* with higher affinity to GC allele than the CTCTAA allele.

To identify the above-mentioned nuclear protein, we performed EMSA using Abs against Sp1 and Sp3; because the nucleotide sequence around -6415 is GC-rich sequence and the similarity of -6415/GC sequence to Sp1-family binding motif is higher in comparison with that of -6415/CTCTAA. The band intensity was markedly reduced and a supershift band with lower mobility than the original one was appeared with the addition of anti-Sp1 Ab to the EMSA reaction mixture (Fig. 1b, lane 2), whereas the band remained in the presence of anti-Sp3 Ab (Fig. 1b, lane 3). Although the reason for the appearance of additional band shift with lowest mobility (marked with asterisk) in the presence of anti-Sp1 Ab is still unclear, a similar profile was observed in a previous study [25]. These results demonstrated that Sp1 binds to the *IL-12B* promoter around -6415 *in vitro* and that -6415/GC allele exhibits a higher affinity to Sp1 than that of -6415/CTCTAA.

In vivo binding of Sp1 around -6415 single nucleotide polymorphism on chromosome DNA in monocytes

To confirm the binding of Sp1 around -6415 single nucleotide polymorphism in the human *IL-12B* gene, ChIP assay was performed with the human monocyte cell line, U937, which was genotyped to be GC/GC homozygous by TaqMan system (data not shown). The amount of genomic DNA including -6415 immunoprecipitated with two anti-Sp1 Ab clones were significantly higher than that of each control Ab (Fig. 2a). This result indicated that Sp1 binds around -6415 of the human *IL-12B* in monocytes *in vivo*.

When Western blotting analysis was performed, the specific band showing appropriate mobility as Sp1 was detected in non-stimulated U937 cells and the band intensity was not changed by stimulation with PMA or LPS (Fig. 2b), suggesting that Sp1 protein is constitutively expressed in U937 cells.

Effect of -6415CTCTAA/GC polymorphism on IL-12B promoter activity

We next examined the role of the -6415 polymorphism for the promoter function using a reporter assay. Each of the two plasmids carrying the luciferase gene under promoter control with -6415CTCTAA or GC sequence [17] were transfected into THP-1 or U937, and luciferase activity derived from each transfectant determined after 24 h

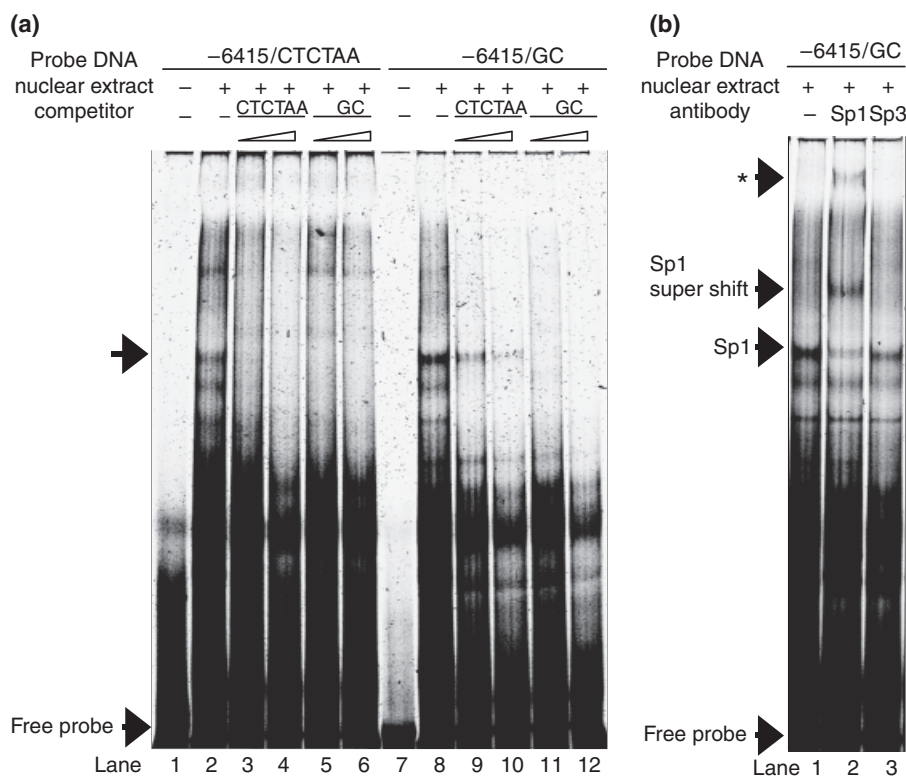


Fig. 1. Identification of nuclear protein binding to the -6415CTCTAA/GC polymorphic site of *IL-12B* gene by electrophoretic mobility shift assay (EMSA). (a) EMSA was performed with FITC-labelled probes (probe for -6415CTCTAA in lanes 1-6 and probe for -6415GC in lanes 7-12) and nuclear extract from THP-1 cells. Lanes 1 and 7, probe only; lanes 2-6 and 8-12, probe with nuclear extract. Lanes 3-6, and 9-12, with competitor. The amount of competitor was 50-fold greater in lanes 3, 5, 9, and 11, and 100-fold greater in lanes 4, 6, 10, and 12. (b) -6415GC probe and THP-1 extract were mixed in the presence or absence of antibodies. Additives are: lane 1, none; lane 2, anti-Sp1; lane 3, anti-Sp3. Specific shifted bands are marked by arrows.

incubation. As shown in Fig. 3a, luciferase activity driven by the -6415GC promoter was significantly higher than that of the -6415CTCTAA promoter. These results suggested that nucleotide GC at -6415 was crucial for showing the strong transcription activity of the *IL-12B* promoter.

To elucidate the transactivating role of Sp1 in *IL-12B* promoter function, the effect of overexpression of Sp1 on transcription activity was assessed using a co-expression study with THP-1, which works better than U937 on co-expression study. The promoter possessing GC sequence was up-regulated by exogenously expressed Sp1 (Fig. 3b). In contrast, exogenous Sp1 exhibited no significant effect against -6415CTCTAA promoter (Fig. 3b). Production of a significantly higher amount of Sp1 in THP-1 co-transfected with Sp1 expression plasmid was confirmed by Western blotting analysis (Fig. 3c). These results suggest that Sp1 transactivates the *IL-12B* promoter via -6415GC.

Relationship of *IL-12B* -6415CTCTAA/GC promoter polymorphism with protein expression of interleukin-12 and interleukin-23

To investigate whether the promoter genotype affected the protein levels of IL-12p40, IL-12p70, and IL-23, we

measured protein production from LPS/IFN- γ -stimulated monocytes by ELISA and compared it in subjects with different genotypes, homozygous for CTCTAA/CTCTAA ($n=6$), heterozygous for CTCTAA/GC ($n=7$), and homozygous for GC/GC ($n=5$). Monocytes with the GC/GC allele exhibited significantly higher expression of IL-12p70 protein in response to LPS than those with CTCTAA/CTCTAA allele ($P=0.009$) (Fig. 4b). There was a tendency toward higher protein production of IL-12p40 ($P=0.126$) and IL-23 ($P=0.082$) in monocytes with the GC/GC allele than those with CTCTAA/CTCTAA allele (Figs 4a and c). These results indicate -6415 polymorphism of *IL-12B* promoter reflects on production levels of IL-12-related cytokines in monocytes.

Discussion

Recent studies have demonstrated crucial roles of IL-12/23p40 in TH₂-type allergic inflammation by mouse model experiments [26, 27]. Genetic analyses investigated that a promoter polymorphism in *IL-12B* were associated with TH₂-type disease, such as atopic asthma [15, 17, 28], however, the role of this polymorphism on susceptibility, severity, and/or IgE levels of asthmatic patients are

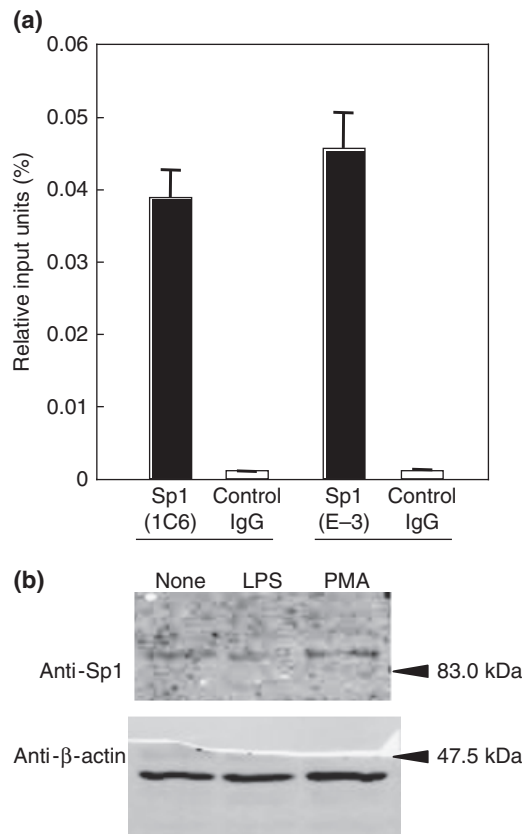


Fig. 2. *In vivo* binding of Sp1 to the human *IL-12B* promoter region containing –6415GC of chromosome DNA in monocytes. (a) Quantitative analysis of Sp1 binding to the promoter region of the *IL-12B* gene in U937 cells using the chromatin immunoprecipitation assay. The results are expressed as mean+SD for three PCRs with duplicate samples. A representative result of two independent experiments is shown. Closed bars, specific antibodies (Abs); open bars, control Abs. (b) Western blotting analysis to confirm the Sp1 protein expression in U937 cells. Whole of U937 cells (4×10^5) was applied in each well. Anti-Sp1 Ab staining (top) and anti-actin Ab staining as control (bottom). Lane 1, non-stimulated; lane 2, 1 µg/mL lipopolysaccharide (LPS)-treatment for 16 h; lane 3, 40 ng/mL phorbol myristate acetate (PMA)-treatment for 16 h.

controversial. Heterozygosity was contributed to severity of childhood asthma in one report [15] but not in others. GC allele was frequently detected in higher IgE population of childhood asthmatic patients [17], whereas GC homozygous resulted in lower serum IgE levels in adult male [16]. Analysis of the genetic effect of the polymorphism on *IL-12B* gene expression would contribute to clarify the aetiology of allergic inflammation.

In this report, we analysed the role of –6415CTCAA/GC polymorphism on *IL-12B* promoter function and found that the –6415GC promoter exhibited stronger transcriptional activity than the –6415CTCTAA promoter in human monocyte cell line. A ubiquitous transcription factor Sp1, up-regulated transcription activity via binding around –6415 with higher affinity to the GC sequence. To date, nuclear factor-κB, Ets protein, C/EBP, and acti-

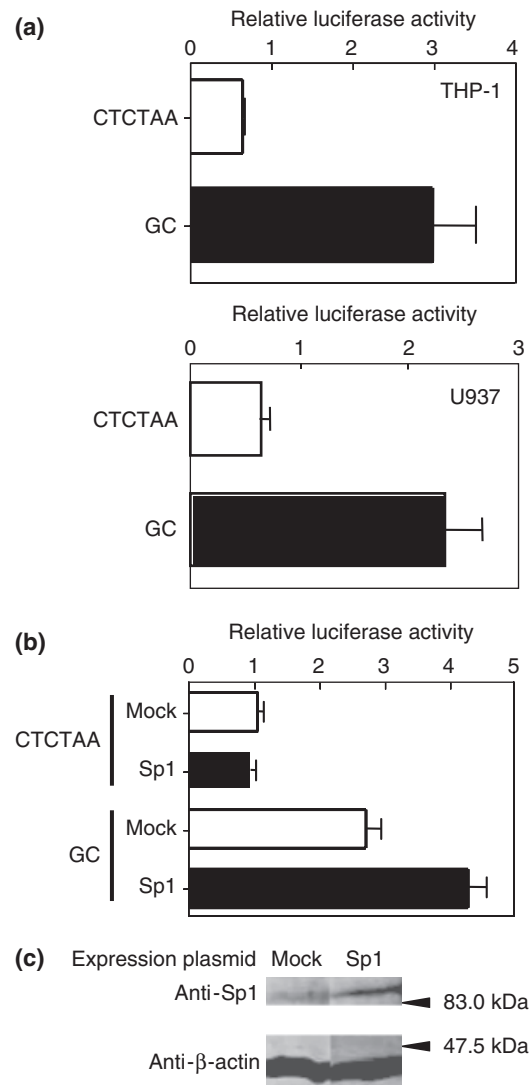


Fig. 3. Involvement of Sp1 on transcription activity affected by the –6415CTCTAA/GC polymorphism. (a) Comparison of *cis*-enhancing activity of –6415CTCTAA and GC sequences. Each reporter plasmid [8] was introduced into human monocyte cell line THP-1 (top) or U937 (bottom). Average values+SD obtained from quadrant samples are shown and similar results were observed in additional two independent experiments in (a) and (b). (b) Transactivation effect of exogenously expressed Sp1 on *cis*-enhancing activity. Plasmids, pCR3.1 (mock) and pCR-Sp1 were used for co-expression. (c) Western blotting analysis to confirm the overexpression of Sp1 in co-expression study. Whole cell lysate of each transfectant prepared in a same way as co-expression study (b) were subjected to western blotting analysis.

vator protein 1 are reportedly involved in LPS-mediated transactivation of *IL-12B* promoter [29–32]. We attempted to detect an additional band shift in EMSA using nuclear extracts from LPS-stimulated monocytes to assess whether another transcription factor recognizing the polymorphic site is involved in *IL-12B* promoter activity that are fully activated by LPS stimulation. However, an additional band was not observed in EMSA with stimulated THP-1, suggesting that Sp1 may affect the potential

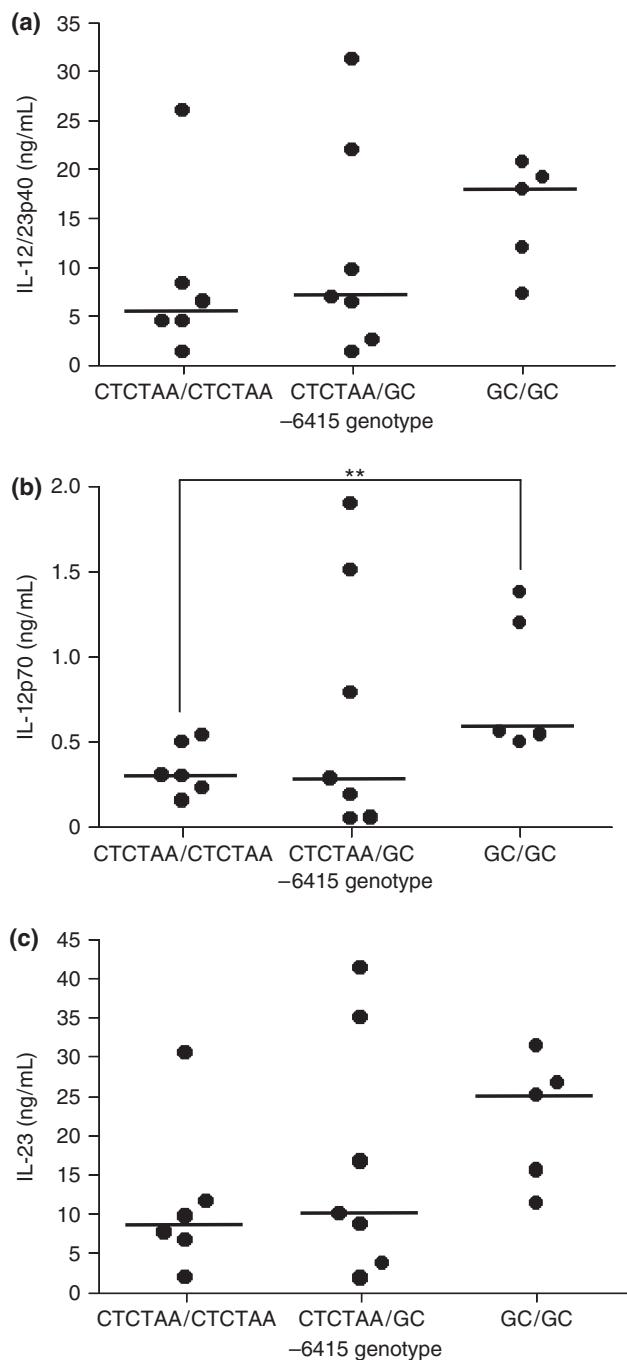


Fig. 4. Effect of -6415CTCAA/GC polymorphism on cytokine production level. The amount of IL-12/23p40 (a), IL-12p70 (b), and IL-23 (c) in culture media of peripheral blood mononuclear cells in response to INF- γ /lipopolysaccharide stimulation was determined by ELISA. Non-parametric Mann-Whitney *U*-tests were used for statistical analysis of cytokine production level. Bars mean medians in each group. ***P* = 0.009.

of *IL-12B* promoter activity but not determine cell-type specificity. Although the relationship between Sp1 and other transcription factors regulating the *IL-12B* promoter is unknown, Sp1 may be involved in this gene regulation through the interaction with other factors as often

observed in many genes. Further detailed analysis is required to clarify the relationship between Sp1 and other transcription factors.

The frequency of GC allele in Japanese childhood atopic asthma is higher than that of healthy controls, suggesting that GC allele is one of risk factors of childhood atopic asthma [17]. In the present study, GC allele promoter exhibited stronger activity via binding of Sp1 resulting in significantly higher amount of IL-12p70 production. Although IL-12p70 is a TH₁ cytokine and IL-12p70 treatment prevents airway hyperresponsiveness, eosinophilia, and TH₂ cytokine production in asthmatic mice model [33, 34], IL-12p70 blockade by anti-IL-12p35 Ab or anti-IL-12p40 Ab during the challenge phase abolishes above asthmatic symptoms in contrast to the aggravating effect of these neutralization of IL-12p70 in the sensitization phase [35]. Therefore, GC allele may be involved in atopic asthma by causing high level of IL-12p70, which affects the sensitization process. Although expression levels of the other cytokines, IL-12/23p40 and IL-23, showed genotype-dependent tendency with following order of the median: GC/GC > CTCTAA/GC > CTCTAA/CTCTAA, the significant differences were not detected in this study. Considering the function of IL-12/23p40 or IL-23 in allergic responses as described below, there is a possibility that high levels of these cytokines are also associated with the risk of atopic asthma. Determination of these cytokine production levels with larger population will be necessary to clarify the effect of a -6415 polymorphism on the expression levels of IL-12/23p40 and IL-23. Such study is also useful to clarify the difference of IL-12p70 expression levels between heterozygous and each homozygous.

Overexpression of IL-12/23p40 is important for the generation of the TH₂ dominant environment in mice with large intestinal TH₂-type allergic diarrhoea [36]. In IL-12/23p40 transgenic mice, p40 functions as IL-12p70 antagonist *in vivo* with forming IL-12p80 (homodimer of p40), and TH₁ responses in p40 transgenic mice are significantly reduced [26]. These findings suggest that IL-12/23p40 plays a crucial role in TH₂ immune responses. Furthermore, IL-12p80 have been recently found to be not only antagonist but exhibit additional functions, including macrophage chemoattractant, dendritic cell migration inducer, and mediator of lung fibrosis [37]. In this study, monocytes from the GC/GC homozygous genotype produced higher amount of IL-12/23p40 compared with other genotypes (Fig. 3a), suggesting a possibility that -6415 polymorphism is involved in allergic diseases by the induction of dominant expression of IL-12p40 monomer and/or p80 dimer. Determination of p80 production level from monocytes of each genotype, which were not performed in this study due to the absence of p80 detection system, may be useful to clarify this. Alternatively, this polymorphism may contribute to allergic disease by affecting IL-23 production level, since we also found that

the amount of IL-23 produced from monocytes is quite higher than that of IL-12p70 (comparison between Figs 3b and c). The median of IL-23 production from examined monocytes was 11.4 ng/mL, and those of IL-12p70 was 500 pg/mL. IL-23 that is mainly produced by macrophages and DCs [8] was recently identified as a cytokine that induces IL-17 expression [38]. IL-23, but not IL-12, induces the proliferation of memory CD4⁺ T cells and the IL-17 production of CD4⁺ T cells termed TH₁₇ [8, 11, 38] and is involved in maintenance differentiated TH₁₇ cells [39–41]. Elevated IL-17 concentrations were found in lung and blood of allergic asthma patients and were linked to asthma severity [42–46]. IL-17 production is also enhanced in acute AD lesions [47] and allergic contact dermatitis [48]. In this study, we could not find significant association between the level of IL-23 and genotype using monocytes derived from 18 subjects. Further analyses of involvement of the promoter polymorphism of *IL-12B* in IL-23 production should be conducted.

Relationship between a –6415 polymorphism and serum IgE level in asthma has been reported in two studies [16, 17]; GC genotype at –6415 was frequently detected in childhood asthma with higher IgE [17], but average of total serum IgE of adulthood asthma (only men but not women) was higher in individuals carrying CTCTAA genotype [16]. Therefore, a –6415 polymorphism may exhibit a different effect on each allergic disease, including childhood asthma, and adult asthma. Different effect of IL-12 on airway hyperresponsiveness depending on the timing, which was reported in mice model [35], may cause these complicated observations. In mice, IL-12 counteracts TH₂ sensitization, but acts as positive regulator for recruitment of CD4⁺ T cells and eosinophils, and TH₂ cytokines production [35]. The enhanced expression of IL-12p70 with GC genotype may induce the childhood asthma by increasing the sensitivity of sensitization. In contrast, the lower expression of IL-12p70 with CTCTAA genotype may cause TH₂ dominance. Considering that the contradictory results are frequently observed in the statistical analysis of polymorphisms, further analyses with large populations should be performed in various nations. Recently, we found that mast cells, which play central roles in IgE-mediated allergic reactions, also produce IL-12/23p40 significantly essential for host defense against polymicrobial infections of mice [49]. The –6415 promoter polymorphism might be involved in IL-12/23p40 production in human mast cells in response to microbial infection. Regardless, further detailed analysis is necessary for clarify the –6415 polymorphism-mediated allergic risk factor.

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