ABSTRACT

Asthma is a complex, heterogeneous disease of the airway leading to difficulty in breathing. Due to its increased prevalence, world over, studies to unravel the mechanisms of its pathogenesis have gained remarkable momentum since it is a serious global health problem. Population genetic studies have lead from the front in the identification of genetic susceptibility factors which in turn have been useful for the understanding of the molecular and immunological aspects of the disease. Candidate gene studies, where an "educated guess" is made on the involvement of gene products, are cost effective and easy to perform and therefore very popular. Also, advent of high-throughput genotyping technologies has given impetus to the research and development in this area.

A number of different subtypes of asthma have been described recently using various classification systems. Atopic asthma, which is also known as extrinsic asthma (accounting for approximately 60% of the total asthma) is one such subcategory that is influenced by external environmental agents/allergens/antigens and supposed to be mediated by IgE, like other atopic disorders. Upon encounter with environmental allergens, a number of different cells/cell types interact with each other in the tissue environment of the lung and bring about the phenotypes that are characteristic feature of asthma. The balance of TH1 and TH2 cytokines during antigen presentation and initiation of the T cell responses has been shown to be critically important in determining the downstream effects of the antigen presentation process and subsequently asthma pathogenesis. The asthma phenotypes are supposed to be mediated by TH2 cytokines while TH1 cytokines have shown to have protective role. A number of pathways other than the classical TH1/TH2 pathways are also being implicated to have important roles in asthma pathogenesis. For example, leukotriene pathway genes, genes of the regulatory T cell development and function and genes regulating cell growth and proliferation have received lot of attention in recent times. The main focus of this study has been on genes regulating TH1/TH2 pathways (for example, IL-4, IFNG and IL-12 signaling pathway genes). We have also included some genes from the regulatory cell development and function, genes involved in the leukotriene biosynthesis, microarray differentially expressed gene in animal model of asthma (these genes may have primary roles in cell cycle and apoptosis) etc. Also
included are some genes that have been identified by positional cloning approach and are widely replicated across different populations/studies.

We have performed case-control and family based candidate gene association studies. Following a set of inclusion and exclusion criteria, we have recruited atopic asthmatics (with family history of asthma) and normal controls for the case-control study while families ascertained through probands were recruited for the family based studies. A total of 393 markers (392 SNPs and 1 microsatellite repeat) have been genotyped in these samples.

We have undertaken a family based and case-control based single marker association analysis and combined the evidence (combined p value) from these two approaches to reject the null hypothesis ($H_0$). This lead to the identification of eight SNPs with evidence of statistical association with asthma [rs1861434A/G ($IFNG$; $p_{corrected}=0.000236$), rs3776944G/A ($LTC4S$; $p_{corrected}=0.0001$), rs3733475A/C ($IRF2$; $p_{corrected}=0.0056$), rs2069832A/G ($IL6$; $p_{corrected}=0.0037$), rs2012075G/A ($IFNGR2$; $p_{corrected}=0.02$), rs1400656G/A ($STAT4$; $p_{corrected}=0.013$), rs1805011C/A ($IL4RA$; $p=0.00000524$) and rs324011G/A ($STAT6$; $p=0.0034$)]. In the single marker association analysis with log_{10} serum total IgE, the p values were not significant after corrections for multiple testing ($p>0.05$).

The single marker association analyses were followed by haplotypic association studies. In genes, where more than five markers have been genotyped, we have performed five locus sliding window analyses to avoid large number of haplotypes with frequency less than 5%. For genes in which five or less than five markers have been genotyped, all the markers have been included in the haplotype based association studies. For the $IFNG$ gene, apart from the five locus haplotypic association analysis (including all the 5 SNPs), a three locus sliding window analysis has been performed where the microsatellite repeat (CA)n was also included. The results of the haplotype based analyses provided strength to the observations made with single marker association studies, besides providing evidence of SNP $\times$ SNP interaction in the $IRF2$ gene. The five locus haplotypes in the region encompassing 5'UTR of $IRF2$ showed very significant association with asthma ($p_{family}=2.9\times10^{-9}$; $p_{case-control}=0.0003$) and log_{10} total serum IgE ($p_{family}=3.68\times10^{-10}$). Specifically, the TAAACG haplotype that has the protective/minor alleles for rs3733475C/A as well as
rs1863314G/A, is highly significantly under-transmitted to asthmatics offsprings in families (p=1.13x10^{-7}) while present with higher frequency in normal controls in case-control analysis (p=9.37x10^{-5}). This haplotype also showed positive association with \log_{10} serum total IgE in families (p=0.000001) as well as case-control study (p=0.0013).

The rs1861494 A/G polymorphisms in IFNG gene, that show highly statistically significant results of association with asthma is located in the intron 3. The IFNG intronic region (from intron 1 to intron 3) has been shown to possess regulatory activity with respect to IFNG gene regulation. Also DNAase hypersensitive sites and a T-Box binding element have been reported in intron 3. A bioinformatic search resulted in identification of various transcription factors that could be regulated due to rs1861494 A→G substitution. Our EMSA results suggest that the wild type allele (A) has a higher binding affinity than the polymorphic allele (G) for binding to putative nuclear factor(s).

We have performed parametric (logistic regression) and non-parametric (Multifactor dimensionality reduction; MDR) gene-gene interaction studies with asthma. In logistic regression (parametric) analysis for detecting gene-gene interaction (two locus) we identified four pairs [rs13170556 (TIM3) \times rs8131980 (IFNGR2) (p=8.045 \times 10^{-6}); rs3024851 (STAT4) \times rs870849 (LAG3) (p=5.22 \times 10^{-5}); rs16863052 (ATF2) \times rs7599504 (STAT4) (p=7.32 \times 10^{-5}) and rs1874791 (IL12RB2) \times rs436857 (IL12RB1) (p=1.89 \times 10^{-5})] of interacting SNPs/genes. We did not find any evidence of interactions, in the linear regression analysis with \log_{10} serum total IgE (p>0.05). In the non-parametric analysis, using MDR, the best two locus model was identified to be rs3827693 (HSPHI) and rs3790558 (IL12RB2) (CVC=6/10; permutation p=0.008). The best three locus model included these two markers and rs6542833 (INPP4A) that improved the efficiency of disease outcome prediction (CVC=9/10; permutation p=0.001). These results were also validated with family based MDR-PDT (Multifactor dimensionality reduction-Pedigree disequilibrium test) and logistic regression analyses.

In brief, using family based and case-control association studies we identified a number of markers/genes to be associated with asthma and/or \log_{10} serum total IgE. The results of our single marker association studies as well as haplotype based and
gene-gene interaction studies are in support of previous reports where TH1/TH2 pathway genes have been shown to play important role(s) in genetic susceptibility to asthma. Importantly, we also reported for the first time, association of IL-6 and LAG3 genes with asthma in single gene and gene-gene interaction analysis, respectively. Both these genes have been shown to be involved in the modulation of TH1/TH2 development and/or function. Furthermore, the results of our MDR analysis suggests that genes modulating cell cycle, apoptosis etc., might as well play significant roles in susceptibility to asthma. Delineation of functional role(s) played by the significantly associated markers/genes should be a step forward in trying to understand the role of these genes in asthma pathogenesis.