

Association study of 15 novel single-nucleotide polymorphisms of the T-bet locus among Finnish asthma families

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Summary

Objective T-box expressed in T cells (T-bet) is a transcription factor regulating the commitment of T helper (Th) cells by driving the cells into the Th1 direction. Abnormal Th1/Th2 balance may lead to complex disorders like asthma or autoimmune diseases. Recent studies have suggested that T-bet might be a candidate gene for asthma. This led us to screen 23 Finnish individuals for single-nucleotide polymorphisms (SNPs) in the T-bet locus and study the association between the SNPs and high serum IgE level and asthma.

Methods We screened all six exons, adjacent intronic areas and 2 kb of the 5'-flanking region from 23 individuals utilizing WAVE™ technology. To explore whether T-bet is associated in serum IgE regulation or asthma we genotyped the SNPs in a Finnish asthmatic founder population. The association analyses were made using haplotype pattern mining.

Results Fifteen novel SNPs were found in the T-bet gene. Within the Finnish asthmatic founder population, there was no association between T-bet SNPs and high serum IgE level or asthma.

Conclusions The genetic variability in the T-bet gene does not play a role in the pathogenesis of human asthma. Our results provide a novel panel of SNPs in T-bet and will help determine whether the SNPs have a functional role in other T cell-mediated diseases.

Keywords asthma, human, SNP, T-bet, Th1/Th2 cells, transcription factors

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Introduction

Allergic asthma and other atopic diseases are characterized by T helper (Th)2-mediated inflammation. Although environmental factors contribute to the development of asthma and atopy, a strong genetic component is evident. Several chromosomal regions have been linked to asthma-related traits [1–5]. Asthma is a multi-factorial disease and, therefore, identification of candidate genes has been difficult.

T-box expressed in T cells (T-bet) (also known as Tbx21 and TBLYM), located in 17q21.32, is a T cell-specific transcription factor regulating in part the differentiation of Th cells. T-bet promotes the differentiation of CD4⁺ T cells to the Th1 direction. Expression of T-bet is up-regulated in response to IFN- γ and activation of the cells through the T-cell receptor (TCR). Up-regulation of T-bet results in enhanced IFN- γ transcription and IL-12R β 2 expression, thus sensitizing the cells for IL-12/STAT4 signalling [6–8]. Th1 cytokines are known to inhibit allergic responses [9, 10]. In a recent study, Finotto et al. [11] observed a reduced expression

of T-bet in human asthmatic airways. In the same study, they demonstrated that T-bet-deficient mice exhibit a phenotype reminiscent of human asthma, even in the absence of allergen exposure. These findings suggest that T-bet might protect from asthma by predisposing the cells to differentiate to the Th1 rather than Th2 direction and further establishing and maintaining the Th1 phenotype. In addition, in one of the several genome-wide screens by Dizier et al. [12] linkage for asthma and asthma-related phenotypes was investigated. In the screen they found linkage to HOX2B gene, which is located close to T-bet in 17q21.32. Taken together these findings suggest that T-bet might be a candidate gene for asthma.

To elucidate the role of T-bet in asthma genetics, we searched for single-nucleotide polymorphisms (SNPs) within the gene in 46 chromosomes. Furthermore, these markers were screened for an association with high levels of serum IgE in asthmatic individuals and controls from a Finnish founder population.

Materials and methods

Patients screened for genetic variation in T-bet

To screen genetic variation in T-bet, we studied two patient groups. Ten patients had the history of allergic asthma (mean

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age 24 years). Their lung function testing showed reversible bronchus obstruction and/or bronchial hyper-reactivity, at least one positive RAST and/or skin prick test (SPT) result, and their mean serum total IgE level was 6065 kU/L ($SD \pm 8429$). Six patients had atopic eczema (mean age 35 years). They had at least one positive RAST and/or SPT result, and their mean serum total IgE level was 12831 kU/L ($SD \pm 4823$). The third group consisted of non-atopic study subjects (mean age 25 years) with no history of asthma or atopy. They were tested in a similar manner and showed normal pulmonary function and no bronchial hyper-reactivity and had negative RAST and SPT results. Their mean serum total IgE level was 143 kU/L ($SD \pm 97$). White blood cells were isolated from peripheral blood using Ficoll-Paque Research Grade (Amersham Pharmacia Biotech, Uppsala, Sweden). Genomic DNA was isolated from white blood cells using propanol/ethanol extraction. Total serum IgE level was determined by Diagnostics CAP FEIA (Kabi Pharmacia, Uppsala, Sweden).

Families in the association study

To study the possible association between found genetic variation and affection status, 120 nuclear families ($N = 360$ study individuals) recruited from central eastern Finland were genotyped. Thirty-two non-overlapping nuclear families (father, mother, and child) were retrieved from large multiplex families used in the genome-wide linkage study [4]. The families were selected through a proband with self-reported asthma. The diagnosis was verified later from hospital records using the following criteria: documented history of asthma symptoms, wheezing by auscultation, and significant reversible airway obstruction and/or bronchial hyper-reactivity. Study subjects who reported asthma symptoms, but did not fulfill our criteria for asthma were classified as phenotype unknown and asymptomatic family members as controls. Serum samples were collected from all individuals and an allergy screening test containing birch, mugwort, timothy, horse, cat, dog, house dust mite, and *Cladosporium herbarum* mould was conducted. If the screening result was positive (>0.3 kU/L), the specific IgE levels for each allergen were measured. In addition, total serum IgE was determined by

Diagnostics CAP FEIA (Kabi Pharmacia) in one batch from all participants. Control for population stratification and clinical evaluation has been described previously in detail [13, 14]. Two phenotypes were analysed: asthma and serum total IgE level. Serum IgE level was analysed as a dichotomized trait: individuals with IgE level >100 kU/L were classified as high responders (affected) and individuals with IgE level ≤ 100 kU/L as low responders (controls).

SNP screening

To find genetic variation in the T-bet locus, PCR products covering all six exons and parts of adjacent introns and 2 kb of 5'-flanking region were analysed with the WAVETM DNA fragment analysis system (Transgenomic, San Jose, CA, USA) among 16 atopic patients and seven controls.

PCR primers were designed for DNA fragments from 200 to 800 bp in length using the Primer Express software (Applied Biosystems, Foster City, CA, USA). Locations of the PCR fragments in the T-bet locus are shown in Fig. 1. PCR fragments were amplified with the following primers: (1) for AACAGCCAAGCACTGAGCAA, rev CAAATAGA GCTGAACCAAGAGGC; (2) for TGTATCCGGTCTC CAACCTCAA, rev ACTGTATAAATCACCCCAAATTC CAC; (3) for GAGGTCTCAGAGGGTGTTCCTGAA, rev CCTGGATGGAAAAGCTGCTTTG; (4) for AAAGC CTCCATCGTCCTATCTC, rev GCTGCATCTTGTAGCT CTAGCC; (5) for TGCTGTGGCTTTATGAAGCTCTC, rev ATGCAGTGCCTTTAAGGAACA; (6) for GGAGA GCTTCATAAAGCCACAGCA, rev AGACGTTCTTGT CCGGCGC; (7) for TGATGTAGGCTTTGCCTGTC, rev TACAGCACACCACTGATGCCT; (8) for GCAAGCCC TACATCACCA, rev TTCATCCCATCTCATCTTCCTC; (9) for CAGGATGACCTCAAGGTGCT, rev CAGGGAG AGGATGTGTCTCAG; (10) for GGTTCTGCTTGTGAC CCGTT, rev GAGGGTACTGGGGTGCCA; (11) for CCC ACCATGTCCTACTACCG, rev CTGTCCTCAGCTTCC TTATCA; (12) for TGACAGCTCCTCCCCTGCT, rev GG ATTAACACACATGCCAACGA; (13) for AGCAGGA GACTCCACTTTCTTC, rev TAAGAGGTAGCATCAAC CAACC; (14) for AGTTACAGCAAGGCCACATGTCC, rev ATCAAGTGCCACCGCCTCAC. PCR assays were

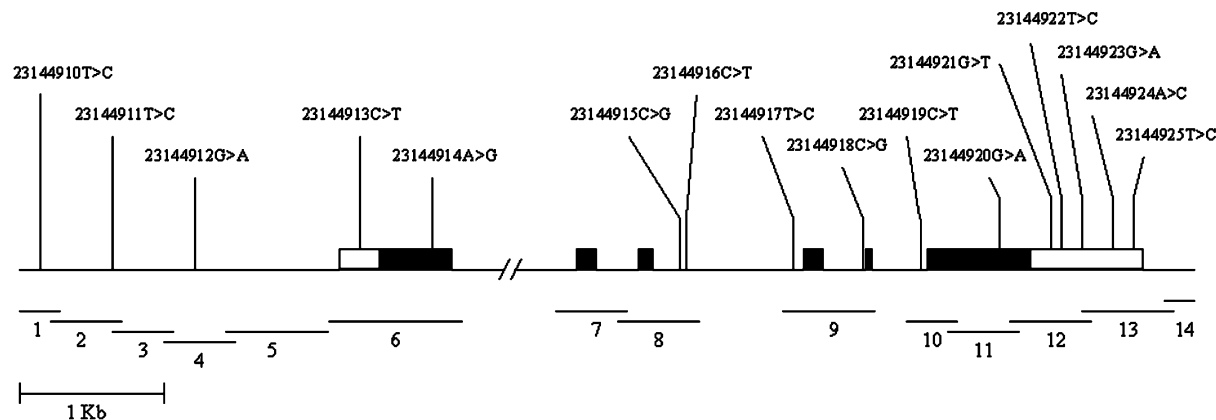


Fig. 1. Genomic location of single-nucleotide polymorphisms (SNPs) identified in the T-bet locus [The SNPs are numbered according to GenBank dbSNP <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp>]. The exons are marked with boxes, in which white areas represents untranslated regions. The length of intron 1 is 8.66 kb. The PCR fragments used for the screening of the locus are marked with lines below the gene. Primers for these fragments are described in Materials and methods.

made in volume of 50 µL containing 100–250 ng of template DNA, 200 µM dNTP (MBI Fermentas, Vilnius, Lithuania), 400–800 nM primers (Medprobe, Oslo, Norway), Dynazyme EXT 1.5–2 U (Finnzymes, Espoo, Finland), 10 × Dynazyme Tritonfree buffer (Finnzymes) (5% DMSO; Finnzymes).

After PCR amplification, mutations were detected in the samples using temperature-modulated heteroduplex analysis (TMHA) based on denaturing high-performance liquid chromatography (DHPLC), WAVETM (Transgenomic) as described previously [15]. The melting behaviour of each amplicon was determined with WAVEMAKER software (Transgenomic) and Melt94 program (<http://web.mit.edu/osp/www/melt.html>). In the case of heteroduplex formation two amplicons of both genotypes were sequenced with the ABI Prism[®] 3100 Genetic Analyzer (Applied Biosystems) to define and verify the specific SNP. Analysis of the sequence data was done with SequencherTM software (Gene Codes Corporation, Ann Arbor, MI, USA).

SNP genotyping

Genotyping was accomplished by the SNaPshotTM method according to the manufacturer's directions (Applied Biosystems) [16]. In a multiplex reaction the primers differed from each other by approximately 10 bp in length to create the size difference between markers (Table 1). The SNPs were genotyped in three panels: (1) 23144910 T>C, 23144911 T>C, and 23144912 G>A; (2) 23144913 C>T and 23144914 A>G; (3) 23144915 C>G, 23144916 C>T, 23144917 T>C, 23144918 C>G, 23144919 C>T, and 23144920 G>A. For PCR, reaction volume was 25 µL containing 20 ng of template DNA and specific PCR primers, which are shown in Table 1. Otherwise the PCR reactions were performed as mentioned above. For primer extension reactions we used 2-U shrimp alkaline phosphatase (SAP) and Exonuclease I (ExoI) (both from UBS, Cleveland, OH, USA), purified PCR-amplified products diluted 1:20–1:100, and extension primers in concentrations of 50–900 nM. After we had demonstrated that diluting SNaPshotTM ready reaction mix did not affect the results we were able to use it in proportion of 2:3 with 1:10 dilution of 20 mM Mg²⁺ and 800 mM Tris-HCl, pH 9.0. After primer extension the products were purified again with SAP and run with the ABI Prism[®] 3100. The results were analysed with GeneScan software (Applied Biosystems).

Since the 3'-untranslated region (UTR) region of *T-bet* contained six SNPs close to each other, we used direct sequencing to genotype them at the same time (Table 1). Purified PCR fragments were sequenced in reverse direction using the ABI Prism[®] 3100 sequencer and dye-terminator chemistry (Applied Biosystems). If the genotype remained ambiguous, sequencing was performed also in forward direction. We assembled the sequence reads using the SequencherTM software (Gene Codes Corporation).

All the markers were in Hardy–Weinberg equilibrium and observed Mendel errors were less than 0.001% [17]. The genotyping success rate was 98.3%.

Statistical analysis

The association analyses were made using haplotype pattern mining (HPM) [18]. Haplotyping was done within each trio

and four independent chromosomes were obtained from each trio. In case of ambiguities (missing genotype data, identical heterozygotic genotypes in all of the family members, or Mendel errors), the alleles were discarded. Haplotyping was done independently for both asthma and for high IgE phenotypes. If the child was affected, the transmitted chromosomes were considered disease-associated and the non-transmitted chromosomes as controls. If one of the parents was affected, his/her chromosomes were considered disease associated and the spouse's chromosomes as controls. If both the parent and the child were affected, only the non-transmitted chromosome of an unaffected parent was considered as the control and the other three as disease associated. These haplotypes were used as input for HPM [18].

The HPM algorithm finds all the haplotype patterns up to a given length whose association to the trait, measured by a χ^2 test, exceeds a given threshold value. Number of allowed gaps in the data can be defined by the user. Based on the found patterns, each marker is given a score based on the number of patterns spanning across the marker. HPM estimates the significances of the obtained scores against the null hypothesis that there is no association. The null distributions are estimated by permuting the trait values and recalculating the scores several times. Comparing the observed scores to these distributions yields marker-wise *P*-values that are comparable with each other.

To assess the power of the sample size to detect a haplotype association to asthma, computer simulations were performed. In the simulations the observed prevalence of asthma in Finland, the size of the study cohort, and the frequencies of potential disease causing haplotype were fixed. Ten hundred thousand iterations were performed. In each iteration the carrier status of all individuals was recomputed and a χ^2 test was performed for the simulated frequencies. The power corresponds to the proportion of iterations where the test yielded the selected significance level (0.05). As a consequence, the power (probability of observing the association between the variant and the disease status) was observed for the best associating haplotypes in the locus.

Results

Identification of 15 novel SNPs in the *T-bet* locus

In order to improve our possibilities to find atopy related and functionally important polymorphisms in *T-bet*, we enriched the pool of screened individuals (*N* = 23) with 10 unrelated asthma patients and six patients with atopic dermatitis who all had high total serum IgE levels (>1143 kU/L) that was used as a surrogate marker of IgE-mediated allergies. The screening was done using the TMHA-based method and included all exons, exon–intron boundaries, and 2 kb of the 5'-flanking region of the gene (Fig. 1), covering altogether 6 kb. Our screen did not cover all intronic sequences. There are three gaps of sizes 8.5 kb, 665, and 103 bases in introns 1, 3, and 5, respectively. TMHA-identified polymorphisms were verified by direct sequencing.

The study resulted in the identification of 15 novel SNPs and verification of one SNP described previously among the

Table 1. The identified SNPs in the T-bet gene and their position in the mRNA NM_013351, primers used in PCR, single base pair extension and sequencing

GenBank SNP id no. (ss#)	Alleles	Location in the T-bet gene	Position in mRNA NM_013351	PCR primers 5' → 3'	SNAPshot primer or sequencing primer 5' → 3'	Genotyping method sequencing (S)/SNAPshot (P)
23144910*	T > C	5'-flanking		CAAGGTACCCCTCTCTTCCAAG AGTCATGGGCACGTGGAAGTAGC	(T ₃₀) TGTTCTTAAGTACGGAGAAATGG	P
23144911	T > C	5'-flanking		CAAGGTACCCCTCTCTTCCAAG AGTCATGGGCACGTGGAAGTAGC	(T ₇) ATACTCTGTCTGTCTGTCTTTGCACAC	P
23144912*	G > A	5'-flanking		CAAGGTACCCCTCTCTTCCAAG AGTCATGGGCACGTGGAAGTAGC	(GACT) GGCACGTGAGGTTGACTTTCA	P
23144913	C > T	5'-UTR	133	GGAGAGCTTCATAAAGCCACAGCA AGACGTTCTTGTTCCGGCGC	(A ₁₈) CGCGTGGGCTGGGATG	P
23144914	A > G	Exon 1	601	GGAGAGCTTCATAAAGCCACAGCA AGACGTTCTTGTTCCGGCGC	(GACT ₆) AGTTCCCGACACCTCCAG	P
23144915*	C > G	Intron 3		GCAAGGCCCTACATCACCA TTCATCCATCTCATCTTCCTC	(T ₄₈) GAAGGTCGTTTTTCTTCTGTCTATAA	P
23144916*	C > T	Intron 3		GCAAGGCCCTACATCACCA TTCATCCATCTCATCTTCCTC	(T ₄₀) AGAAACAGCCAGAGTTTAGGAAGGA	P
23144917	T > C	Intron 3		CAGGATGACCTCAAGGTGCT CTGCTTTCAGCTTCCTTATCA	(T ₂₅) GGGGAGGAAGGATTTCGAGG	P
23144918	C > G	Intron 4		CAGGATGACCTCAAGGTGCT CTGCTTTCAGCTTCCTTATCA	(T ₇) GACTCAGGTGACTCTATTTCCCTTCTCT	P
23144919*	C > T	Intron 5		CAGGATGACCTCAAGGTGCT CTGCTTTCAGCTTCCTTATCA	GGTTCTGTCTTGACCCGTTTT	P
23144920	G > A	Exon 6	1666	CAGGATGACCTCAAGGTGCT CTGCTTTCAGCTTCCTTATCA	(T ₄₁) GCCCCCATCCGGCC	P
23144921	G > T	3'-UTR	1975	AGAACACCTTGGGTACACCAGC GACTAATTTGGGAAACGGATGAA	GACTAATTTGGGAAACGGATGAA	S
23144922*	T > C	3'-UTR	1988	AGAACACCTTGGGTACACCAGC GACTAATTTGGGAAACGGATGAA	GACTAATTTGGGAAACGGATGAA	S
23144923	G > A	3'-UTR	2122	AGAACACCTTGGGTACACCAGC GACTAATTTGGGAAACGGATGAA	GACTAATTTGGGAAACGGATGAA	S
23144924*	A > C	3'-UTR	2314	AGAACACCTTGGGTACACCAGC GACTAATTTGGGAAACGGATGAA	GACTAATTTGGGAAACGGATGAA	S
23144925	T > C	3'-UTR	2549	AGAACACCTTGGGTACACCAGC GACTAATTTGGGAAACGGATGAA	GACTAATTTGGGAAACGGATGAA	S

*SNPs published whilst this work was under review.

SNP, single-nucleotide polymorphism; UTR, untranslated region.

Japanese (exon 1, ss23144914). Two of the SNPs were found in the coding region, both silent. One was located in the 5'-UTR, five in 3'-UTR, five in introns, and three in the 5'-flanking region (Fig. 1). Another SNP (rs2240017) potentially causing an amino acid change His33Glu, which has been described also among the Japanese with the allele frequency of 13%, could not be found among the Finns.

Association study among the Finnish asthma families

We genotyped all 15 SNPs among the Finnish asthma families. The dataset contained 120 nuclear families (father, mother, offspring) with full-phase and phenotype information. The association analysis was done in the family dataset independently for both asthma and high serum total IgE level. For asthma, these families revealed 199 disease-associated chromosomes and 161 family-based control chromosomes; and for high IgE, 277 trait-associated chromosomes, and 203 family-based control chromosomes [13, 14]. No allele associations were found between T-bet polymorphisms in either of the study groups compared with the family-based control chromosomes (Table 2). The frequencies of the minor alleles varied between 1% and 30% in the pools of control chromosomes.

Together these SNPs form a dense marker map across the T-bet locus spanning 15 kb in size. Phase information allowed us to continue with the haplotype analysis, which is more informative than single-allele association. For haplotype analysis we used the HPM algorithm [18]. All haplotype associations for both phenotypes remained weak and statistically non-significant ($\chi^2 < 3.7$ for IgE and < 1.3 for asthma, data not shown), when the maximum length of the haplotype pattern was defined to be 15 markers and two to four gaps in the haplotype patterns were allowed. For high IgE, one haplotype (23144911*C-23144915*C-23144916*C-23144917*T-23144918*C-23144919*C-23144920*G-2314492

1*T-23144922*T) was found in five trait-associated chromosomes, but not in any of the control chromosomes. For asthma haplotype (23144914*G-23144915*G-23144916*T-23144917*T-23144918*C-23144920*G-23144921*G-23144922*C-23144923*G-23144924*A) was found in six trait-associated chromosomes and in two control chromosomes (population frequency 1.2%). Based on simulations, our study design had high power ($> 85\%$) to exclude a relative risk of more than 5.9 for these rare haplotypes in the T-bet locus at a P -level of 0.05.

To study potential linkage disequilibrium across the T-bet locus indifferent towards the studied phenotypes, we applied again the HPM algorithm [18]. We allowed 30% missing data, but no errors in the haplotype patterns of 15 markers. A total 460 chromosomes filled the criteria. Four most common haplotypes explained 88% of all haplotypes (Table 3). Ten rare haplotypes explained the rest of the haplotypes occurring among the Finns with the frequency from 1% to 3%.

Discussion

T-bet is a transcription factor regulating the commitment of Th lymphocytes. T-bet induces IFN- γ production and establishes functional IL-12/STAT4 signalling and thus drives the cells to the Th1 direction [6–8]. Finotto et al. [11] have shown reduced expression of T-bet in CD4⁺ cells in the lungs of asthmatic patients. They also described that T-bet-deficient mice develop spontaneous airway hyper-reactivity. These findings suggest that impaired function of T-bet might be associated with asthma. In addition, the chromosomal region where T-bet is located, 17q21.2, has been linked to asthma and atopy [12]. Therefore, T-bet can be considered as a functional candidate gene for asthma, and a regulator of Th1/Th2 differentiation.

We found 15 novel SNPs in the T-bet locus by screening all six exons, exon–intron boundaries, and 2 kb of the 5'-flanking region. In addition, a previously known marker ss23144914 was detected in our screening population (Fig. 1. and Table 2). The expected frequency of SNPs in the human genome is 1/750–1000 bases [19], but the variation is high (reviewed in ref. [20]). SNPs showed strong linkage disequilibrium across the locus (Table 3). Interestingly, no markers coding for a change in amino acid sequence were found even in as large material as 44 chromosomes screened. We believe that the proper function of T-bet is highly essential for the immune system and this explains why little divergence is allowed in its protein structure.

At this stage it is impossible to estimate whether the SNPs have some biological function. There are several examples of SNPs altering the production or the function of the particular protein [21–25]. Among the panel of novel SNPs described here there are many interesting candidates for further functional studies, especially the SNPs in the UTR regions and ss23144918 C > G, which is located in the splice site at the border of intron 4 and exon 5.

Despite the fact that T-bet is functionally a potential candidate gene for asthma, our study representing still a relatively small sample of the Finnish asthma families found no evidence of an association between the common variants

Table 2. Major allele frequencies of T-bet SNPs among high IgE and asthma-related chromosomes

GenBank SNP id no.*	Allele	High IgE		Asthma	
		Affected (%), <i>n</i> = 277	Control (%), <i>n</i> = 203	Affected (%), <i>n</i> = 199	Control (%), <i>n</i> = 161
23144910	T > C	26.5	29.5	27.8	30.8
23144911	T > C	19.1	19.9	18.4	20.8
23144912	G > A	9.0	8.9	9.8	9.7
23144913	C > T	1.8	2.0	2.5	1.9
23144914	A > G	27.0	29.9	28.2	31.3
23144915	C > G	20.7	21.1	21.6	20.8
23144916	C > T	21.4	22.2	21.6	22.7
23144917	T > C	1.8	2.0	2.5	1.9
23144918	C > G	1.8	2.0	2.5	1.9
23144919	C > T	18.8	20.0	18.6	21.2
23144920	G > A	5.8	7.9	7.5	8.1
23144921	G > T	1.8	0.5	1.5	1.9
23144922	T > C	21.4	21.8	21.6	22.2
23144923	G > A	6.7	9.5	8.3	9.0
23144924	A > C	18.0	19.1	17.5	19.6
23144925	T > C	2.5	3.0	2.0	0.6

*Alleles numbered according to GenBank dbSNP (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp>).

Table 3. Four most frequent haplotypes among the Finnish asthma families

Percentage of all haplotypes	23144910	23144911	23144912	23144913	23144914	23144915	23144916	23144917	23144918	23144919	23144920	23144921	23144922	23144923	23144924	23144925	χ^2
65.0	1*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.7
8.5	2†	2	1	2	2	1	2	1	2	1	1	2	1	2	2	1	-0.0
7.8	2	2	1	2	2	1	2	1	2	1	1	2	1	2	2	1	-0.1
6.5	2	1	1	2	2	1	1	1	1	2	2	1	2	1	1	1	-0.3

Alleles numbered according to GenBank dbSNP.

*Major allele. †Minor allele.

of T-bet and asthma or serum IgE level, which is an important indicator and mediator in allergic inflammation. Our study population represents a population isolate. For common alleles (frequency >5%), however, the overall frequencies in Finland are known to be similar to other European countries and therefore, our results, showing no association, most likely are comparable with results in populations of more mixed origin [26]. The fact that no association was found in this population suggests that if T-bet plays a role in the pathogenesis of human asthma it is mediated by mechanisms other than genetic variability in the gene. T-bet is one of the key regulators of Th1/Th2 commitment and therefore its role is worth studying also in immunological disorders other than asthma. One disorder of interest is Th1-dependent colitis, e.g. Crohn's disease, in which T-bet has been shown to regulate the Th1-type inflammation in the intestinal mucosa [27]. We have optimized an effective system for genotyping T-bet SNPs, which enables us to rapidly genotype any larger phenotypically well-defined patient population of interest.

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References

- 1 Cookson WMM. Genetics of asthma and allergic disease. *Hum Mol Genet* 2000; 9:2359.
- 2 Haagerup A, Bjerke T, Schiotz PO, Binderup HG, Dahl R, Kruse TA. Asthma and atopy – a total genome scan for susceptibility genes. *Allergy* 2002; 57:680.
- 3 Hakonarson H, Bjornsdottir US, Halapi E et al. A major susceptibility gene for asthma maps to chromosome 14q24. *Am J Hum Genet* 2002; 71:3.
- 4 Laitinen T, Daly MJ, Rioux JD et al. A susceptibility locus for asthma-related traits on chromosome 7 revealed by genome-wide scan in a founder population. *Nat Genet* 2001; 28:87.
- 5 Van Eerdedewegh P, Little RD, Dupuis J et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002; 418:426.
- 6 Afkarian M, Sedy JR, Yang J et al. T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells. *Nat Immunol* 2002; 3:549.
- 7 Lighvani AA, Frucht DM, Jankovic D et al. T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. *Proc Natl Acad Sci USA* 2001; 98:15137.
- 8 Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000; 100:655.
- 9 Gavett SH, O'Hearn DJ, Li X, Huang SK, Finkelman FD, Wills-Karp M. Interleukin 12 inhibits antigen-induced airway hyperre-

- sponsiveness, inflammation, and Th2 cytokine expression in mice. *J Exp Med* 1995; 182:1527.
- 10 Li XM, Chopra RK, Chou TY, Schofield BH, Wills-Karp M, Huang SK. Mucosal IFN- γ gene transfer inhibits pulmonary allergic responses in mice. *J Immunol* 1996; 157:3216.
 - 11 Finotto S, Neurath MF, Glickman JN et al. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science* 2002; 295:336.
 - 12 Dizier MH, Besse-Schmittler C, Guilleud-Bataille M et al. Genome screen for asthma and related phenotypes in the French EGEA study. *Am J Respir Crit Care Med* 2000; 162:1812.
 - 13 Kauppi P, Laitinen LA, Laitinen H, Kere J, Laitinen T. Verification of self-reported asthma and allergy in subjects and their family members volunteering for gene mapping studies. *Respir Med* 1998; 92:1281.
 - 14 Laitinen T, Kauppi P, Ignatius J et al. Genetic control of serum IgE levels and asthma: linkage and linkage disequilibrium studies in an isolated population. *Hum Mol Genet* 1997; 6:2069.
 - 15 Kuklin A, Munson K, Gjerde D, Haefele R, Taylor P. Detection of single-nucleotide polymorphisms with the WAVE DNA fragment analysis system. *Genet Test* 1997; 1:201.
 - 16 Turner D, Choudhury F, Reynard M, Railton D, Navarrete C. Typing of multiple single nucleotide polymorphisms in cytokine and receptor genes using sNaPshot. *Hum Immunol* 2002; 63:508.
 - 17 O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 1998; 63:259.
 - 18 Toivonen HT, Onkamo P, Vasko K et al. Data mining applied to linkage disequilibrium mapping. *Am J Hum Genet* 2000; 67:133.
 - 19 Wang DG, Fan JB, Siao CJ et al. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science* 1998; 280:1077.
 - 20 Chakravarti A. Population genetics – making sense out of sequence. *Nat Genet* 1999; 21:56.
 - 21 Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP. Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. *J Immunol* 2001; 166:3915.
 - 22 Shen LX, Basilion JP, Stanton VP Jr. Single-nucleotide polymorphisms can cause different structural folds of mRNA. *Proc Natl Acad Sci USA* 1999; 96:7871.
 - 23 Nickel RG, Casolaro V, Wahn U et al. Atopic dermatitis is associated with a functional mutation in the promoter of the C–C chemokine RANTES. *J Immunol* 2000; 164:1612.
 - 24 Wong PM, Yuan Q, Chen H, Sultzner BM, Chung SW. A single point mutation at the 3'-untranslated region of Ran mRNA leads to profound changes in lipopolysaccharide endotoxin-mediated responses. *J Biol Chem* 2001; 276:33129.
 - 25 Tzetis M, Efthymiadou A, Doudounakis S, Kanavakis E. Qualitative and quantitative analysis of mRNA associated with four putative splicing mutations (621+3A $\rightarrow$ G, 2751+2T $\rightarrow$ A, 296+1G $\rightarrow$ C, 1717-9T $\rightarrow$ C-D565G) and one nonsense mutation (E822X) in the CFTR gene. *Hum Genet* 2001; 109:592.
 - 26 Kere J. Human population genetics: lessons from Finland. *Annu Rev Genom Hum Genet* 2001; 2:103.
 - 27 Neurath MF, Weigmann B, Finotto S et al. The transcription factor T-bet regulates mucosal T cell activation in experimental colitis and Crohn's disease. *J Exp Med* 2002; 195:1129.