STUDIES OF ASTHMA GENETICS IN INDIAN POPULATION

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Unique asthma research group involving medical and research community across the country
Identification and characterization of novel polymorphic loci with asthma and related disorders

Prevalence of Asthma in India

Affects 11-12% of the Indian population, with man days loss equal to 34%.

In a study on adolescent school children in Chandigarh, north India, presence of one or more respiratory symptoms was reported by 31% students. 2.6% of the boys while 1.9% of the girls were found to be suffering from asthma.

One fourth of the total Indian population is suffering from various allergic diseases including Asthma, Allergic and other respiratory disorders.

Major Biochemical Pathways Involved

Major biochemical pathways involved in asthma include Allergen, Histamine, Leukotrienes, Prostaglandins, Histamine, Leukotrienes, Prostaglandins, etc.

OBJECTIVES

- To identify polymorphisms and repetitive sequences within and around the asthma susceptibility genes.
- To establish association of polymorphic loci within these genes with asthma.
- To functionally validate the association of polymorphic loci with asthma.
- To identify novel gene(s) associated with asthma.
Inclusion criteria:
1. Age of the proband should be between 6-35 years.
2. History of asthma as per questionnaire and verified by clinicians.
3. Clinical improvement with inhaled steroids.
4. Reversibility testing with β-2 agonist (more than 15%).
5. Skin test.
6. Clinical parameters:
   - Total and specific IgE.
   - Absolute eosinophil counts (more than 500/cu. mm.).
   - X ray of chest and PNS.

Exclusion criteria:
1. Proband must be a non-smoker.
2. Proband is in advanced stages of pregnancy.
3. Diagnosis of ABPA.
4. Patients taking drugs known to produce Eosinophilia.
5. Patients with tropical pulmonary eosinophilia.

For the recruitment of the asthma proband, American Thoracic Society (ATS) guidelines are followed. All participants are required to sign informed consent.

Association of IFNG gene polymorphism with asthma in the Indian population.


Established a significant association of a CA repeat marker in IFNG locus on 12q21 with total serum IgE levels.

A previously reported promoter polymorphism at the -333 base pair position was not detected in our population.

This is the first report on the association of a candidate gene with asthma from the Indian subcontinent.

Summary

- Identified a novel polymorphic CA repeat (R1) in the STAT6 promoter.
- Three novel SNPs found in the intronic regions.
- Established an association of the polymorphic CA repeat R3 with asthma in the Indian population.
- Established a significant association of the 17_15 (R1_R3) haplotype with asthma in the Indian population (p<0.003).


Original Article

A_16_C haplotype in the FceRIβ gene confers a higher risk for atopic asthma in the Indian population

A three locus haplotype, A_16_C, in Fc epsilon RI beta gene confers higher risk for atopic asthma


A three locus haplotype, A_16_C, in Fc epsilon RI beta gene confers higher risk for atopic asthma

Schematic map of the FcRIR Gene with polymorphic sites

Development of Functional Assay to Validate the Haplotypic Associations obtained

Histamine Release from leukocytes obtained from peripheral blood of patients and controls of selected haplotypes.

Functional validation of polymorphisms/haplotypes in Fc epsilon RI beta gene involved in asthma pathogenesis

Human Basophils + anti-IgE/PMA

Histamine

MAJOR BIOCHEMICAL PATHWAYS INVOLVED

TRANSFORMING GROWTH FACTOR β1 (TGFβ1)

- An important profibrotic secretory growth factor implicated in airway remodeling and pulmonary fibrosis
- Can be generated by macrophage, epithelial cells, fibroblast and eosinophils.

MAJOR BIOCHEMICAL PATHWAYS INVOLVED

CONCLUSIONS

- A novel (CT)_{n=1}(CA)_{n=2} repeat was identified 24.9 Kb upstream of the +1 site.
- Alleles 21 & 23 were positively and alleles 22 & 24 were negatively associated with asthma for this repeat.
- Genotype CC was the protective genotype for –509C/T polymorphism.
- The haplotypes 21_G_C and 23_G_T were major risk and 22_G_C and 24_G_C were major protective haplotypes for asthma.
- The allelic, genotypic and haplotypic association of TGFβ1 with asthma was correlated with the expression of TGFβ1 in serum.

Interleukin-10 promoter polymorphisms and atopic asthma in North Indians:

Polymorphism at 1082 A/G, 819 C/T and 592 C/A nucleotides were genotyped in a case-control and a family-based studies.

Provided evidence that the ATA haplotype is positively, whereas GCC and a novel ATC haplotypes are negatively associated with asthma in Indian population.


β2-Adrenergic Receptor Polymorphisms and Response to Salbutamol among Indian Asthmatics.

First report of association of promoter and downstream SNPs of the AMCase Gene with atopic asthma and total serum IgE. C to T change at the promoter SNP –1261 leads to abolished binding of transcription factor Oct 1 and thus could be differentially regulating the expression of AMCase leading to asthma.


A Case-control study and family based association study have been conducted. An intronic SNP rs1861494 A/G was found to be associated with asthma. Differential affinity of transcription factors on the promoter of the SNP was demonstrated.

Kumar A and Ghosh B. Genes Immun., 2008, 9(4); 294-301

Significant transmission distortion to the asthmatic probands was seen for allele 3 of the AFM311ZB1 (p=0.006). Allele 3 of M3 is also found to be significantly associated with percentage blood eosinophil (p=0.0006) and asthma severity (p=0.04). Moreover, it was functionally correlated with high serum NO levels (p=0.007).


Identification of INPP4A as a Novel Candidate Gene Associated with Asthma:

Genomewide-candidate gene approach by combining:

a) Extracted information from microarray datasets;

b) Experimental genomic and functional genomic approaches.

Ghosh B et al AJRCCM, 177,712-719, 2008

Identification of a novel asthma associated gene, Inositol polyphosphate 4-phosphatase (INPP4A)

Sharma M et al. 2007, Am J Respir Crit. Care Med..

Genomic Organization of INPP4A Locus

Chromosome 2q11.2 in humans and chromosome 1 in mice

27 Exons spanning 142.7 kb

Ghosh B et al AJRCCM, 2008

Loss-of-function of inositol polyphosphate-4-phosphatase reversibly increases the severity of allergic airway inflammation.

Ghosh et al, Nature Commun. 3:877 doi: 10.1038/ncomms1880 (June 2012)

Discovery of a novel link between metabolic diseases and asthma

- Inositol polyphosphate-4-phosphatase A (INPP4A) is the terminal enzyme in PI3K-Akt Pathway.
- Excess activation of PI3K-Akt pathway causes inflammatory diseases like asthma.
- Calpain mediated loss of INPP4A leads to severe asthma by activating PI3K-Akt Pathway.
- Stability of INPP4A critically determines the PI3K-Akt signaling.
- Stability of INPP4A could be altered by immune or non-immune conditions (metabolic diseases).
- INPP4A could serve as a novel target for steroid resistant asthmatics.

Restoration of INPP4A reverses severity of asthma
Emerging Interface Between Metabolic Syndrome and Asthma.


INNP4A could be a novel link between metabolic diseases and asthma

Asthma-like symptoms are increased in the metabolic syndrome. Lee et. al. J Asthma 46, 339-42, 2009

**Key findings:**
1) Let-7 family of microRNA regulates IL-13 post-transcriptionally.
2) Let-7 microRNA is down regulated under allergic inflammatory conditions.
3) Exogenous administration of let-7 microRNA mimics alleviates asthmatic Phenotypes in mice model of allergic asthma.

**SIGNIFICANCE**

- Developing genetic finger prints of atopic asthmatics in Indian population.
- Identifying responsive population/individuals to drugs for better efficacy and limited side effects.
- Developing better diagnostic and therapeutic modalities for asthma.
- Identification of INPP4A gene establishes a novel link between metabolic diseases and asthma.

**ACKNOWLEDGEMENTS**

Rana Nagarkatti
C.B.Rao
Manita Sharma
Shilpy Sharma
Sarvesh Kumar
Jyotirmaya Ray
Rajeshwar Chatterjee
U. Mahabir
ek
Anuvrat Kumar
Sameer Nahid
Arunaw Agrawal
S.K.Brahmachari
FGU Sequencing Facility, IGB

Clinical Collaborators
V.Vijayan, Director VPCU
Raj Kumar, Asst. Prof., VPCU
S.K.Brahmachari, Prof., Medicine, AIIMS
E.V. Niphadkar, Mumbai
J.B.Rishi, SMS Hospital, Jaipur
Vivek Atahya, SMS Hospital, Jaipur
Rajendra Prasad, KG’s Hospital, Lucknow
Brajen Lahkar, Downton Hospital, Guwahati

All Participating Volunteers (Patients and Controls)
Leads from GEO Database

Gene Datasets screened:
- GDS 347 (5 samples): Lungs of ovalbumin sensitized/challenged C3H (ovalbumin resistant) mice compared with controls.
- GDS 348 (6 samples): Lungs of ovalbumin sensitized/challenged C57BL/6 (ovalbumin resistant) mice compared with controls.
- GDS 349 (6 samples): Lungs of ovalbumin sensitized/challenged A/J (ovalbumin sensitive) mice compared with controls.

Individual RNA Samples (5/6) from OVA-sensitized/challenged mouse lungs Vs Pooled RNA Samples (6) from Saline treated mouse lungs

Gene Datasets
- GDS 347
- GDS 348
- GDS 349

Differential expressed genes (at least in 5 samples)

Differential expressed genes (3 fold) in each sample tested

Working Strategy:

Filters:
- Filter 1: 3 fold difference in expression in at least 5 mice per data set
- Filter 2: Genes mapped to epigenetic regions in the human genome and linked to autoimmune or related phenotypes
- Filter 3: Novelty and potential polymorphic repeats
- Filter 4: Genes with non-polymorphic repeats were defined from the filter set 2

Statistical Analysis:
- Western blot evidence & Protein BLASTES/fold measurement

Loss-of-function of inositol polyphosphate 4-phosphatase reversibly increases the severity of allergic airway inflammation.


This finding establishes a novel link between metabolic diseases and asthma.
Suggestive evidence of association of C-159T functional polymorphism of CD14 gene with atopic asthma in the North and Northwest Indian population.


Allelic and genotypic distribution of C-159T polymorphism in case vs control groups (P=1.87, C=2.27).

<table>
<thead>
<tr>
<th>Allele</th>
<th>Transmitted</th>
<th>Non-Transmitted</th>
<th>Total</th>
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<tbody>
<tr>
<td>C</td>
<td>54</td>
<td>57</td>
<td>87</td>
</tr>
<tr>
<td>T</td>
<td>33</td>
<td>54</td>
<td>87</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>87</td>
<td>174</td>
</tr>
</tbody>
</table>

χ^2 TDT = 5.069, DF = 1, p < 0.025

Chemokine Receptor 5 (CCR5) A32 deletion and atopic asthma in Indians.


Controls 11/367 were heterozygous
Patients 17/215 were heterozygous
(p = 0.0009)

36 families from North-east & 48 families from North-west 2 members from a North-west family were found homozygous

Association of an Intragenic Microsatellite Marker in CC16 gene with Asthma in Indian Population.


p = 0.016

PATENT FILED


iNOS Gene Polymorphisms in Indian Population

NOS2A is composed of twenty seven small exons and spans approximately 43 kb on chromosome 17q11. Region having promoter and first six exons is enlarged.

Bioinformatics approach: use of Repeatmasker to identify any microsatellite repeat in and around inducible Nitric Oxide Synthase.
Association of iNOS polymorphisms with asthma

- Four polymorphic microsatellite repeats viz; a CCTTT repeat 2.5 kb upstream of transcription start site (M1), a novel GT repeat (BV680047) in intron 2 (M2), a GT repeat (AFM311ZB1) in intron 4 (M3) and a CA repeat (D17S1878) in intron 5 (M4) are identified in iNOS gene.

- A significant transmission distortion to the asthmatic probands was seen for allele 3 of the AFM311ZB1 (p=0.006).

- Allele 3 of M3 is also found to be significantly associated with percentage blood eosinophil (p=0.0006), asthma severity (p=0.04) and high serum NO levels (p=0.007).

- The promoter repeat (M1) is found to be associated with serum total IgE (p=0.028). Individuals carrying allele 4 of this repeat has high serum IgE (p<0.0001) as well as NO levels (p=0.004).


Chemokine Receptor 5 (CCR5) A32 deletion and atopic asthma in Indians.

- Controls 11/367 were heterozygous
- Patients 17/215 were heterozygous
- (p = 0.0009)

- 36 families from North-east & 48 families from North-west
- 2 members from a North-west family were found homzygous


Eotaxin

- We observed for the first time a highly significant association of the newly studied (GAAGGA)n hexanucleotide repeat with asthma (p=1x10^-6). The frequency of occurrence of this hexanucleotide is very low in control populations.

- We further identified C-A-C-S as an important risk haplotype associated with high IgE and plasma eosinophil levels.


C/EBP-d

- Different binding of nuclear proteins to the A and C allele of TNFA-863C>A polymorphism was also demonstrated by electrophoretic mobility shift assay (EMSA).


C481T was found to be associated with asthma binary trait, log10 serum total IgE and percentage of peripheral blood eosinophil counts.

- Fast acetylators have protective advantage over the slow acetylators against asthma and atopy and could serve as a genetic marker

Batra et al. Pharmacogenomics, Jul 2006, Vol 7, No 5

Genomic Organization of the STAT6 Gene

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- We further identified C-A-C-S as an important risk haplotype associated with high IgE and plasma eosinophil levels.


Statin

- A study cohort of 219 atopic asthmatic unrelated patients and 210 normal healthy controls.

C481T was found to be associated with asthma binary trait, log10 serum total IgE and percentage of peripheral blood eosinophil counts.

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Batra et al. Pharmacogenomics, Jul 2006, Vol 7, No 5


Fast acetylators have protective advantage over the slow acetylators against asthma and atopy and could serve as a genetic marker

Batra et al. Pharmacogenomics, Jul 2006, Vol 7, No 5

Nagarkatti et al. Am J Respir Cell Mol Biol. 2004 Sep;31(3):317-21

Transforming growth factor β1 (TGFβ1) and asthma: a foe or a friend ????

Role as a Foe:
- Produced in exaggerated quantities by eosinophils and fibroblasts from patients with severe and mild asthma.
- TGFβ1 mRNA levels in eosinophils are increased in patients with severe asthma.
- TGFβ1 is implicated in several aspects of fibrosis:
  - Increases synthesis by fibroblasts of many components of extracellular matrix (ECM)
  - Decreases synthesis of enzymes and increases synthesis of inhibitors of these enzymes that degrades the ECM

Role as a Friend:
- Indirectly inhibits T-cell activation by modulating of antigen presentation function and deactivating macrophages
- Inhibits IgE synthesis
- Inhibits proliferation of mast cells, eosinophils and basophils
- Abrogates established asthma conditions in mice.
• Considered as a prime candidate gene for asthma and atopy
• Chromosomal position 19q13.1-13.2, linked to mite sensitivity (a major risk factor for asthma)

Schematic map of the TGFβ1 Gene

exon 7
exon 6
exon 5
exon 4
exon 3
exon 2
exon 1

24.9 Kb

21_G_C and 23_G_T were the most frequent in the patient population
OR = 2.49 with 99% CI = [1.58, 3.94] [p = 0.00001],
OR = 2.64 with 99% CI = [1.51, 4.59] [p = 0.00001]

21_G_C/21_G_C
21_G_C/Non21_G_C
Non21_G_C/Non21_G_C

22_G_C and 24_G_C were the most frequent in the control population
OR = 0.18 with 99% CI = [0.10, 0.31] [p = 0.00001],
OR = 0.48 with 95% CI = [0.24, 0.98] [p = 0.00001]

22_G_C/22_G_C
22_G_C/Non22_G_C
Non22_G_C/Non22_G_C

TGFβ1 LEVELS CORRESPONDING TO ASSOCIATED ALLELES, GENOTYPES AND HAPLOTYPES

Classification of Drugs
• Quick relief medications
  • β-adrenergic agonist
  • Methylxanthine
  • Anticholinergics
• Long term control medications
  • Glucocorticoids
  • Leucotrine inhibitors
  • Mast cell stabilizing agents
  • Receptor antagonists
• Miscellaneous

Quick Relief Medications

- Inhibit smooth muscle contraction
  - Adrenergic stimulants
  - Drugs - catecholamines, resorciols and saligenins
- Mechanism of action: stimulation of ß-adrenergic receptors
  - Activation of G-proteins
  - Formation of cyclic AMP
  - Smooth muscle relaxation
- Limitations
  - Effective only when administered either inhalation or parenteral
  - Considerable chronotropic and ionotropic cardiac effects

Agents with highly selective action
- Resorcinol – metaproterenol, terbutaline, fenoterol
- Saligenin – commonly used albuterol (Salbutamol)
  - Highly selective for respiratory tract
  - Little cardiac side effects except high doses
  - Active by all routes
  - Bypass metabolic processes
  - Side-effect – tachycardia

Saltmeterol
- Congener of albuterol
- Long acting
- Effective in nocturnal and exercise induced asthma
- Limitations – non for acute use, cumulative side effects

Publications on Asthma Genetics


Newly developed drugs are predicted to be equally ineffective in some patients. Genotyping and haplotyping of target genes will help in identifying responsive populations/individuals to drugs. Identifying polymorphisms in genes encoding interacting proteins may develop better predictive value. Identifying protective and risk alleles, genotypes and haplotypes in asthma-associated genes may help in better management of asthma. Functionally correlating the expression profile of the genes with the excess activation of PI3K may serve as a novel target for inactivating PI3K. Overexpression of INPP4A could serve as a novel target for inhibiting smooth muscle contraction. Identifying polymorphisms in genes encoding interacting proteins may develop a causal relationship with the disease and thus may help to develop better modalities for therapeutic intervention.

**SIGNIFICANCE**

- Current therapy is symptomatic relief of asthma and is not equally effective in all asthma patients and having side effects.
- Newly developed drugs are predicted to be equally ineffective in some patients.
- Genotyping and haplotyping of target genes will help in identifying responsive populations/individuals to drugs.
- Identifying polymorphisms in genes encoding interacting proteins may develop better predictive value.
- Identifying protective and risk alleles, genotypes and haplotypes in asthma-associated genes may help in better management of asthma.
- Functionally correlating the expression profile of the genes with the excess activation of PI3K could serve as a novel target for inhibiting smooth muscle contraction.
- Overexpression of INPP4A could serve as a novel target for inhibiting smooth muscle contraction.
- Limitations
  - Inadequate population/individuals to drugs.
  - Considerable chronotropic and ionotropic cardiac effects.
  - Specific effect only when administered either inhalation or parenteral.
  - Considerable chronotropic and ionotropic cardiac effects.

**Conclusion**

INPP4A is significantly decreased in allergic airway inflammation.
- Calpains degrade INPP4A in vivo and in vitro.
- Calpain inhibitor, calpeptin, alleviates asthma features.
- siRNA-mediated knockdown of INPP4A worsens asthmatic phenotype.
- Overexpression of INPP4A attenuates asthma.
- Loss of INPP4A was sufficient to induce asthma like features in naive mice without any sensitization and challenge.

**Asthma Medication-Role of β2-Adrenergic receptor**

Commonly used drugs - Salbutamol and its derivatives
- Inhibit smooth muscle contraction
  - Adrenergic stimulants
    - Drugs: catecholamines, resorcinols and saligenins
    - Mechanism of action:
      - Activation of β2-adrenergic receptors
      - Activation of G proteins
      - Formation of cyclic AMP
      - Smooth muscle relaxation
    - Limitations
      - Effective only when administered either inhalation or parenteral
      - Considerable chronotropic and ionotropic cardiac effects

**INPP4A was identified to be novel asthma gene using population genetic studies**

**VARIANT** | **PEST SCORE** | **PEST SCORE**
--- | --- | ---
α1 | 573 VLYPLLLTLTDCVAMMDSK508 | +5.36
α2 | Absent | Absent
α3 (604 Thr) | 584 PPSCPCPTMSVACH607 | +7.49 (∗∗∗)
α3 (604 Ala) | 584 PPSCPCPTMSVACH607 | +4.95 (∗∗)

Fine mapping and association studies on INPP4A led to the identification of A+110832G Thr/Ala polymorphism associated with asthma.

The allele A is transmitted more to the asthmatic individuals whereas the allele G is protective.

**Discovery of a novel genetic component of airway remodeling and asthma**

- Apeal: overexpression of INPP4A is linked to allergic airway inflammation.
- Calpain-mediated loss of INPP4A leads to severe airway remodeling.
- INPP4A knockdown attenuates allergic airway inflammation.
- Restoration of INPP4A(584) reverses severity of asthma.

Medwire news: Caroline Price, 16 January 2008, Inositol pathway linked to asthma susceptibility.

**INPP4A** was identified to be novel asthma gene using population genetic studies.
Allergy, metabolic syndrome, environmental stress, and infections have all been linked to asthma.

Excessive PI3/Akt signaling may be one point of integration.

Agrawal et al, Physiological Genomics, 40, 1-7, 2009

Abnormal signaling in airway epithelium (PI3/Akt pathway) and organelle stress (ER-mitochondria) may determine asthma severity or risk.

INPP4A:
- Magnesium independent enzyme
- Dephosphorylates 4-position phosphate of Inositol 1,3,4-triphosphate, Inositol 3, 4 bisphosphate and Phosphatidylinositol 3,4 bisphosphate
- Important regulator in PI3-AKT pathway

Mouse Inpp4a null mutant:
- One base pair deletion in the exon 10
- Neuron loss and severe locomotor instability (wobble phenotype)

Loss of INPP4A associated with cancer, acute myeloid leukemia

Inositol Polyphosphate 4 Phosphatase Type I (INPP4A)

Developments of Transfient assay to validate the haplotypic associations obtained in FcεRIb

Kumar A and Ghosh B. Genes Immun., 2008

A case control study and family based association study have been conducted. A novel 3-locus haplotype, 23_G_T, was significantly associated with asthma (P = 0.00001 in cohorts A and B) as well as with higher serum TGF-β1 level (P = 0.01)

Sharma S, Clin Genetics, 2004
Sharma et al, JACI, 2006
Sharma S, Ghosh B, IAAI, 2009
Nagpal K et al, JACI, 2005

β2-Adrenergic Receptor Polymorphisms and Response to Salbutamol among Indian Asthmatics

Kukrati et al, Pharmacogenomics, 2005