Asthma Phenotyping: The Evolution from Clinical to Molecular

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Asthma: Traditional concepts

- Often thought of as disease of children
  - In fact, more adults than children have asthma
  - About 30% begins in adulthood
- Allergic component often considered most critical
  - Led to concept that all asthma “Th2 process”
  - 4-5x as many people with “allergies” and no asthma
- One size fits all
  - Medications used across “all” asthma

The 3 “A”s: Arthritis, anemia and asthma

- How are they all alike?
  - They all are nonspecific and general characteristics of disease, describing joint swelling, low RBC numbers or reversible airway obstruction
  - They shed almost no light on what caused these characteristics to develop
    - No self respecting rheumatologist would ever diagnose a patient with “arthritis”
2015: Asthma has an umbrella definition

“Asthma”

Symptoms
Exacerbations
FEV1

Clinical Phenotypes: 5 Clusters defined by age asthma onset and lung function

Mild atopic Asthma → Mild to moderate atopic asthma (largest) → Severe atopic asthma

Late onset Nonatopic 1º female

1º late onset/females Severe obstruction/less atopy Highest HCU/lowest QOL

Moore et al. AJRCCM 2010
Severe asthma can occur in 4 of 5 of these phenotypic clusters.


The Transition to Mechanisms and Inflammatory/Molecular Phenotypes

Phenotypes
Overlapping clinical physiologic hereditary characteristics

Molecular phenotypes
Incorporation of associated pathobiologic MECHANISMS, ideally at molecular level, to molecularly define a clinically recognizable phenotype
SARP #2: Incorporation of sputum/blood variables

- Based on original clinical clusters, 2nd clustering of 423 subjects with sputum
- 15 variables from factor analysis identified 4 subject clusters of varying severity
- Presence of neutrophils in sputum, esp with eos defined most severe asthma, but other clinical characteristics less clear

Moore, JACI 2014

SARP Clusters #3: MULTIPLE inflammatory-immune variables

- Clustering: 378 SARP subjects who had undergone bronchoscopy, using 112 immuno-inflammatory variables Wu, et al JACI 2014
  - Hierarchical clustering used to cluster variables
  - Machine learning approach identified most important variables
  - K means approach identified 6 subject clusters
    - 1 healthy cluster and 5 asthma clusters
Unsupervised clustering of 112 clinical variables

- Variable clustering identified 10 variable clusters
- Six subject clusters
- 51 nonredundant variables identified
  - Age at onset, clinical symptoms, medication use, lung function, FeNO, WBC

Subject clusters

- K means identified 6 subject clusters
  - Cluster 1: HCs
  - Cluster 2: Mild early onset asthma, largest cluster
  - Cluster 3: Preserved lung function, more severe, 60% Hispanic, little inflammation
  - Cluster 4: Early onset, severe allergic, strong family history, over 40% African American
  - Cluster 5: Late onset, nasal polyposis, eosinophilic only male dominant cluster
  - Cluster 6: Most severe, highest OCS use, lowest lung function and greatest reversibility, most complex inflammation
Confirmation of age at onset/allergy connection

- Age of onset most informative variable
- Clustering identifies differences related to allergy, incorporating both specific IgE responses and allergic symptoms as related to age at onset

Identified adult onset-nasal polyp/eos + disease

Wu, JACI 2014
Identified complex inflammation associated with most severe

<table>
<thead>
<tr>
<th></th>
<th>BAL Eosinophil %</th>
<th>P&lt;0.0001</th>
<th>BAL Neutrophil %</th>
<th>P&lt;0.0001</th>
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</thead>
<tbody>
<tr>
<td>Subject Clusters</td>
<td>1 2 3 4 5 6</td>
<td>1 2 3 4 5 6</td>
<td>1 2 3 4 5 6</td>
<td>1 2 3 4 5 6</td>
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Comparison of Clinical and Inflammatory Clusters

- Age at onset critical for both
- Characteristics of most severe asthma similar
- Additional features allowed identification of late onset eosinophilic asthma and nasal polyposis
- Obese late onset female Cluster 3 “lost”
- Majority of mild asthmatics indistinguishable from each other
  - Is phenotyping less important for mild asthma?
Incorporation of molecular signatures

3 genes expressed in vitro in epithelial cells in response to IL-13 applied to ex vivo epithelial cells—“cluster” of mild asthmatics with:

- More BHR, atopy, eosinophils
- More Type 2 cytokines and better response to corticosteroids
- But only 50% of mild asthma

Type-2 Hi: Differential non-specific (CS) Rx response

We think we are so smart….
Mechanistically: Type 2 vs Not

"Asthma"

Symptoms
Exacerbations
FEV1

Type 2 inflammation
No/less Type 2 inflammation

Type-2 subtypes: IL-4/13 blockade decreased allergen induced exacerbation

Similar efficacy of Anti-IL-13 antibodies, with no effect on sputum eos
gauverau Am J Resp Crit Care Med 2011

Wenzel, Lancet, October 2007
Type-2 subtype: eosinophilic adult onset severe asthma

Higher urinary LTs, LESS atopy, more Aspirin sensitive-like disease
Miranda JACI 2004

Can we get more granular?: Type-2 immunity and FeNO

- Murine models and cell lines did not support FeNO as Type-2 mediated pathway
- In primary human epithelial cells, Type-2 cytokines induced iNOS and Type-2 blockade in vivo decreases FeNO
  - But also induced by IFNγ
- FeNO strong predictor of chronic oral CS use in Severe asthma Wysocki JACI 2014
  - But also can be high in allergic rhinitis

Chibana, K Clin Exp Allergy 2008
Corren et al, NEJM 2011
Clustering epithelial cell samples by FeNO level

- 155 epithelial brushings from range of asthmatics and healthy controls
  - Agilent microarrays performed
- Identified genes strongly correlated with FeNO
  - 589 genes identified with rho values >0.4, all passing FDR<0.05
  - iNOS most strongly correlated rho=0.71
- K-means clustering of genes identified 5 patient clusters, 3 FeNO Hi and 2 FeNO Lo, with distinct characteristics
- Expanded to 1349 genes differentially expressed across the 5 clusters

K-means clustering identifies 5 molecular participant clusters

Subject Clusters (SCs)

<table>
<thead>
<tr>
<th>SC1</th>
<th>SC2</th>
<th>SC3</th>
<th>SC4</th>
<th>SC5</th>
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<tbody>
<tr>
<td>Less atopic, 78% HCs or mild controlled asthma</td>
<td>Early onset, 73% moderate to severe, low lung function, eosinophilia</td>
<td>Later onset majority severe. Mixed inflammation, nasal polyps, sinus surgery</td>
<td>Earliest age at onset, longest disease duration 100% atopic, but low FeNO</td>
<td>Youngest, early onset, 50% African American, strong FH/highest IgE (most classic “traditional” asthma)</td>
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Hierarchical clustering of 1349 additional genes distinguishes 9 gene clusters

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<thead>
<tr>
<th>Highest in SC</th>
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Speculations from microarrays

- Increasingly severe disease associated with increasingly complex immunity
  - Type-2 immunity does not define severity
  - Considerable support for Type-1 immunity and innate immune pathways (little for Th/Type-17)
- Strong evidence for dysregulated wound repair (which comes first??)
Complex Type 2: Asthmatic Granulomatosis

- First reported 2012
- 10 “severe asthma” pts (now ~40) who met asthma diagnosis (reversibility or + methacholine)
  - All on systemic corticosteroids (10 mg or above)
- Often adult onset or adult worsening
- Modest obstruction with some evidence for decrease in FVC and DLCO
- Hi FeNO (and blood eos) despite systemic CSs
- Associated with autoimmune family history in 70%

_Wenzel AJRRM 2012_

Complex Type 2: Small airway inflammation and granulomas

Can respond well to azathioprine/mycophenolate, more so if personal or FH of autoimmunity
**Type 2 Molecular Phenotypes 2015:**
Will Rx responses also differ?

- **Eosinophilia**
- **Periostin**
- **FeNO**

**Type-2 Asthma**

- Mild CS naïve/CS responsive early onset asthma (IL-4/13)
- Moderate-Severe Early onset CS treated atopic asthma (persistent IL-4/13)
- Late onset eosinophilic (IL-5, ?13)
- Type-2 + Very severe, autoimmunity, eosinophils, neutrophils IL-4, 5, 13 and Type 1 factors

“Type 2-Lo Asthma”

- Much less well defined than Type-2 HI
  - Defined as “apparent” absence of Type 2
  - No definite endotypes yet, but likely confounders including obesity-related, post infectious, smoking, long disease duration
  - ‘omics may provide some clues
- *All* associated with poor CS response
  - Macrolides, thermoplasty, weight loss

Type 2-Lo late onset obese asthma responds to weight loss

- 23 obese asthmatics evaluated before and 12 mos after bariatric surgery
- Roughly phenotyped patients by median IgE levels (25 vs 305 IU/ml)
  - Late onset asthma=Lower IgE
- Later onset/low IgE obese asthmatics improved PC20 while no effect seen in low IgE/early onset
- Suggest weight loss will be more effective in some phenotypes

Dixon AE et al J Allergy Clin Immunol 2011
Azithromycin for Type-2 Lo asthma?

- Macrolide antibiotics inconsistent efficacy
  - Suggestion that azithro improves asthma quality of life in neutrophilic asthma
    - Simpson AJRCCM 2009
  - Azithro not efficacious in 100+ severe subjects with Type-2 Lo asthma as defined by Lo FeNO alone
    - But suggestion of response in those with low blood eos and FeNO

Type 2-Lo: Bronchial Thermoplasty

- Heat airways to 65°C
- 3 bronchoscopies
- Severe asthmatics excluded on basis of FEV1, sinus disease (rules out many Type 2- Hi)
- High # of exacerbations in 1st 3 mos post
- Short term efficacy modest with long term data flawed
- ATS-ERS task force recommends only doing in setting of IRB approved registry or clinical trial
Conclusions

• Asthma phenotyping has evolved from clinical to molecular
  ▪ ‘Omics platforms have increased granularity
  ▪ Incorporation of molecularly targeted therapies, multiple cell types will further advance our understanding
• However, incorporation of targeted biologic therapies to these molecular phenotypes required to confirm “causality” and endotypes

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