Gene-environment interactions in pediatric asthma

Feige Kaplan, Ph.D.

Israel Pediatric Pulmonary Society

July 10, 2013

- **Asthma is the most common chronic disease of children, affecting 7-10 % of the pediatric population in North America. Ministry of Health data in Israel - ~7% 13-14yr olds (2003).**

- **The prevalence of asthma in children under 4 has increased by 74% in the last 2 decades.**

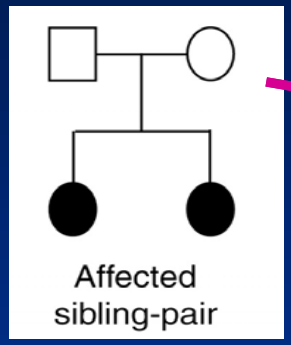
- **Ninety percent of *all* asthma has its origins in childhood.**

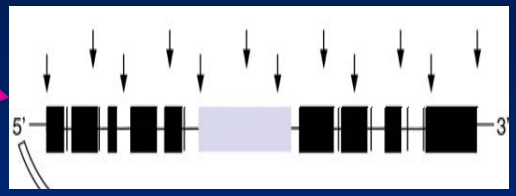
- **Maternal exposure to environmental insult contributes to impaired lung function in newborns that can persist throughout life.**

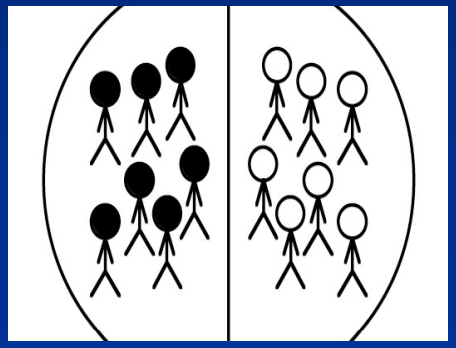
Yet it remains unclear how allergen exposures interact with predisposing genotype leading to disease.

Discovering susceptibility genes for asthma and allergy

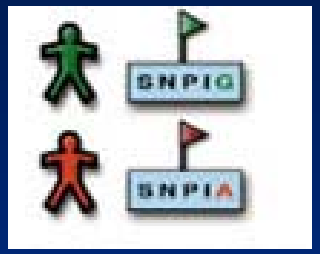
A. Linkage analysis in families







B. Genome wide association studies



A. Closely related individuals tend to share large regions of the genome. This approach depends on a priori hypotheses regarding *specific candidate genes*.

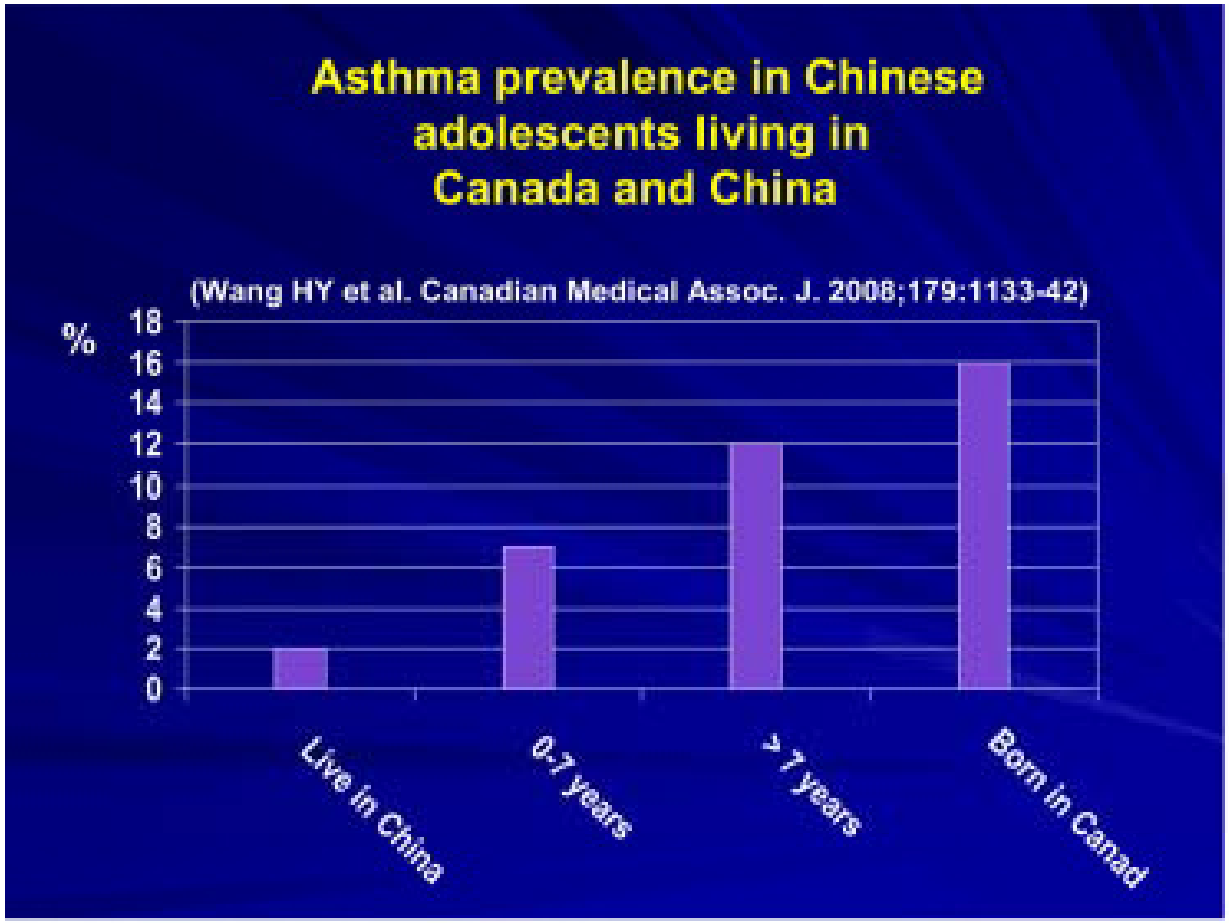
B. GWAS relies on poymorphisms across the genome. These studies survey common genetic variants for a role in disease susceptibility. GWAS have the potential to identify *novel candidate genes*

Bottom line

Approximaely 100 confirmed genetic loci have been implicated in asthma. These genes account for a very limited contributon of current asthma pathology.

Gene-environment interactions in asthma

Epidemiogical studies



Asthma prevalence in Chinese adolescents born in China and either still living in China or having migrated to Canada for more or less than 7 years compared with being born in Canada. The study demonstrates the effects of early life influences on asthma prevalence.

Henderson, JA Seminars in Fetal and Neonatal Medicine, 17, I2, 82-91, 2012

Asthma and allergy phenotypes in Cochin Jews living in Israel

	Jerusalem Mountain Region		
Variable	**Cochins** (N=481)	**Non-Cochins** (N=149)	**Pvalue**
Allergy	166 (34.5)	10 (6.7)	<.001
Asthma	132 (27.4)	13(8.7)	<.001

	Cochins		
Variable	**Desert (N=353)**	**Jerusalem mountains (N=481)**	**Pvalue**
Allergy	80 (22.7)	166 (34.5)	<.001
Asthma	66 (18.7)	132 (27.4)	<.003

Yaari et al 2009, *Ann Allergy Asthma Immunol*

Asthma in Haifa children more than double the national average

Researchers link high incidence of asthma to area's severe air pollution and growing problem of obesity among schoolchildren.

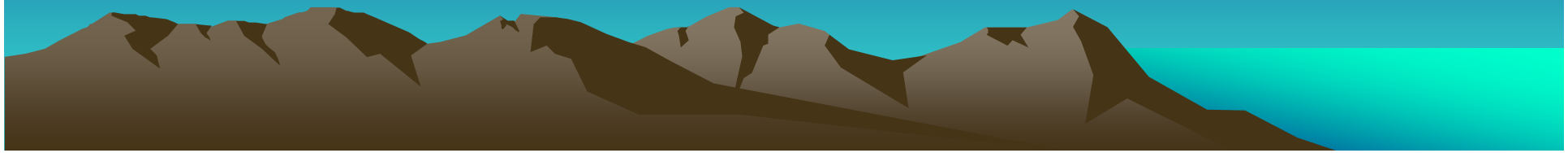
Ha'aretz Feb.25, 2010

The study, conducted by the Health Ministry, Clalit and Haifa U, examined **3,922 children aged 6 to 14** living in the Haifa area .

Of these, 16% had asthma - more than double the national average of 7%.

The highest incidence - 21% - was found in Kiryat Yam, and the lowest, 9%, in Kiryat Bialik.

The high incidence of asthma was linked to severe air pollution and increased obesity among schoolchildren.



Association between Single-Nucleotide Polymorphisms on Chromosome 17q21 and Asthma, Early-Onset Asthma, and Late-Onset Asthma

Gene and SNP	P Value for Total Subjects†		
	Asthma (N=1511)	Early-Onset Asthma (N=1270)	Late-Onset Asthma (N=1282)
GSDML (or *GSDMB*)			
rs2305480	1.4×10^{-4}	9.3×10^{-6}	0.02
rs2305479	0.005	6.6×10^{-6}	0.22
rs4795400	9.4×10^{-4}	3.0×10^{-4}	0.06
rs9303281	0.005	4.4×10^{-5}	0.18
rs7219923	0.009	9.7×10^{-5}	0.17
ORMDL3			
rs8076131	0.003	1.0×10^{-4}	0.03

Bouzigon E et al. N Engl J Med 2008;359:1985-1994

The NEW ENGLAND JOURNAL of MEDICINE

Analysis of the Association between 11 Single-Nucleotide Polymorphisms (SNPs) on Chromosome 17q21 and Early-Onset Asthma, according to Status with Respect to Exposure to Environmental Tobacco Smoke in Early Life

Gene and SNP	Family Sample with Offspring Exposed to Tobacco Smoke (N=627)‡		Family Sample with Offspring Not Exposed to Tobacco Smoke (N=460)‡	
	SNP Effect§	P Value¶	SNP Effect§	P Value¶
GSDML (or *GSDMB*)				
rs2305480	2.77	8.7×10^{-6}	1.40	0.17
rs2305479	2.64	2.4×10^{-5}	1.56	0.07
rs4795400	2.50	5.3×10^{-5}	1.30	0.27
rs9303281	2.46	1.1×10^{-4}	1.59	0.07
rs7219923	2.29	3.3×10^{-4}	1.59	0.06
ORMDL3				
rs8076131	2.36	1.7×10^{-4}	1.38	0.21

Bouzigon E et al. N Engl J Med 2008;359:1985-1994

The NEW ENGLAND JOURNAL of MEDICINE

Timing of exposures

Early influences on lung development contribute to asthma susceptibility

- *In utero* exposure to allergen increases risk of atopy in children.
- Mechanical ventilation, GC and CLD increase risk of childhood asthma.
- Boys have a higher incidence of / more severe asthma.
- Airway biopsies in asthmatic children show tissue restructuring *up to 4 years before* onset of symptoms.
- Infant wheeze often resolves with growth. A subset of children develop inflammatory airway damage leading to long-term asthma.
- Infants of smoking mothers have impaired lung function which tracks into adolescence and beyond.

Intrinsic and induced phenotypic changes in the lung in infancy (or earlier) may be programmed into the long-term functional phenotype of the respiratory system, conferring asthma susceptibility.

HYPOTHESES

1. Innate genetic differences in the pattern of gene expression in developing lung may contribute to individual variation in airway hyperresponsiveness (AHR) and asthma susceptibility.

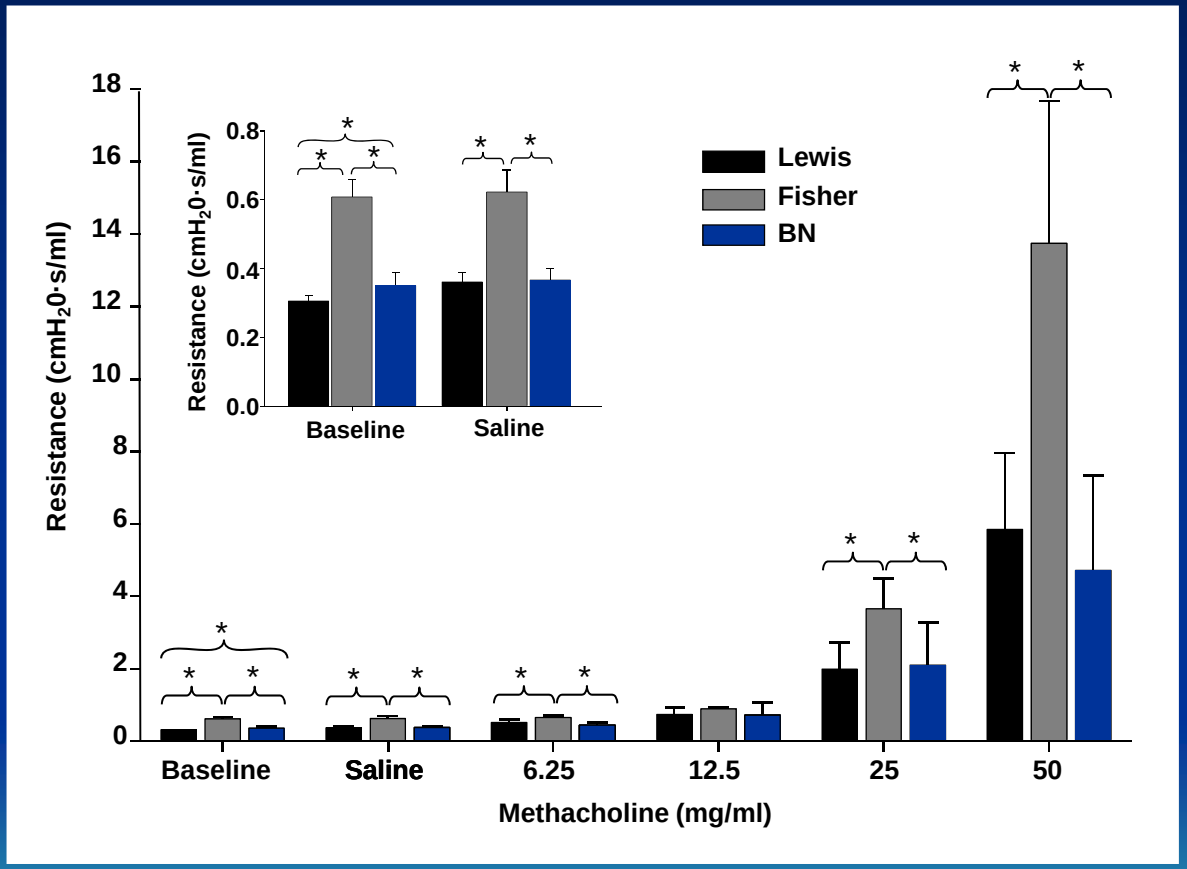
2. Allergen exposure during early development can influence asthma pathogenesis by reprogramming these primary patterns of pulmonary gene expression.

Animal models of asthma susceptibility and airway hyperresponsiveness

Brown Norway		Fisher (compared to Lewis rat)
High IgE producer		Hyperresponsive to MCh and serotonin
Early and late allergic airway responses to allergen challenge		More smooth muscle in the airways
Late responses adoptively transferred with CD4+ and CD8+ T cells		ASM contracts with greater force (at submaximal dose of agonist) and with greater velocity
Eosiniphilia and Th2 cytokines following allergen challenge		Greater quantity of fast isoform of myosin
Susceptible to respiratory viral bronchitis		Greater calcium responses to contractile agonists

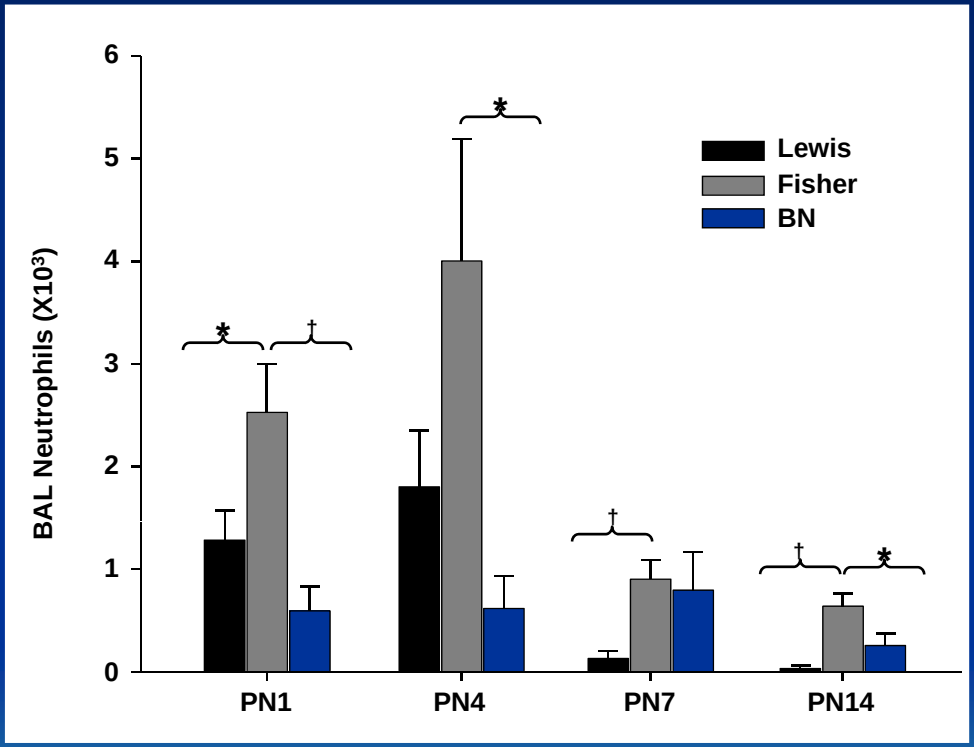
* Adapted from: Martin, J. and D. Ramos-Barbon. 2003. Airway smooth muscle growth from theperspective of animal models. *Respir.Physiol.Neurobiol.* 137:251-261.

Lung resistance in normoresponsive (Lewis), hyperresponsive (Fisher) and asthma-susceptible (Brown Norway) rats at postnatal day 14.



Am J Res Cell Mol Biol 2010

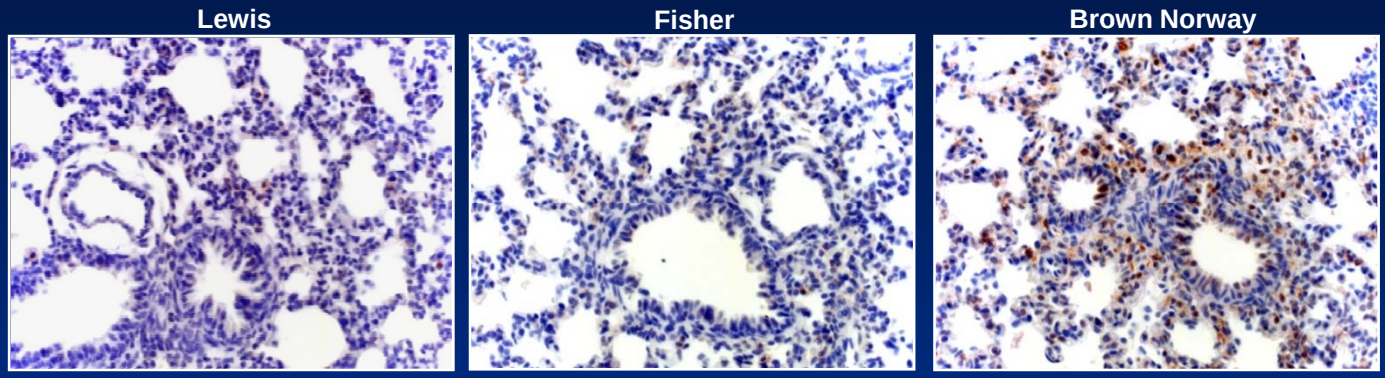
Postnatal Fisher pups show increased neutrophils in bronchoalveolar lavage



Am J Res Cell Mol Biol 2010

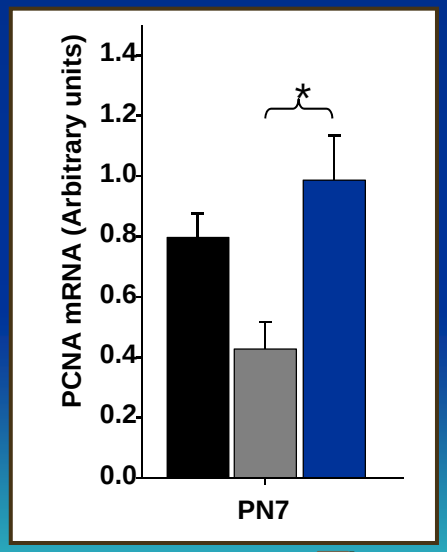
Brown Norway rats show increased cell proliferation at PN7

A

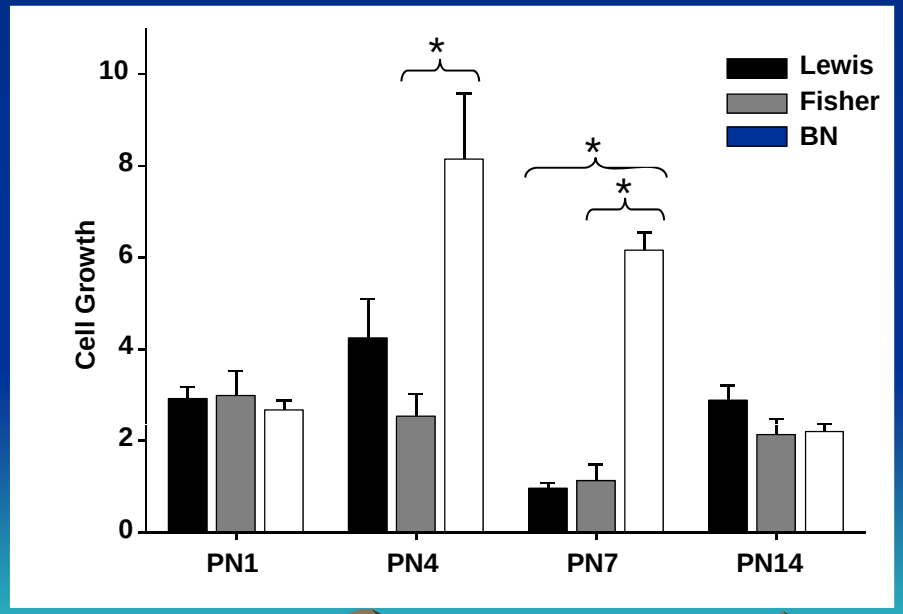


Increased PCNA immunostaining, a measure of cell proliferation, is observed in the lungs of BN compared to Lewis and Fisher pups at PN7. (n=4, 40X)

B



C



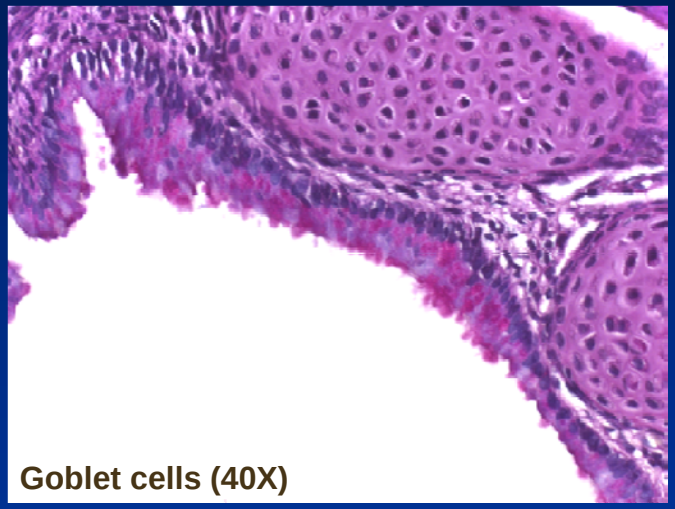
B) Real-time RT-PCR showing increased PCNA mRNA in lungs of BN compared to Lewis pups at PN7 (n=4) n≥4, *p<0.05.

C) Proliferation of primary lung cell cultures isolated from BN, Fisher and Lewis rat pups at PN7 as measured by cell counts (n=9, *p<0.05.)

Am J Res Cell Mol Biol 2010

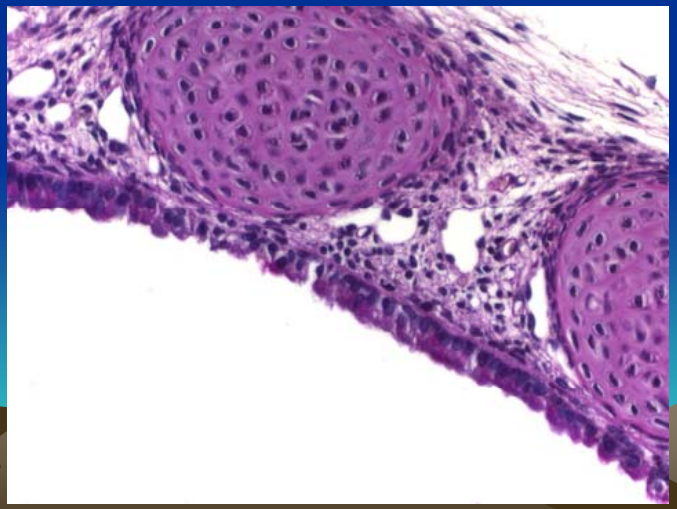
Brown Norway rats have increased numbers of tracheal goblet cells, a marker associated with inflammation, at postnatal day 1.

Brown Norway

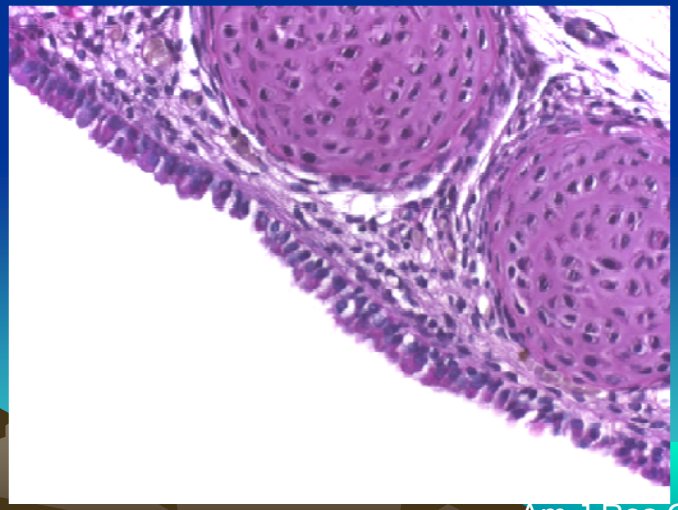


Goblet cells (40X)

Lewis

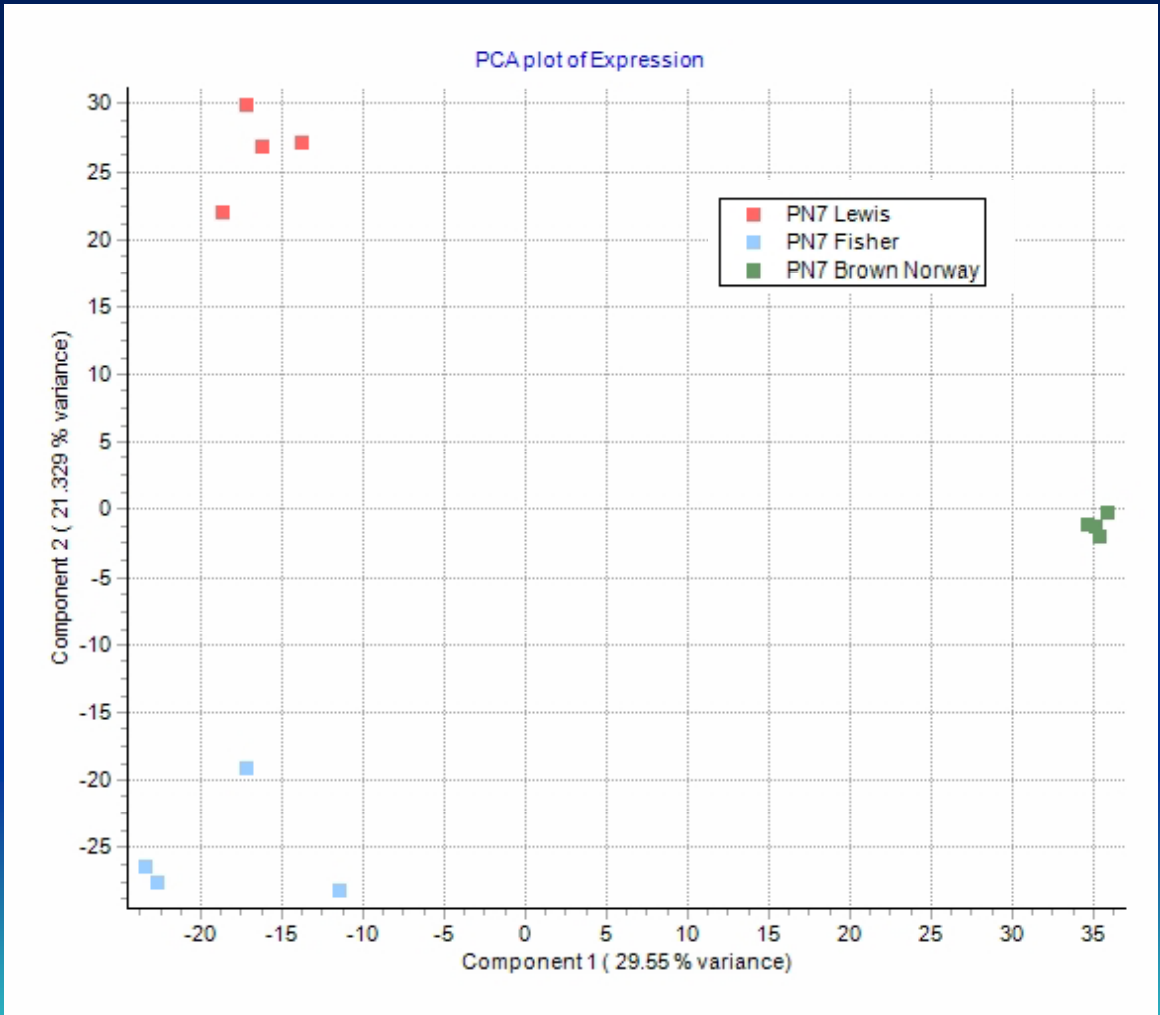


Fisher



Am J Res Cell Mol Biol 2010

Microarray analysis identifies highly distinct profiles of gene expression in postnatal lung from Lewis, Fisher and BN rats.



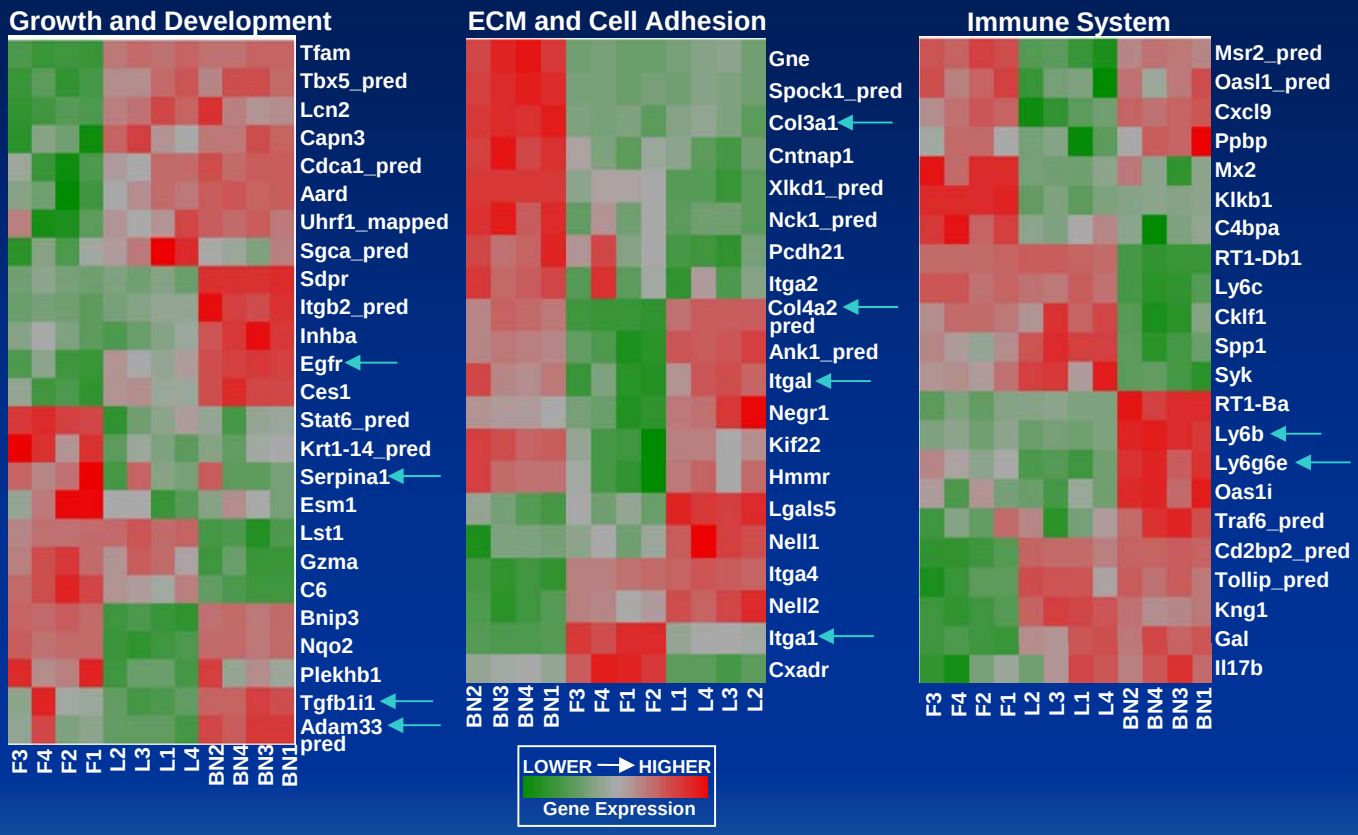
Representative PCA plot:

Principal Components Analysis is used to find patterns in data of high dimension such as microarrays and *highlights the differences and similarities in the data.*

Representative PCA plot shows that BN, Fisher and Lewis rat lungs (n=4) have clearly distinguishable expression profiles at PN7. Data are tightly clustered. The data also show that Fisher rats more closely resemble Lewis rats than BN rats. Similar results are obtained at PN1 and PN14.

Am J Res Cell Mol Biol 2010

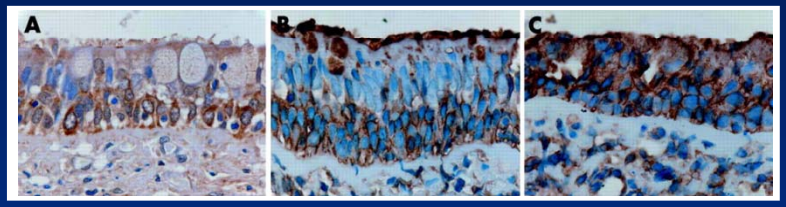
Heat maps illustrating biological processes heavily represented among variably expressed genes at PN7.



Processes highlighted are growth and development, ECM and cell adhesion and immune function. Genes of interest previously associated with asthma are represented by blue arrows.

Am J Res Cell Mol Biol 2010

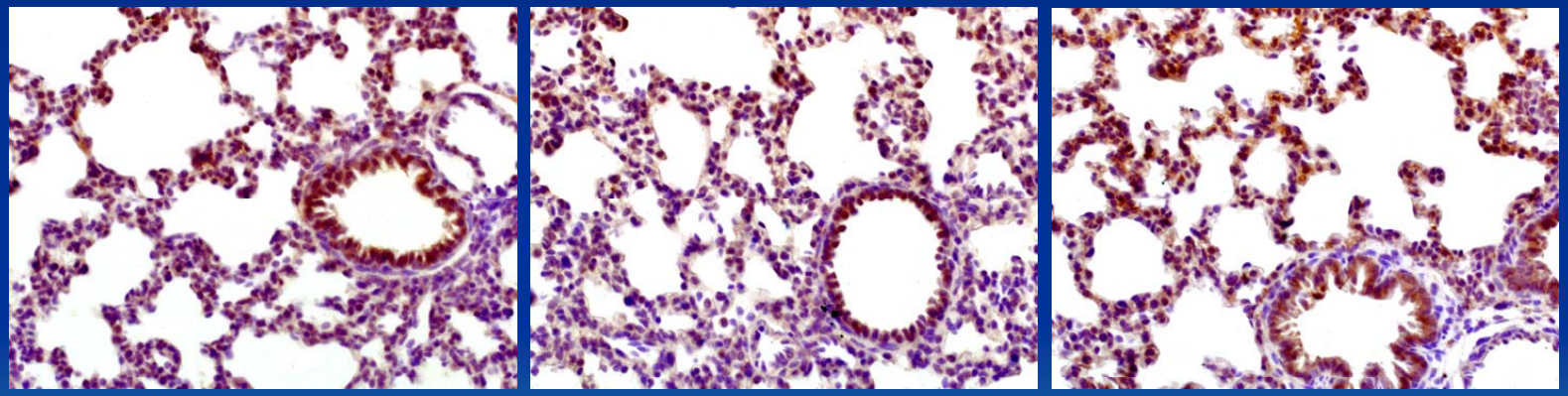
Developmental differences in expression of EGFR in developing lungs of Lewis, Fisher and BN rats



Epithelial EGFR in bronchial mucosa: (A) normal subject (B) moderate asthma C) severe asthma .

Fedorov et al. Thorax 2005

Lewis | Fisher | Brown Norway



Increased EGFR immunostaining is observed in the lungs of BN compared to Lewis and Fisher rats at PN7. (n=4, 40X)

The Epidermal Growth Factor Receptor Gene and Fetal Origin of Asthma and Airway Responsiveness

1. ***Does EGFR* gene sequence variation influence asthma susceptibility in children?**

2. **Do *EGFR* DNA variants influence *EGFR* expression in the developing human lung?**

ATS 2010

Genetic Association Study

403 Caucasian parent-child trios participating in the Childhood Asthma Management Program (CAMP) on whom genome-wide data was available.

Table 2: Genetic Association Results in CAMP

Phenotype	SNP	Minor Allele (fequency)	# Informative Families	p value
Asthma*	**rs2293348**	T (0.36)	202	0.0004
	rs12535482	A (0.38)	201	0.01
	rs7801956	A (0.10)	107	0.02
	rs12536061	C (0.41)	205	0.02

*Asthma association analyses unadjusted for covariates

Genomic Analysis of *In Utero EGFR* Expression

We tested the association of SNP rs2293348 with *in utero* expression of *EGFR* in samples isolated from 36 human

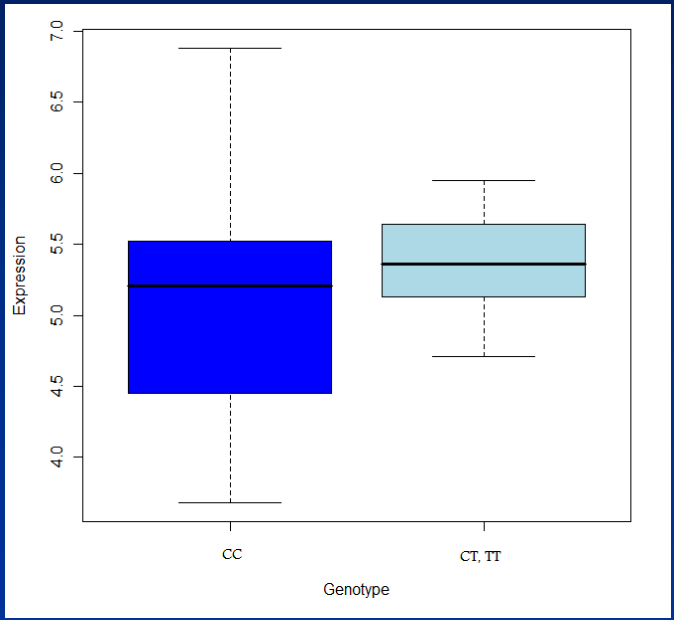
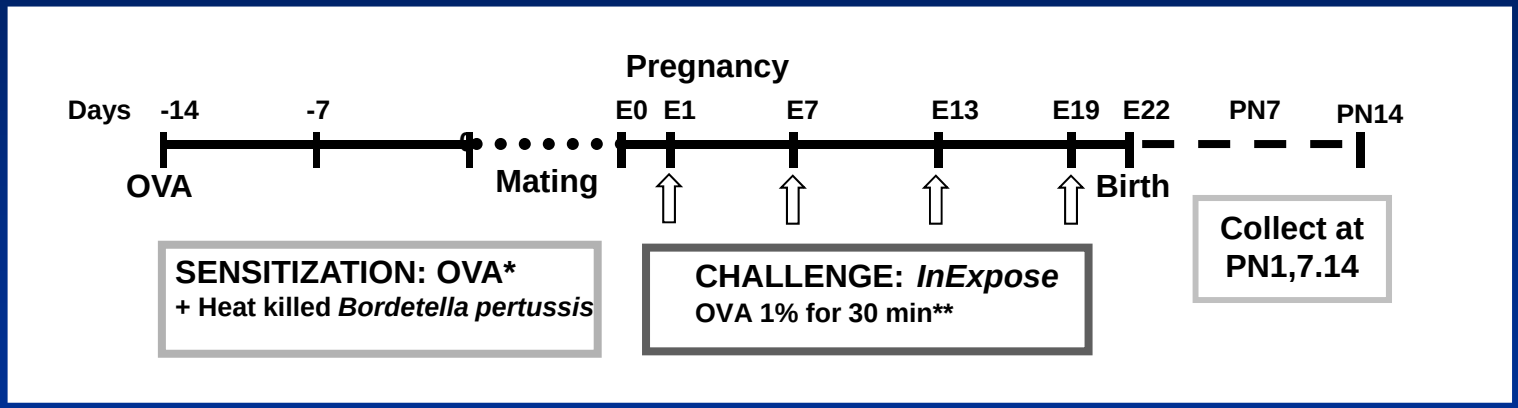


Figure 1: The effect of intronic SNP rs2293348 on *in utero* gene expression during human lung development demonstrating that that T allele is associated with increased *EGFR* expression (p=0.04).

Regulatory variation in EGFR that is associated with increased *in utero EGFR* expression is associated with asthma susceptibility and airway responsiveness in asthmatic children.

Do gene environment interactions in developing lung influence asthma susceptibility?

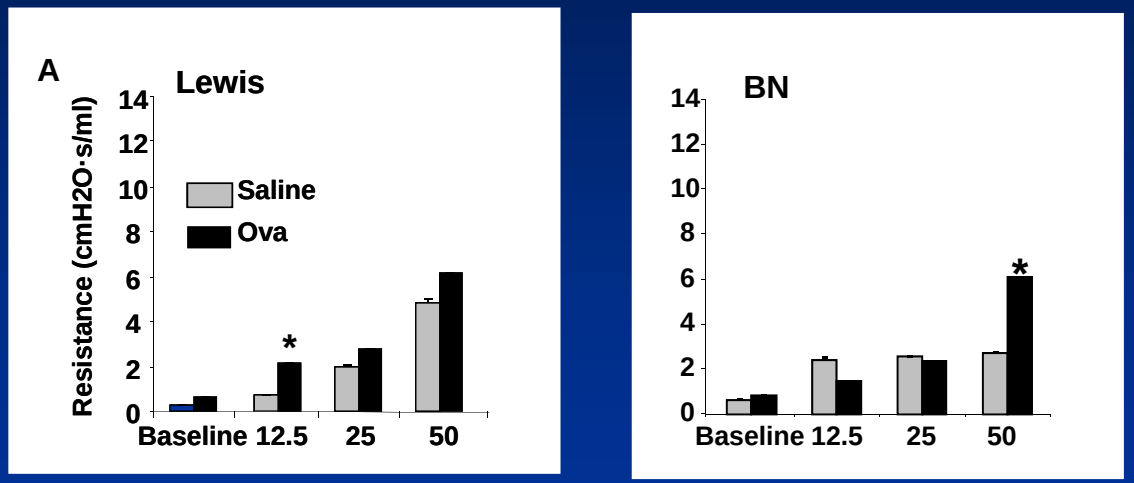


Female rats were given a 1mg dose of OVA s.c. 2 weeks before breeding. During pregnancy, dams were challenged with 1% OVA using the *InExpose* system (Scireq) at embryonic day (E)1,E7,E13 and E19. At PN7, lung mechanics, BAL and tissue collection were performed.

*** Control animals received saline (SAL) injections.**
**** Control animals were exposed to SAL.**

Am J Physiol Lung 2012

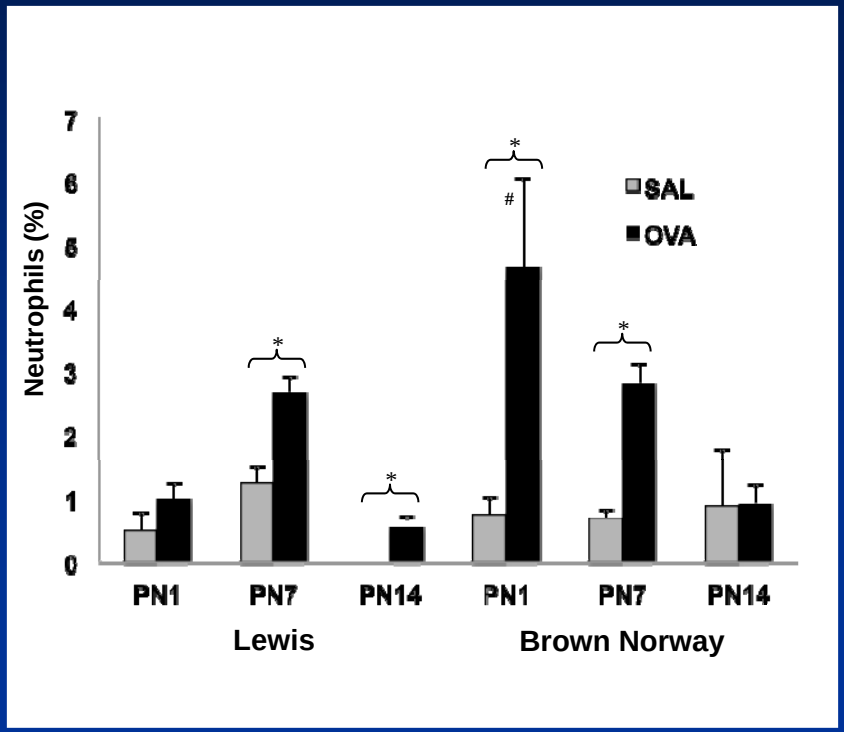
Lung resistance is elevated in BN and Lewis pups following maternal allergen exposure

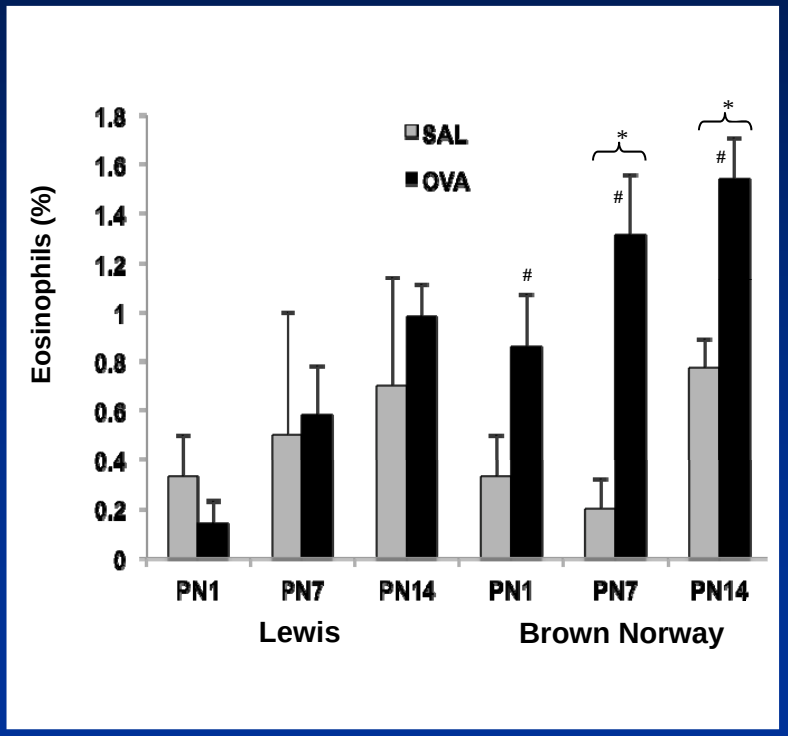


Airway resistance is significantly increased after OVA exposure in (A) Lewis pups after 12.5mg of MCh (*p<0.05, n=4 SAL, n=15 OVA), (B) in BN after 50 mg/ml of MCh (*p<0.05, n=5 SAL, n=16 OVA). All measurements were taken at PN14.

Am J Physiol Lung 2012

Inflammatory cell infiltrates in BAL fluid isolated from rat pups at PN1, 7 and 14.

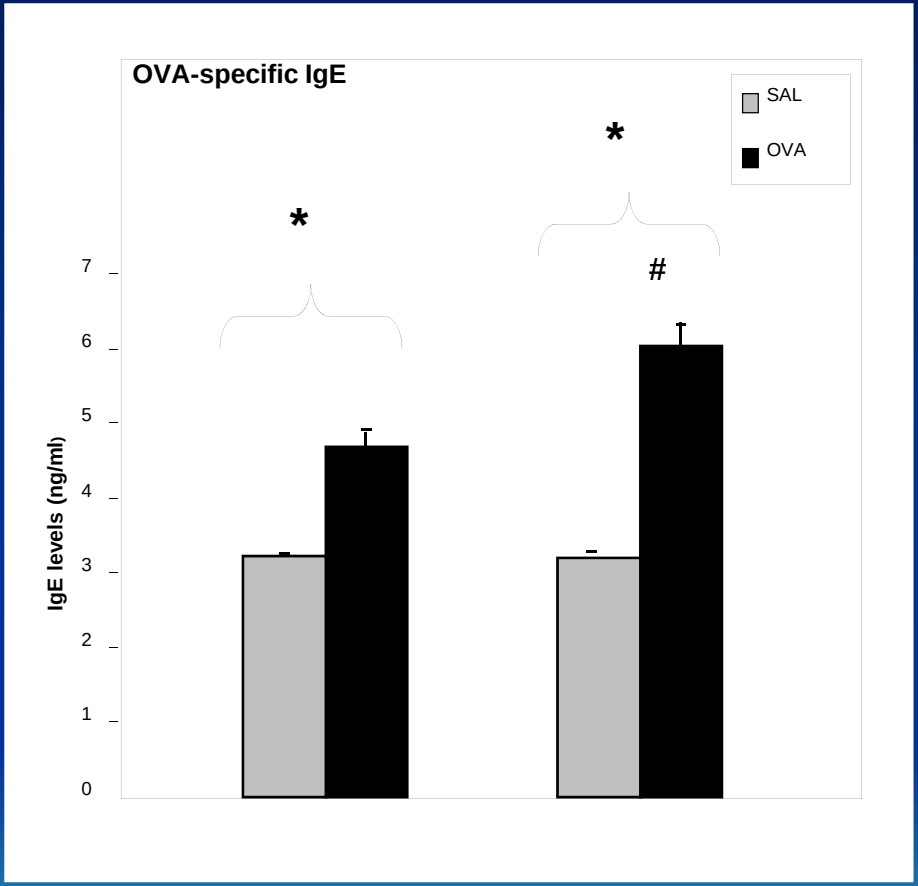




The percentage of neutrophils was elevated in OVA-exposed Lewis pups at PN7 and 14 and BN pups at PN1 and 7. Following *in utero* OVA exposure BN pups display higher neutrophils than Lewis at PN1. Eosinophil percentage was significantly increased in BN pups after *in utero* OVA exposure at PN7 and 14 and was higher than Lewis pups at all three timepoints

Am J Physiol Lung 2012

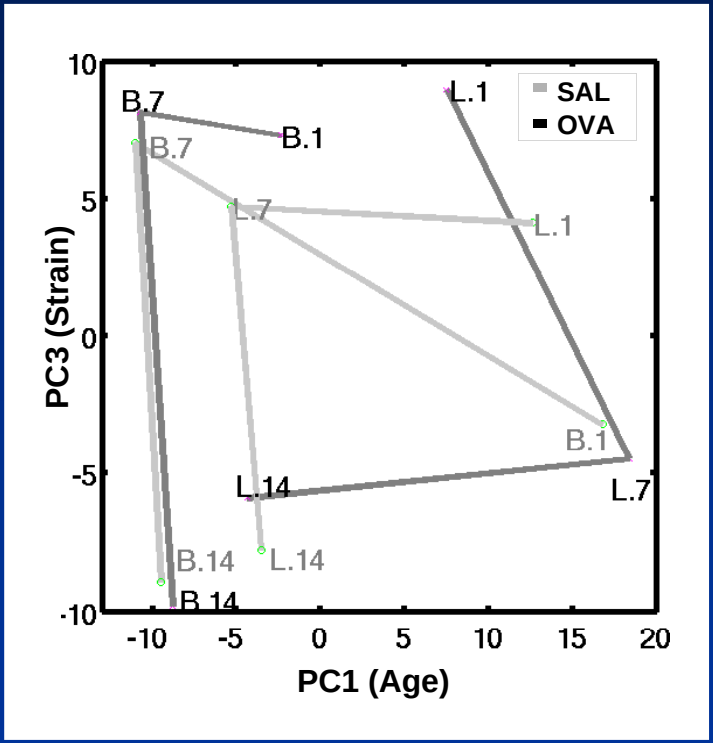
Serum IgE and cytokine levels are increased after maternal *OVA exposure*



OVA exposure is associated with increased serum IgE in BN and Lewis pups at PN14 (n=4, *p<0.05 OVA *vs.* SAL, #p<0.05 *vs.*. Lewis).

Am J Physiol Lung 2012

Principal component analysis of whole lung samples in 22,523-gene probe expression space



# Probes	B (SAL-OVA)	L (Sal-OVA)
112	B	L
176	B	-
511	-	L
1090	-	-
Total / strain	288	623

Two replicate samples were selected per condition – 2 strains x 3 ages x 2 exposures. The principal components 1-3 (PC1-3) coordinate centroids are plotted for each condition. Samples are marked Strain.Age. Connecting lines are colored grey for SAL and black for OVA.

Of the 3,693 reproducible probes 1,889 were in the top 5% which correspond to age-strain variation, and 799 of these are affected by OVA exposure – OVA vs. SAL correlation < -0.1 – for either strain.

Am J Physiol Lung 2012

Gene ontology enrichment analysis highlights distinct biological processes affected by *in utero* OVA exposure in developing lungs of BN and Lewis rat.

In utero OVA vs. SAL-exposed BN rat.	No of genes	Human Genes*	Fold Enrichment **
Lipid metabolism	2	ORM1, ORM2	43.50
	3	PPAP2A, PTEN, PP3R1	20.75
Oxidative stress	2	CLN8, SOD2	41.50
	3	PDIA4, PTGIS,GREM1	11.61
	3	EDNRB, SFTPD, CKLF	13.83
Immune regulation and function	3	CXCL12, IL6R, GREM1	11.32
	4	CALR, PTEN, SFTPD, MFGE8	10.37
	10	EPHX2, IL6R, C5, ORM1, C1QC, ORM2, CASP6, KLKB1,	4.34
	3	CASP7, CFLAR,CASP6	10.18
	8	MYD88, CYCS, CASP7, CFLAR,	3.00
Cell death	24	BIRC2, CASP6, PPP3CA, EDNRB, PTEN, BCL2A1, CLN8,	2.98

In utero OVA vs. SAL -exposed Lewis rat.	No of genes	Human Genes*	Fold Enrichment **
Lipid metabolism	3	CRYL1, HADH, HADHA	18.84
	4	ITPKA, IPMK, IP6K1, ITPKC	14.36
	4	HADH,MECR,HADHA,PPT	9.74
Angiogenesis /angiotensin	3	ECE1, ACE, ATP6AP2	13.48
	6	RUNX1, BTG1, NOS3, GATA2, ANGPTL4, SPHK1	5.18
Cell cycle	3	CDK4, CDKN1B, CDC2	11.93
	6	CDC25B, CCNA2, MKI67, RBL2, CDK4, CDC2	5.16
		ORC6L, MCM3, GINS4, POLD1, POLD2, RRM2, RFC3, RFC2, DSCC1,	

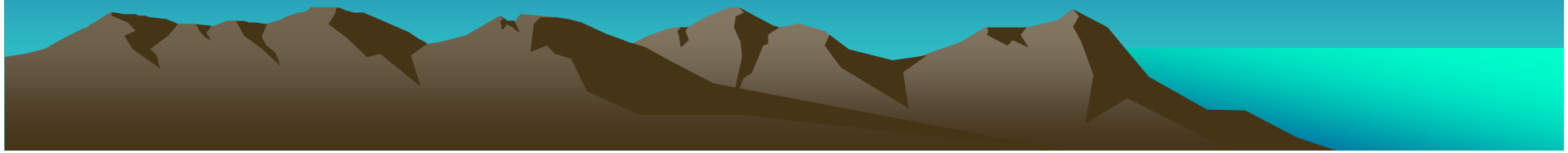
* Not all genes in larger ontology groups are listed. * * Fold enrichment represents odds ratio that the difference in developmental pattern of expression for genes in the ontology group are a consequence of *in utero* OVA exposure.

Among the human homologues corresponding to the OVA-affected probes; ***24.3% of those affected in BN rat and 18.7% of those affected in Lewis rat were differentially expressed*** *in* ***lymphoblastoid cells from asthmatic vs. non-asthmatic human sibling pairs***

Am J Physiol Lung 2012

OVA impacted gene sets were also strongly enriched for characteristic genes of the developing human fetal lung (Kho et al, AJRCCM, 2010). ***Moreover, affected genes in BN and Lewis rat had distinct patterns of lung developmental expression*****.**

ATS 2012



Conclusions

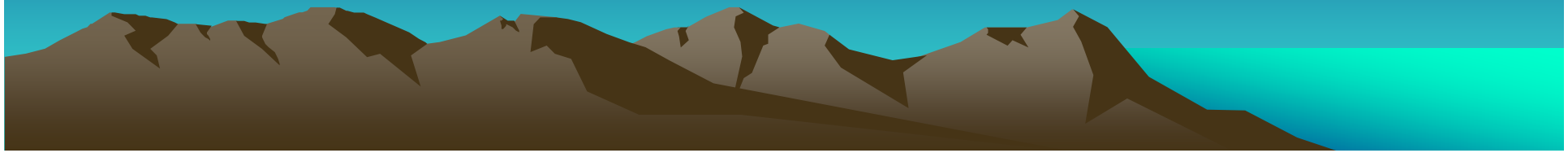
Gene sets that are most profoundly influenced by allergen exposure are *almost entirely different* from those that confer innate genetic susceptibility.

Maternal OVA exposure profoundly impacts developmental lung gene expression and respiratory phenotype in a *normo-responsive* rat.

Unique patterns of altered gene expression point to specific roles for environmental exposures in influencing fetal respiratory development in offspring of atopic vs. non-atopic mothers.

There is a need to describe a set of *"allergen" sensitive genes* that constitute a unique class of genes not identified by GWAS studies. GXE genes

Israel as an ideal setting to study gene-environment interactions In pediatric asthma



Within 300 kilometers, distinct Environments

1.

Haifa – high humidity
heavy pollution
gulf
refineries

Tel Aviv – high humidity
dense population
cars, diesel fuel

Jerusalem – low humidity
cleaner air
mixed population

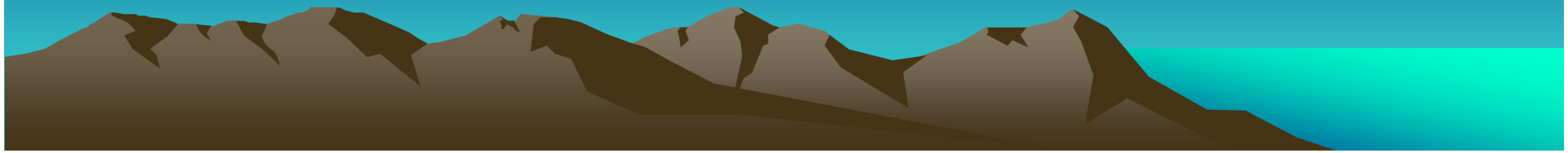
Negev – arid
Ramat Chovav
Dimona

2. Nearly universal access to high quality medicine.

3. Relatively minimal migration into and out of communities facilitates

    a. patient compliance
    b. Less variability in medication
    c. greater liklihood for follow-up to longterm studies

The impact of maternal exposure on asthma in children *in the absence of pre-existing genetic predisposition* emphasizes the need to identify children born to mothers in settings of dramatically increased exposures.



Acknowledgements

Montreal Children's Hospital Research Institute

Nicole Carpe
Isabel Mandeville
Salomon Cornejo
Feige Kaplan

Meakins-Christie Laboratories

James Martin

Channing Laboratory, Harvard medical School

Alvin T. Kho
Jen-Hwa Chu
Sunita Sharma
Kelan Tantisira
Benjamin A. Raby
Scott T. Weiss