Association of *TGF-β_1*, *IL-4* and *IL-13* gene polymerphisms with asthma in a Chinese population

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Summary

Background: **Asthma is a common respiratory disease caused by genetic and environmental factors. It has been suggested that *TGF-β_1*, *IL-4* and *IL-13* play important roles in asthma.**

Objectives: **We attempted to confirm the roles of *TGF-β_1*, *IL-4* and *IL-13* polymorphisms in asthma in a Chinese population.**

Methods: **Five SNPs (rs1800469, rs2241712, rs2070874, rs20541 and rs1800925) in *TGF-β_1*, *IL-4* and *IL-13* were genotyped using the MassArray SNP genotyping system. Allelic and genotypic associations between these SNPs and asthma were evaluated using logistic regression analysis.**

Results: **The CT genotype of rs1800469 and T allele of rs20541 were significantly associated with asthma. Among atopic subjects, the CT genotype of rs1800469 and GA genotype of rs2241712 decreased the risk of asthma, while the CC genotype of rs2070874 showed a decreasing trend of asthma risk with a borderline significance. No significant association was found between rs1800925 and asthma.**

Conclusion: **In the present study, we confirmed the association of rs1800469 in *TGF-β_1* and rs20541 in *IL-13* with asthma and found a trend toward association between rs2241712 in *TGF-β_1* and rs2070874 in *IL-4* with asthma among atopic subjects, suggesting *TGF-β_1*, *IL-4* and *IL-13* may be associated with the susceptibility and development of asthma in this Chinese population.** ***(Asian Pac J Allergy Immunol 2011;29:273-7)***

Key words: ***TGF-β_1; IL-4; IL-13****; polymorphism; asthma*

Abbreviations

TGF-β_1	=	Transforming growth factor-β_1
IL4	=	Interleukin 4
IL13	=	Interleukin 13
SNP	=	Single nucleotide polymorphisms
SD	=	Standard Deviation
GINA	=	Global Initiative for Asthma
HWE	=	Hardy-Weinberg equilibrium
OR	=	Odds ratio
95% *CI*	=	95% confidence intervals
B9D2	=	B9 protein domain 2

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Introduction

Asthma is a common respiratory disease characterized by intermittent airway obstruction and respiratory symptoms that are caused by acute and chronic bronchial inflammation. The development of asthma appears to be determined by genetic and environmental factors. Many genes have been shown to be involved in its pathogenesis[1].

Transforming growth factor-β_1 (*TGF-β_1*) is a multifunctional cytokine that may influence asthma by modulating allergic airway inflammation and airway remodeling[2]. It is important in growth, transformation, tissue repair, fibrosis and the modulation of immune inflammatory responses[3]. Interleukin 4 (*IL-4*) and Interleukin 13 (*IL-13*) are immuno-regulatory cytokines produced by activated T helper cells and result in the production of immunoglobulin E by B cells[4]. They share a receptor component and signaling pathways and both play an important role in the development of allergic airway inflammation[5]. To date, some SNPs in the *TGF-β_1*, *IL-4* and *IL-13* genes have been

Table 1. Characteristics of asthma patients and healthy controls

Variable	Case subjects (n=202)	Control subjects (n=205)
Mean age (years)[a]	41.59±15.94	41.16±20.31
Male % (n)	50.00 (101)	75.60 (155)*
FEV1 (mean SD)[a]	61.44±24.00	ND
FVC (mean SD)[a]	67.86±20.24	ND
Smoking (%)	62.16	ND

ND = not done, [a] Mean (±SD), * $P< 0.05$ by χ^2 test

Table 2. Markers genotyped in the current study

Gene	Polymorphism	Other name	Location	Alleles
TGF-β_1	rs1800469	C-509T	Promoter	C/T
TGF-β_1	rs2241712	A-10807G	Promoter	G/A
IL-4	rs2070874	C-33T	Promoter	C/T
IL-13	rs20541	R110Q	Exon 4	C/T
IL-13	rs1800925	C-1024T, C-1111T, C-1055T	Promoter	C/T

reported to be associated with the development of asthma[6-10]. However, not all associations have been replicated in other populations. To address the question as to whether some genetic variations in *TGF-β_1* (rs1800469, rs2241712), *IL-4* (rs2070874) and *IL-13* (rs20541, rs1800925) are associated with asthma, we performed a case-control association study in a Chinese population.

Methods

Study subjects

All subjects in this study were recruited from the outpatient and inpatient clinics of Nanfang Hospital, Guangdong province, southern China, between 2006 and 2009.

Patient samples. Two hundred and two patients with asthma were recruited. The mean age of the asthmatic patients was 41.59 years (SD 15.94; range 11-80 years), and 50.0% of subjects were males. The diagnosis of asthma was based on clinical symptoms and the criteria of the Global Initiative for Asthma (GINA)[11]. Atopic information was derived from the responses to questions about allergy (house dust mite, cat and dog dander, mixed grass pollen and mixed moulds) asked by research nurses at diagnosis.

Normal donors. A total of 205 healthy unrelated subjects who had neither respiratory symptoms nor a history of asthma-related disease were recruited after being interviewed by physicians regarding whether they has been diagnosed with asthma or other lung disease. The mean age of the healthy controls was 41.16 years (SD = 20.31; range 18-89 years), and 75.6% of healthy subjects were males.

The study was approved by the NanFang Hospital Ethics Committee and written informed consent was obtained from all participants.

Genotyping

Genomic DNA was extracted from 200 μl of peripheral blood using the Genomic DNA Purification Kit from Tianamp Biotech, Beijing, China according to the manufacturer's instructions and then stored at -70ºC until use. All SNPs were genotyped using SEQUENOM MassARRAY matrix-assisted laser desorption ionization-time of flight mass spectrometry platform (Sequenom, USA). Primers were designed using a semiautomatic method (Assay Design 3.1, Sequenom).

Statistical analysis

A significant departure of genotype frequency from the Hardy-Weinberg equilibrium (HWE) for each SNP was estimated using Haploview 4.2 software (www. broadinstitute.org/haploview/ haploview). Significant differences in genotype and allele frequency between cases and controls were assessed using the chi-squared test. The associations between polymorphisms and asthma risk were estimated by odds ratio (*OR*) and 95% confidence intervals (95% *CI*), using binary logistic regression analysis for age and sex as covariates under a co-dominant model. Statistical analyses were performed using SPSS 13.0. The significance level was set at 0.05.

Results

The characteristics of the healthy controls and patients with asthma are shown in Table 1. The control subjects were well matched to asthma cases. In age and were in Hardy-Weinberg equilibrium for all polymorphisms addition, the gene symbol, position and alleles for each SNP are present in Table 2. The genotype distributions and allele frequencies of five SNPs in healthy controls and patients with asthma are summarized in Table 3.

In the present study, significant differences were found in the genotype distributions of rs1800469 and rs2241712 in *TGF-β_1* between patients and controls (P = 0.030 and P = 0.008, respectively). The CT genotype of rs1800469 was associated with decreased risk for asthma (*OR*=0.56, *95% CI*: 0.35-0.90, P = 0.016). After adjusting for sex and age, the significance disappeared for rs2241712. The GA genotype

Table 3. Risk associated with the five polymorphisms

SNP	Genotype /Allele	Control n (%)	Case n (%)	P^a	adjusted[b]OR (95% *CI*)	P^c
rs1800469	TT	73 (37.4)	54 (27.4)	0.030*	1.00	
	CT	88 (45.1)	115 (58.4)		0.56 (0.35-0.90)	0.016*
	CC	34 (17.4)	28 (31.2)		0.91 (0.48-1.71)	0.763
	T	234 (60.0)	223 (56.6)	0.334	1.00	
	C	156 (40.0)	171 (43.4)		0.87 (0.65-1.17)	0.363
rs2241712	GG	80 (40.2)	51 (27.4)	0.008*	1.00	
	GA	89 (44.7)	112 (60.2)		0.57 (0.30-1.08)	0.086
	AA	30 (15.1)	23 (12.4)		1.11 (0.56-2.17)	0.772
	G	249 (62.6)	214 (57.5)	0.154	1.00	
	A	149 (37.4)	158 (42.5)		1.19 (0.88-1.61)	0.250
rs2070874	TT	131 (63.9)	132 (65.3)	0.192	1.00	
	CT	67 (32.7)	56 (27.7)		1.33 (0.85-2.09)	0.213
	CC	7 (3.4)	14 (6.9)		0.50 (0.19-1.32)	0.163
	T	329 (80.2)	320 (79.2)	0.713	1.00	
	C	81 (19.8)	84 (20.8)		0.99 (0.69-1.41)	0.943
rs20541	CC	90 (44.3)	105 (52.2)	0.197	1.00	
	CT	80 (39.4)	73 (36.3)		1.31 (0.84-2.05)	0.232
	TT	33 (16.3)	23 (11.4)		1.78 (0.95-3.34)	0.073
	C	260 (64.0)	283 (70.4)	0.054	1.00	
	T	146 (36.0)	119 (29.6)		1.38 (1.02-1.88)	0.040*
rs1800925	CC	148 (72.5)	144 (74.6)	0.871	1.00	
	CT	50 (24.5)	43 (22.3)		1.21 (0.75-1.97)	0.436
	TT	6 (2.9)	6 (3.1)		1.06 (0.32-3.50)	0.925
	C	346 (84.8)	331 (85.8)	0.707	1.00	
	T	62 (15.2)	55 (14.2)		1.15 (0.76-1.73)	0.507

[a] *P* values for differences between controls and patients by χ^2 analysis;

[b] adjusted for age and sex;

[c] *P* values for differences between controls and patients by logistic regression analysis;

* $P < 0.05$.

of rs2241712 showed a trend towards association with the risk of asthma (*OR*=0.57, *95% CI*: 0.30-1.08, *P* = 0.086). Another significant difference was observed in the allele frequency of rs20541 in the *IL-13* gene. The T allele of rs20541 showed a 1.38-fold significantly increased risk for the development of asthma (95% *CI*: 1.02-1.88, *P* = 0.040) and its TT genotype showed a trend towards an association with increased risk for asthma (*OR*=1.78, 95% *CI*: 0.95 to 3.34, *P* = 0.073). As to rs1800925 in *IL-13*, no significant differences were found both in genotype and allele frequencies between patients and controls (*P* = 0.871 and *P* = 0.707, respectively). For rs2070874 in *IL-4* gene, we also did not find any significant differences in genotype and allele frequencies between patients and controls (*P* = 0.192 and *P* = 0.713, respectively) as well.

The genotype distributions of five SNPs in asthmatic patients with non-atopic and atopic status, as compared with controls, are shown in Table 4. Significant differences were observed in genotype distribution for rs1800469 and rs2241712 in *TGF-β_1* between atopic patients and controls. The CT genotype of rs1800469 and GA genotype of rs2241712 were associated with a protective effect for the development of asthma (*OR* = 0.36 and *OR* = 0.19, respectively). Although no significant differences were found for rs2070874 of *IL-4*, its CC genotype was associated with increased risk of asthma with a borderline significance (*P* = 0.054). As to *IL-13*, no significant differences were found for its two SNPs (rs20541, rs1800925) between atopic asthmatic patients and controls.

Discussion

In the present study, we investigated the association between five single nucleotide polymorphisms located in three candidate genes, *TGF-β_1* (rs1800469, rs2241712), *IL-4* (rs2070874) and *IL-13* (rs20541, rs1800925), and asthma in a Chinese population.

The association between rs1800469 polymorphism and asthma was found in our study, which is consistent with the results of previous studies in India[6] and Mexico[12] as well as another Chinese study[13], but contrary to the results of studies in Brazil[14] and the Czech Republic[15]. To date, only one article has reported an association of rs2241712 in *TGF-β_1* with asthma[16]. In this study, the G allele of rs2241712 was associated with decreased asthma, but this difference was not statistically significant (*OR* =0.84;

Table 4. Risk associated with the five polymorphisms of *TGF-β_1*, *IL-4* and *IL-13* in patients with asthma compared with controls by atopic status

SNP/Genotype		Controls n (%)	Non-atopic n (%)	Atopic n (%)	Adjusted[a] *OR*[1] (95% *CI*)	Adjusted[a] *OR*[2] (95% *CI*)
rs1800469	TT	73 (37.8)	18 (38.3)	9 (22.0)	1.00	1.00
	CT	86 (44.6)	25 (53.2)	28 (68.3)	0.82 (0.41-1.66)	0.36 (0.15-0.86)*
	CC	34 (17.6)	4 (8.5)	4 (9.8)	2.11 (0.65-6.87)	1.06 (0.29-3.92)
rs2241712	GG	80 (43.5)	17 (37.0)	6 (16.7)	1.00	1.00
	GA	74 (40.2)	25 (54.3)	27 (75.0)	0.63 (0.31-1.29)	0.19 (0.07-0.53)*
	AA	30 (16.3)	4 (8.7)	3 (8.3)	1.66 (0.50-5.48)	0.70 (0.15-3.24)
rs2070874	TT	131 (63.9)	29 (61.7)	22 (52.4)	1.00	1.00
	CT	67 (32.7)	14 (29.8)	16 (38.1)	1.19 (0.57-2.50)	0.85 (0.40-1.83)
	CC	7 (3.4)	4 (8.5)	4 (9.5)	0.32 (0.08-1.24)	0.26 (0.07-1.03)
rs20541	CC	90 (44.3)	19 (40.4)	20 (48.8)	1.00	1.00
	CT	80 (39.4)	24 (51.1)	15 (36.6)	0.71 (0.35-1.14)	1.20 (0.55-2.61)
	TT	33 (16.3)	4 (8.5)	6 (14.6)	1.53 (0.47-4.97)	1.59 (0.52-4.82)
rs1800925	CC	148 (72.5)	28 (62.2)	29 (70.7)	1.00	1.00
	CT	50 (24.5)	17 (37.8)	10 (24.4)	0.56 (0.28-1.14)	1.12 (0.49-2.55)
	TT	6 (2.9)	0 (.0)	2 (4.9)	-[b]	1.17 (0.13-10.77)

[a] Adjusted for age and gender;

-[b] Odds ratio not calculated due to no cases with TT genotype;

OR^1 = controls vs. non-atopic; OR^2 = controls vs. atopic.

* P <0.05

95% *CI:* 0.62-1.13; P = 0.250), which is consistent with the results reported by Sharma et al[16]. It is interesting that a significant association was found between rs2241712 and asthma among atopic subjects and that the GA genotype was associated with decreased asthma (*OR* =0.19; 95% *CI*: 0.07-0.53; P = 0.002). Rs2241712 is not only located in the promoter region of *TGF-β_1* but also in the intron of the B9 protein domain 2 (*B9D2*), which is localized within the *TGF-β_1* promoter and encodes a B9 domain protein that is exclusively expressed in ciliated organisms. Data from the International HapMap consortium suggests that *TGF-β_1* and *B9D2* are organized in a large haplotype block in Caucasians (HapMap public release number 7)[17]. Another study also reported that rs2241712 was strongly linked with rs1800469[18]. Therefore, *B9D2* might play similar pathogenic role in asthma as *TGF-β_1*.

Studies on the association between rs2070874 polymorphisms in *IL-4* and asthma are very limited[8,9,19]. Our present study did not find a significant association with asthma, but found that its CC genotype among atopic subjects could increase the risk of asthma with a borderline significance (P = 0.054), similar to several previous studies[8,9,19]. With respect to *IL-13*, the association between rs20541 polymorphism and asthma has been extensively reported[9,10,20-24]. We also found that the T allele of rs20541 conveys a significantly increased risk of asthma, which is consistent with other studies.[9,20-22] For the second SNP, rs1800925 in *IL-13*, six studies reported its association with asthma[9,22-26]. However, we did not find a significant association between rs1800925 polymorphism and asthma. Palikhe et al.[23] also were unable to detect significant difference in the genotype of this SNP between asthma patients and controls and our results confirm this finding. This is in contrast to results for the white populations[9,22,24,25] and a separate study in a Chinese population[26]. Inconsistency in the same population is not surprising. Mitsuyasu et al.[27] described an association of the ile50 allele with atopy, whereas a study by Noguchi et al.[28] did not show any association with any allele on the locus.

Taken together, the associations between two SNPs (rs1800469 in *TGF-β_1* and rs20541 in *IL-13*) and asthma risk were confirmed in the present study. A trend toward association was found between other two SNPs, rs2241712 in *TGF-β_1* and rs2070874 in *IL-4*, and asthma, especially among atopic subjects. The association of rs1800925 in *IL-13* with asthma wasn't confirmed. These findings suggest that polymorphisms in *IL-13*, *IL-4*, and *TGF-β_1* genes are associated with asthma susceptibility and development in this Chinese population.

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References

1. Sandford A, Weir T, Paré P. The genetics of asthma. Am Resp Crit Care Med. 1996; 153: 1749-65.
2. Duvernelle C, Freund V, Frossard N. Transforming growth factor-beta and its role in asthma. Pulm Pharmacol Ther. 2003; 16: 181-96.
3. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. N Engl J Med. 2000; 342: 1350-8.
4. Shirakawa T, Deichmann KA, Izuhara K, Mao XQ, Adra CN, Hopkin JM. Atopy and asthma: genetica variants of IL-4 and IL-13 signalling. Immunol Today. 2000; 21: 60-4.
5. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. Science. 1998; 282: 2258-61.
6. Nagpal K, Sharma S, B-Rao C, Nahid S, Niphadkar PV, Sharma SK, et al. TGFbeta1 haplotypes and asthma in Indian populations. J Allergy Clin Immunol. 2005; 115: 527-33.
7. Mak JC, Leung HC, Ho SP, Law BK, Ho AS, Lam WK, et al. Analysis of TGF-beta (1) gene polymorphisms in Hong Kong Chinese patients with asthma. J Allergy Clin Immunol. 2006; 117: 92-6.
8. Kabesch M, Tzotcheva I, Carr D, Höfler C, Weiland SK, Fritzsch C, et al. A complete screening of the IL4 gene: novel polymorphisms and their association with asthma and IgE in childhood. J Allergy Clin Immunol. 2003; 112: 893-8.
9. Black S, Teixeira AS, Loh AX, Vinall L, Holloway JW, Hardy R, et al. Contribution of functional variation in the IL13 gene to allergy, hay fever and asthma in the NSHD longitudinal 1946 birth cohort. Allergy. 2009; 64: 1172-8.
10. López KI, Martínez SE, Moguel MC, Romero LT, Figueroa CS, Pacheco GV, et al. Genetic diversity of the IL-4, IL-4 receptor and IL-13 loci in mestizos in the general population and in patients with asthma from three subpopulations in Mexico. Int J Immunogenet. 2007; 34: 27-33.
11. GINA Workshop Report. Global strategy for asthma Management and Prevention. NIH Publication No 02-3659. Updated 2006. [cited 2010 Sep 9]. Available from: www.ginasthma.com.
12. Li H, Romieu I, Wu H, Sienra-Monge JJ, Ramírez-Aguilar M, del Río-Navarro BE, et al. Genetic polymorphisms in transforming growth factor beta-1 (TGFB1) and childhood asthma and atopy. Hum Genet. 2007;121(5): 529-38.
13. Lv J, Liu Q, Hua L, Dong X, Bao Y. Association of five single nucleotide polymorphism loci with asthma in children of Chinese Han nationality. J Asthma. 2009; 46: 582-5.
14. de Faria IC, de Faria EJ, Toro AA, Ribeiro JD, Bertuzzo CS. Association of TGF-beta1, CD14, IL-4, IL-4R and ADAM33 gene polymorphisms with asthma severity in children and adolescents. J Pediatr (Rio J). 2008;84:203-10.
15. Buckova D, Izakovicová Hollá L, Benes P, Znojil V, Vácha J. TGF-beta1 gene polymorphisms. Allergy. 2001; 56: 1236-7.
16. Sharma S, Raby BA, Hunninghake GM, Soto-Quirós M, Avila L, Murphy AJ, et al. Variants in TGFB1, dust mite exposure, and disease severity in children with asthma. Am J Respir Crit Care Med. 2009; 179: 356-62.
17. The International HapMap Consortium. The International HapMap Project. Nature. 2003; 426: 789–96.
18. Celedón JC, Lange C, Raby BA, Litonjua AA, Palmer LJ, DeMeo DL, et al. The transforming growth factor-beta1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD). Hum Mol Genet. 2004; 13: 1649-56.
19. Liu DF, Liu RM, Cui TP, Wu JM: Correlation between polymorphisms in the IL-4 promoter region and tumor necrosis factor alpha gene and susceptibility of allergic asthma in Chinese children of Han nationality in Hubei province. J Huazhong Univ Sci Tech (Health Sci). 2004; 33: 196-8.
20. Heinzmann A, Mao XQ, Akaiwa M, Kreomer RT, Gao PS, Ohshima K, et al. Genetic variants of IL-13 signalling and human asthma and atopy. Hum Mol Genet. 2000; 9: 549-59.
21. Xi D, Pan S, Cui T, Wu J. Association between IL-13 gene polymorphism and asthma in Han nationality in Hubei Chinese population. J Huazhong Univ Sci Technol Med Sci. 2004; 24: 219-22.
22. Howard TD, Whittaker PA, Zaiman AL, Koppelman GH, Xu J, Hanley MT, et al. Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population. Am J Respir Cell Mol Biol. 2001; 25: 377-84.
23. Palikhe NS, Kim SH, Cho BY, Choi GS, Kim JH, Ye YM, et al. IL-13 gene polymorphisms are associated with Rhinosinusitis and Eosinophilic inflammation in aspirin intolerant asthma. Allergy Astham Immunol Res. 2010; 2:134-40.
24. Beghé B, Hall IP, Parker SG, Moffatt MF, Wardlaw A, Connolly MJ, et al. Polymorphisms in IL13 pathway genes in asthma and chronic obstructive pulmonary disease. Allergy. 2010; 65: 474-81.
25. van der Pouw Kraan TC, van Veen A, Boeije LC, van Tuyl SA, de Groot ER, Stapel SO, et al. An IL13 promoter polymorphism associated with increased risk of allergic asthma. Genes Immun. 1999; 1: 61-5.
26. Wu B, Liu JL, Chen M, Deng RQ, Wu D. Correlation of interleukin-13 gene -1112C/T polymorphisms with asthma and total plasma IgE levels. Chin J Tubere Respir Dis. 2004; 27: 668-71.
27. Mitsuyasu H, Izuhara K, Mao XQ, Gao PS, Arinobu Y, Enomoto T, et al. Ile50Val variant of IL4R alpha upregulates IgE synthesis and associates with atopic asthma. Nat Genet. 1998; 19: 119-20.
28. Noguchi E, Shibasaki M, Arinami T, Takeda K, Yokouchi Y, Kobayashi K, et al. No association between atopy/asthma and the Ile50val polymorphism of IL-4 receptor. Am J Respir Criti Care Med. 1999; 160: 342-5.