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REPORT

Association analysis of *STAT6* SNP with asthma in Pakistani population

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Abstract: Signal transducer and activator of transcription 6 gene (*STAT6*) is an important player of inflammatory pathways. The role of single nucleotide polymorphisms (SNPs) of *STAT6* to the risk of asthma or IgE has been studied in several populations. We examined *STAT6* SNP in relation to asthma risk among Pakistani individuals, suffering from asthma. We genotyped *STAT6* SNP rs1059513 using SNaPshot minisequencing assay. SNP association analysis was statistically confirmed by linkage disequilibrium calculator and online SHEsis software. Significance of differences between asthmatics and control group was tested for genotypes and allele frequencies. Our results indicated that *STAT6* polymorphism rs1059513 has no association with asthma in Pakistani population as any difference in allele frequency and genotype was not observed between asthmatics and controls. The present study to date is very first of genetic variation in *STAT6* and asthma risk, designed for evidence of the *STAT6* involvement in asthma risk in Pakistani population.

Keywords: *STAT6*, childhood asthma, inflammatory pathways, transcription factors, rs1059513.

INTRODUCTION

Asthma and asthma like allergic conditions show strong genetic susceptibility. A number of asthma susceptible genes have been identified that may be potential determinants for asthma therapy (Henderson *et al*., 2007). Chromosomal locus 12q13-24 association to asthma or elevated IgE level has been observed in many populations (Wilkinson *et al*., 1998; Immervoll *et al*., 2001). Signal transducer and activator of transcription 6 (*STAT6*), map to chromosomal location 12q13.3-q14.1 that contributes towards asthma and other inflammatory diseases (Barnes *et al*., 1999). Human *STAT6* gene (a member of STAT-family of transcription factors) is 19 kb in length and comprises of 23 exons (Patel *et al*., 1998).

A number of studies focused on the *STAT6* polymorphisms have shown association to allergic conditions (Duetsch *et al*., 2002; Schedel *et al*., 2004). *STAT6* gene plays an important role in signaling pathways of interleukin 4 (*IL-4*), interleukin 13 (*IL-13*) cytokines (Lin *et al*., 2000; Takeda *et al*., 1996; Tekkanat *et al*., 2001; Shimoda *et al*., 1996). All these cytokines play an important role in atopic asthma and other allergic conditions (Takeda *et al*., 1996; Tekkanat *et al*., 2001; Miyata *et al*., 1999). They are also important for various biological functions (Punnonen *et al*., 1993), like proliferation of T-lymphocytes, the differentiation of Th2-cells (Mosmann and Coffman, 1989), activation of B lymphocytes (Li *et al*., 2015).

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Several single nucleotide polymorphisms (SNPs) in *STAT6* have been studied for association with asthma, atopy and IgE level, including rs1059513 polymorphism. The rs1059513 polymorphism in 3' UTR may influence asthma and IgE levels. Present study was designed to evaluate *STAT6* polymorphism rs1059513 associated with asthma in Pakistani population. We performed SNaPshot minisequencing based genotyping of this reported polymorphism in *STAT6* gene from blood samples of 100 childhood asthma patients to examine possible association of this polymorphism with asthma.

MATERIALS AND METHODS

Study design and clinical characterization

Asthma patients and control subjects were selected based upon family history, the analysis of clinical data, and environmental stimuli using a standardized questionnaire based studies. The diagnosis of persistent bronchial asthma was established in 100 individuals aged 4-45 years (median 24.5 years), referred to the Asthma Clinic Gulab Devi Hospital and Children Hospital Lahore during 2011-2012. Asthma patients were selected with a positive family history of asthma with at least one first-degree asthma affected relative. A total of 100 normal healthy individuals were also included in this study as control subjects. The study was approved by the Centre for Applied Molecular Biology, Lahore. Signed informed consent was taken from all patients. Atopic status of all patients was also evaluated based upon family history, the evaluation of clinical data and environmental details.

While collecting samples geographic origin of all patients and migration status of asthma patients and controls also observed.

Primers selection for PCR amplification

PCR primers were designed to achieve a product size of 120 bps to obtain a melting temperature of 60°C. The designed primer pairs were checked for specificity of binding sites and melting temperature. Primer homology and complementation to other primers was also checked.

Polymerase chain reaction amplification of polymorphic regions

PCR conditions were optimized by using 20µl reaction mixture in a thermocycler ABI GeneAmp 2700. For each PCR, 2.0µl of genomic DNA (10ng/µl), 2.0µl of PCR buffer, 2.4µl of 25mM $MgCl_2$, 2.4µl of dNTP mix (2.5 mM each dNTP), 0.4µl of each of forward and reverse primer (10µM) (Forward Primer: AGGGGTCTGGA CATGTGGT, Reverse Primer: ACGTATCAGAAGCC TCCACCT) and 2.0µl of Taq DNA polymerase (2U/µl) were mixed. 7.6µl of deionized water was finally added to mixture to make total reaction mixture of 20µl. For polymerase chain reaction amplification, DNA was incubated in thermocycler at 94°C for 5 min, 95°C for 30 sec, 57°C for 50 sec and extension at 72°C for 30 sec for 35 amplification cycles. Final extension was done at 72°C for 7 min.

SNaPSHOT minisequencing assay

5.0µl of amplified PCR product, 1.66µl of SAP enzyme (1U/ µl) and 1µl of Exonuclease1 (1U/µl) enzyme were incubated at 37°C for 60 min and 80°C for 15min. For Single Base Extension PCR 2.5µl of ready to use reaction mix (ABI PRISM SNaPSHOT Multiplex Kit), 1.5µl of PCR (SAP, Exo1 treated), 0.5µl of single base extension primer (6 µM) (SBE: TTTTTTAACTATACAVGAATCT CAGCCCT) were incubated for 10 sec at 96°C, for 5 sec at 56°C, for 30 sec. at 60°C for 25 cycles of amplification. 0.5 µl of SAP (0.5U/µl) was finally added to mixture and incubated for 60 min. at 37°C and for 15 min. at 80°C respectively. After the SNaPSHOT reaction capillary electrophoresis was done on ABI PRISM™ 3730 Genetic Analyzer.

STATISTICAL ANALYSIS

Genotypes and Allele frequencies of the *STAT6* gene polymorphisms were determined by online SHEsis software and counting method. Hardy-Weinberg equilibrium (HWE), linkage disequilibrium analysis was also done by using online SHEsis software facility (Shi and He, 2005; Li *et al*., 2009). Significance of differences between asthma patients group and controls was checked for alleles and genotype frequencies.

RESULTS

A case control study was performed for association analysis of *STAT6* polymorphism rs1059513 with asthma risk among Pakistani individuals. To determine the allele and genotype frequency of polymorphism rs1059513 (A/G) in *STAT6* gene among asthma patients and control group, genomic DNA samples were genotyped from 100 asthmatics and 100 control subjects as illustrated in Materials and Methods. The frequency of this locus was consistent with Hardy-Weinberg equilibrium. The analyzed results of rs1059513 polymorphism with asthma risk in Pakistani population are presented in table 1 and table 2. The results showed that *STAT6* rs1059513 polymorphism was not associated with asthma risk in Pakistani population.

The allele and genotype frequency of rs1059513 polymorphism in *STAT6* did not vary between asthmatic and control group (Presented in table 1). Our results indicated that the *STAT6* gene polymorphism rs1059513 has no association with asthma among case and control groups in Pakistani population.

DISCUSSION

Single nucleotide polymorphisms in asthma candidates' gene may affect response to asthma medications and therapy (Henderson *et al*., 2007). *STAT6* gene is one of the asthma susceptible genes (Weidinger *et al*., 2004; Yamauchi *et al*., 1995). *STAT6* gene involvement in asthma and asthma related atopic diseases have been studied in many populations. Association of rs1059513 polymorphism with high level of IgE has been noticed (Duetsch *et al*., 2002; Weidinger *et al*., 2004; Daley *et al*., 2009).

Role of *STAT6* polymorphism rs1059513 in causing asthma in Pakistani population was studied. In current study a non-significant association between asthma and asthma control for rs1059513 polymorphisms in *STAT6* gene in Pakistani population was noticed. We observed no significant difference in allele frequency of A or G alleles between case and the control groups. Similarly, no differences in genotypes frequency were observed in asthma patients and control (healthy) individuals.

Despite the fact that many studies indicated the involvement of *STAT6* as asthma susceptible/prone gene, and a number of studies showed association between asthma, high IgE level and *STAT6* polymorphisms (Duetsch *et al*., 2002; Weidinger *et al*., 2004; Daley *et al*., 2009), but in the present study we did not observe rs1059513 polymorphism influence for asthma risk in Pakistani populations.

STAT6 gene susceptibility to asthma aetiology has been observed in a number of populations but ambiguous

results for *STAT6* polymorphisms association to asthma risk has left many reports inconclusive (Li *et al*., 2013). Asthma is a heterogeneous disease (Fitzpatrick *et al*., 2011). Differences in the results of association analysis may be due to variability in the pathogenesis of heterogeneous asthma phenotypes (Hoffjan and Ober, 2002). Current study involves a small sample number but the asthmatic and control subjects were involved from the same geographic locations.

CONCLUSIONS

Results of current experiments showed that SNP rs1059513 in *STAT6* gene has no influence on asthma risk in Pakistani population, therefore, possible role of parental imprinting in *STAT6* regulation need to be investigated in further studies.

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