STAT6 - polymorphisms, haplotypes and epistasis in relation to atopy and asthma

Marek Godava, Radek Vrtel, Radek Vodicka

Background. STAT6 has an important role in the IL-4 / IL-13 signalling pathway. Genome - wide association studies have shown that particular polymorphism (SNP) or haplotype variants of *STAT6* as well as epigenetic gene modifications are associated with IgE level and asthma in childhood.

Methods. A review of the available literature was performed to map out the function and signalling pathway of STAT6, studies of STAT6 SNPs association with susceptibility to asthma and atopy, covering the years 1997 – 2012 were summarized, and the value of epigenetic and epistatic influences on *STAT6* and their relevance to the development of the studied phenotype (atopy or asthma) were determined.

Results. There are 2 SNPs (rs71802646 and rs320411) with clinical association and proven functional effect on *STAT6* expression. The effect of *STAT6* SNPs cumulates in haplotypes and more potently during interaction with SNPs in the genes from the signalling pathway (*IL4*, *IL4Ra*, and *IL13*). Expression of *STAT6* is also influenced by DNA methylation. Atopy is traditionally believed to be maternally inherited but there is one report about paternally overtransmitted *STAT6* haplotype (TCA haplotype, built from rs324011, rs3024974 and rs4559 SNPs).

Conclusions. *STAT6* polymorphisms and their combinations have an important influence on IgE level and development of asthma. However, the interaction between SNPs in the IL-4 / IL-13 signalling pathway is of greater impact. Hypermethylation of the *STAT6* promoter is also significant in the regulation of *STAT6* expression and this fact opens possibilities for targeting therapy in asthma.

Key words: IgE, atopy, asthma, polymorphisms, haplotypes, STAT6, epistasis

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INTRODUCTION

Allergic diseases, such as asthma, atopic dermatitis and hay fever, are associated with atopy[1]. The main feature of atopy is elevation of IgE level, which occurs after IL-4 stimulation and ligation of CD40 ligand to CD40 that is required for isotype switching to IgG1 and IgE (ref.[2]). The high occurrence of atopy in families implies that genetic factors have an important role in its control. Several papers have reported significant association of maternal atopy with elevated cord blood IgE level and some follow-up studies have suggested that an elevated IgE level is a good marker for prediction of early atopy onset, better than maternal atopy[3-5]. Canfield et al.[6] showed a strong association of parent's total IgE level (both maternal and paternal) with elevated total IgE levels in 4-year old children; in addition, specific IgE levels in children were associated with their mother's specific IgE levels. Cookson et al.[7] linked the 11q13 locus with maternally transmitted atopy. Genome wide association analysis (GWAS) of total IgE level on a population of children with asthma in Costa Rica revealed modest linkage with 7p15 for both genders and significant association with the 20q12 locus in boys. Subsequent analysis found two of the *JAG1* polymorphisms to be significantly associated with total IgE

in males[8]. Weindiger's et al.[9] GWAS found *FCER1A* to be another locus for susceptibility to elevated IgE levels.

The central pathways for IgE production are orchestrated by IL-4 and IL-13 and their shared receptor. The cytokines are located on 5q31 – a region repeatedly linked with atopy and asthma[10-13]. Total IgE level has been also shown to be influenced by the 12q locus[14-16]. This locus contains, among others, the gene for STAT6 which is signalling downstream of the IL-4/13 receptor. *STAT6* is one of the most common candidate genes associated with IgE level and asthma in childhood. To search for publications on polymorphisms and haplotypes of *STAT6* and their relation to the level of IgE, we scanned pubmed using the key words: *STAT6*, polymorphisms, haplotypes, IgE, asthma, atopy interaction, maternal, paternal, parental and inheritance. These papers spanned years 1997 - 2012.

Structure of the STAT6 and its product

STAT6 was successfully identified and cloned for the first time by Hou et al.[17] and independently by Quelle et al.[18]. The gene maps to 12q13.3-q14.1, encompasses over 19 kb and consists of 23 exons[19]. The first two and part of third exons belong to 5´UTR (untranslated region) and a large part of the last exon is untranslated as well (Fig. 1). *STAT6* gives to rise to several mRNA isoforms: STAT6
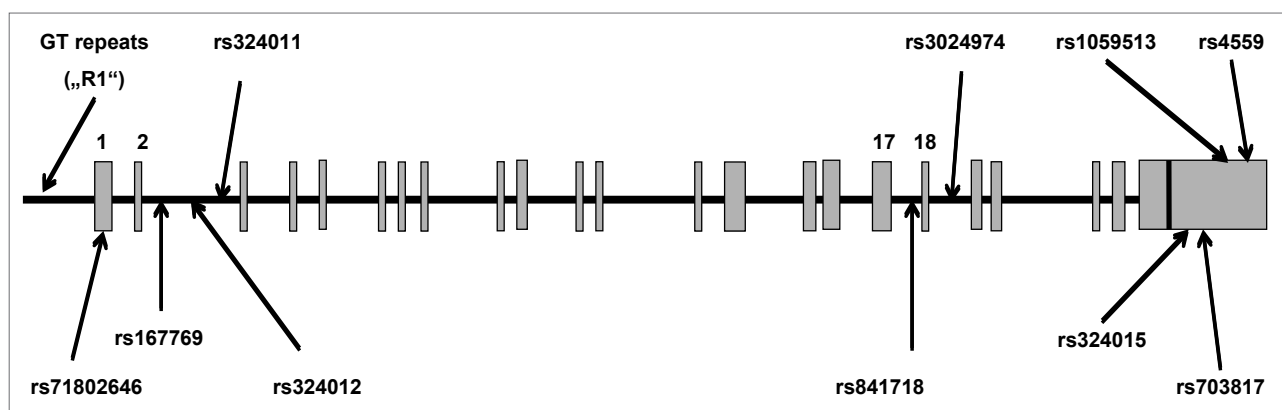
Fig. 1. Structure of *STAT6*. The STAT6 gene contains 23 exons. Localization of polymorphisms reviewed in this article is depicted according to their position in the gene.
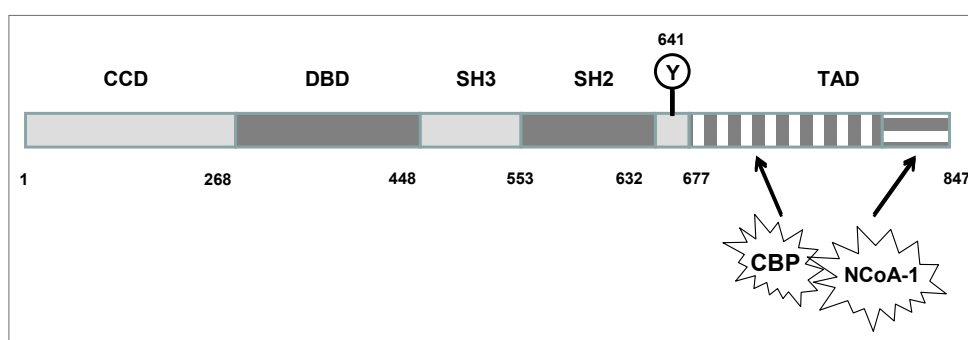

Fig. 2. Structure of STAT6. CCD - coiled coil domain (N - terminal domain), DBD - DNA binding domain, SH3 - Src Homology 3 domain, SH2 - Src Homology 2 domain, Y - tyrosine residue, TAD - transactivation domain; CBP and NCoA-1 - coactivators of STAT6; numbers mark position of amino residues.

(assembled from all of the exons), STAT6a (missing part of the 3´UTR), STAT6b (missing exon 4), STAT6c (part of the exon 16 is included into intron) (ref.[20-22]). Finally, there are 2 isoforms created by intron retention: in addition to exonic regions, STAT6d is made up of intron 17 and STAT6e contains introns 17 and 18 (ref.[23]).

STAT6 shares structural similarity with the other STAT proteins (Fig. 2) and is composed of the N-terminal domain (coiled coil domain), DNA binding domain (DBD), SH3- like domain, SH2 domain and transactivation domain (TAD) (ref.[24]). The N-terminal domain is composed of 8 helices showing coiled coil structural motif. The first 130 amino acids are conserved among all of the STAT proteins and this part of the protein has an important role in dimer – dimer interactions, tetramerization of STAT dimers and consequently cooperative DNA binding[25,26]. The region of the DBD is highly conserved amongst the STATs and serves for binding of specific GAS sequences[25]. SH3 domains are generally known as "Molecular Velcro" because of their ability to interact with proteins having a proline – rich region[27,28]. The conserved Src homology 2 (SH2) domain has the ability to associate with cytokine receptor and interact with phosphotyrosine – containing proteins, thus dimerizes with the other STAT and influences DNA binding[29,30]. The TAD has two distinct regions, the presence of both

of them is important for transactivation. The N-terminal interacts with CBP (CREB-binding protein) and the distal C-terminal interacts with NCoA1 (nuclear receptor coactivator 1, a member of the p160/SRC coactivator family) - specific STAT6 coactivator[31].

STAT6 function and signalling pathway

STAT6 was initially identified as a transcription factor induced by IL-4 signalling[17] and it was suggested that activation of STAT6 is required in IL-4 promoted IgE isotype switching[32]. Next, the physiological role of STAT6 was assessed on animal models. Stat6 null mice were viable; however, they were unable to undergo class switching to IgE antibodies. Additionally, they were unable to upregulate IL-4 responsive gene in B cells and their T cells failed to acquire T_{H2} phenotype in response to IL-4 (ref.[33,34]).

IL-4 may signal through two receptors – type I is composed of IL-4Rα and γc subunit and in type II, the IL-4Rα associates with IL-13a. The type I complex is expressed predominantly in hematopoietic cells and binds specifically to IL-4 only. After IL-4 binding, the subunits of the receptor dimerize, bringing the JAK kinases associated with the receptor (JAK1 with IL-4Rα and JAK3 with γc subunit) in close vicinity (Fig. 3). This leads to their auto- or cross-phosphorylation. Activated JAKs then phosphorylate specific thyrosine residues in the cytoplasmatic
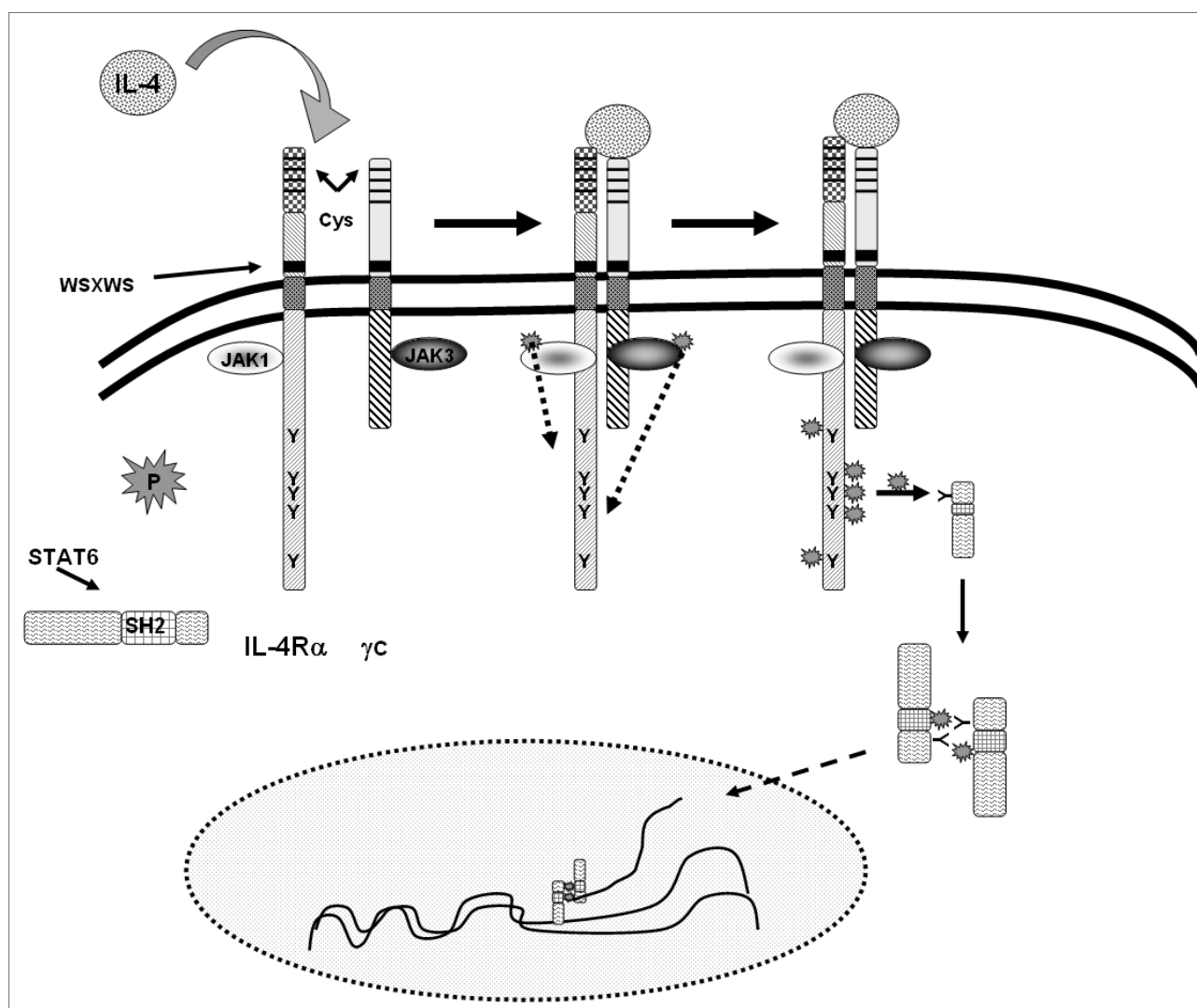
Fig. 3. STAT6 signalling pathway. IL4 binding to receptor activates JAKs, they phosphorylate receptor and STAT6 binds to the activated receptor. STAT6 becomes tyrosine phosphorylated, STAT6 monomers dimerize, tanslocate to the nucleus and bind to GAS elements on target genes – for details see the text. Cys - positionally conserved cysteine residues; WSXWS - motif, that is characteristic for type I cytokine receptors; JAK1 - Janus kinase 1; JAK3 - Janus kinase 3; P - phosphorylation; Y - conserved tyrosine residues; SH2 - SH2 domain of STAT6; IL-4Rα, γc - subunits of IL4 receptor

domain of IL-4Rα, namely Y575, Y603 and Y633 of the human receptor. The thyrosines provide a docking site for the STAT6 which associates with the receptor through its SH2 domain and STAT6 becomes tyroxine phosporylated at conserved Y641. Activated STAT6 monomers dimerize by interaction of phosphorylated Y641 of one monomer and the SH2 domain of the other monomer. Dimers of STAT subsequently translocate to the nucleus and bind to the GAS elements (γ-IFN activated sequence) – in the case of STAT6, TTCN4GAA motifs are recognized[30,35], reviewed in[36,37].

POLYMORPHISMS IN STAT6 - IGE LEVEL AND ASTHMA

Since the first description of the 12q locus association with asthma or IgE level, and since the discovery of the role of STAT6 in the IL-4 signalling pathway, great attention has been directed to the role of *STAT6* polymorphisms on IgE level or asthma development in GWA studies[14,16,38].

5´UTR of the gene contains several polymorphisms. The GT repeat polymorphism (rs71802646) was described by Tamura et al.[39] and contains 7 different alleles, according to the number of GT repeats, ranging in size from $(GT)_{12}$ to $(GT)_{18}$ (ref.[39-41]). In the Indian population, a wider spectrum of GT repeats was observed, from 13 to 24 repeats[42]. The $(GT)_{13}$ allele was much more frequent in children with allergic disease (bronchial asthma, atopic dermatitis and/or food-related anaphylaxis) than controls in the Japanese population (P=0.0158), and $(GT)_{13}$ / $(GT)_{15}$ heterozygotes were strongly associated with those diseases (P=0.0002), but not with IgE level[39]. Hence the $(GT)_{17}$ repeats showed a trend to increase IgE level in a study of the German population, there was no significant association of GT repeats (13-17 repeats) with IgE levels in asthma phenotype or bronchial hyperresponsiveness

(after methacholine challenge test) in the Caucasian population. However, a significant effect (P=0.001) was observed on eosinophil count in case of 17 GT repeats[40]. In a study on the British population, the 13 GT repeats were significantly more frequent in atopic asthmatics compared to healthy persons (OR 1.52, 95% confidence interval 1.02 to 2.28, P=0.027) and conversely, the frequency of 16 GT repeats was significantly increased in persons with non-atopic asthma (OR 0.39, 95% confidence interval CI 0.17 to 0.92, P=0.018) (ref.[41]). Nagarkatti and Ghosh[43] focused on 5´UTR and promoter regions of *STAT6* and identified a novel GT repeat region (denoted as R1) in the proximal STAT6 promoter region (2,3kb upstream of the 5´UTR) and they described the allelic frequency for this polymorphism and for the GT repeats polymorphism in exon 1 (denoted by them as R3 region) in the Indian population. In a subsequent study, Nagarkatti et al.[42] found a positive association of 16 GT repeats in the R1 locus (OR 1.48, 95% CI 1.09 to 2.00, but 99% CI 0.99 to 2.20; P=0.01) and 15 GT repeats in the R3 locus with asthma incidence (OR 1.76, Wald's 99% CI 1.18 to 2.60; $P<10^{-4}$). The 16 GT repeats of R3 were negatively associated with asthma in the Indian population (OR 0.33, 99% CI 0.19 to 0.57; $P<10^{-5}$). They concluded that the R3 region has a greater influence on asthma incidence than the R1 locus[42].

Gao et al.[41] analyzed promoter activity of *STAT6* and they showed that an allele with 13 GT repeats had a significantly higher transcriptional activity compared to the other alleles (with 12, 16 and 17 GT repeats). However, the promoter activity was not proportional to the number of GT repeats, based on the repeatedly highest promoter activity of $(GT)_{13}$ allele compared to the other alleles (both longer and shorter). The reciprocal gel shift test obtained a result suggesting more stable binding of the 16 GT repeats allele to the putative nuclear factor(s) compared with the allele with 13 GT repeats. The test for the alleles with 12 and 17 GT repeats gave a similar result; the shorter allele once again had a similar binding property compared to the 13 GT repeats allele. They hypothesized that the allele with longer GT repeats may have a relatively more stable binding to the nuclear factor[41]. These results suggest that GT repeats are important for regulation of gene expression. Buerger et al. showed that the number of intron 1 GT repeats in the gene for epithelial growth factor receptor (EGFR) correlated with the expression of EGFR (ref.[44]). The GT repeats are capable of forming a non-B-form of DNA (such as Z-, H- and cruciform DNA), they can be found particularly around promoter regions and therefore they can participate in biological events, such as DNA replication and transcription. According to the close proximity of GT repeats in intron 1 to the translation start of *STAT6*, Gao et al.[41] speculated that the repeats may favour formation of a secondary structure (Z-DNA) and thus affect the binding of transcription factors in the vicinity of the exon 1 region. Additionally, other repeat sequences in some gene could influence the transcription activity, but the activity may not be (as seen in case of GT repeats in *STAT6* exon 1) proportional to the length of the repeats[45-47]. Therefore the impact of the GT repeats on transcription could be caused by their binding to inhibitors of transcription, or by forming various non-B form conformation of DNA, or by their combination as hypothesized by Gao et al.[41].

The 5´flanking region of the *STAT6* contains several potential regulatory sequences. Duetsch et al.[40] screened all of the *STAT6* exons and adjacent exon/intron junctions and they identified two polymorphisms (among others) in the promoter region. The first (rs2598483) is located 77 bp upstream of CCAAT box, suggested to be an enhancer, and 1771 bp upstream of a prominent transcriptional initiation site. The second lies closer to the site of transcription initiation, 1216 bp upstream of it, and 474 bp downstream of the CCAAT box[19]. Although they are relatively close to the CCAAT box and therefore could putatively have impact on transcription activity of the gene, they were not associated with asthma, peripheral eosinophil cell count or total IgE level in the Caucasian population[40,48,49].

Duetsch et al.[40] identified 3 common polymorphisms (SNP) in intron 2 – rs167769, rs324012 and rs324011. Although none of the SNPs were linked to the occurrence of the asthma phenotype, the rs324011 was associated with total IgE level[40,48,49]. By contrast, the rs324011 was not associated with IgE level in the Finish population[50]. Surprisingly, Huang et al.[51] described an association between the C allele (wild type allele) of this polymorphism with penicillin allergy and urticaria, but the polymorphism has no effect on specific IgE level.

Schedel et al.[48] tried to investigate the putative role of the rs320411 in regulation of *STAT6* so they performed a computer analysis of the second intron sequence. The analysis revealed two putative binding sites for transcription factor (TFB) – nuclear factor κB (NF- κB). The NF-κB role in IL4 induced responses has been known for a longer time. It binds to specific sequences in promoter regions of target genes, in the vicinity of STAT6 specific sequences. Both the STAT6 and the NF- κB binding to target sequence is required to induce IgE production. This event occurs most probably in the protein complex made by other transcription factors, such as PU.1[52]. The two TFB sites in the *STAT6* second intron mentioned above are very close to each other and the T allele of rs324011 gives rise to the distal one. The sequence at the site of this SNP is quite strongly conserved among primates[48]. Because the STAT6 synergic cooperates with NF- κB by IL4 mediated IgE production, Schedel and al.[48] made an assumption that the nucleotide sequence change in this second TFB site caused by rs324011 could contribute to elevated IgE production. To confirm this hypothesis they performed an in vivo and ex vivo experiment focused on the effect of *STAT6* In2 and its rs324011 SNP on *STAT6* function[23]. They found that the In2 itself has a silencing influence on *STAT6* promoter activity (and therefore on the *STAT6* transcription), no matter if the intron 2 has sense or antisense orientation (in used promoter constructs). This fact indicates that silencing elements are also in the promoter region (approximately 1.1 – 5.6 kb before transcription start). This theory supports the observation that the transcription activity of constructs contain-

ing only promoter (without intron 2 sequence) depends on the length of sequence located before the start of translation. When intron 2 was introduced into the used constructs, there was a great decrease in promoter activity in the presence of the wt allele of rs324011. Contrarily, the T allele of rs324011 SNP resulted in restoration of potent increase in *STAT6* promoter activity – however, this effect was observed only in constructs containing the longest *STAT6* promoter sequence (5643 bp). These observations point out to putative physical interaction of intron 2 with promoter region. Allele specific binding of NF-κB responsible for promoter activity increase was proved by EMSA experiments (electrophoretic mobility shift assay), since NF-κB binding onto specific DNA/protein complexes was observed only in the presence of the T allele of rs324011.

Schedel et al.[23] wanted to verify the *in silico* observation in *ex vivo* condition, they decided to measure *STAT6* mRNA activity in children with asthma. Surprisingly, they revealed two new *STAT6* mRNA isoforms containing In17 or In17+In18 (STAT6d a STAT6e, respectively). They supposed their presence was caused by intron retention or alternative site of translation start. Patients with asthma bearing the TT genotype in rs324011 had a mild, but not significant increase in the known STAT6 form and a significantly increased level of STAT6d and STAT6e mRNA. However, the role of the two isoforms on IgE production is still unclear. Shortly, Schedel et al. confirmed the T allele of rs324011 increased *STAT6* promoter activity by creating a new site for NF-κB specific binding. According to their results, they presumed physical interaction of intron 2 with promoter region and further change of *STAT6* 3D structure or its epigenetic signature caused by NF-κB binding[23].

Leung et al.[53] investigated the effect of *STAT6* together with five other candidate genes on asthma occurrence. They assessed the effect of T1309C (rs841718) and C1570T (rs3024974) single nucleotide polymorphisms (SNP) in 17th and 18th introns, respectively, on functional lung parameters and they discovered the C1570T SNP significantly influenced FEV_1 decline (spirometric measurements were repeated in one year delay). This SNP, located approximately in the middle of the 18th intron, was not associated with total IgE level in the Chinese population in a previous study of these authors and in a study performed on a Caucasian population of German origin[48,54]. However, a study on another German Caucasian population described a weakly significant effect of the T1309C (*P*=0.026) and strong effect of C1570T (*P*=0.007) polymorphisms on IgE level[40]. Interestingly, the C1570T SNP was associated with annual FEV1 change ("growth") in Chinese children with asthma[53].

Hence the 3´ UTR could also have an important role in appropriate gene expression by affecting mainly mRNA stability and localisation of the mRNA in the cytoplasm. This region was also screened for polymorphisms with possible effect on asthma disease incidence. Duetsch et al.[40] studied 5 polymorphisms in this region and found association of 3´UTRSNP3 (rs1059513) with bronchial responsiveness to methacholine challenge and 3´UTRSNP4

(rs4559) with IgE level. The T allele of the rs4559 SNP was more common in Chinese children with asthma but there was no association of any genotype bearing T allele with asthma bronchiale or IgE level[55]. In spite of this lack of association for single SNPs in their population, Wang et al.[55] observed clear interaction of GT repeats in intron 1, C258T (rs4559) and T710C (rs324015) polymorphisms with susceptibility to develop asthma. In a GWAS in the Framingham Heart Study, the rs1059513 was shown as one of the most significant SNPs associated with IgE dysregulation[56].

The 2964 G>A SNP (rs324015), identified in the year 2000 in the healthy UK population[57], is also located in the 3´UTR region of the *STAT6* and its role in atopy and asthma development was relatively extensively studied. Gao et al.[58] reported an association of this SNP with mild adult atopic asthma (OR 3.19, 95% CI 1.40 to 7.08; *P*=0.0043) in the Japanese population. They observed no association of this SNP with either IgE level or marked asthma (groups of children or adults) in the Japanese and British populations. Negoro et al.[59] described the association of this SNP with IgE level and eosinophil count in Japanese children with atopic eczema and food allergy. Although there was no association with total or specific IgE in the UK population, the G allele of rs324015 was significantly more frequent in patients suffering from nut allergy compared to both health controls (without sufficient information about their atopy status) and atopic persons without nut allergy. The GG homozygotes were at increased risk of developing a severe nut allergy reaction (OR = 3.9, 95% confidence interval 1.9-8.3) (ref.[60]). Conversely, no association was found with atopy or asthma for this SNP in German, Chinese or Finish populations[40,50,61]. In addition, distribution of 2964 G>A SNP was also examined in patients with inflammatory bowel disease and the G allele was significantly more frequent in patients with Crohn disease without variation in the *CARD15* (ref.[62]). Therefore, the polymorphisms in 3´UTR of *STAT6* may have a rather minor effect on allergic disease development, and their direct impact on *STAT6* expression, as suggested by Duetsch et al.[40], could be excluded.

HAPLOTYPES

Each polymorphism could have a small effect which may reach statistical significance in samples of thousands of people. Therefore, the association studies of haplotypes assembled from studied SNPs are very helpful because they do not require huge numbers of persons to be examined.

Haplotype analysis of GT repeats in the promoter region and in the first exon of *STAT6* (rs71802646) in the Indian population showed that the 16_15 and 17_15 haplotypes (assembled from R1 and R3, respectively) were linked to an elevated total IgE level (OR 1.886, *P*=0.001; OR 2.6284, *P*=0.0031; respectively). In contrast, 17_14, 24_16 and 23_16 haplotypes were negatively associated with total IgE level (OR 0.1007, OR 0.0881, OR 0.0902, respectively, $P<10^{-5}$ for all of the 3 haplotypes) (ref.[42]).

Table 1. Frequency of the most frequent haplotypes from *STAT6* SNPs common to studies performed on Czech and German Caucasian populations.

Haplotype	Godava et al.[64]	Weidinger et al.[49]	Duetsch et al.[40]	Schedel et al.[48]
TCA	43.68	37.51	42.82	36.65
CCG	26.84	26.00	15.87	26.34
CCA	20.52	25.04	23.30	13.00

We compared our study[64] (Czech population) with studies of German populations, and we built haplotypes from STAT6 SNPs common to all of the studies only - rs324011, rs3024974 and rs4559, respectively. Subsequently, we compared frequency of STAT6 SNP haplotypes in our population to frequency of haplotypes in German populations.

Homozygosity for 15 GT repeats of rs71802646 together with homozygosity for wild type of 2964 G>A SNP was significantly associated with allergic subjects (*P*=0.0382) in the Japanese population[63]. Duetsch et al.[40] analysed the relation of haplotypes (obtained from a combination of 13 examined SNPs) with atopy or asthma in the German population but they did not find any significant association, not even for the two haplotypes containing all alleles of the polymorphisms with weak or significant effects on the examined traits. Weidinger et al.[49] conducted a study of 6 SNPs spanning the entire *STAT6* in the German population and by using the haplotype analysis, they found that the most frequent haplotype was associated with an elevated IgE level for IgE cut-off 100 kU/l (OR 1.7, 95% CI 1.11-2.6, *P*=0.015) and subsequently for the 50th, 66th and 90th percentiles of IgE level (OR 1.54, *P*=0.032; OR 1.6, *P*=0.025; OR 2.54, *P*=0.007; respectively). Another two haplotypes were found as a significant risk for specific sensitization (OR = 2.0, *P*=0.03) and asthma development (OR = 2.17, *P*=0.01), respectively. Schedel et al.[48] performed the third study of *STAT6* polymorphisms on the German Caucasian population, haplotypes were inferred from the 6 SNPs spanning the entire gene. They observed that the most frequent haplotype (haplotype no. 3) was significantly associated with IgE level. In contrast, haplotype no. 4 showed a borderline protective effect against elevation of IgE. We also conducted an association study of *STAT6* SNPs with IgE level and asthma on a group of Czech Caucasian children[64]. We did not find any association of single SNP with the examined traits, but the most frequent haplotype (s1 haplotype) in our population was associated with an elevated total IgE level in patients. We compared our results to results of studies performed on the German population[40,48,49] and we found that the frequency of haplotypes assembled from polymorphisms common to our and German studies were similar (Table 1). According to this observation, we suggested that these *STAT6* SNPs have the greatest effect on IgE level and therefore the TCA haplotype alone (built from rs324011, rs3024974 and rs4559 SNPs, respectively) in *STAT6* is associated with total IgE elevation.

STAT6 AND EPISTASIS

According to the theory of epistasis, several SNPs in combination could have an additive effect on gene function. The SNPs may interact with each other in the signalling pathway and this could lead to more than an additive effect, especially in the case of interaction between SNPs of different candidate genes. The effect of simple SNPs or haplotypes built from the SNPs in one gene could be noteworthy, but genes work and interact together in pathways. Therefore the interaction of functionally important polymorphisms in the genes of one pathway may have greater value on dysregulation of the pathway.

Japanese children suffering from atopic dermatitis or food allergy (complicated by another allergy disease such as asthma) with AG genotype for rs324015 SNP (2964 G>A) in combination with AA genotype of IL-4R polymorphism (1652 A>G) or TC genotype of IL-4 polymorphism (590C>T) were significantly more likely to have an increased eosinophil count or IgE level, respectively[59]. Kabesch et al.[13] studied 18 SNPs in four genes – *STAT6*, *IL4*, *IL13* and *IL4Ra*. For analysis of interaction among these genes, they chose only one SNP per gene because all of the four polymorphisms (rs324011 in *STAT6*, rs1805010 in *IL4Ra*, rs1800925 in *IL13* and rs2243250 in *IL4*) were previously reported to lead to functionally relevant alterations. They found that combinations of these SNPs in pairs or triplets increased the risk of elevated IgE or asthma development more potently than did the single allele of the respective SNPs. Risk of developing asthma or elevated serum IgE was significant in the presence of T/T and G/T alleles of *STAT6*/*IL13* and *IL4Ra*/*IL4* pair combinations, respectively. Among these combinations, the maximal risk for elevated IgE was 10.8 times higher (*P*=0.02) and risk of developing asthma was 16.8 times higher compared to the maximal effect of a single polymorphism (1.73 times higher in case of *STAT6* SNP). Although the risk of developing atopic asthma was not included in the statistical correlation, the authors commented that rs1800925 (in IL-13) and rs324011 (in STAT6) seemed to be overrepresented in SNP combinations conferring the greatest risk for asthma and/or elevated IgE (in other words for atopic asthma). Wu et al.[65] performed analysis of 8 SNPs in 5 susceptibility genes (*STAT6*, *IL4*, *IL4Ra*, *IL13* and *CD14*). They discovered significant interaction of rs324011 in *STAT6*

and 5 of the other loci on asthma development in children. Li et al.[66] observed significant gene-gene interaction between *STAT6* (rs324015), *IFNG* and *STAT4* on risk of asthma development in the Chinese population.

STAT6 AND EPIGENETICS

In recent years, researchers have pointed out that environmental and epigenetic factors have an important impact on the final atopy or asthma phenotype[67]. The histone acetylation and DNA methylation are main epigenetic mechanisms[67,68]. For example, the Th2 cells showed complete demethylation in the RHS7 (*rad50* hypersensitive site 7) of the Th2 LCR (locus control region) due to almost complete methylation (96%) of the third CpG residue of RHS7 in naïve T cells and this occurred in a STAT6-dependent manner[69]. Furthermore, the parental origin of atopy or asthma has been discussed for a long time. Transmission of atopy from the maternal line was observed for locus 11q13 (ref.[70]). Infants with elevated cord blood IgE had a higher risk of developing asthma and elevated IgE in cord blood was associated with maternal atopy[3].

Kim et al.[71] examined the *STAT6* promoter and reported that its hypermethylation leads to repression of *STAT6* expression. They also found that the 13 GT repeats in exon 1 of *STAT6* appeared more frequently than 15 GT repeats in patients with asthma and rheumatoid arthritis than in controls. Subsequently, they performed expression analysis of these repeats in constructs containing minimal functional promoter. The expression was significantly higher than in the case of promoter alone, but there was no significant difference in expression between those GT repeats. This result was in contradiction to the results of Gao et al.[41] so they concluded, that DNA hypermethylation rather than promoter polymorphisms influences *STAT6* expression. However, Kim et al.[71] used constructs with a much shorter part of promoter compared to the report of Gao et al.[41] and so interaction of GT repeats polymorphism with the distal part of the promoter could not be excluded. Moreover, Schedel et al.[23] reported the above interaction of *STAT6* intron 2 with the distal region of *STAT6* promoter and proved the influence of rs324011 polymorphism on STAT6 expression.

At the present time the maternal influence is believed to have the greatest influence on atopy or asthma development. In an association study of *STAT6* polymorphisms on the Czech population, we also attempted to examine the role of parental origin of *STAT6* haplotypes on total IgE levels in the patients. Surprisingly, we observed that haplotype associated with elevation of total IgE levels was significantly more often inherited from fathers and showed a higher tendency to increase the total IgE level than did the haplotype transmitted from mothers. This difference in IgE level could point to a distinct imprinting pattern of the haplotype. Some genes are able to fully or partially escape epigenetic reprogramming during prenatal development[67,72] and thus they can preserve their special DNA methylation pattern. Moreover, the specific epigenetic pattern can be transmitted to the descendant, in other words the epigenetic changes may be transmitted transgenerationally[72,73]. These processes could at least partially explain the difference in total IgE levels. We hypothesize that paternally inherited s1 haplotype by itself confers a greater risk to the elevation of total IgE. This effect is modulated *in utero* by the mother, as e.g. maternal behaviour and diet could influence the epigenetic programming of the fetus[72].

CONCLUSION

STAT6 has a crucial role in the IL-4/IL-13 signalling pathway and previous studies have showed linkage of *STAT6* to atopy or asthma. Association studies searching for relation of polymorphisms in *STAT6* with IgE level or asthma discovered a few polymorphisms significantly associated with examined traits. All of these polymorphisms are in introns or in untranslated regions of *STAT6* and therefore they affect *STAT6* expression rather then the aminoacid sequence. Only two polymorphisms showed repeatedly significant clinical association and/or functional effect on *STAT6* function (GT repeats in exon 1 and rs324011 polymorphism in intron 2). The haplotype studies showed that the effect of *STAT6* polymorphisms cumulates in haplotypes and according to studies on the Caucasian population (Czech and German) there is obviously one potent haplotype build from the 3 polymorphisms. STAT6 gene does not work alone, it cooperates with other genes in IL-4 / IL-13 signalling pathway therefore the study of gene – gene interactions (with regard to relevant polymorphisms of those genes) is more important than examination of the only particular polymorphisms in a single gene. Research of methylation or generally imprinting pattern of regulatory region of *STAT6* and other genes is also very interesting due to their effect on gene expression. Furthermore, influencing gene expression represents a very exciting field for research with a promising perspective for targeted therapy, as was outlined by Darcan-Nicolaisen et al.[74] in a study demonstrating inhibition of allergy inflammation and hyperreactivity by *STAT6* siRNA in mice.

CONFLICT OF INTEREST STATEMENT

Author's conflict of interest disclosure: *None declared.*

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