Prenatal and early postnatal exposures and asthma risk- How???

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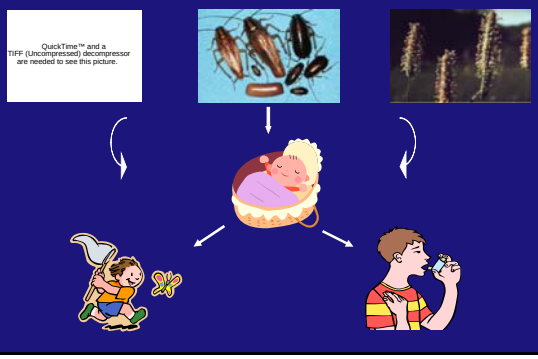
Asthma is a complex disease

- Mediated by
  - genetic predisposition
  - environmental exposures
  - host factors eg obesity, psychosocial
  - infections

Prenatal and early postnatal exposures modify asthma risk



How???

- What does the literature tell us so far?
- How is CCCEH cohort study* addressing this question?

Exposure Assessment	Biomarkers of Exposure/ Effect/Susceptibility	Outcomes
• Monitoring • Questionnaire • GIS	• PAH metabolites • PAH-DNA adducts • Cotinine • Pesticides • IgE, Cytokines • T cell proliferation • Gene expression • Genetic polymorphisms • Lead, mercury • Chromosomal aberrations	• Asthma • Allergic sensitization • Obesity • Growth & neurobehavioral development • Cancer risk

*Pregnancy through childhood: Repeat measures on women and children

Epidemiological support: Prenatal exposure to ETS

- ETS is associated with
  - impaired respiratory function
  - transient wheeze,asthma, and/or respiratory infections in infants, young children, and adolescents

Magnusson L, et. al. *Clin Exp Allergy* 2005;35:1550-1556
Alati R et. al. *Epidemiology* 2006;17:138-144

Additional prenatal exposures

- Increase asthma risk?
  - Low maternal intake of vitamin E, zinc
  - Use of antibiotics
  - Respiratory infections during pregnancy
  - Ambient air pollution eg PAHs
- Decrease asthma risk?
  - Probiotics
  - Multiple pregnancies

Devereux G et. al. *Am J Respir Crit Care Med* 2006;174:499-507.
Jedrychowski W. et. al. *Int J Occup Med Environ Health* 2006;19:70-76.
Kukkonen K et. al. *J Allergy Clin Immunol* 2007;119:192-198.
Hughes et. al. *Clin Exp Allergy*; 1999;29 (10): 1378-81

Epidemiological support: Early postnatal exposures

- Dust mite allergen during infancy
  - determinant for later childhood asthma
- Dog, cat allergen
  - associated with protection from later childhood wheeze
- Combustion-related pollutants
  - associated with later childhood sensitization to dust mite
  - reduction in FEV_1

Sporik R et. al. *N Engl J Med* 1990;323:502-507
Remes et. al. *J of Allergy and Clinical Immunol* 2001;108:509-515
Ponsonby et. al. *Clin Exp Allergy* 2001;31:1544-1552

Prenatal PAH, postnatal ETS and respiratory score (CCCEH)

	Analysis for Main Effects			Analysis for Interactions		
	Exposure	B	P value	Exposure	B	P value
12 months (n=263)	Intercept	1.04		Intercept	0.73	
	Pre ETS	-0.02	0.90	Pre ETS	-0.01	0.92
	PAH	0.01	0.72	PAH	0.09	**0.018**
	ETS	0.15	0.29	ETS	-0.23	0.27
				PAH x ETS	**0.11**	**0.014**
24 months (n=169)	Intercept	0.68		Intercept	0.30	
	Pre ETS	-0.07	0.67	Pre ETS	-0.08	0.65
	PAH	0.03	0.28	PAH	0.13	**0.002**
	ETS	-0.09	0.60	ETS	-0.63	**0.011**
				PAH x ETS	**0.15**	**0.003**

Miller, R.L., et. al., *Chest* 2004, 136: 1071-78.

Potential mechanisms?

- Immune
- Epigenetic

Immune-mediated mechanisms

- Altered cytokine regulation
- Generation of antigen-specific T cell immune responses

Altered cytokine regulation

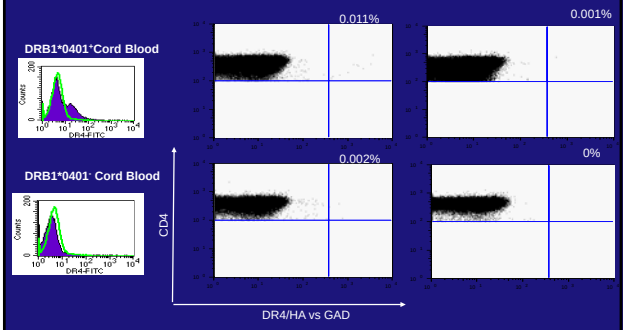
Description of Study		Methods and Measures			Major Findings
Source	Study Population	Age,Sample	Cytokines	Antigens	
Contreras JP et al. Boston, MA J Allergy Clin Immunol, 2003	112 children with parental history of asthma or allergy Birth to age 2 yrs	Age 2 years: PBMCs	IFN-γ TNF-α IL-10 IL-13	Bla g 1 (cockroach) Der f 1 (HDM) Fel d 1(cat)	Children w/ atopic disease, repeated wheeze had **lower IFN-γ levels** in response to HDM and cockroach allergen.
Prescott SL et al. Perth, Australia Allergy, 2003	60 children Birth to age 6 yrs All born by elective C-section	Birth: CBMCs Age 6, 12, 18, 24 mos: PBMCs	IFN-γ IL-4, IL-5 IL-6 IL-9 IL-10 IL-13	HDM Ovalbumin Fel d 1 PHA Tetanus toxoid	Children w/ family history of allergy had **lower IFN-γ resp** to PHA stimulation of CBMCs Children with atopy at 6 yrs had 1) incr in **IL-5 mRNA** in response to HDM age 2 yr 2) **incr IL-13** resp to HDM at 1 yr.
Neaville WA et al. Madison, WI J Allergy Clin Immunol, 2003	285 Children Birth to age 1 yr Parent w/ allergies, asthma	Birth: CMBCs Age 1 yr: PBMCs	IFN-γ IL-5 IL-10 IL-13	PHA	**Lower IL-10** production in response to PHA-stimulated CBMCs risk factor for egg sensitization at age 1
Kondo N et al. Gifu, Japan CEA,1998	21 children Birth to age 6 yrs Full term, vag birth	Birth: CBMCs	IFN-γ IL-2	Ovalbumin BSA	Children with allergy by age 6 yrs had **lower IFN-γ** in response to ovalbumin or BSA

Modified from Chung, E.K., R. L. Miller, M. T. Wilson, S. J. McGeady, J. F. Culhane. *Arch. Dis. in Child. Fetal Neonatal Ed.* 92: 68-73, 2007

Cord blood proliferation in response to indoor antigens (CCCEH)

Miller et al *Am. J. Respir. Crit. Care Med*, 2001, 164 (6), 995

Influenza-specific T cells identified in cord blood following prenatal vaccination of mother



Rastogi, D., C. Wang, X Mao, C. Lendor, P. B. Rothman, R. L. Miller. *Journal of Clinical Investigation*, 2007, 117 (6):1637-46

Epigenetic-mediated mechanisms

- Heritable changes* in gene expression that occur in the absence of alterations in DNA sequences

* at least between cells

Epigenetic regulation

- DNA methylation
  - covalent addition of a methyl group to cytosines in CpG dinucleotides
- Chromatin packaging of DNA via post-translational modifications of histones
  - egs. acetylation,methylation,phosphorylation
- Believed to occur predominantly prenatally and shortly after birth
- May influence gene expression differentially throughout lifespan

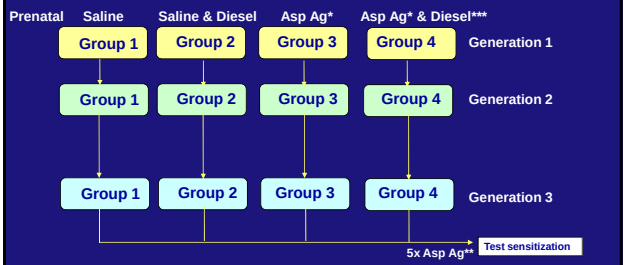
Eg: T helper cell differentiation

- Proallergic IL-4 production and Th2 differentiation
  - demethylation of sites at the prox promoter and conserved intronic regulatory element (CIRE) in 1st intron of the IL-4 gene
  - hypermethylation of sites in the counterregulatory IFNγ promoter
- Th1 differentiation
  - methylation of a highly conserved DNaseI-hypersensitive region at the 3' end of the IL-4 locus

Lee DU et. al. *Immunity* 2002;16:649-660.
Agarwal S,et al. *Immunity* 1998;9:765-775.
Tykocinski LO, et. al. *J Biol Chem* 2005;280:28177-28185.
Jones B et. al. *The EMBO Journal* 2006;25:2443-2452

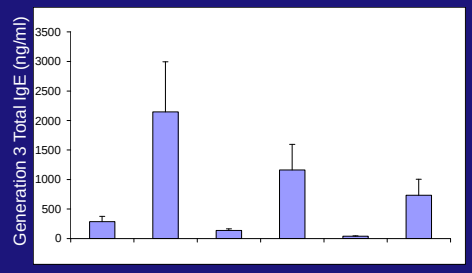
CCCEH work-in-progress

Multigenerational effects of exposure to mold *aspergillus*, diesel



*5 x 1.25 mg Asp Ag prior to mating; 2x during pregnancy
**5 x 125µg Asp Ag
***last week of pregnancy 6 hr/day

Grandparental exposure to mold protected against development of IgE



In vivo study of methylation of IL4 gene in asthma-like mouse models

Saline | Asp Ag | Diesel | Asp Ag & Diesel

Group 1 | Group 2 | Group 3 | Group 4

saline or Asp Ag (62.5 µg/dose) X 5
diesel 6 hr/day x 3 weeks

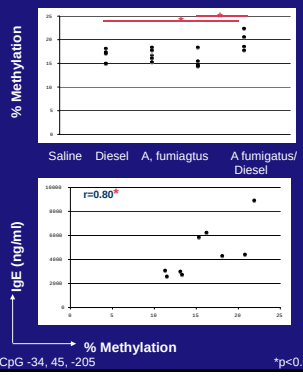
→ IgE

Collect CD4+ cells

Isolate genomic DNA

Bisulfite conversion
PCR amplification
Pyrosequence of IL4, IFNγ gene:
Quantify methylation level

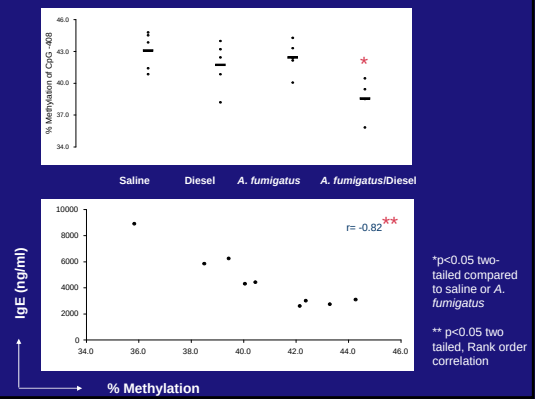
In vivo A. fumigatus and DEP exposure are associated with increased methylation of IFNγ promoter at CpG-53 site and increased IgE



Significant correlations also with CpG -34, 45, -205

*p<0.05 two tailed t test

In vivo A. fumigatus and DEP assoc with hypomethylation of IL-4 at CpG^{-408} and decreased IgE



*p<0.05 two-tailed compared to saline or *A. fumigatus*

** p<0.05 two tailed, Rank order correlation

Prenatal exposure to airborne PAHs and alterations in DNA methylation

Bench ⟶ Bedside and beyond

Question: Does prenatal PAH exposure affect asthma risk via epigenetic mechanisms, such as CpG methylation?

F.P. Perera, D. T.Tang, J. Herbstman, S. C.Edwards, R. Whyatt, P. Kinney, R.L. Miller (CCCEH) in collaboration with Drs. Shuk0mei Ho and W.Tang of the University of Cincinnati

Methylation profiling using methylation sensitive restriction fingerprinting

- Study population/samples: CCCEH cord blood
- PAH exposure
  - high prenatal PAH exposure: highest quartile prenatal air PAHs/high PAH-DNA adducts
  - low exposure: lowest quartile PAHs/low PAH-DNA adducts

Pilot study (n=60): Identification of candidate genes

50+ clones were chosen for sequencing and *in silico* analysis

34 candidate clones were identified

7 clones contained 5' CpG islands

6 were homologous (>90%) to known genes expressed in lung or lymphoid tissue

- *DSP2* → cell signaling
- *CCL17 (TARC)* → Th cell recruitment
- *RAD21* → oxidative stress
- *SFMB2*, *WWOX* → gene-transcription related chromatin remodeling
- *ACS3* → fatty acid oxidation

Perera, Ho et al., ISEE, 2007

Differential ACS3 methylation status confirmed by MSPCR

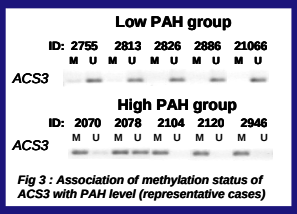


Fig 3 : Association of methylation status of ACS3 with PAH level (representative cases)

ACS3 unmethylated in low PAH group

ACS3 methylated in high PAH group

•Trend suggesting that methylated ACS3 is associated with parental report of childhood asthma at age 5 (OR: 3.76, 95% CI: 1.04, 13.65)

Perera, Ho et al., ISEE, 2007

Conclusion

- Prenatal and early postnatal exposures impact risk for later asthma
- Both immune-mediated and epigenetically-regulated mechanisms likely contribute
- Much more cohort-driven mechanistic research needed to assess:
  - dose of specific exposures
  - time periods of increased susceptibility
  - interactions btw genetics and epigenetics

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