Developmental Disorders and Allergic Disease in the United States

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Disclosures

• I have no financial relationships or affiliations to disclose
Objectives

• Examine the association between developmental disorders (DD) and allergic disease in the U.S. population

• Discuss a potential novel mechanism of IgE-independent mast cell stimulation by food-derived peptides

DD and Eosinophilic Esophagitis (EoE) at UVA

• Of the 250 patients under 18yo at UVA, 30 have a diagnosis of Autism Spectrum Disorder (ASD) (12%)

• 50 have a diagnosis of ASD, Asperger’s syndrome, pervasive developmental disorder, or developmental disorder of speech and language (20%)
Reported an association between neurodevelopmental disorders and eosinophilic esophagitis (EoE)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental Delay</td>
<td>48</td>
<td>12.4</td>
</tr>
<tr>
<td>Prematurity</td>
<td>28</td>
<td>7.3</td>
</tr>
<tr>
<td>Depression/anxiety</td>
<td>63</td>
<td>16.3</td>
</tr>
<tr>
<td>Autism/behavioral disorder</td>
<td>32</td>
<td>8.3</td>
</tr>
<tr>
<td>Mitochondrial disorder</td>
<td>3</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Developmental Disorders (DD)

- Individuals with Disabilities Education Act (IDEA)
- 6.5 million children
- Children with DD may have an increased odds of asthma, respiratory allergies, eczema and food allergies
ASD and Allergies

Association of Food Allergy and Other Allergic Conditions With Autism Spectrum Disorder in Children

Gufeng Xu, MD; Linda G. Snetselaar, PhD; Jin Jing, MD, PhD; Buyun Liu, MD, PhD; Lane Strathearn, MBBS; FRACP, PhD; Wei Bao, MD, PhD

- **Design**: Cross-sectional study of NHIS (National Health Interview Survey)
- **Population**: 199,520 children 3-17 yo with self-reported food allergy
- **Results**: Children with ASD reported higher food, respiratory and skin allergies, adjusted for age, sex, race, SES, geographic region and other allergic conditions
Limitations

• Broad question on self-reported food allergy

• No lab data collected ∴ no IgE

• Residual confounders

Objectives

• To examine the association between developmental delay (DD) and allergic disease, including specific food allergies and allergen-specific IgE (sIgE)
National Health and Nutrition Examination Survey

- Survey conducted by the CDC designed to be nationally representative
- ~5000 individuals from 15 counties across the United States annually
- Interviews in homes, physical exams and blood draws in mobile centers
- 80% response rate among those interviewed

Methods

- **Study design:** Cross-sectional study of children ages 1-17 years enrolled in NHANES
- **Developmental disorders:** Received special education or early intervention services
- **Allergic outcomes:** Self-reported asthma (NHANES 2005-2010), food allergy (2007-2010), hay fever (2005-2010) and sensitization (sIgE≥0.35 kU/L) to aeroallergen or foods (2005-2006)
- **Statistical analysis:** Multivariable logistic regression, adjusted for race, age, sex, household smokers/income/insurance/education, number of annual healthcare visits, and LBW
Demographics

<table>
<thead>
<tr>
<th></th>
<th>DD (n = 799)</th>
<th>Controls (n=9,386)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>10.2 ± 0.20</td>
<td>9.63 ± 0.09</td>
<td>0.015</td>
</tr>
<tr>
<td>Male (%)</td>
<td>67.0</td>
<td>49.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Race/ethnicity (%)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Caucasian</td>
<td>59.7</td>
<td>58.1</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>18.7</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>13.9</td>
<td>20.5</td>
<td></td>
</tr>
<tr>
<td>Household smokers</td>
<td>24.0</td>
<td>14.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>College educated</td>
<td>53.3</td>
<td>56.2</td>
<td>0.203</td>
</tr>
<tr>
<td>Annual healthcare visits ≥2</td>
<td>73.3</td>
<td>64.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low birth weight (%)</td>
<td>18.1</td>
<td>10.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Self-reported Respiratory Allergic Disease

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Adjusted OR* (95% CI)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>7335</td>
<td>1.54 (1.13 – 2.09)</td>
<td>0.007</td>
</tr>
<tr>
<td>Current asthma</td>
<td>7322</td>
<td>1.45 (1.00 – 2.11)</td>
<td>0.05</td>
</tr>
<tr>
<td>Recent attack</td>
<td>6874</td>
<td>1.64 (1.11 – 2.44)</td>
<td>0.02</td>
</tr>
<tr>
<td>ED/urgent care</td>
<td>6480</td>
<td>1.39 (0.73 – 2.44)</td>
<td>0.31</td>
</tr>
<tr>
<td>Wheezing</td>
<td>10179</td>
<td>1.54 (1.11 – 2.13)</td>
<td>0.01</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>10169</td>
<td>1.51 (0.95 – 2.35)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

manuscript under review
Self-reported Food Allergy

<table>
<thead>
<tr>
<th>n</th>
<th>Adjusted OR* (95% CI)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-reported FA</td>
<td>5915</td>
<td>1.06 (0.67 – 1.70)</td>
</tr>
<tr>
<td>Peanut</td>
<td>2.13 (0.68 – 6.65)</td>
<td>0.19</td>
</tr>
<tr>
<td>Milk</td>
<td>2.30 (1.18 – 4.46)</td>
<td>0.02</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.71 (0.09 – 5.49)</td>
<td>0.73</td>
</tr>
<tr>
<td>Egg</td>
<td>3.58 (1.09 – 11.8)</td>
<td>0.04</td>
</tr>
<tr>
<td>Soy</td>
<td>3.64 (0.40 – 33.1)</td>
<td>0.24</td>
</tr>
<tr>
<td>Fish</td>
<td>0.53 (0.11 – 2.54)</td>
<td>0.42</td>
</tr>
<tr>
<td>Shrimp</td>
<td>0.78 (0.26 – 2.28)</td>
<td>0.56</td>
</tr>
<tr>
<td>Corn</td>
<td>2.14 (0.87 – 5.26)</td>
<td>0.12</td>
</tr>
<tr>
<td>Tree nuts</td>
<td>2.04 (0.43 – 9.66)</td>
<td>0.35</td>
</tr>
<tr>
<td>Other</td>
<td>0.37 (0.21 – 0.66)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Sensitization (sIgE)

<table>
<thead>
<tr>
<th>n</th>
<th>Adjusted OR* (95% CI)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitization (sIgE)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>3706</td>
<td>1.83 (1.04 – 3.22)</td>
</tr>
<tr>
<td>Milk</td>
<td>3661</td>
<td>1.05 (0.58 – 1.93)</td>
</tr>
<tr>
<td>Egg</td>
<td>3696</td>
<td>0.85 (0.45 – 1.62)</td>
</tr>
<tr>
<td>Shrimp</td>
<td>2779</td>
<td>0.45 (0.24 – 0.84)</td>
</tr>
<tr>
<td>Aeroallergens</td>
<td>2948</td>
<td>0.95 (0.48 – 1.96)</td>
</tr>
</tbody>
</table>
Results

- Children with DD had an increased odds of **self-reported** asthma, milk and egg allergy, and a trend towards increased allergic rhinitis
- There was no association between DD and specific IgE to milk, egg, or aeroallergens

What do these results mean?

- Immune dysregulation is thought to play a role in the development of both atopic disease and DD

However our findings suggest the discrepancy could be from:
- Misdiagnosis
- Increased perception of allergic disease
- A true increase in non IgE-mediated allergic disease
As we were thinking of this in our EoE cohort, a patient brought up A2 milk...

A1 versus A2 milk

- EoE is notably increasing in prevalence in countries where A1 milk is consumed
Beta-casomorphin 7 (BCM7)

- Beta-casomorphin 7 (BCM7) - opioid receptor agonist peptide that cross the blood brain barrier
- Association with ASD
- Shown to stimulate rat peritoneal mast cells but this has not been reported in humans

BCM7 and G7
Mas-related G Protein Coupled Receptor 2 (MRGPRX2)

- The MRGPRX2 receptor on mast cells recognizes a range of cationic substances, including opiates
- Implicated in numerous diseases including atopic dermatitis, asthma, chronic idiopathic urticaria, and non-IgE mediated drug reactions

Objectives

- To determine if BCM7 and G7 stimulate mast cells (MCs)
- To investigate if this response is via MRGPRX2
Methods

Cells:
• LUVA
• HEK293 +/- MRGPRX2
• Peripherally-differentiated MCs (CD34+)

Stimulation: Commercially-available BCM7 and G7

Measurements:
• Prostaglandin D2 (PGD2) by ELISA
• Transcript expression by RT-PCR
• Calcium flux by microscopy

PGD2 release in LUVA and CD34+
Gene Transcript Expression in LUVA

Calcium flux in LUVA
Presence of MRGPRX2

Gene Transcript Expression in HEK293+MRGPRX2
Conclusions

• LUVA and peripherally-differentiated MCs are stimulated by BCM7 and G7 as evaluated by PGD2 release, induction of gene transcript expression, and calcium flux

• This appears to be mediated by the MRGRPX2 receptor, as we showed increased gene transcripts in HEK293 cells transfected with MRGRPX2

Role in EoE?

• Despite the name, eosinophilic inflammation alone is not sufficient to cause EoE

• Although EoE is triggered by food antigens, it is not mediated by IgE

• MCs are increasingly recognized as being important in the pathogenesis of EoE and are thought to drive fibrosis in asthma

• Milk and wheat are the most common triggers in EoE
Working Model of EoE

BCM7 and G7 activation of MCs offers a novel model for the development of inflammation and fibrosis in EoE.

Future Directions

- Validate findings for MRGPRX2
- Look for MC mediators that contribute to eosinophil recruitment and fibrosis
- Quantify MCs and extracellular tryptase in esophageal biopsies
Conclusions

• Children with DD were more likely to have self-reported asthma, milk, and egg allergy, but not sensitization to either these foods or aeroallergens
• Whether patients with DD have a true increased risk of atopic disease, a higher rate of misdiagnosis or access to care, or a higher prevalence of non-IgE mediated disorders warrants further study
• Human MCs are stimulated by BCM7 and G7, possibly through the MRGPRX2 receptor
• Further investigations into whether BCM7 and G7 are driving the inflammation and fibrosis seen in EoE (and possibly ASD) are ongoing

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References


2. Guifeng Xu, M.L.G.S., PhD; Jun Jung, MD, PhD; Buyun Liu, MD, PhD; Lane Strathearn, MBBS, FRACP, PhD; Wei Bao, MD, PhD, Association of food allergy and other allergic conditions with autism spectrum disorder in children. JAMA Network Open, 2018.


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Methods

- Determine expression of MRGPRX2 on LUVA and HEK293 MCs transfected with the MRGPRX2 receptor (HEK293+MRGPRX2) via flow cytometry
- Incubate LUVA and peripherally-derived MCs (CD34+) with commercially-available BCM7 and G7, and measure PGD2 by ELISA as a marker of activation
- Incubate LUVA and HEK293 cells with and without transfected MRGPRX2 receptor with BCM7 and G7 and measure transcript expression by RT PCR
- Incubate LUVA and HEK293+MRGPRX2 cells with Fluo-4 and stimulate with BCM7 to detect calcium flux
DD and immune dysregulation

• Mutations in several immunoregulatory genes have been identified in association with ASD, and maternal autoimmune disease and immune system activation have been shown to be risk factors for the development of DD.
• Additionally, and most important for the development of allergic disease, children with ASD have been shown to have an imbalance of T cell subsets and elevated levels of Th2 associated cytokines (IL-4, IL-5 and IL-13, compared to matched controls)

Misdiagnosis or over-reporting?

• Self-reported food reactions overestimate the true prevalence of food allergy confirmed by ingestion challenge
• Patients with DD have increased health care utilization leading to possible reporting bias
• Patients with DD have comorbid conditions and more frequent respiratory infections that can mimic allergies but are not associated with allergic sensitization
Non-IgE-mediated allergic type diseases

- Diagnoses of food intolerances and eosinophilic esophagitis are more common in children with ASD
- Milk and wheat are the two most common food triggers in EoE and have also been implicated in IBS, MCAS and non-celiac gluten sensitivity
- Improvement in communication, attention and hyperactivity with gluten and casein-free diet