

Age-Related Changes of Transforming Growth Factor β 1 in Japanese Children

Masahiro Morimoto¹, Eiko Matsui¹, Norio Kawamoto¹, Satomi Sakurai¹, Hideo Kaneko¹, Toshiyuki Fukao¹, Shinichi Iwasa², Makoto Shiraki², Kimiko Kasahara¹ and Naomi Kondo¹

ABSTRACT

Background: Transforming growth factor β 1 (TGF β 1) is an important factor in immunomodulation. The expression of TGF β 1 has been shown to be influenced by the C-509T polymorphism in the TGF β 1 gene. We investigated age-related changes of plasma TGF β 1 levels in a birth-cohort study. In addition, the genotypes of the C-509T polymorphism were investigated in allergic and non-allergic subjects.

Methods: Sixty-four neonates who met the following criteria were enrolled in this cohort study: 1) full-term vaginally delivery; 2) underwent DNA polymorphism analysis; and 3) questionnaire forms were filled out by parents at 0, 6 and 14 months of age. The umbilical cord blood at 0 months and peripheral blood at 6, and 14 months were collected. Plasma TGF β 1 levels were measured at 0, 6 and 14 months of age. Genomic DNA was extracted from their umbilical cord blood. The genotype of the subjects was examined for the presence of C-509T.

Results: The plasma TGF β 1 level at 6 months was the highest of the 3 measurements (at 0, 6, and 14 months of age). The TGF β 1 levels at 14 months in allergic subjects were significantly higher than those in non-allergic subjects ($p = 0.03$). All subjects with bronchial asthma ($n = 3$) had the TT genotype of the C-509T polymorphism.

Conclusions: The plasma TGF β 1 levels change with age. In addition, TGF β 1 may play a role in the pathogenesis of bronchial asthma.

KEY WORDS

bronchial asthma, single nucleotide polymorphism (SNP), transforming growth factor β 1 (TGF β 1), umbilical cord blood

INTRODUCTION

Transforming growth factor β 1 (TGF β 1) is a 25 kDa disulfide-linked homodimeric multifunctional cytokine. TGF β 1 may be the most important growth factor related to immunomodulatory effects because knockout mice died of massive inflammatory lesions.¹ The role of TGF β 1 in allergic diseases have been reported.^{2,4} The levels of TGF β 1 concentration were found to be higher in the bronchoalveolar lavage fluid of patients with asthma compared with subjects without asthma.³ The expression of TGF β 1 was found to be influenced by polymorphisms in the TGF β 1 gene, some of which may be associated with bronchial

asthma and other diseases.^{4,5} In particular, the T allele of the C-509T polymorphism within the TGF β 1 gene was associated with elevated serum TGF β 1 levels and also associated with elevated levels of total IgE in allergic asthma patients.^{5,6}

In this study, we investigated the age-related changes of TGF β 1 levels in a birth-cohort study. In addition, C-509T within the TGF β 1 gene in subjects with asthma was compared with non-allergic subjects.

METHODS

SUBJECTS

Sixty-four neonates born at Iwasa Maternity Hospital in Gifu Prefecture between November 2005 and July

¹Department of Pediatrics, Graduate School of Medicine, Gifu University and ²Iwasa Maternity Hospital, Gifu, Japan.

Correspondence: Eiko Matsui, MD, PhD, Department of Pediatrics, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1193, Japan.

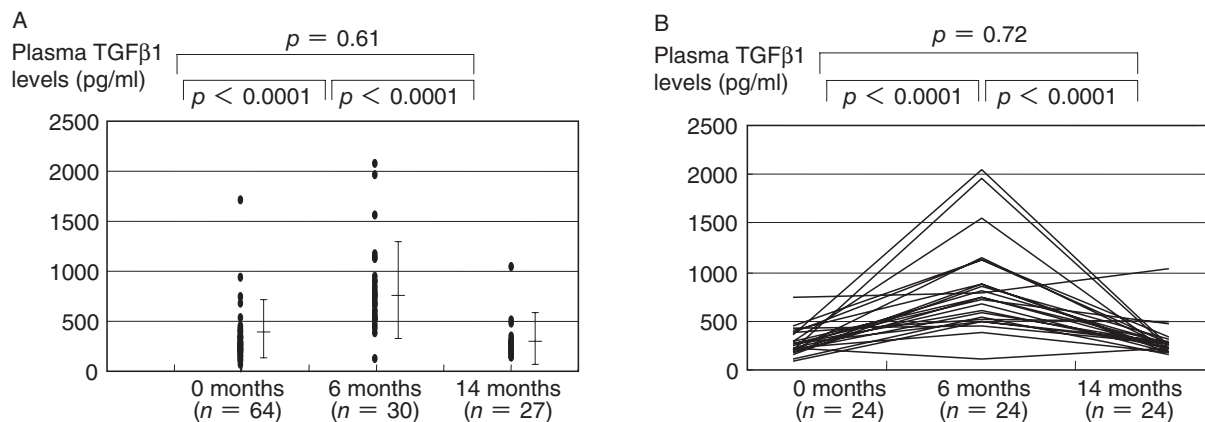
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Table 1 Characteristics of subjects

Gender	Male	32	Female	32
Birth body weight (g)	Mean±SD	3137.1 ± 400.5 (range: 2410–4026g)		
Gestational week	Mean±SD	39w3d ± 1w2d (range: 37w6d–41w1d)		
Family history of allergy	Father	With allergy 25		
		Without allergy 32		
		No record 7		
	Mother	With allergy 34		
		Without allergy 24		
		No record 6		
Family smoking history with 1 or both parents still smoking	Present	16	Absent	48

**Fig. 1** (A) Plasma TGFβ1 levels at 0, 6, and 14 months of age. (B) Changes of plasma TGFβ1 levels in 24 subjects whose data were available at all three points.

2006, and satisfying the following criteria were enrolled in this cohort study: 1) full-term vaginally delivery; 2) underwent DNA polymorphism analysis; and 3) questionnaire forms were filled out by parents at 0, 6 and 14 months of age. Informed consent, including DNA analysis, was obtained from the parents of the neonates during their stay in the hospital. This cohort study was approved by the Ethical Committee of the Graduate School of Medicine of Gifu University. Umbilical cord blood samples were collected from 64 neonates and peripheral blood samples were also collected for analysis from 30 subjects at 6 months and from 27 subjects at 14 months of age. Subjects who were given a diagnosis of atopic dermatitis and bronchial asthma during the follow-up over a period of 14 months after birth, were classified as having those diseases. Allergic subjects were defined as those who were given a diagnosis of atopic dermatitis and/or bronchial asthma and non-allergic subjects were defined as those having neither atopic dermatitis nor bronchial asthma during the 14-month observation period. Some data of the recruited population are summarized in Table 1.

ASSAYS FOR PLASMA IgE LEVELS AND PLASMA TGFβ1 LEVELS

Plasma samples obtained from heparinized blood were kept at -30°C . Plasma IgE levels were determined by chemiluminescent enzyme immunoassay and plasma TGFβ1 levels were measured with a human ELISA kit (R & D Systems, Minneapolis, MN, USA); the detection range was 31.2–2000 pg/ml.

DETECTION OF C-509T WITHIN THE TGFβ1 GENE

Genomic DNA was extracted from the heparinized umbilical cord blood using a Sepagene kit (Sanko Junyaku, Tokyo, Japan). The TGFβ1 gene was amplified and sequenced using the PCR technique and an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). The primers used for amplification of the DNA fragments were the forward primer at position -737– -718 (5'-CAGACTCTAGAGACTGTC AG-3') and the reverse primer at position -320– -338 (5'-GTCACCAGAGAAAGAGGAC-3').

STATISTICAL ANALYSES

The relations between age and plasma in TGFβ1 lev-

Age-Related Changes of Transforming Growth Factor β 1

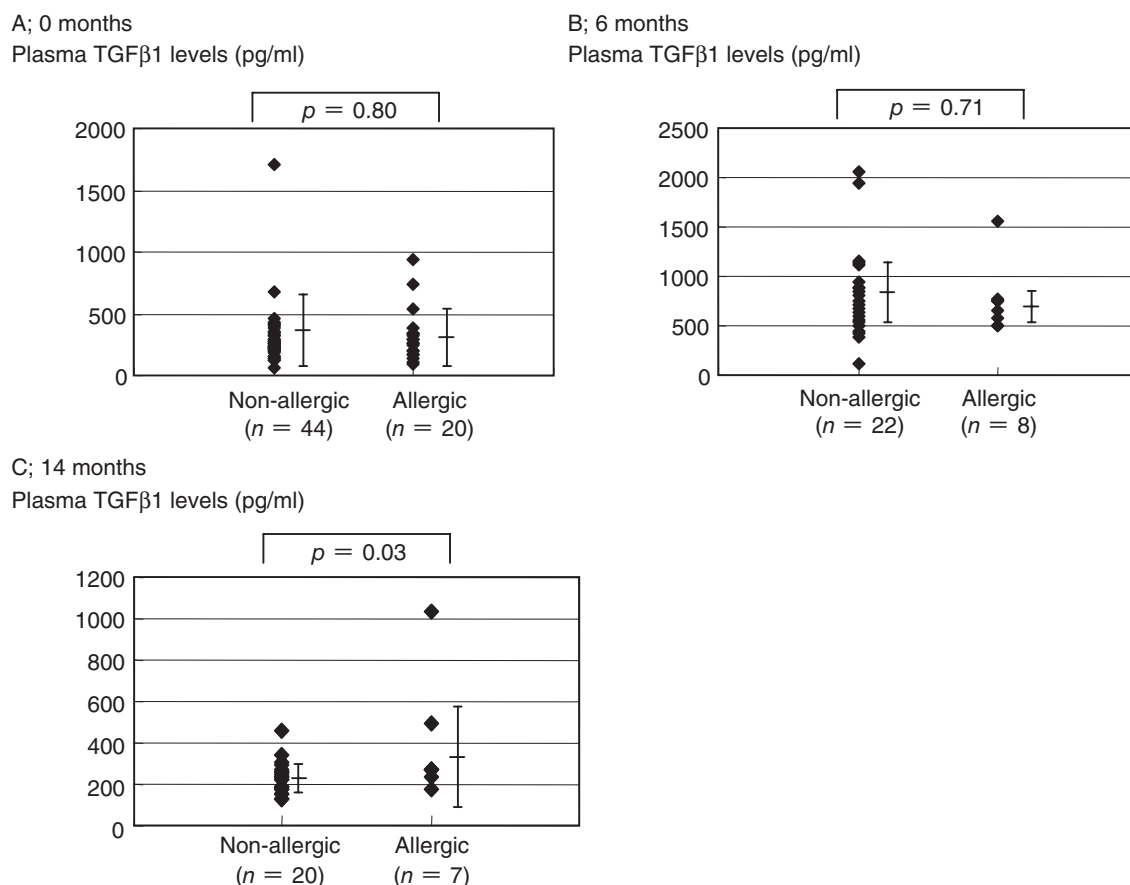


Fig. 2 Plasma TGF β 1 levels of non-allergic and allergic subjects at 0 months (A), 6 months (B) and 14 months (C) are shown. The plasma TGF β 1 levels at 14 months of age in allergic subjects were significantly higher than those in non-allergic subjects ($p = 0.03$, C).

els were determined by using the Mann-Whitney unpaired U-test. Pearson's correlation coefficient was used for statistical analysis of plasma IgE levels and TGF β 1 levels. The chi-square test was performed for statistical analysis of allergic symptoms and C-509T within the TGF β 1 gene. Probability (p) values of less than 0.05 were considered to indicate statistical significance.

RESULTS

ALLERGIC DISORDERS AND SYMPTOMS IN SUBJECTS

By the age of 14 months, 20 of the 64 subjects were allergic and 44 were non-allergic as defined in the Methods. Of the 64 subjects, 19 had atopic dermatitis and 3 had bronchial asthma, while two subjects suffered from both atopic dermatitis and bronchial asthma. Peripheral blood samples were collected at 6 months of age in 30 subjects and at 14 months of age in 27 subjects.

CHANGES IN PLASMA TGF β 1 LEVELS BY AGE

Plasma TGF β 1 levels at 0, 6, and 14 months of age

are shown in Figure 1A. Plasma TGF β 1 levels were 306.2 ± 230.9 pg/ml ($M \pm SD$) at 0 months ($n = 64$), 805.3 ± 428.4 pg/ml at 6 months ($n = 30$), and 273.6 ± 175.8 pg/ml at 14 months ($n = 27$). Plasma TGF β 1 levels at 6 months of age were significantly ($p < 0.0001$ for each) higher than those in both 0 months and 14 months.

TGF β 1 levels were measured at all 3 points in all 24 subjects (data shown in Fig. 1B). The plasma TGF β 1 levels at 6 months of age were significantly ($p < 0.0001$ for each) higher than at both 0 and 14 months. This pattern was observed in 22 of the 24 subjects whose plasma TGF β 1 levels were measured at all 3 points.

RELATIONSHIP BETWEEN PLASMA TGF β 1 LEVELS AND ALLERGIC DISEASES OR PLASMA IgE LEVELS

Figure 2A, B, C show plasma TGF β 1 levels at 0, 6, and 14 months of age in non-allergic and allergic subjects. The plasma TGF β 1 levels at 14 months of age in allergic subjects were significantly higher than those in the non-allergic subjects ($p = 0.03$, Fig. 2C).

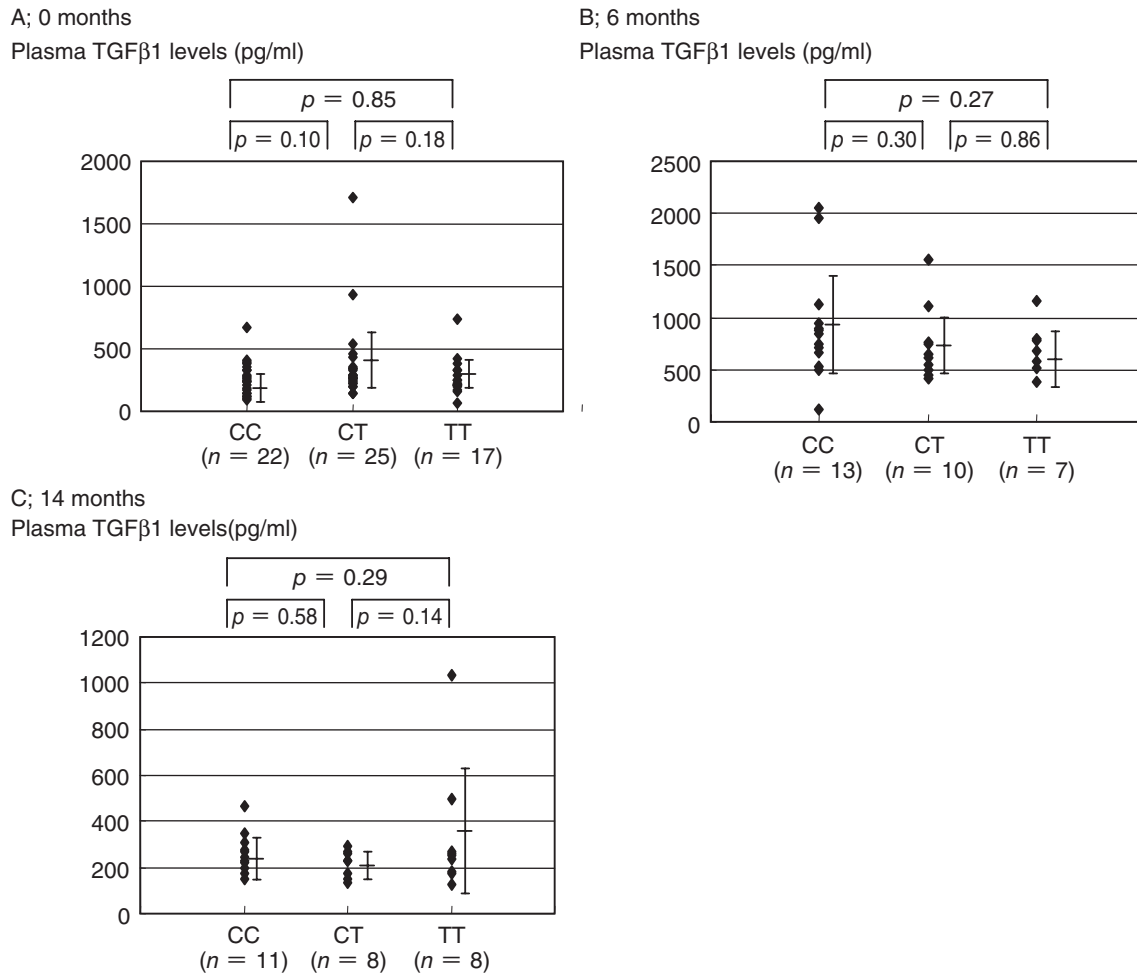


Fig. 3 Relationship between C-509T SNP of TGFβ1 gene and plasma TGFβ1 levels at 0 months (A), 6 months (B) and 14 months (C) is shown.

We also investigated the relationship between plasma IgE levels and TGFβ1 levels at 0, 6 and 14 months of age and found no significant relations (0 months; $p = 0.368$, 6 months; $p = 0.778$, 14 months; $p = 0.818$, data not shown).

PLASMA TGFβ1 LEVELS AND TGFβ1 C-509T POLYMORPHISM

Among the 64 subjects, 22, 25 and 17 were classified as genotype CC, CT, and TT, respectively. As shown in Figure 3, these genotypes were not significantly related with plasma TGFβ1 levels at either 0, 6, or 14 months of age.

TGFβ1 C-509T POLYMORPHISM AND ALLERGIC DISEASES

The genotypes of TGFβ1 C-509T polymorphism were not associated with the prevalence of atopic dermatitis at 6 months of age (Table 2) and 14 months of age (Table 3). However, the TT genotype was present in the TGFβ1 gene (C-509T) in all 3 patients with

asthma at 14 months of age (Table 4). Furthermore, the TGFβ1 levels ($M \pm SD$; 651.42 ± 544.79 pg/ml) in patients with asthma having the TT genotype in the TGFβ1 gene (C-509T) were higher than the TGFβ1 levels ($M \pm SD$; 243.75 ± 89.19 pg/ml) in subjects without asthma (CC, CT or TT genotypes) at 14 months.

DISCUSSION

TGFβ1 is a multifunctional cytokine that has immunomodulatory effects produced by airway epithelial cells, eosinophils, helper T type 2 lymphocytes, macrophages, and fibroblasts. An important finding in this study is the changing pattern of plasma TGFβ1 levels over time at 0, 6, and 14 months of age. Plasma TGFβ1 levels at 6 months of age were the highest among these 3 measured age points. This pattern was observed in 22 of the 24 subjects whose plasma TGFβ1 levels were available at all 3 points. Statistically, the plasma TGFβ1 level at 6 months of age was significantly higher than that at 0 months and at 14

Table 2 Genotype frequency of C-509T *TGF- β 1* promoter SNP in subjects with/without atopic dermatitis at 6 months

	CC	CT	TT	Total
Without atopic dermatitis	18	17	12	47 (74%)
With atopic dermatitis	4	8	5	17 (26%)

 $(p = 0.551)$ **Table 3** Genotype frequency of C-509T *TGF- β 1* promoter SNP in subjects with/without atopic dermatitis at 14 months

	CC	CT	TT	Total
Without atopic dermatitis	18	19	12	49 (77%)
With atopic dermatitis	4	6	5	15 (23%)

 $(p = 0.865)$

months of age ($p < 0.0001$ for each). We believe this is the first time this unique change in the plasma TGF β 1 level has been reported.

A previous study showed that the range of plasma TGF β 1 levels was 2000–4000 pg/ml in adult population.⁵ In our study, the range of plasma TGF β 1 levels at 0, 6, and 14 months of age was 0–2500 pg/ml, and was lower than that of adult populations. At present, we do not know what causes the plasma TGF β 1 level changes, but one possible explanation could be that TGF β 1 is necessary for dramatic changes in immunological response or maturation at around 6 months of age. In other studies, the CD4 or CD8 cells producing cytokines, including TGF β 1, increased with age.^{7,8} An increase in the number of TGF β 1-producing cells may partially explain why TGF β 1 production at 6 months was higher than that at 0 months. We are planning to observe further changes in plasma TGF β 1 levels from 14 months of age to 5 years of age or older.

We could not find any association of IgE with TGF β 1 in this study. TGF β 1 is produced by various cells including airway epithelial cells, eosinophils, lymphocytes, macrophages, and fibroblasts.^{9,10} Various cell factors should be considered to evaluate the level of plasma TGF β 1.

It has been hypothesized that the T allele of the C-509T SNP enhances the Yin Yang 1 (YY1) transcription factor consensus binding site (-CCATCTC/TG-) on the TGF β 1 promoter and is responsible for increased TGF β 1 transcription.¹¹ In the present study there were no significant differences in plasma TGF β 1 levels at 0, 6, and 14 months of age regarding genotypes of *TGF β 1* C-509T. Pulley has shown that the T allele of the C-509T SNP is associated with the diagnosis of asthma and asthma severity.¹² In this study, only 3 subjects were given a diagnosis of bronchial asthma by the age of 14 months. Interestingly, the TT genotype was present in these 3 subjects (Table 4, $p = 0.016$). Although the prevalence of bron-

Table 4 Genotype frequency of C-509T *TGF- β 1* promoter SNP in subjects with/without bronchial asthma at 14 months

	CC	CT	TT	Total
Without bronchial asthma	22	25	14	61 (96%)
With bronchial asthma	0	0	3	3 (4%)

 $(p = 0.016)$

chial asthma at 14 months of age is low we need to obtain data at 5 years of age for an accurate evaluation of the role of the *TGF β 1* polymorphism in bronchial asthma. We are planning to increase the number of subjects to participate in future studies and to conduct a follow up for a longer time span.

In conclusion, this birth-cohort study suggests that plasma TGF β 1 levels are influenced by age and that the C-509T SNP of the TGF β 1 gene is an important susceptibility locus for asthma in infants at 14 months of age, despite the fact that the number of subjects who participated in this study was limited.

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