Asthma is a multi-factorial complex disorder characterized by reversible obstruction of the airways. Genetic studies, particularly population genetics studies, have identified a number of genes that could modulate asthma susceptibility. Population genetics studies could be performed using linkage studies and association studies. We have performed candidate gene association studies with asthma and serum total IgE. For the candidate gene association studies we have primarily focused on genes modulating differentiation, development and/or functions of TH1/TH2 cells since dysregulation of this pathway has been proposed to be one of the major cause of asthma pathogenesis. We have also included some genes from leukotriene pathway, genes involved in regulatory T cell development and function, some popular positionally cloned asthma genes and genes regulating cell cycle and apoptosis (found to be differentially expressed in microarray datasets). We have performed family based and case-control association studies wherein single marker analysis, haplotype based studies and gene-gene interaction studies have been undertaken. The summary of the observations of this study are as follows:-

**Single marker association studies:**

- We identified a number of novel markers [rs1861494 (IFNG), rs3733475 (IRF2), rs2012075 (IFNGR2), rs1400656 (STAT4) and rs2069832 (IL-6)] to be associated with asthma and showing minor associations with log10 serum total IgE.
  - To the best of our knowledge, association of IL-6 SNP (rs2069832) is the first report of association of this gene with asthma.
  - The other genes (such as IFNGR2, IRF2, STAT4 etc.), found to be associated with asthma in our study, have rarely been investigated for their involvement in genetic susceptibility to asthma. Our focused gene selection strategy resulted in inclusion of these genes for the association studies. These genes are ideal candidate genes for asthma due to the important roles played by them in TH1/TH2 cell polarization and our results suggest that these could play important role in asthma susceptibility.
We also found two previously reported asthma (or related trait) associated polymorphisms [rs1805011 (IL4RA) and rs324011 (STAT6)] to be associated in our study population.

- These polymorphisms may have important functional role(s); rs1805011 (IL4RA) is a coding region non-synonymous polymorphism, and rs324011 (STAT6) has been shown to alter NFkB binding which modulate its promoter activity and mRNA expression. Therefore, our study supports the role of these polymorphisms/genes in genetic susceptibility to asthma.

The minor alleles for all the associated polymorphisms [rs1861434A/G (IFNG), rs3776944G/A (LTC4S), rs3733475A/C (IRF2), rs2069832A/G (IL-6), rs2012075G/A (IFNGR2), rs1400656G/A (STAT4), rs1805011C/A (IL4RA) and rs324011G/A (STAT6)] show negative association with asthma and hence may have protective role(s).

- Number of previously (other studies/populations) associated SNPs [for example, rs2069812 (IL-5), rs20541 (IL-13), rs2243250 (IL-4) etc.] were not found to be associated with asthma in our study, which could be due to any of the reasons such as phenotypic heterogeneity, ethnic differences etc. It could also be possible that some of these may have minor effects and require larger sample size than what have been used in this study, for any conclusive inferences.

The microsatellite (CA)n repeat in IFNG gene, that has been shown to be associated with asthma in other population as well as in Indian population in a case-control study design, was not found to be associated with either asthma or log10 serum total IgE in this study.

Functional studies for the rs1861494 A→G (IFNG) substitution.

- We showed, in this study, that the rs1861494 A→G (IFNG) substitution may alter the binding of some nuclear/transcription factor(s).
  - Wild type (A) allele was found to have higher binding affinity than the polymorphic allele (G).
This differential binding may have important functional consequences in the modulation of IFNG gene expression.

Identification of factors binding at this locus would definitely be an important step forward in understanding the role of this loci/gene in asthma pathogenesis.

**Haplotypic association studies**

- The haplotypic association studies added further strength to the single markers association results.

  - In this analysis, in all the genes, the haplotypes (omnibus, multiallelic analysis) which included the significantly associated markers (single marker association analysis) showed association with asthma.

- Importantly, in the sliding window haplotypic analysis of IRF2 gene, the five locus haplotypes (rs1863316T/A, rs3733475C/A, rs1863314G/A, rs1059492C/A, rs768213A/G) in the region encompassing 5'UTR, showed highly significant statistical association with asthma and log10 serum total IgE.

- The rs3733475 C/A (IRF2) SNP, as mentioned previously, showed highly significant association with asthma in single marker association analysis while the rs1863314G/A (IRF2) SNP showed weak association signal (not significant after multiple testing corrections). Interestingly, when these two SNPs are combined together in the haplotypic analysis a very significant association signal is observed.

- An increased association signal in the haplotypic analysis may be indicative of SNP x SNP interaction between the two markers (rs3733475C/A and rs1863314G/A).

- Importantly, the TAACG haplotype that has the protective alleles for rs3733475 C/A as well as rs1863314 G/A, is highly significantly under-transmitted to asthmatics offsprings in family analysis while present with higher frequency in normal controls in case-control analysis. In fact, this haplotype has almost negligible frequency in cases (<1%) while
comparatively higher frequency among the controls (>7%), indicating
the protective role played by it with respect to asthma susceptibility.

In the three marker sliding window haplotypic analysis of IFNG gene where
the microsatellite marker repeat was included, the most significant p value was
scored with the CA repeat in intron 1, rs1861494 A/G and rs2069718 C/T in
intron 3, in our family based study and in case-control analysis.

- This indicates that individually the repeat may not have any significant
effect, but in combination with other polymorphisms, viz., rs1861494
A/G and rs2069718 C/T, it might assume significant role.

Together, the results of our haplotypic association analysis and EMSA support
previous studies where the region encompassing the intron1 to intron3 has
been shown to possess important role in regulation of IFNG gene.

Gene-gene interaction studies

- The gene-gene interaction analyses also lead to the identification of some
interesting candidate genes to be associated with asthma.

In logistic regression analysis for detecting gene-gene interaction using the set
of SNPs with significant main effect (p≤0.1), we identified three pairs
[rsl3170556 (TIM3) x rs8131980 (IFNGR2); rs3024851 (STAT4) x rs870849
(LAG3); rs16863052 (ATF2) x rs7599504 (STAT4)] of interacting SNPs/genes
after corrections for multiple testing. Each of these pairs has important role in
TH1-cell development and function.

The identification of LAG3 gene as potential asthma susceptibility gene is
novel and interesting since it plays important roles in T helper cell
development and function. The marker, rs870849G/A, identified in this gene
to be associated with asthma is a non-synonymous polymorphism and
therefore could have functional consequences.

The role of STAT4 in asthma susceptibility is further validated through the
gene-gene interaction results. The rs3024851 SNP identified by this analysis,
lies in vicinity of the rs1400656 SNP that showed association in our single gene association studies and rs3024861 SNP that has been shown to be associated with asthma in a previous report. This led us to speculate that the intronic regions harboring these polymorphisms may have important role in STAT4 gene regulation.

- When logistic regression analysis was performed with SNPs from signaling pathways/biological categories, we identified significant statistical interaction between rs1874791 (IL12RB2) × rs436857 (IL12RB1) in the IL-12 signaling pathway. Since both these SNPs did not have a significant main effect (p>0.05), this might be a purely epistatic interaction.
  - Both these genes form heterodimers upon engagement of IL-12 and mediate its downstream signaling, therefore, a possible biological role for these polymorphisms (or other functional polymorphisms in LD with each of these) is indicated.

- In the non-parametric analysis, using MDR, the best two locus model was identified to be rs3827693 (HSPH1) and rs3790558 (IL12RB2). The best three loci model included these two markers and rs6542833 (INPP4A) that enhance the efficiency of disease outcome prediction.
  - The MDR-PDT analysis, as well, suggested epistatic interactions between the three markers rs3827693 (HSPH1), rs3790558 (IL12RB2) and rs6542833 (INPP4A).
  - The logistic regression analysis further validated the MDR results showing statistically significant interactions among these markers.

- The results of the non-parametric gene-gene interaction analysis support previous reports of involvement of INPP4A, from our lab and others, in asthma susceptibility.

- The role of HSPH1, an important gene involved in maintaining cellular stress and homeostasis, apoptosis etc., is indicated in asthma pathogenesis.

- This should encourage genetic association studies wherein apart from genes involved in modulation of classical TH1/TH2 paradigm, genes involved
regulation of other vital cellular functions particularly cell cycle, cellular stress, apoptosis etc., are also brought in the ambit of potential asthma candidate genes.

The parametric and non-parametric gene-gene interaction studies support the view that a better understanding, with respect to complex disease genetic susceptibility, is possible through these approaches.

- In this study, gene-gene interaction analyses led to the identification of different set of genes, for example, TIM3 (rs13170556) and LAG3 (rs870849) for their involvement in asthma. Both these genes showed minor independent main effects in our study population but showed highly statistically significant results with their interacting partners.

In brief, this study was conducted with an aim to identify asthma susceptibility genes using single marker association analysis and gene-gene interaction studies. We identified a number of loci which might be relevant to asthma pathogenesis due to their molecular/biochemical roles. The IL-6 and LAG3 genes have not been previously reported to be associated with asthma and therefore this is a novel finding. A number of the associated markers (in this study) have been shown to possess functional roles in the regulation of the respective genes. In this study a functional role for the rs1861494 A→G (IFNG) is indicated. Delineation of the functional role of the other polymorphisms (for which there are no functional clues) or identification of functional polymorphisms in LD with these markers, would add important dimension to the understanding of involvement of these genes/polymorphisms in asthma pathogenesis. Another important finding of this study is gene-gene interaction among the genes of the T cell polarization and cellular apoptosis pathway. Thus, our results may encourage future genetic association studies with candidate genes/factors responsible for maintaining cellular signaling, homeostasis, apoptosis etc.