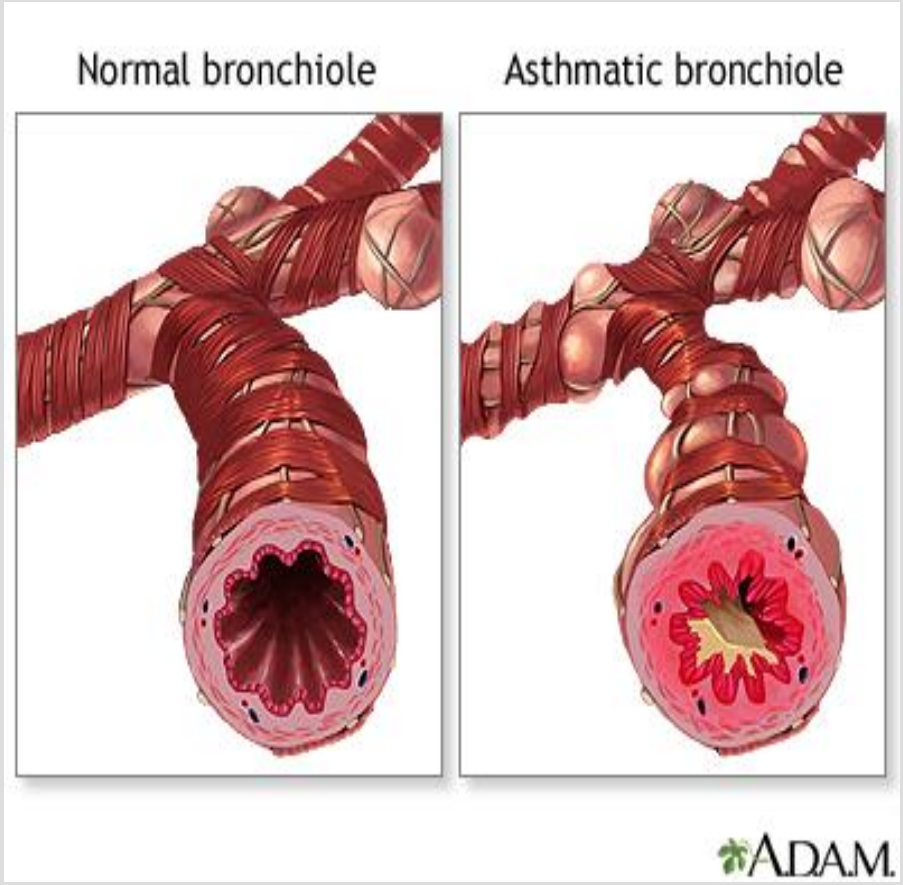
AMERICAN INDIAN CHILDREN WITH ASTHMA: IMMUNOLOGIC AND GENETIC FACTORS

6/10/15

WHAT IS ASTHMA?



- **BRONCHO-SPASM**
  - **MUSCLES IN AIRWAYS**
  - **Rx: ALBUTEROL**
- **INFLAMMATION**
  - **INCREASED MUCUS**
  - **SWELLING IN LINING OF AIRWAYS**
  - **Rx: STEROIDS**
  - **ANTI-HISTAMINES**

ASTHMA

- AMERICAN INDIAN POPULATIONS HAVE A HIGH PREVALENCE OF ASTHMA
- ETIOLOGY OF ASTHMA REMAINS UNKNOWN
  - Immune sensitization an important factor
- TREATMENT RELIES ON:
  - Bronchodilators
  - Anti-inflammatory
    - Eg: glucocorticoid steroids, inhaled or systemic
  - mast cell stabilizers, eg: Chromolyn sodium
  - Allergen desensitization or avoidance

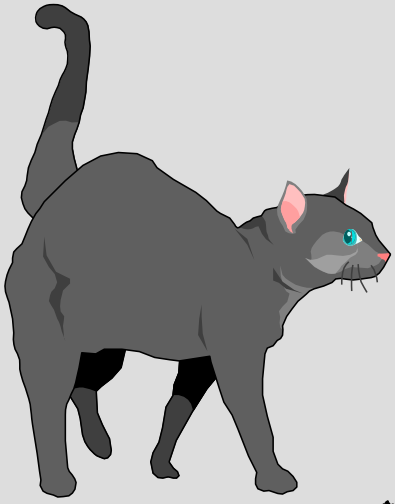
CASE/CONTROL STUDY

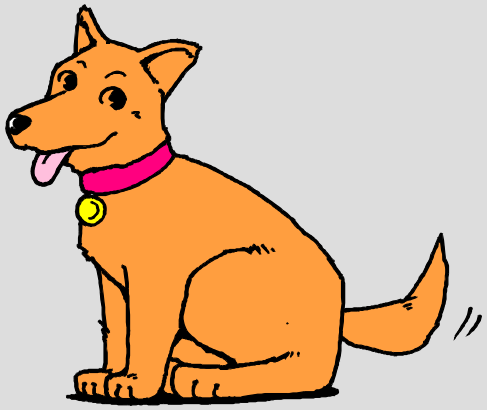
- 108 CHILDREN BETWEEN 6 AND 17 YO WITH A DIAGNOSIS OF ASTHMA
- EACH MATCHED WITH 2 CHILDREN WITHOUT ASTHMA
- AIR ENVIRONMENT TESTS
- BLOOD TESTS FOR ALLERGIES, INFLAMMATION
- DNA TESTING

CASE DEFINITION

- Cases chosen by search of asthma diagnosis within the electronic medical record system
- Criteria confirmed by medical record review
- Between 4 and 17 years of age and at least 2 of:
  - A diagnosis of asthma on 2 occasions
  - Asthma treatment refills X 2, within 2 years
  - FEV1 improvement of 20% with bronchodilator
- No history of prematurity, ventilator requirement, or significant congenital heart disease
- Controls: Do not meet criteria

ENVIRONMENTAL TESTS





- **“HYGIENE THEORY” OF ASTHMA**
  - **? TOO CLEAN??**
- **TESTS FOR:**
  - **DUST**
  - **ANIMAL DANDER**
  - **INSECT PARTS**
  - **POLLEN**
  - **CARBON MONOXIDE**
  - **SMOKE**

ASSAY METHODS

- Serum measures (Sanford clinical lab):
  - hsCRP (C-reactive Protein)
    - Turbidimetric (Antigen/Antibody Complex)
  - IgE
    - Fluroenzymeimmunoassay
  - Antigen specific IgE
    - Fluoroallergrosorbent
  - CBC
    - Coulter Counter
- Genotyping
  - TaqMan assay (Life Technologies) using real-time PCR (Bio-Rad)

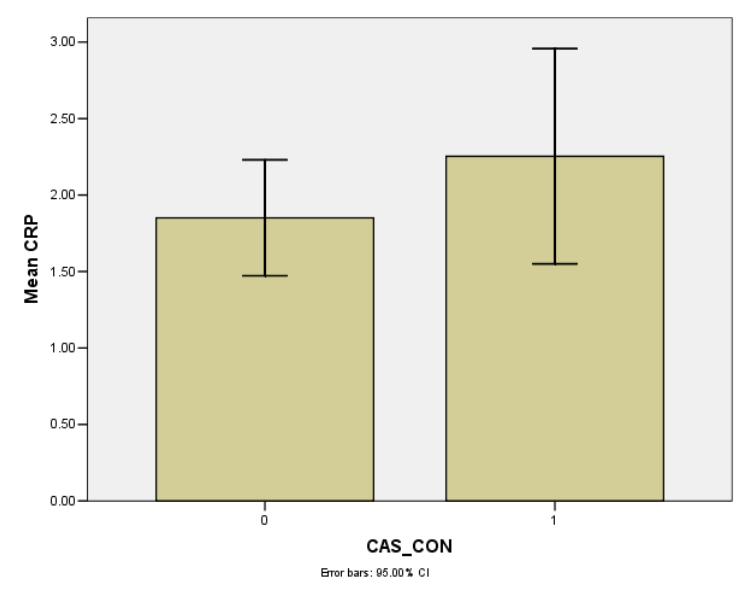
ANALYSIS METHODS

- **STATISTICAL ANALYSIS:**
- **Student t-test for independent means**
- **Chi squared test for prevalence of variant in case/controls**
- **Logistic regression to adjust for covariates (age, gender, body mass index) influencing dependent variable of asthma**

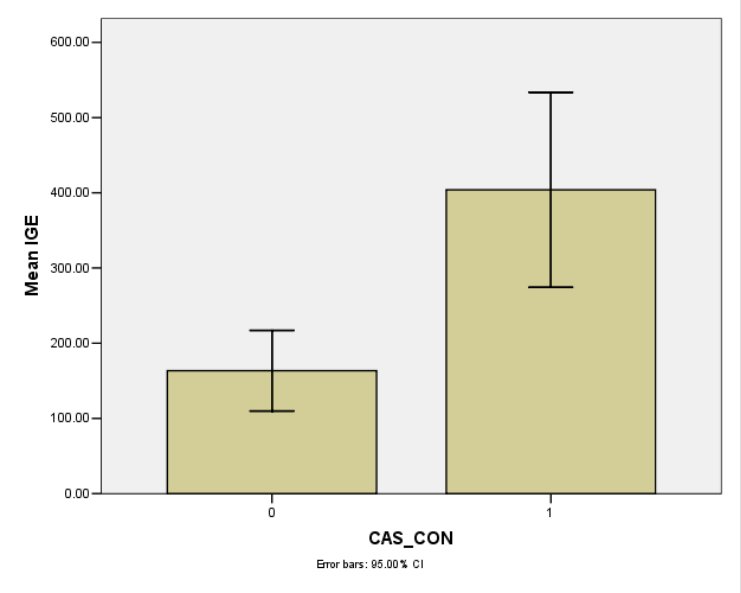
Immune Measures Case/Control

	Case		Control		P value
	Mean	SD	Mean	SD	
Participants	108		216		
Male, N(%)	57 (53%)		109 (51%)		0.694
Age, yr	11.79	3.20	12.13	3.18	0.358
WBC* (X 1000)	7.54	2.39	6.89	1.81	0.006
PMN (% of WBC)**	54.8	9.96	56.0	10.42	0.334
Lymphocytes***	31.6	8.49	31.4	8.34	0.874
Basophils***	1.5	0.73	1.7	1.41	0.200
Eosinophils***	5.0	3.99	3.8	3.45	0.005
hsCRP (mg/L)	2.6	3.12	2.8	7.94	0.781
IgE (total, kU/L)	486	705.2	219	370.1	0.001

IMMUNE MEASURES



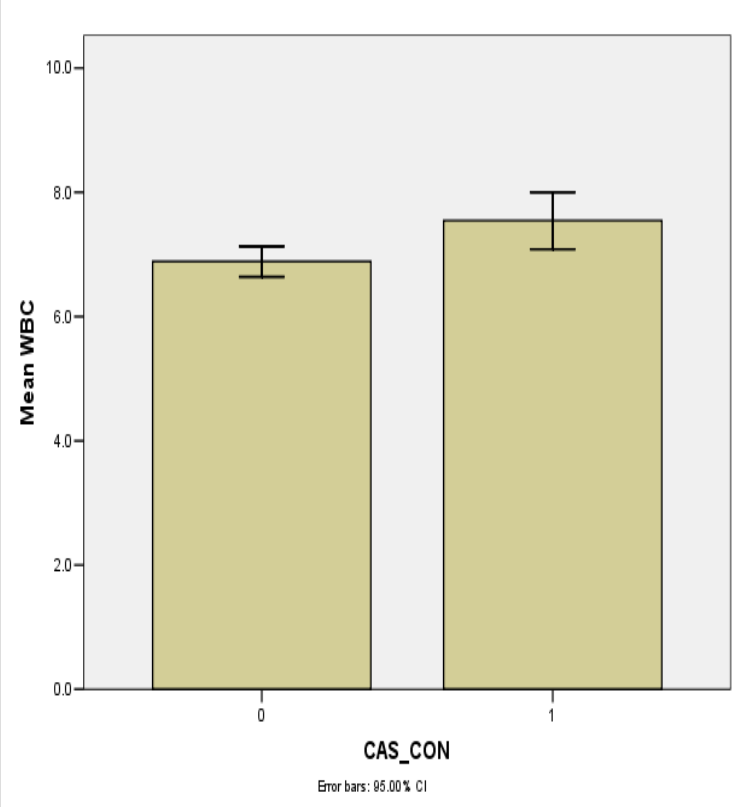
hsCRP



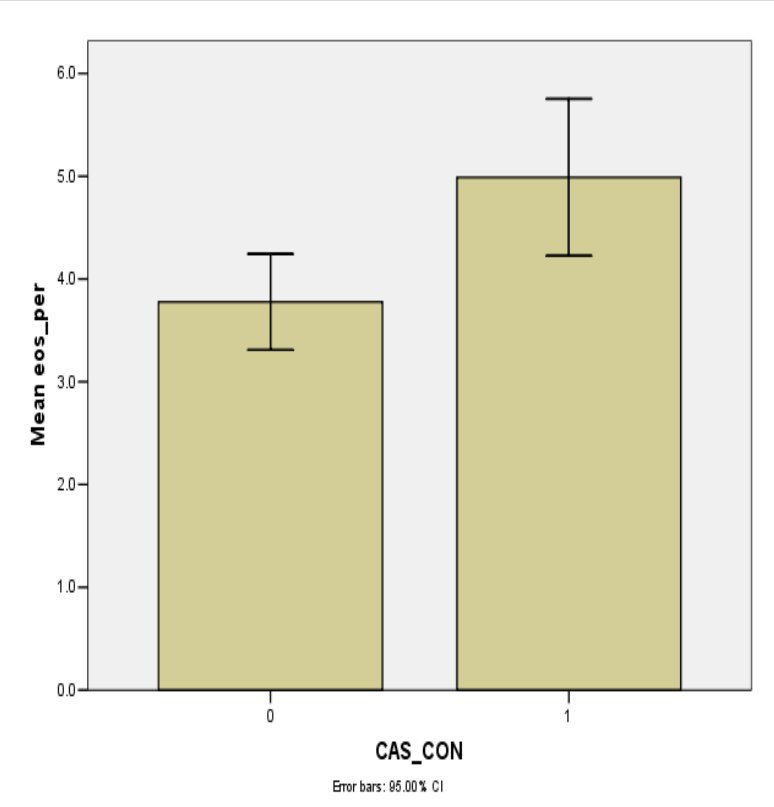
IgE

- Sensitivity to all tested individual allergens: mold, cat/dog, mite, cockroach was greater among cases compared with controls

IMMUNE MEASURES

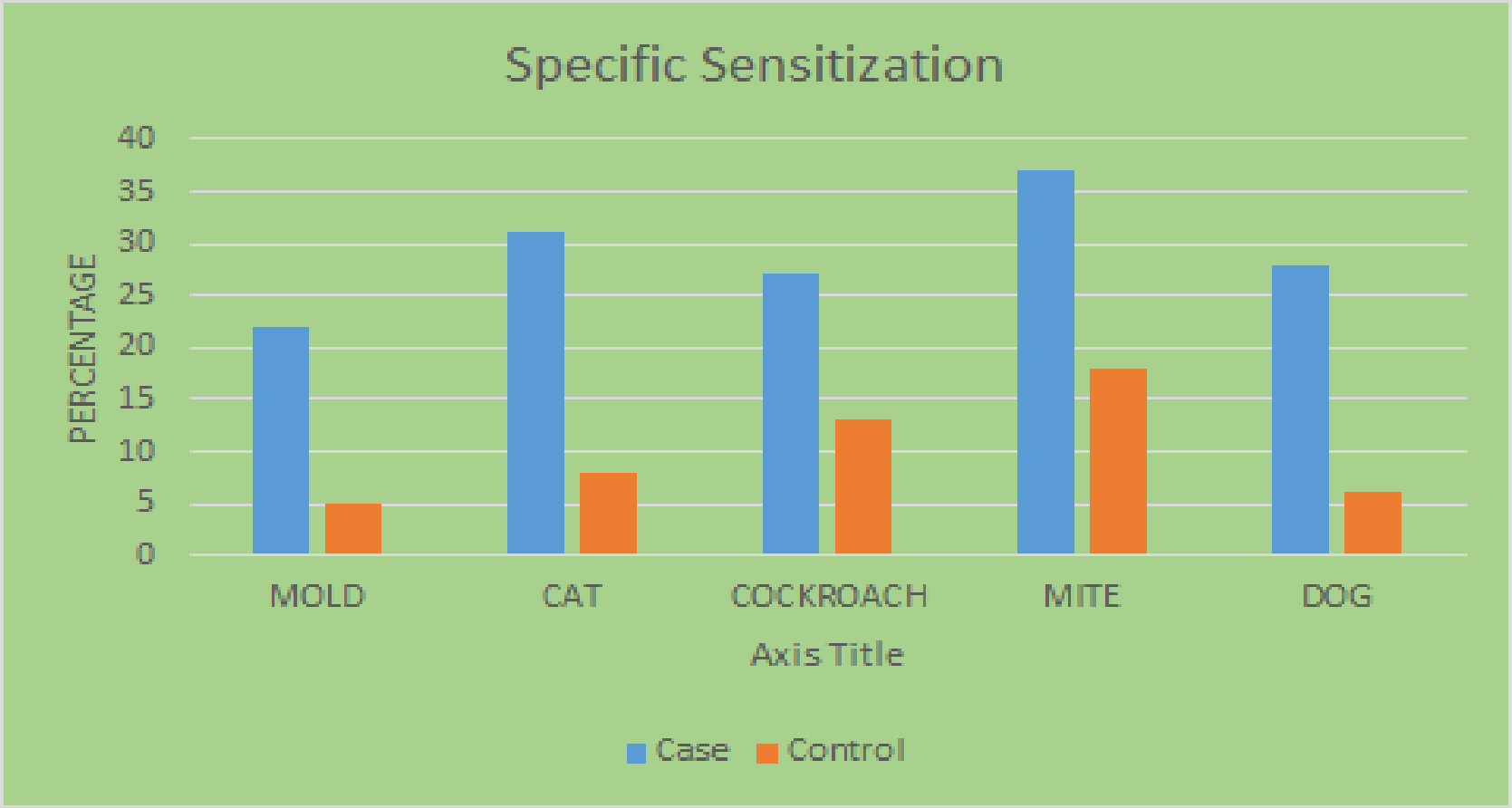


WBC

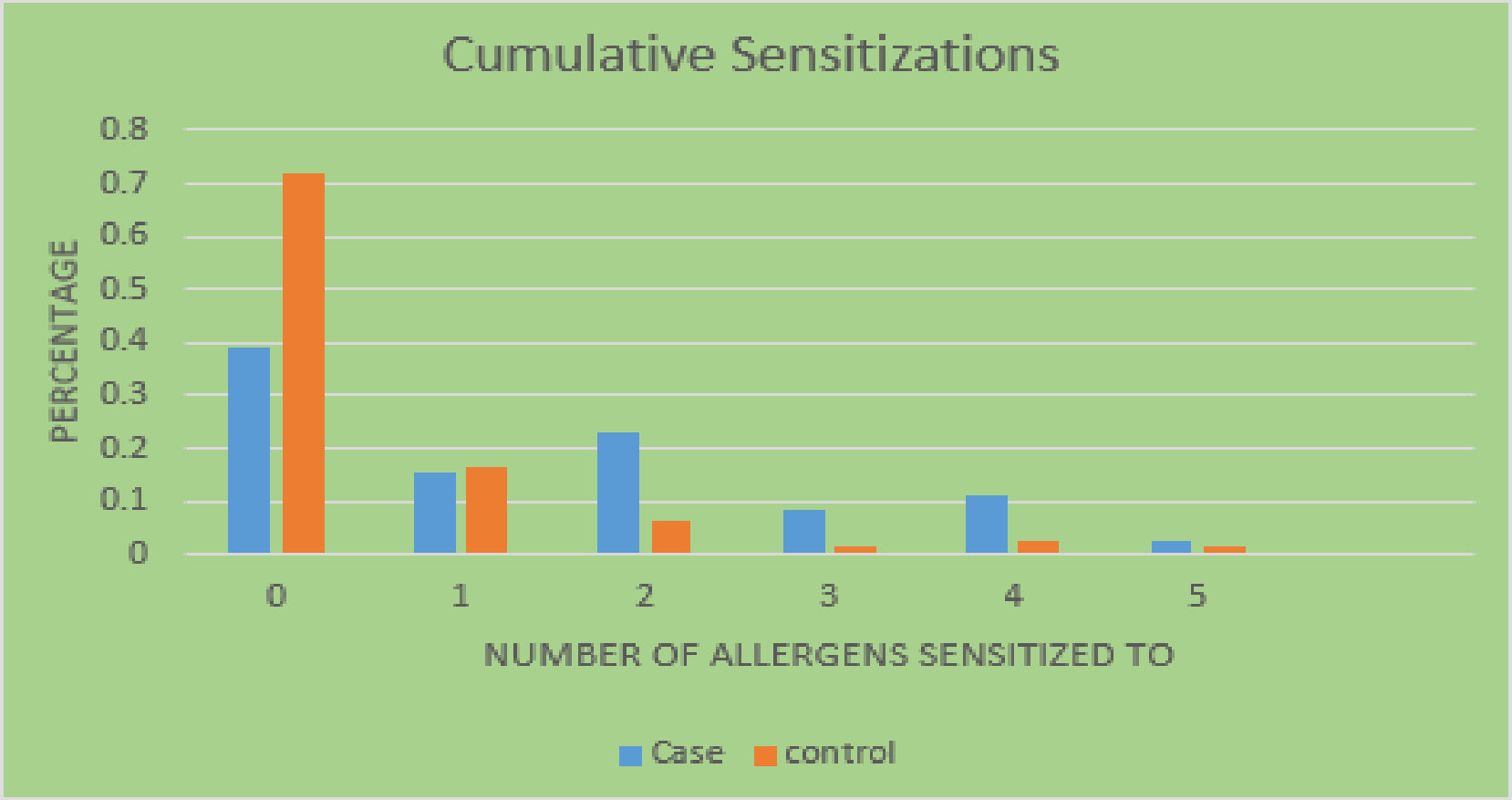


Eosinophiles

IMMUNE MEASURES



IMMUNE MEASURES



DISCUSSION

- **Increased IgE levels are associated with atopy and asthma severity.**
  - **Curr Opin Allergy Clin Immunol. 2010 Oct;10(5):408-17. doi:10.1097/ACI.0b013e32833d7d2d.**
  - **Clin Immunol. 2007 Jul;120(1):137-43. PubMed PMID: 17498790.**
- **Genetic linkage and GWAS have also identified loci and variants affecting IgE expression.**
  - **Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. Genes Immun. 2006 Mar;7(2):95-100. Review. PubMed PMID: 16395390.**
- **Anti-IgE antibodies have shown effectiveness in treating asthma**
  - **J Allergy Clin Immunol. 2001 Aug;108(2):184-90. PubMed PMID: 11496232.**

DISCUSSION

- **Neutrophils (polymorpholeuckocytes, PMNs) are found to be increased in children with asthma, in both blood and bronchial fluids**
  - **J Investig Allergol Clin Immunol, 19:340, 2009.**
  - **Some postulate a sub-type of asthma (“neutrophilic asthma”)**
- **Eosinophils are also increased in many people with asthma**
  - **Possibly another sub-type of asthma (“eosinophilic asthma”)**
  - **J Asthma Allergy. 2014 Apr 11;7:53-65. doi: 10.2147/JAA.S39119.**
  - **N Engl J Med. 1990 Oct 11;323(15):1033-9. PubMed PMID: 2215562.**

DISCUSSION

- **Role of biomarkers in evaluation and research of asthma**
  - **J Allergy Clin Immunol. 2012 March ; 129(3 Suppl): S9–23. doi:10.1016/j.jaci.2011.12.979.**
  - **Multi-allergen screening recommended as a “core” biomarker**
  - **CBC**
  - **eosinophil count**
  - **IgE, total and allergen specific antibody levels**
  - **genomics considered an “emerging” outcome measures**
- **Increased levels and increased number of allergen-specific IgE assays associated with increased risk of wheezing in children**
  - **J Allergy Clin Immunol. 2005; 116(4):744–9.**

ALLERGEN SENSITIZATION

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Randomized Trial of Peanut Consumption in Infants at Risk for Peanut Allergy

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ABSTRACT

BACKGROUND

The prevalence of peanut allergy among children in Western countries has doubled in the past 10 years, and peanut allergy is becoming apparent in Africa and Asia. We evaluated strategies of peanut consumption and avoidance to determine which strategy is most effective in preventing the development of peanut allergy in infants at high risk for the allergy.

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GENETICS AND ASTHMA

- **The sibling of a dizygotic twin with asthma will have a 3X increased risk of asthma, compared with the general population.**
- **The sibling of a monozygotic twin with asthma will have a 6X increased risk of asthma, compared with the general population.**
- **Multiple studies have shown association between asthma and a number of genes in the 17q21 region**
- **EVE Consortium meta-analysis (multi-ethnic, but not AI)**
  - ***IL1RL1 (2q12), TSLP (5q22)*, *IL33 (9p24), ORMDL3/GSDML (17q21)***
  - **Possible unique variant of *PHYHIN1* in African-Americans**

PROGRESS TO DATE

- **108 Cases** and **216 controls enrolled**
- **3 SNPs from 17q21 locus genotyped for approximately one half of participants**
  - **rs7216389**
    - **GSDMB gene, "gasdermin B", (1st intron)**
      - **Regulation of apoptosis in epithelial cells**
  - **rs2305480**
    - **GSDMB gene, "gasdermin B", (6th exon)**
    - **"missense", proline to serine amino acid coding**
  - **rs8076131**
    - ***ORMDL3* gene, "sphingolipid biosynthesis regulator 3"**
      - **(3rd intron)**
      - **possible role in Ca^{++} signaling**

CASE/CONTROL CHARACTERISTICS

Characteristic	Cases	Controls	p value
Number (N)	115	219	N/A
Age, mean years (SD)	23.97 (6.38)	23.97 (5.37)	0.988
Gender (female)	51/108 (47%)	107/216 (49.5%)	0.783
BMI	25.43 (8.16)	23.77 (7.46)	.070
rs7216389 allelic frequency (C)	44/132 (33%)	90/218 (41%)	0.372
rs2305480 allelic frequency (A)	31/114 (27%)	77/208 (37%)	0.247
rs8076131 allelic frequency (A)*	82/114 (72%)	106/156 (68%)	0.841

* Risk Allele

CHI-SQUARE RESULTS

SNP	Model	Allele	Chi Square	DF	P value
rs7216389	Additive	C	2.55	2	0.280
	Dominant	C	1.80	1	0.179
	Recessive	C	0.46	1	0.496
rs2305480	Additive	A	3.54	2	0.170
	Dominant	A	1.53	1	0.217
	Recessive	A	1.82	1	0.177
rs8076131	Additive	A	2.36	2	0.307
	Dominant	A	1.38	1	0.240
	Recessive	A	0.00	1	1.000

LOGISTIC REGRESSION RESULTS

UNIVARIATE					
SNP	**Model**	**Allele**	**Odds Ratio**	**95% CI**	**P value**
Age	**Additive**		**0.944**	**0.87 - 1.02**	**0.147**
Gender	**Additive**		**1.011**	**0.63-1.62**	**0.962**
BMI	**Additive**		**1.037**	**1.004-1.071**	**0.030**
rs7216389	**Additive**	**C**	**0.679**	**0.42 - 1.10**	**0.117**
rs2305480	**Additive**	**A**	**0.624**	**0.37-1.04**	**0.072**
rs8076131	**Dominant***	**A**	**2.647**	**0.7-10.1**	**0.154**
MULTIVARIATE (age, gender and BMI + following SNPs)					
rs7216389	**Additive**	**C**	**0.653**	**0.40-1.08**	**0.095**
rs2305480	**Additive**	**A**	**0.588**	**0.35-1.0007**	**0.050**
rs8076131	**Dominant***	**A**	**2.806**	**0.701-11.24**	**0.145**

NOTE: Only models with lowest p value within category are shown.
NOTE: * indicates risk allele

Discussion

- **Obesity and Asthma**
  - **? Decreased thoracic/lung volume, reduced airway diameter**
  - **? Systemic, chronic, mild inflammatory state**
  - **? Potential common etiologies, genetic, environmental**
  - **? Adiponectin, leptin imbalances**
  - **Shore SA. Obesity and asthma: possible mechanisms. J Allergy Clin Immunol 2008; 121:1087–1093.**
- **Similar associations between asthma and obesity among Blackfeet Tribe in Western Montana**
  - **Noonan CW, Brown, BD. 2010. Journal of Asthma, 47:496–500.**

DISCUSSION

- These results are clearly preliminary, since only about half of the participants have been genotyped and entered into analysis
- Chi-Square analyses tend to be conservative by nature, but all three SNPs show allelic prevalence bias in the expected direction
  - ie increased prevalence of risk alleles among cases
- Logistic regression analyses show borderline significance for the rs2305480 allele (p=0.07) in univariate analysis and right at significance (p=0.05) with adjustment for demographic and anthropomorphic variables
- The other two SNPs also show trends in the expected direction that are also strengthened with multivariate adjusment

TRANSLATIONAL EFFORTS

- **Planned (as conducted for pre-eclampsia research)**
  - **PRESENTATION OF FINDINGS TO THE LOCAL MEDICAL STAFF**
  - **REVIEW AND APPROVAL OF MANUSCRIPTS BY TRIBAL GOVERNMENT**
  - **PRESENTATION AT AAIP, LOCAL HEALTH FAIRS**
  - **INCLUSION OF OTHER TMCC STUDENTS IN LAB DEMONSTRATIONS**
  - **ENGAGEMENT WITH INMED, HIGH SCHOOL AND SUMMER ENRICHMENT PROGRAMS**
  - **RADIO PROGRAMS, NEWSLETTERS**

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- **Cheyenne River Sioux Tribal Health Department**
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