STUDIES OF ASTHMA GENETICS IN INDIAN POPULATION

Balaram Ghosh, PhD, FNA, FNASc, FASc

J.C.Bose National Fellow

Head, Genomics & Molecular Medicine
IGIB, Delhi.

Unique asthma research group involving medical and research community across the country

Nat Immunol. 2008; 9(12):1319-22

Identification and characterization of novel polymorphic loci with asthma and related disorders

Prevalence of Asthma in India

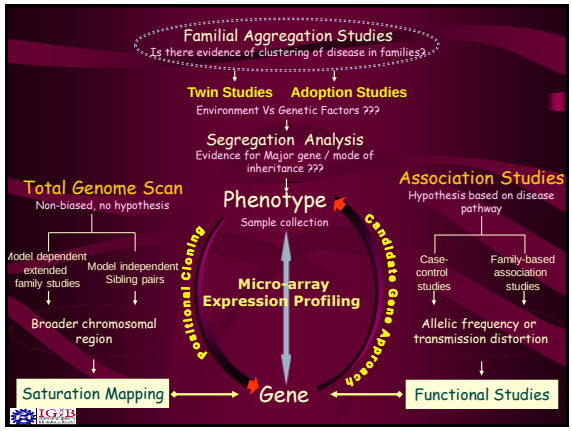
Affects 11-12% of the Indian population, with man days loss equal to 34%. PNAS, US 2002

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.

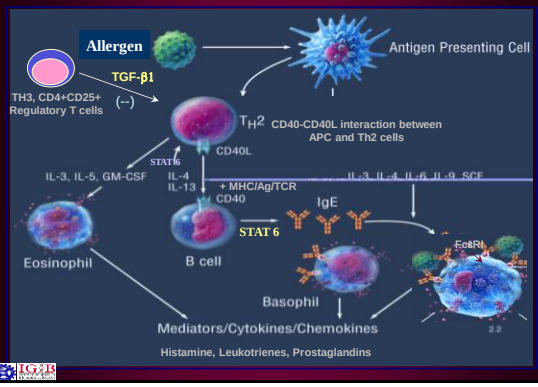
J Asthma. 2001 Sep;38(6):501-7

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

Times of India Nov 28,2004



MAJOR BIOCHEMICAL PATHWAYS INVOLVED



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.

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

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.).
   - Xray 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.

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

Nagarkatti et al. (2002) J. Allergy Clin. Immunol. 110:410-2.

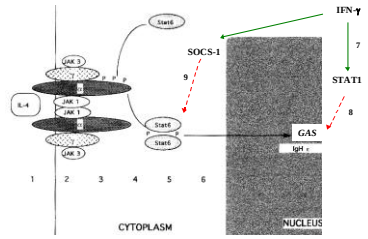
Established a significant association of a CA-repeat marker in *IFNG locus* on 12q21 with total serum IgE levels.

A previously reported promoter polymorphism at the –333 base pair positionwas not detected in our population.

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

SIGNAL TRANSDUCER & ACTIVATOR OF TRANCRIPTION 6 (STAT6)

IL-4 R α Mediated Signal Transduction: Pathway Approach



1. IL4 Binding
2. Receptor dimerization
3. Transphosphorylation of JAKs
4. JAKs phosphorylate tyrosine of IL4 receptor
5. STAT 6 phosphorylation and dimerization.
6. STAT6 translocation to nucleus binding to *GAS* site.
7. STAT1 activation by IFN-g.
8. Competition between STAT1 and STAT6 for *GAS* site.
9. SOCS1 activation by IFN-g and inhibition of STAT6.

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).

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

Clin Genet 2004: 66: 417–425
Printed in Denmark. All rights reserved

CLINICAL GENETICS
doi: 10.1111/j.1399-0004.2004.00333.x

Original Article

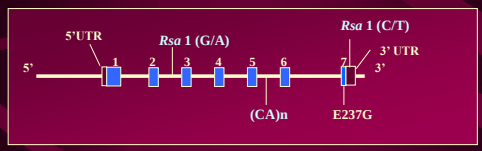
A_16_C haplotype in the *FcεRIβ* gene confers a higher risk for atopic asthma in the Indian population

Sharma S, Nagarkatti R, B-Rao C, Niphadkar PV, Vijayan V, Sharma SK, Ghosh B. A_16_C haplotype in the *FcεRIβ* gene confers a higher risk for atopic asthma in the Indian population. Clin Genet 2004: 66: 417–425. © Blackwell Munksgaard, 2004

S Sharma[a], R Nagarkatti[a], C B-Rao[a], PV Niphadkar[b], V Vijayan[c], SK Sharma[d] and B Ghosh[a]

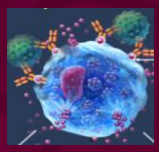
A three locus haplotype, A_16_C, in Fc epsilon RI beta gene confers higher risk for atopic asthma

Sharma, S. et al (2004). Clin. Genetics.;66(5):417-25.



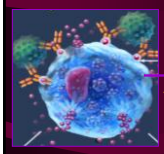
Schematic map of the FcεRIβ Gene with polymorphic sites

Development of Functional Assay to Validate the Haplotypic Associations obtained



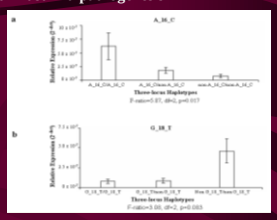
Histamine Release from leukocytes obtained from peripheral blood of patients and controls of selected haplotypes.

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



FceRIb

Human Basophils + anti-IgE/PMA → Histamine

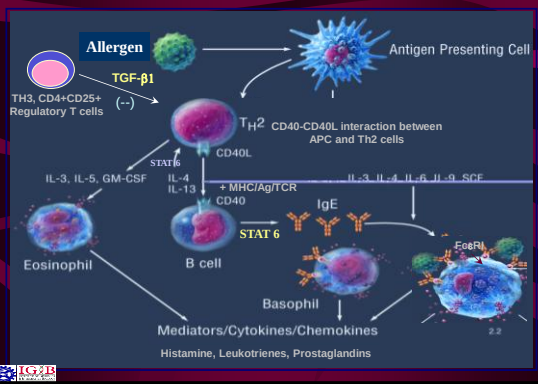


Real-time PCR

Genotypes/Haplotype	Number	HR-anti-IgE (%)	HR-A23187 (%)
A_16_C/A_16_C	6	11.01 ± 5.69	13.30 ± 9.37
Non A_16_C/A_16_C	30	4.87 ± 3.74	14.75 ± 15.03
G_18_T/G_18_T	10	3.52 ± 2.22	20.33 ± 20.26
Non G_18_T/G_18_T	26	9.48 ± 6.70	12.81 ± 10.85

Sharma *et al*, J Allergy Clin Immunol. (2007) 18(4):960-3.

MAJOR BIOCHEMICAL PATHWAYS INVOLVED



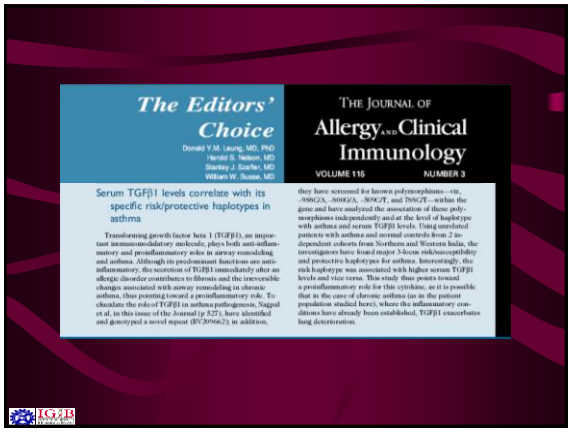
TRANSFORMING GROWTH FACTOR β1 (TGFβ1)

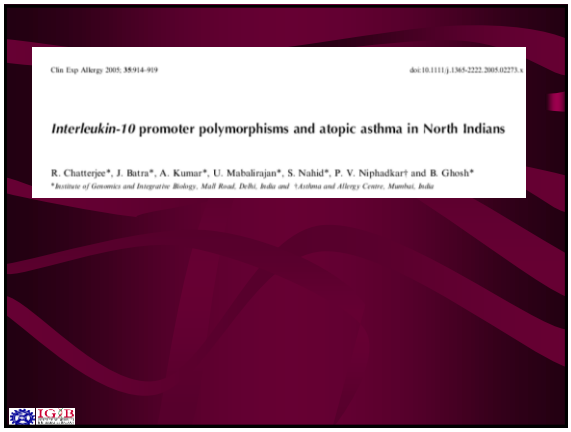
- An important profibrotic secretory growth factor implicated in airway remodeling and pulmonary fibrosis
- Can be generated by macrophage, epithelial cells, fibroblast and eosinophils.

CONCLUSIONS

- A novel $(CT)_N(CA)_M$ 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.

Ghosh B et al (2005) J Allergy Clin Immunol. 115(3):527-33.





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.

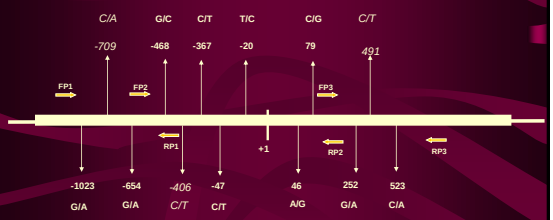
Provided evidence that the ATA haplotype is positively, whereas GCC and a novel ATC haplotypes are negatively associated with asthma in Indian population.

Chatterjee et al (2005) Clinical and Exptl. Allergy 35, 914-919

β_2-Adrenergic Receptor Polymorphisms and Response to Salbutamol among Indian Asthmatics.

Kukreti, R. -- **Ghosh, B.** (2005) **Pharmacogenomics 6(4), 399-410**

β2AR gene and localization of the gene polymorphisms



Kukreti et al. Pharmacogenomics 2005 Jun;6(4):399-410.

SNP Position	Genotype Good Responders	%	Genotype Poor Responders	%	χ^2	P value
-1023	GG (n=18) GA (n=9) AA (n= 4) (N=31)	58.0 29.0 13.0	GG (n=18) GA (n=5) AA (n=2) (N=25)	72.0 20.0 8.0	1.18	0.554
-654	GG (n=10) GA (n=23) AA (n=2) (N=35)	28.6 65.7 5.7	GG (n=7) GA (n=20) AA (n= 9) (N=36)	19.4 55.6 25.0	5.18	0.075
-468 SNP Block	GG (n=2) GC (n=19) CC (n=14) (N=35)	5.7 54.3 40.0	GG (n=1) GC (n=12) CC (n=23) (N=36)	2.7 33.3 64.0	4.09	0.129
46	AA(n=3) AG(n=27) GG(n=11) (N=41)	7.3 65.9 26.8	AA(n=13) AG(n=22) GG(n=4) (N=39)	33.3 56.4 10.3	9.98	0.0068
252	GG (n=13) GA (n=20) AA (n=2) (N=35)	37.2 57.1 5.7	GG (n=18) GA (n=16) AA (n=2) (N=36)	50.0 44.5 5.5	1.23	0.538
523	CC (n=14) CA (n=19) AA (n= 2) (N=35)	40.0 54.3 5.7	CC (n=19) CA (n=16) AA (n=1) (N=36)	52.8 44.4 2.8	1.33	0.513

NITRIC OXIDE SYNTHASE 2

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).

Batra J *et al.* Thorax (2007), 62(1): 16–22.

INTERFERON GAM

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 factor(s) for binding to the SNP was demonstrated.

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

ACID MAMMALIAN CHITINASE (AMCase)

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.

Chatterjee R et al. J Allergy Clin Immunol. 2008, 122(1): 202–8.

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

Genomewide-candidate gene approach by combining:

a) Extracted information form microarray datasets;

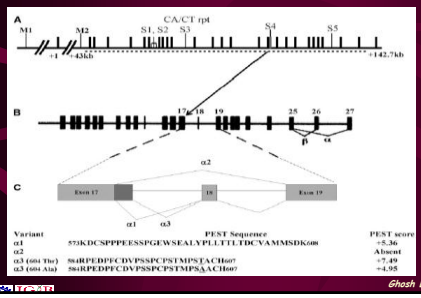
b) Experimental genomic and functional genomic approaches.

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

Genomic Organization of *INPP4A* Locus

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

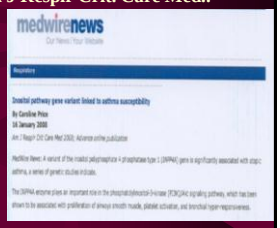
27 Exons spanning 142.7 kb



Ghosh B et al AJRCCM, 2008

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

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

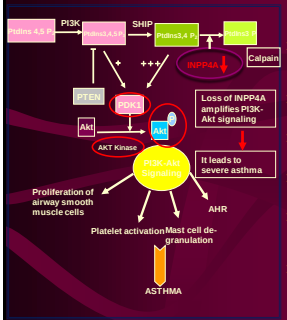


Opening new avenues for therapeutic intervention by modulating PI3K-Akt pathway

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

INNP4A could be a novel link between metabolic diseases and asthma

Emerging Interface Between Metabolic Syndrome and Asthma.

Agrawal et al, Am J Respir Cell Mol Biol Vol 44. pp 270–275, 2011

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

PNAS

Posttranscriptional regulation of interleukin-10 expression by hsa-miR-106a

Amit Sharma[a], Manish Kumar[a], Jyotirmoi Aich[a], Manoj Hariharan[b], Samir K. Brahmachari[b], Anurag Agrawal[a], and Balaram Ghosh[a,1]

[a]Molecular Immunogenetics Laboratory and [b]G. N. Ramachandran Knowledge Centre for Genome Informatics, Institute of Genomics and Integrative Biology, Mall Road, Delhi 110007, India

Edited by David W. Talmage, University of Colorado Health Sciences Center, Denver, CO, and approved February 11, 2009 (received for review September 3, 2008)

nature INDIA

doi:10.1038/nindia.2009.103; Published online 15 April 2009

Research highlight

Gene stopper spotted

Geneticists have identified a new mechanism that stops the synthesis of the Interleukin-10 (IL-10) gene, a key orchestrator of the immune system[1]. This could provide new insights into managing various immunological diseases such as cancer, rheumatoid arthritis, asthma and infectious disorders.

IL-10 plays an important role in immune-regulatory events. Its dysregulation means one could suffer from any of these immunological diseases.

Using a combination of bioinformatics and molecular approaches, they found that microRNA (hsa-miR-106a) regulates IL-10 expression. They identified a novel microRNA mediated regulatory mechanism for IL-10, in which expression of hsa-miR-106a inhibits the synthesis of IL-10 through post-transcriptional regulation. A cluster of six microRNAs including hsa-miR-106a, present on chromosome X, is over expressed in 40% of human T-cell leukemias.

Co-authors Amit Sharma (left) and Balaram Ghosh.

Welcome Institute of Genomics and Integrative Biology

FACULTY OF 1000 BIOLOGY
MAJOR ADVANCES. EXPERT OPINIONS.

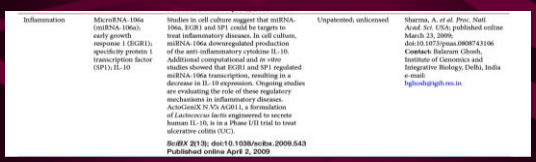
BIOLOGY REPORTS
NEW from Faculty of 1000
Fast reports on hot topics

My F1000 | Browse | Top 10s | Advanced Search | My Details | About | Faculty Members | Associate FMs | F1000 Reports NEW

Recommended
F1000 Factor 3.0
EndNote
Download citation
Send page by email

Posttranscriptional regulation of interleukin-10 expression by hsa-miR-106a.
Sharma A, Kumar M, Aich J, Hariharan M, Brahmachari SK, Agrawal A, Ghosh B
Proc Natl Acad Sci U S A 2009 Apr 7 **106**(14):5761-6 [abstract on PubMed]
[citations on Google Scholar] [related articles] [FREE full text]

Selected by | Richard L. Stevens NEW
Evaluated 20 Apr 2009
Relevant Sections



Inflammation	MicroRNA-106a (miRNA-106a); early growth response 1 (EGR1); specificity protein 1 transcription factor (SP1); IL-10	Studies in cell culture suggest that miRNA-106a, EGR1 and SP1 could be targets to treat inflammatory diseases. In cell culture, miRNA-106a downregulated production of the anti-inflammatory cytokine IL-10. Additional computational and *in vitro* studies showed that EGR1 and SP1 regulated miRNA-106a transcription, resulting in a decrease in IL-10 expression. Ongoing studies are evaluating the role of these regulatory mechanisms in inflammatory diseases. ActoGeniX N.V's AG011, a formulation of *Lactococcus lactis* engineered to secrete human IL-10, is in a Phase I/II trial to treat ulcerative colitis (UC).	Unpatented; unlicensed	Sharma, A. *et al. Proc. Natl. Acad. Sci. USA*; published online March 23, 2009; doi:10.1073/pnas.0808743106 **Contact:** Balaram Ghosh, Institute of Genomics and Integrative Biology, Delhi, India e-mail: bghosh@igib.res.in

SciBX 2(13); doi:10.1038/scibx.2009.543
Published online April 2, 2009

Let-7 microRNA–mediated regulation of IL-13 and allergic airway inflammation

Manish Kumar, MSc, Tanveer Ahmad, MSc, Amit Sharma, PhD, Ulaganathan Mabalirajan, MBBS, PhD, Ankur Kulshreshtha, MTech, Anurag Agrawal, MD, PhD, and Balaram Ghosh, PhD *Delhi, India*

(*J Allergy Clin Immunol , November 2011;128:1077-85.*)

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
Mamta Sharma
Shilpy Sharma
Sarvesh Kumar
Jyotsna Batra
Rajshekhar Chatterjee
U. Mabalirajan
Amrendra kumar
Sanober Nahid

Anurag Agrawal
S.K.Brahmachari
FGU Sequencing Facility, IGIB

Funding from CSIR, DBT, DST, Govt. of India, Indo-European Bilateral Program

Clinical Collaborators

V.Vijayan, Director VPCI
Raj Kumar, Asst. Prof.. VPCI
S.K.Sharma, Prof.. Medicine AIIMS
P.V. Niphadkar, Mumbai
J.P.Rishi, SMS Hospital, Jaipur
Vivek Atahya, SMS Hospital, Jaipur
Rajendra Prasad, KG's Hospital, Lucknow
Brajen Lahkar, Downtown Hospital, Guwahati

&

All Participating Volunteers (Patients and Controls)

THANK YOU

Leads from GEO Database

Gene Dataset

Individual RNA Samples (5 nos.) OVA-sensitized/challenged mice VS Pooled RNA Samples (6) from Control mice

Differential expressed genes (3 fold) in each sample tested

Genes differentially expressed in at least 5 samples

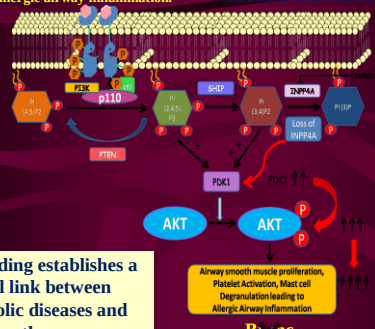
Gene Datasets screened:

- 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.

Working Strategy:

	Datasets	GDS 347	GDS 348	GDS 349
Candidate Gene Selection	**Filter 1** 3 fold difference in expression in at least 5 mice per data set		111 Genes	
	Filter 2 Genes mapped to syntenic regions in the human genome and linked to asthma/atopy or related phenotypes. Novelty and potential polymorphic repeats		69/111 Genes	
	Filter 3 Genes with non-polymorphic repeats were deleted from the filter set 2		26/69 Genes	
Association Studies	**Filter 4** Genes associated in Trio Samples		21/26 Genes	
	Screening for SNP's / c SNP's in trios and Case-controls in *INPP4A* Haplotype Generation / Prediction Detection of SNP/ Haplotypes associated with asthma Detection of SNP in PEST Sequence		2/21 Genes (*INPP4A, HSP105*)	
			A>G SNP in PEST sequence	
Functional Studies	Protein stability and expression studies in human and mice Platelet downstream assay		Western blot Evidence & Plasma RANTES level 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.

Bronchial Asthma

Suggestive evidence of association of C-159T functional polymorphism of *CD14* gene with atopic asthma in the North and Northwest Indian population.

Sharma, M., Batra, J., Mabalirajan, U., Goswami, S., Ganguly, D., Lahkar, B., Kumar, A., Bhatia, N K . and Ghosh, B. (2004). Immunogenetics ;56(7):544-7.

Allelic and genotypic distribution of C-159T polymorphism in case vs control groups (P=187, C=227).

p values: allelic = 0.0048 , genotypic= 0.0146

The transmission disequilibrium test (TDT)

Allele	Transmitted	Non- Transmitted	Total
C	54	33	87
T	33	54	87
Total	87	87	174

χ^2TDT = 5.069, DF = 1, p < 0.025

Chemokine Receptor 5 (*CCR5*) Δ32 deletion and atopic asthma in Indians.

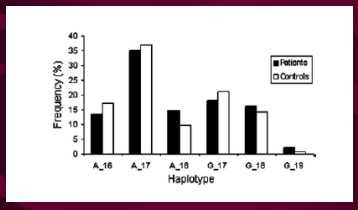
J Batra, M Sharma, R Chatterjee, S Sharma, U Mabalirajan and B Ghosh (2004) Thorax 2005;60(1):85.

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.

Sharma, S. and Ghosh, B. (2004) J. Human Genetics 2004;49(12):677-83.



p = 0.016

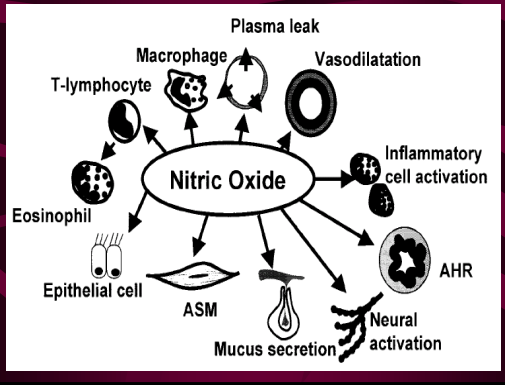
PATENT FILED

Ghosh B, Sharma S and Nagpal K. Genetic variants of human Transforming Growth Factor Beta1 (TGFβ1) and prediction of susceptibility for immunological disorders. Submitted for PCT patent, June 2004.

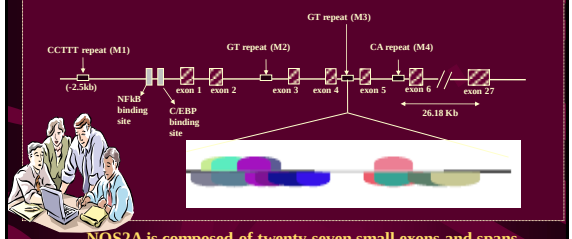
Ghosh, B., Rao, C-B., Brahmachari, S.K., Guleria, R., Das, C., Bhatnagar, P. and Kukreti, R A method for predicting an individual's bronchodilatory response to a beta agonist. Submitted for PCT patent, March 2004.

Ghosh., B., Nagarkatti R., and Rao, C.-B. Detection of predisposition to atopic disorders by screening for human Signal transducer and Activator of Transcription-6 (STAT-6) gene variants. Submitted for PCT patent, November, 2002.

Pulmonary Effects of Nitric Oxide



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 micro-satellite repeat in and around inducible Nitric Oxide Synthase.

Batra et al, Thorax 2007 Jan;62(1):16-22.

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=0.0046).

Batra et al, Thorax 2007 Jan;62(1):16-22.

Chemokine Receptor 5 (*CCR5*) Δ32 deletion and atopic asthma in Indians.

J Batra, M Sharma, R Chatterjee, S Sharma, U Mabalirajan and B Ghosh (2004) Thorax 2005;60(1):85.

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

Eotaxin

We observed for the first time a highly significant association of the newly studied (GAAGGA)n hexanucleotide repeat with asthma (p=3x10⁻⁶), log10TsIgE (p=0.0058) and eotaxin levels (p=0.004).

We further identified G_A_C_8 as an important risk haplotype associated with high TsIgE and plasma eotaxin levels.

Batra *et al.* JMG 2007 June

N-ACETYLTRANSFERASE (NAT) 2

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.

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

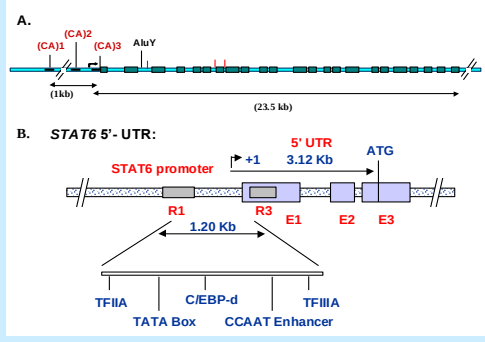
TUMOR NECROSIS FACTOR (TNF)

AACACG is the most frequent haplotype in the control population [p=0.0016].

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

Sharma *et al.* Am J Respir Cell Mol Biol 2006 May 25

Genomic Organization of the *STAT6* Gene



Frequency (relative frequency) of R1_R3 Haplotypes in patients and controls estimated by PHASE. Haplotypes with relative frequencies > 0.025 (2.5 % of sample size) in either of the groups have been depicted below.

Haplotype (R1_R3)	Patients N (relative frequency)	Controls N (relative frequency)	Odds-ratio (OR)	p-value
16_14	21 (0.047)	17 (0.040)	1.1719	0.8339
16_15	84 (0.187)	46 (0.108)	1.886	0.001
16_17	28 (0.062)	21 (0.050)	1.2733	0.41
17_14	2 (0.004)	18 (0.042)	0.1007	0
17_15	32 (0.071)	12 (0.028)	2.6284	0.0031
22_17	21 (0.047)	21 (0.050)	0.939	0.8433
23_16	2 (0.004)	20 (0.047)	0.0902	0
23_17	50 (0.111)	38 (0.09)	1.2697	0.2905
24_15	12 (0.027)	2 (0.005)	5.7808	0.0063
24_16	3 (0.007)	30 (0.071)	0.0881	0
24_17	46 (0.102)	47 (0.111)	0.9133	0.679
24_18	8 (0.018)	11 (0.026)	0.6796	0.4075
25_16	4 (0.009)	11 (0.026)	0.3367	0.0523
25_17	21 (0.047)	9 (0.021)	2.2572	0.036
Sub-total	334 (0.743)	303 (0.715)		
Other haplotypes	116	121		
Total	450	424		

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 degrade the ECM

Role as a Friend:

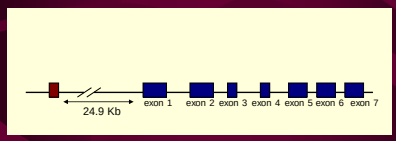
- Indirectly inhibits T-cell activation by modulation of antigen presentation function and deactivating macrophages
- Inhibit IgE synthesis
- Inhibits proliferation of mast cells, eosinophils and basophils
- Abrogates established asthma conditions in mice.

TGFβ1

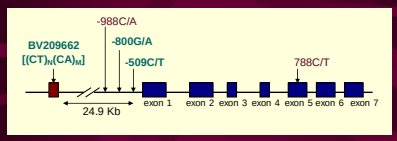
- Considered as a prime candidate gene for asthma and atopy
- Chromosomal position-19q13.1-13.2, linked to mite sensitivity (a major risk factor for asthma)

Blumenthal MN et al. Genes Immun. 2004 May;5(3):226-31.

Schematic map of the TGFβ1 Gene

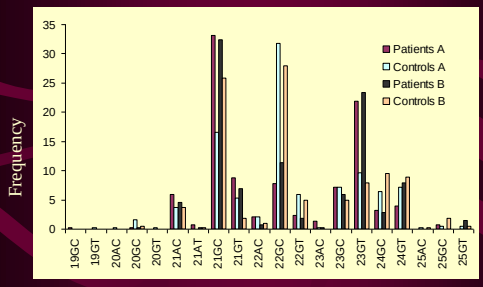


TGFβ1 GENE POLYMORPHISMS IDENTIFIED IN INDIAN POPULATION



Nagpal et al, *J Allergy Clin Immunol. 2005; 115(3):527-33*

COMPARISON OF HAPLOTYPE FREQUENCIES IN TWO INDEPENDENT COHORTS



Nagpal et al, *J Allergy Clin Immunol. 2005; 115(3):527-33*

HAPLOTYPE ANALYSIS

- 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]

- 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]

Nagpal et al, *J Allergy Clin Immunol (In Press).*

TGFβ1 LEVELS CORRESPONDING TO ASSOCIATED ALLELES, GENOTYPES AND HAPLOTYPES

$(CT)_n(CA)_{nt}$ Allele	Number	Log TGFβ1 levels (±SE)	F-ratio	DF	P-value
21	50	1.73(±0.03)	4.59	3	0.004
22	33	1.59(±0.04)			
23	37	1.76 (±0.04)			
24	15	1.62 (±0.06)			
-509 C/T Genotype					
CC	28	1.62 (±0.06)	9.63	2	0.0002
CT	46	1.79 (±0.05)			
TT	7	2.24 (±0.13)			
Haplotype					
22_G_C/22_G_C	5	1.41 (± 0.13)	6.87	2	0.0019
22_G_C/Non22_G_C	20	1.61 (± 0.07)			
Non22_G_C/Non22_G_C	47	1.83 (± 0.04)			
23_G_T/23_G_T	3	2.09 (±0.15)	4.77	2	0.01
23_G_T/Non23_G_T	23	1.79 (±0.05)			
Non23_G_T/Non23_G_T	46	1.66 (±0.04)			

Nagpal et al, *J Allergy Clin Immunol. 2005; 115(3):527-33*

Classification of Drugs

- Quick relief medications
  - ß-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, resorcinols 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 parentral
      - Considerable chronotropic and ionotropic cardiac effects

Quick Relief Medications

- Agents with highly selective action
  - Resorcinol – metaproterinol, terbutaline, fenoterol
  - Saligenin – commonly used albuterol (Salbutamol)
    - Highly selective for respiratory tract
    - Deviod of cardiac side effects except high doses
    - Active by all routes
    - Bypass metabolic processes
    - Side effect – tremor
  - Salmeterol
    - Congener of albuterol
    - Long acting
    - Effective in nocturnal and exercise induced asthma
    - Limitations – non for acute use, cumulative side effects

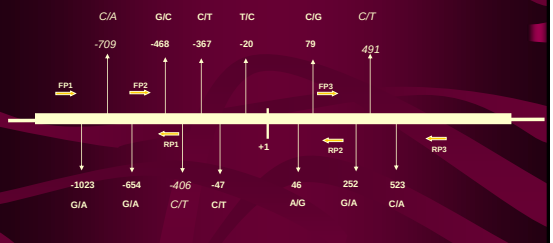


Figure 1. Physical representation of the β2AR gene and localization of the gene polymorphisms. The SNPs marked in bold are polymorphic and the SNPs marked in etalics are not polymorphic in the studied Indian population. Nucleotide positions of the SNPs are with reference to the first nucleotide of the start codon being +1. β2AR gene is amplified and sequenced by appropriate synthetic oligonucleotides marked in this figure.

SNP Position	Genotype Good Responders	%	Genotype Poor Responders	%	χ^2	P value
-1023	GG (n=18) GA (n=9) AA (n= 4) (N=31)	58.0 29.0 13.0	GG (n=18) GA (n=5) AA (n=2) (N=25)	72.0 20.0 8.0	1.18	0.554
-654	GG (n=10) GA (n=23) AA (n=2) (N=35)	28.6 65.7 5.7	GG (n=7) GA (n=20) AA (n= 9) (N=36)	19.4 55.6 25.0	5.18	0.075
-468 SNP Block	GG (n=2) GC (n=19) CC (n=14) (N=35)	5.7 54.3 40.0	GG (n=1) GC (n=12) CC (n=23) (N=36)	2.7 33.3 64.0	4.09	0.129
46	AA(n=3) AG(n=27) GG(n=11) (N=41)	7.3 65.9 26.8	AA(n=13) AG(n=22) GG(n=4) (N=39)	33.3 56.4 10.3	9.98	0.0068
252	GG (n=13) GA (n=20) AA (n=2) (N=35)	37.2 57.1 5.7	GG (n=18) GA (n=16) AA (n=2) (N=36)	50.0 44.5 5.5	1.23	0.538
523	CC (n=14) CA (n=19) AA (n= 2) (N=35)	40.0 54.3 5.7	CC (n=19) CA (n=18) AA (n=1) (N=36)	52.8 44.4 2.8	1.33	0.513

Publications on Asthma Genetics

Nagarkatti R. and Ghosh, B. (2002) Identification of single-nucleotide and repeat polymorphisms in two candidate genes, interleukin 4 receptor (*IL4RA*) and signal transducer and activator of transcription protein 6 (*STAT6*), for Th2 mediated diseases. J. Human Genetics 47 (12): 684-687.

Ghosh, B., Sharma, S and Nagarkatti, R. (2003) Genetics of asthma: Current research paving the way for development of personalized drugs. Ind. J. Med. Res. 117, pp185-197.

Nagarkatti, R., Raj Kumar, Sharma, S. K. and Ghosh, B. (2004) Polymorphisms in the Interleukin 4 (IL4) gene and asthma in the North Indian population. Int. Arch. Allergy & Immunol. 134, 206-212.

Nagarkatti, R., Rao, C. B, Vijayan, V., Sharma, S. K. and Ghosh, B. (2004) *Signal Transducer and Activator of Transcription 6* haplotypes and asthma in the Indian population. Am. Jr. Resp. Cell Mol Biol; 31(3):317-21.

Sharma, S, Nagarkatti, R, B-Rao C, Niphadkar, P.V., Vijayan, V, Sharma, S.K. and Ghosh, B (2004). A three locus haplotype, A_16_C, in Fc epsilon RI beta gene confers higher risk for atopic asthma Clin. Genetics.;66(5):417-25.

Publications on Asthma Genetics

Sharma, M., Batra, J., Mabalirajan, U., Goswami, S., Ganguly, D., Lahkar, B., Kumar, A., Bhatia, N K . and Ghosh, B. (2004) Suggestive evidence of association of C-159T functional polymorphism of *CD14* gene with atopic asthma in the North and Northwest Indian population. Immunogenetics ;56(7):544-7.

Sharma, S. and Ghosh, B. (2004) Association of an Intragenic Microsatellite Marker in *CC16* gene with Asthma in Indian Population. J. Human Genetics (In press).

J Batra, M Sharma, R Chatterjee, S Sharma, U Mabalirajan and B Ghosh (2004) *Chemokine Receptor 5* (*CCR5*) Δ32 deletion and atopic asthma in Indians. Thorax (In press).

Batra, J. Niphadkar, P.V., Sharma, S. K. and Ghosh, B. (2004) *Uteroglobin-Related Protein1* (*UGRP1*) gene polymorphisms and atopic asthma in the Indian population. . Int. Arch. Allergy & Immunol. (In Press)

Nagpal, K., Sharma, S., B-Rao, C., Nahid, S., Niphadkar, PV., Sharma, S.K. and Ghosh, B. (2004) Transforming Growth Factor beta1 (TGFb1) Haplotypes and Asthma in Indian Population. J Allergy Clin Immunol (In Press).

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 population/individuals to drugs.
- Idenfifying polymorphisms in genes encoding interacting proteins may develop better predictive value.
- Identifing 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 associated haplotypes and genotypes may develop a causal relationship with the disease and thus may help to develop better modalities for therapeutic intervention.

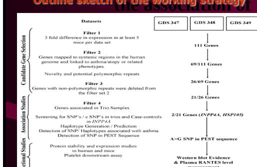
Asthma Medication-Role of β2-Adrenergic receptor

Commonly used drugs - Salbutamol and its derivatives

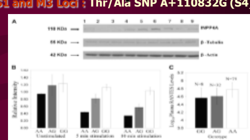
- Inhibit smooth muscle contraction
  - Adrenergic stimulants
    - Drugs – catecholamines, resorcinols and saligenins
    - Mechanism of action
      - stimulation of ß-adrenergic receptors ↓ Activation of G-proteins ↓ Formation of cyclic AMP ↓ Smooth muscle relaxation
    - Limitations
      - Effective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

Using a genomewide candidate gene approach, we demonstrate the association of INPP4A with atopic asthma.

Outline sketch of the working strategy

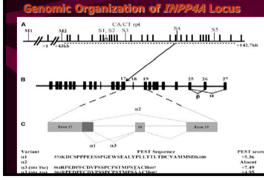


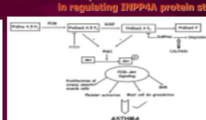
A Functional Polymorphism is Present in LD with S1 and M3 Loci : Thr/Ala SNP A+110832G (S4)



Western blot analyses indicated a functional role of this SNP in regulating INPP4A protein stability

Genomic Organization of *INPP4A* Locus





Proposed Role of INPP4A in ASTHMA Patho--genesis

Sharma M et al, 2007, Am J Respir Crit. Care Med..
Medwire news: Caroline Price, 16th January 2008, Inositol pathway gene linked to asthma susceptibility.
FUNDING: Project Head SMM 0006 and NWP 0033

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

VARIANT	PEST SEQUENCE	PEST SCORE	
α1	573SEALYPLLTTLTDCVAMMSDK608	+5.36	
α2		Absent	
α3 (604 Thr)	584VPSSPCPSTMPSTACH607	+7.49	('A')
α3 (604 Ala)	584VPSSPCPSTMPSAACH607	+4.95	('G')

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

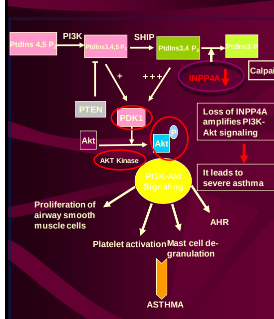
The allele A is transmitted more to the asthmatic individuals whereas the allele G is protective.

Ghosh B et al AJRCCM, 177,7

Conclusions

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

Discovery of a novel link between metabolic diseases and asthma



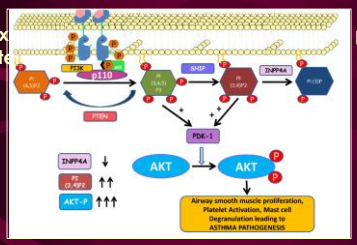
- Inositol polyphosphate-4-phosphatase A (INPP4A) is the terminal enzyme in PI3K-Akt Pathway.
- Excess activation of PI3K-AKT pathway causes inflammatory diseases like asthma.
- Calpain mediated loss of INPP4A leads to severe asthma by activating PI3K-Akt Pathway.
- Stability of INPP4A critically determines 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

nature COMMUNICATIONS 2012

Excessive PI3/Akt signaling and asthma

- Allergy, metabolic syndrome, environmental stress, and infections have all been linked to asthma.
- Ex… point of int…



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

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

Inositol Polyphosphate 4 Phosphatase Type I (INPP4A)

INPP4A:

- Magnesium independent enzyme
- Dephosphorylates 4-position phosphate of Inositol 1,3,4 triphosphate, Inositol 3, 4 bisphosphate and Phosphatidyl inositol 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 (weeble phenotype)
Neuron, 32, 203-212, 2001; Nature Letters, 32, 1-5, 2010

Loss of INPP4A associated with cancer, acute myeloid leukemia
***Nature*. 458, 97-101, 2009; *J. Virol*. 78, 1971–1980, 2004**

ICGEB

FCεR1β

Development of Functional Assay to Validate the Haplotypic Associations obtained in FcεRIβ
Sharma S, Clin Genetics , 2004
Sharma et al, JACI, 2006
Sharma S, Ghosh B, IAAI, 2009

INTERFERON GAI

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 factor(s) for binding to the SNP was demonstrated.
Kumar A and Ghosh B. Immun., 2008,

Transforming Growth Factor-β(TGFβ)

A novel (CT)n(CA)m repeat polymorphism (BV209662) 24.9 kb upstream of *TGFβ1* was identified
A novel 3-locus haplotype, 23_G_T, was found to be significantly associated with asthma (*P* = 0 .00001 in cohorts A and B) as well as with higher serum TGF-β1 level (*P* = .01)
Nagpal K et al, JACI, 2005

β2 Adrenergic Receptor Polymorphisms and Response to Salbutamol among Indian Asthmatics
Kshitija et al, Pharmacogenomics, 2005