

Short communication

STAT1 gene variations, IgE regulation and atopy

Background: Signal transducer and activator of transcription 1 (STAT1), an intracellular signal transducer and activator of transcription centrally involved in many inflammatory pathways, was recently suggested to play an important role in allergy related immune responses.

Aim: Thus, we investigated the effect of polymorphisms in the *STAT1* gene on the development of atopic sensitization and allergic diseases.

Methods: Haplotype tagging single nucleotide polymorphisms (SNPs) previously described in the *STAT1* gene were genotyped by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry technology in a cross-sectional study population of 3099 German children recruited and phenotyped by the International Study of Asthma and Allergy in Childhood, phase II (ISAAC II). Effects of single SNPs and haplotypes were studied using SAS/Genetics and Haploview.

Results: The polymorphism C39134A (rs3771300), located in a potentially *cis* acting regulatory element in STAT1 intron 24, was inversely related to atopy measured by skin prick test, total and specific serum IgE levels while no effect on atopic disease risk was observed.

Conclusion: Our results indicate that *STAT1* SNP C39134A may protect from atopic sensitization. Because of its location in a highly conserved noncoding sequence near a putative GATA3 binding site, this polymorphism represents an interesting target for further studies.

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Numerous mediators, such as cytokines, chemokines, and adhesion molecules contribute to the development of allergic inflammation. The expression of these very diverse mediators involved in allergic disease mechanisms is determined by a limited number of redundant intracellular transcription factors (TF). The STAT (signal transducer and activator of transcription) family represents a very prominent group of TF regulating various genes important for cellular and humoral immunity (1). It has been known for some time that STAT4 and STAT6 are centrally involved in regulating T-cell differentiation (2) and STAT6 is necessary for IgE switching in B cells (3). Only recently, the role of other STAT molecules in allergy was determined. Thus, it was shown that the blockade of STAT1 signalling inhibits the development of allergen-induced eosinophilic airway inflammation in a mouse model and decreases *in vivo* airway reactivity (4). Previous studies indicated that genetic variations in *STAT4* and *STAT6* significantly affect IgE regulation and the development of atopy and asthma (3, 5). Thus, we investigated if polymorphisms in the *STAT1* gene may also influence atopic diseases and studied *STAT1* haplotype tagging single nucleotide polymorphisms (SNPs) in 3099 German children.

Methods

Population description

Between 1995 and 1996, a cross-sectional study was performed in Munich and Dresden, Germany, as part of the International Study of Asthma and Allergies in Childhood (ISAAC phase II), to assess the prevalence of asthma and allergies in school children, aged 9–11 years ($n = 5629$). All children of German origin with DNA available were included in this analysis ($n = 3099$). Informed written consent was obtained from parents and the study methods were approved by the respective ethics committees. The population and phenotyping methods have been described in detail before (6). Self-administered questionnaires included the ISAAC core questions. Children whose parents reported a physician's diagnosis of asthma, spastic bronchitis or recurrent asthmatic bronchitis were classified as asthmatics. Children's classification of hay fever or atopic eczema was also based on parental report of a physician's diagnosis of the respective diseases. A child was considered atopic if a wheal reaction of ≥ 3 mm occurred in a skin prick test (SPT) (Alk Scherax GmbH, Wedel, Germany) to one or more allergens (*Dermatophagoides pteronyssinus*, *Dermat-*

Table 1. *STAT1* polymorphisms and their respective position within the gene, rs numbers, primers used for genotyping, minor allele frequency (MAF), successful genotyping call rate (Call R, %) and the *P*-value for deviation from Hardy–Weinberg Equilibrium (pHWE)

<i>STAT1</i> Variations*	Location	rs number	1 st PCR primer; 2 nd PCR primer; Extension primer	MAF	Call R (%)	pHWE
1_T-5741C	Promotor	1467198	ACGTTGGATGCCCATGGATATGCCTATATG; ACGTTGGATGTGTGAGGGTGCAATTTGTGAC; TGTGCAAAAGAAAAAACTCA	0.31	97.99	<i>P</i> = 0.190
2_G-4831A† (G-5773C/G-3925C/AG-642del)	Promotor	10195683	ACGTTGGATGAGAGGTGTGGACGGGATAAG; ACGTTGGATGTTCTCCTAAACGCTGTGCTG; AACGCTGTGCTGTGATGACCT	0.20	97.99	<i>P</i> = 0.233
3_T-4410C	Promotor	N/A\$	ACGTTGGATGTTCTGCAACACAGCACGTC; ACGTTGGATGTGTTGCCACGCTGGGAACG; TGGGAACGCGCTTCTGTTTAC	0.13	96.86	<i>P</i> = 0.912
4_A-3758G	Intron1	N/A\$	ACGTTGGATGAGTGACGGTAAATGGGAAGG; ACGTTGGATGATTGTCCTTGCCAGTCGTGC; ccACTTGTGAAATATAATTTCTCTCT	0.10	93.45	<i>P</i> = 0.630
5_G-306A	Intron 2	N/A\$	ACGTTGGATGAGGTGACAGTGGTGGTGAAG; ACGTTGGATGTAAACTGCAACACCTCCC; TCCCACCTGGAAACATG	0.12	98.20	<i>P</i> = 0.774
6_T63C‡	Exon 3	2066802	ACGTTGGATGTCAGTGGTACGAACTTCAGC; ACGTTGGATGGCCAGGTACTGTCTGATTTC; ATGGGAAAACGTGCATCATA	0.06	98.50	<i>P</i> = 0.094
7_del1569ATT† (G-4688A /C-3387del)	Intron 3	N/A\$	ACGTTGGATGTAACGTGATAGGTGTTCTAC; ACGTTGGATGGGGACCCCTTCACTTTCTATG; GGACGTTTTTAAATTAGCAAATAA	0.42	98.86	<i>P</i> = 0.313
8_T1177A	Intron 4	10199181	ACGTTGGATGCTTATGCTATATTTACTGATGC; ACGTTGGATGATTGAGCACACACTTATTGG; AATAGAGACTAACTCCATTATGTT	0.32	97.53	<i>P</i> = 0.766
9_G19209A	Intron 11	7562024	ACGTTGGATGCTAGGCCATTTACTGTGGTG; ACGTTGGATGCTGATGGATGGATTATAGC; TATAGCTTTCATCAGTTTCTCA	0.36	98.20	<i>P</i> = 0.628
10_T19856C	Intron 11	11887698	ACGTTGGATGAGCGTCCCATCTATAGTCAC; ACGTTGGATGCTTCTCAAAGTCTCCAGCTC; ggGCCCTCCATCTTTCCA	0.13	97.01	<i>P</i> = 0.486
11_T23964G	Intron 14	2280232	ACGTTGGATGTTTCCCTCAGCAGAGTCTC; ACGTTGGATGTGACAGCTCAGAGATTGACG; GTCCACTTGAGGGCAG	0.24	98.40	<i>P</i> = 0.090
12_G24164A	Intron 14	2280233	ACGTTGGATGTGATTCCAAGAGATCCCTCC; ACGTTGGATGACAAATTGGCCCTCGTTCAG; GAGCAGAAGCACGTTTCC	0.48	98.76	<i>P</i> = 0.693
13_C24631T	Intron 15	2280234	ACGTTGGATGGACACTCATGGACAACCTCAG; ACGTTGGATGTTACCCGTAAGATGCCAAG; AAGAATACAAAAGCCACA	0.35	98.71	<i>P</i> = 0.624
14_del128944G	Intron 18	5837215	ACGTTGGATGAAGCAAAGTCAGCTGATGG; ACGTTGGATGCTCCTCTAAAGAGAGGACAG; GGA CAG TCC TTT TAA TGC C	0.35	98.45	<i>P</i> = 0.756
15_G29005C	Intron 18	1547550	ACGTTGGATGAAGGACTGCTCTCTCTTAG; ACGTTGGATGAGGGAAGCGGAAACATCAC; AAACATCACAAAGCCTTACCATCTGCT	0.32	98.61	<i>P</i> = 0.527
16_G34754A† (T30900C/T32851C/C32971T)	Intron 23	1914408	ACGTTGGATGTCTCTGGTGCTTTTCTGTCC; ACGTTGGATGTA AGCCTAGGTGTGAGCATG; TCACTACCTGAGATGACAATGCCT	0.20	97.84	<i>P</i> = 0.941
17_C35266T† (T28593 G/C31285T)	Intron 24	2066793	ACGTTGGATGTGAGCACTGCACTCTCCTTG; ACGTTGGATGTAAGTGTGTGCTCAGAGG; ATCCCGGGCCTGTCTG	0.12	98.14	<i>P</i> = 0.763
18_G38812A	Intron 24	7575823	ACGTTGGATGTAAGTTGCTCTGGTCAAGGC; ACGTTGGATGGCAGAACTACTAAGAACCCC; GCAGAATTACTAAGAACCCTTACC	0.11	99.02	<i>P</i> = 0.768
19_C39134A	Intron 24	3771300	ACGTTGGATGAAGGTCTTGATTAGCCCAGG; ACGTTGGATGATACCGTGAATAAGAGGGC; ITGGAATAAGAGGGCCTTCAGCA	0.46	97.94	<i>P</i> = 0.587

Table 1. (Continued)

STAT1 Variations*	Location	rs number	1 st PCR primer; 2 nd PCR primer; Extension primer	MAF	Call R (%)	pHWE
20_T39455C‡	3'UTR	N/A§	ACGTTGGATGCAGTAAGATGCATGATGCCC; ACGTTGGATGAGCAAATTCGCTGCAACCTG; TGTGATAGCAAGTGAATTTTC	0.03	98.55	P = 0.735

*Based on the NCBI GenBank sequence (accession number NM_139266), chr2:191665770–191704442.

†The tagging SNPs representing a SNP block are marked in bold letters.

‡Infrequent SNPs located in coding sequences.

§N/A: not available in dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>).

ophagoides farinae, *Alternaria tenuis*, cat dander, mixed grass and tree pollen) after subtraction of the negative control. Total serum IgE levels were measured using the Imulite system (DPC Biermann GmbH, Bad Nauheim, Germany). Specific IgE against inhalative allergens (local grass pollen, birch pollen, mugwort pollen, Der p, cat dander, dog dander, *Cladosporium herbarum*) were measured in a range between 0.35 and 100 IU/ml, with specific IgE measurements considered positive when values were >0.35 IU/ml (*Sx1* from Phadia AB, Uppsala, Sweden).

Polymorphisms selection and genotyping

The *STAT1* gene was screened for mutations by the Innate Immunity Programs for Genomic Applications (<http://innateimmunity.net/>; accessed 9 July 2007). Using *Haploview* software, 18 haplotype tagging SNPs were selected for genotyping from all 28 *STAT1* polymorphisms with a minor allele frequency >0.10. In addition, two less frequent polymorphisms, however, located in the coding region, were also genotyped. Genomic DNA was extracted from whole blood (7) and preamplified as described before (8). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom Inc., San Diego, CA, USA) was used for genotyping. Polymerase chain reaction assays and associated extension reactions were designed with the SpectroDESIGNER software (Sequenom Inc.). All amplification and extension reaction conditions have been previously described (8) and specific primers are given in Table 1. Deviations from Hardy–Weinberg Equilibrium (HWE) were assessed for quality control of genotyping procedures.

Bioinformatics and statistical analysis

Association between polymorphisms and dichotomous outcomes were tested using chi-square tests in an allelic model (9). Total serum IgE concentrations were log-transformed and the differences between IgE levels were tested by *t* test. All tests were two-sided and the differences were considered significant with *P* < 0.05. Calculations were carried out with the SAS software (version 9.1.3; SAS Institute Inc., Cary, NC, USA). Haplotype frequencies were estimated with an expectation maximization (EM)

algorithm and associations of the common haplotypes with atopic sensitization were calculated with Haploview. Significant associations were replicated and validated in a second independent population (Munich and Dresden) to control for spurious associations. Transcription factor binding analyses and phylogenetic comparisons were performed using *MatInspector* (10) and the *VISTA* Genome Browser (11), respectively. Conserved noncoding sequences (CNS) were defined as a sequence of more than 100 bp showing more than 70% homology with mouse sequence.

Results

Initially, 20 haplotype tagging polymorphisms were genotyped in the ISAAC II study population from Dresden (*n* = 1940). All polymorphisms showed call rates of at least 93% and no significant deviation from HWE (Table 1). No associations with asthma, hay fever, atopic dermatitis were found. However, the minor allele of SNP G-306A and C39134A were inversely related to atopy measured by SPT and elevated total serum IgE levels (Table 2). When these two polymorphisms were genotyped in the Munich population (*n* = 1159), SNP 39134A showed a protective effect against atopy (Table 2). In the combined dataset, associations with SNP C39134A were inversely related to elevated total and specific serum IgE levels and atopy measured by SPT (Table 2). Haplotype analyses did not contribute additional information to the single gene analysis (data not shown). The gene variation C39134A, located in the last intron of *STAT1* (Fig. 1), was further analyzed for indications of putative role in *STAT1* regulation. *In silico* bioinformatic tools indicated that SNP C39134A is located within a highly conserved CNS (Fig. 2) and TF binding analysis predicted that the C39134A polymorphism is located three base pairs away from the core sequence of a highly conserved GATA motif.

Discussion

Signal transducer and activator of transcription molecules represent a bottleneck in the signaling of many

Table 2. Geometric means (IU/ml), log means, standard error and *P*-values for total serum IgE. Odds ratios (and 95% confidence intervals) for associations with specific IgE and atopy (SPT ≥ 3 mm) comparing major and minor alleles for all polymorphisms genotyped in Dresden (significant associations in bold)

STAT1 Polymorphisms	Total serum IgE levels*			Specific serum IgE† OR (CI); <i>P</i> -value	Atopy measured by SPT OR (CI); <i>P</i> -value
	Major allele (geometric mean; mean log IgE $\pm$ SEM)	Minor allele (geometric mean; mean log IgE $\pm$ SEM)	<i>P</i> -value (<i>t</i> -test)		
Dresden, <i>N</i> = 1940				<i>n</i> = 1217/721	<i>n</i> = 1366/493
G-306A	72.24; 4.28 $\pm$ 0.024	66.69; 4.20 $\pm$ 0.066	0.295	0.93 (0.76–1.14); 0.494	0.76 (0.59–0.96); 0.022
C39134A	73.70; 4.30 $\pm$ 0.031	67.35; 4.21 $\pm$ 0.033	0.039	0.88 (0.77–1.01); 0.066	0.93 (0.80–1.08); 0.335
Munich, <i>N</i> = 1159				<i>n</i> = 696/463	<i>n</i> = 859/284
G-306A	64.07; 4.16 $\pm$ 0.033	73.70; 4.30 $\pm$ 0.092	0.137	1.16 (0.90–1.49); 0.264	1.01 (0.75–1.35); 0.948
C39134A	65.37; 4.18 $\pm$ 0.045	63.43; 4.15 $\pm$ 0.044	0.618	0.87 (0.73–1.03); 0.101	0.80 (0.65–0.97); 0.022
Pooled data, <i>N</i> = 3099				<i>n</i> = 1913/1184	<i>n</i> = 2225/777
G-306A	68.72; 4.23 $\pm$ 0.020	68.72; 4.23 $\pm$ 0.054	0.940	1.01 (0.87–1.19); 0.855	0.85 (0.70–1.02); 0.075
C39134A	70.28; 4.26 $\pm$ 0.026	66.02; 4.19 $\pm$ 0.026	0.043	0.88 (0.79–0.98); 0.017	0.88 (0.78–0.99); 0.027

*An allelic model has been used for all comparisons. IgE levels represent the geometric means for the major allele vs the minor allele. Considering that IgE levels are not normally distributed in the population, total serum IgE concentrations were log-transformed. Differences between geometric means of IgE levels were tested by *t*-test in an allelic model comparing polymorphic alleles to the wild type alleles and values of log IgE were retransformed and presented as international units per ml.

†Specific serum IgE against inhalative allergens (local grass pollen, birch pollen, mugwort pollen, *Dermatophagoides pteronyssinus*, cat dander, dog dander, *Cladosporium herbarum*).

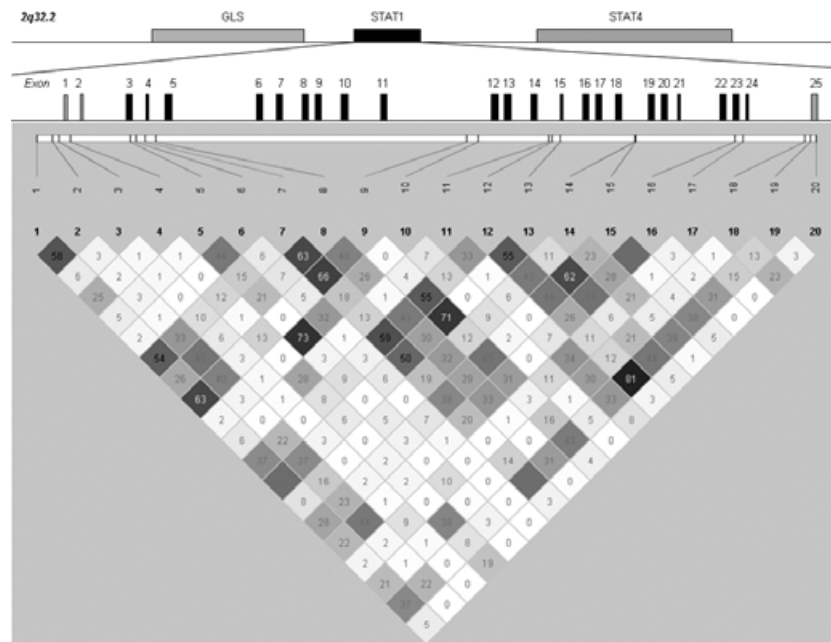


Figure 1. Position of *STAT1* on chromosome 2q32 and description of genetic linkage disequilibrium pattern (r^2 plots). Position of the *STAT1* gene on chromosome 2q32 in relation to adjacent genes (upper panel), gene structure showing exons (middle panel) and LD (r^2) between genotyped *STAT1* polymorphisms.

pathways contributing to immune responses as they carry the signal from specific ligand receptor interactions to the cell nucleus, activating transcription of numerous immune genes. While genetic variants in *STAT4* and *STAT6* had previously been identified and their effect on

the genesis of atopic disease was investigated (3, 5), this study contributes with new knowledge to the field by systematically and extensively studying the role of *STAT1* polymorphisms in atopic diseases. In our large population sample, SNP C39134A showed a small but significant

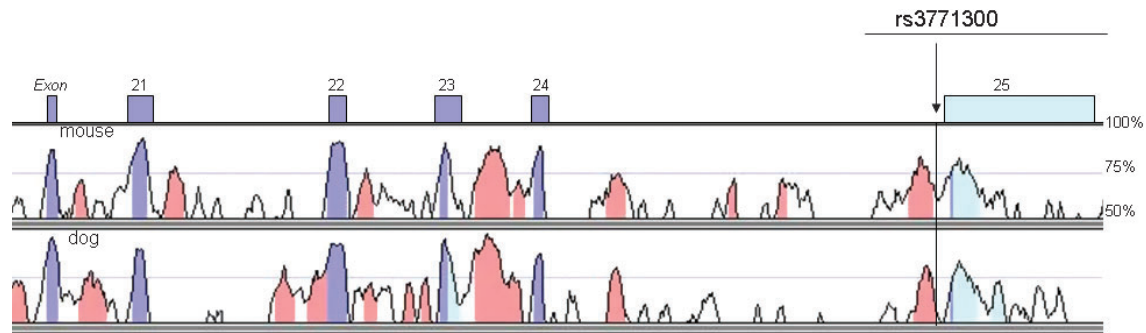


Figure 2. Position of C39134A in the *STAT1* gene and the degree of conservation in this region. *VISTA* plot displaying evolutionarily conserved sequences identified by comparison of *STAT1* human sequence and *STAT1* sequences from mouse and dog. The height of the peaks on the vertical axis indicates the level of conservation in percent identity. Conserved sequences (defined as regions > 100 bp and showing > 70% conservation) in coding exons (dark blue), UTR (light blue) and noncoding (red) are shown. Five conserved noncoding sequences (CNS) with both mouse and dog were identified in this region. SNP C39134A is located in an intronic CNS.

inverse relation with atopic sensitization, which may be a result of its location in a highly conserved *cis* regulatory element in intron 24, which was identified by *in silico* analyses. However, no significant effect of *STAT1* polymorphisms on atopic diseases was observed.

The region harbouring the *STAT1* gene on chromosome 2q32-q33 (Fig. 1), had previously been linked to elevated total serum IgE (12). In addition to *STAT1*, a number of potential allergy candidate genes map to the same region, such as *STAT4* (15 Kb from *STAT1*), *CD28* (12.6 Mb) and *CTLA4* (12.8 Mb). However, association signals from neighbouring genes (*CTLA4* and *STAT4*) with total IgE levels (13) and specific sensitization to house dust mites (5) do not influence our finding. Using HapMap data, linkage disequilibrium (LD) between C39134A and these SNPs in other candidate genes could also be excluded.

The location of polymorphism C39134A, which showed associations with atopic sensitization in our study, is interesting. It is positioned at the boarder of a highly conserved CNS in intron 24, as indicated by inter-species sequence comparisons performed with the Vista Genome Browser (Fig. 2). These comparisons have proven to be a useful tool for identifying functionally important regions in the human genome, as these regions tend to stay conserved throughout evolution. Based on the location of the CNS element, a *cis* regulatory effect on *STAT1* is suggested. Within the CNS element, polymorphism C39134A is located three base pairs from the evolutionary highly conserved core sequence of a GATA motif [CAGGATAATACATAGTAC(C/A)TGC], potentially capable of binding GATA-3, the master regulatory TF for the differentiation and perpetuation of human Th2 cells.

However, it is not yet clear how polymorphism C39134A affect the role of *STAT1* in allergy as *STAT1* may not only have pro-allergic effects (4) but is also involved in the IL-15 induced *STAT1* binding to *IFN-γ*

regulatory sites (14). Slight discrepancies in the effect of *STAT1* polymorphism C39134A on total IgE regulation and specific sensitization were observed between both study populations, which may be explained by modifications of the genetic effects by different environmental factors. Notably, children from Dresden spent their first 3 years of live in the former German Democratic Republic, exposed to very different life style factors (e.g. higher number of siblings, early day care, different outdoor pollutants, pet keeping) which were previously associated with the development of or the protection against atopy (6). Clearly, further studies are needed to better understand the role of *STAT1* in these mechanisms.

In conclusion, our results suggested that the minor allele of SNP C39134A in the *STAT1* gene is inversely related to total IgE levels and atopic sensitization in German children. We suggest that this association may be as a result of C39134A altering a *cis* regulatory element in *STAT1*, making C39134A an interesting polymorphism for further genetic and functional studies.

Acknowledgments

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