UTHealth-Houston Research Team

- PI: Mohammad H. Rahbar, PhD (Biostatistics; Epidemiology)
- Co-I: Katherine Loveland, PhD (Psychology)
- Co-I: Eric Boerwinkle, PhD (Genetics)
- Co-I: Jan Bressler, PhD (Genetics)
- Co-I: Deborah Del Junco, PhD (Epidemiology)
- Co-I: Deborah Pearson, PhD (Psychology)
- Manouchehr Ardjomand-Hessabi, MD, MPH (Research Assistant)
- Aisha Dickerson (PhD Candidate, Epidemiology)
- Megan Grove (Manager, Genetics Lab)
- Consultant: Julie Wirth, PhD (Microbiology; Epidemiology)
UWI Research Team and Collaborators

- PI: Maureen Samms-Vaughan, MD (Pediatrics), PhD (Epidemiology)
- Wayne McLaughlin, PhD (Molecular Biology)
- Gerald Lalor, PhD (Chemistry)
- Roxanne Melbourne – Chambers, MD (Pediatric Neurology)
- Judy Tapper, MD (Pediatric Neurology)
- Sydonnie Pellington (Research Assistant/Coordinator)
- Compton Beecher (Manager, Genetics Lab)
- Ann – Marie Graham (Data Manager)
- Samantha Foote (Data Entry Operator)
Epidemiological Research on Autism in Jamaica (ERAJ)

• In June 2009 our R21 grant was funded by NICHD and Fogarty International Center (FIC) as part of PA, “Brain Disorders in the Developing World: Research Across the Lifespan”.

• The partners in this collaboration include UTHealth, University of West Indies (UWI) and a consultant from Michigan State University (MSU)

• The no-cost extension period will end on December 31, 2012
Specific Aims of the R21 project

1. To develop increased collaboration among teams at UTHealth and the UWI in order to build capacity for collecting genetic data and conducting epidemiologic research on Autism Spectrum Disorders (ASDs) in Jamaica

2. To conduct pilot studies to compare ASD case-finding and case-ascertainment approaches that will be used in future studies

3. To conduct a pilot study to investigate whether environmental exposures to mercury, lead, arsenic and cadmium play a role in autism and to assess the role of select polymorphisms in glutathione-S-transferase (GST) genes, and their potential interactions with these heavy metals in relation to ASD
Study Design
(Matched Case-Control)

- Cases include about 150 children with ASD, 2-8 years
- Controls include age- and sex-matched children without ASD
- Parents/guardians of both cases and controls are also included in this study
- We administered a questionnaire to assess demographic and SES information, parental history, parental education levels, medical history of children, and potential exposure to heavy metals through food with a particular focus on the types and amount of vegetables and seafood consumed by children or parental occupation
- We draw 5 mL of whole blood from each child, 2 mL of saliva (parents and children), and hair samples (only from children with long hair, at least 3 inches) to be analyzed for a variety of environmental and genetic exposures
METHODS…

❖ Cases:

- Recruited from Dr. Maureen Samms-Vaughan’s (PI –UWI) existing database of ASD cases identified through a composite process involving family history (comprehensive family, developmental and behavioral), clinical observation based on the Diagnostic Statistical Manual of Mental Disorders (DSM-IV-TR) criteria and the Childhood Autism Rating Scale (CARS)

- To confirm cases, we administer the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview-Revised (ADI-R) to children and parent/guardian, respectively

❖ Controls:

- Recruited from well-child clinics, community churches, and schools.

- To confirm non-ASD status in controls, we administer the Social Communication Questionnaire (SCQ). Children with major neurodevelopmental disability or major congenital malformation are excluded from the study
Capacity Building and Training

- Our UWI colleagues visited UTHealth for a 3-day workshop, conducted by Dr. Katherine A. Loveland, for training in the administration of ADI-R
- Our UWI colleagues were trained in the administration of ADOS at the University of Michigan (U of M)
- Dr. Eric Boerwinkle and Megan Grove visited UWI to tour the facilities, and provide training for handling of genetics specimens
- Dr. Rahbar visited the colleagues at the UWI in 2009, 2010, and 2011
- Dr. Rahbar conducted a week-long biostatistics workshop at the UWI in March 2011
Enrollment
Enrollment completed on March 31, 2012

Projected Enrollment

Number enrolled

Research Assistant/Coordinator was on Maternity Leave
Demographic and socioeconomic characteristics of children by ASD case status based on n = 150 Matched-Pairs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Categories</th>
<th>Case (n=150)</th>
<th>Control (n=150)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>129 (86)</td>
<td>129 (86)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>21 (14)</td>
<td>21 (14)</td>
</tr>
<tr>
<td>Race *</td>
<td>Black (African)</td>
<td>139 (92.7)</td>
<td>147 (99.3)</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>11 (7.3)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Age of Child (months)</td>
<td>Age &lt; 48</td>
<td>43 (28.7)</td>
<td>41 (27.3)</td>
</tr>
<tr>
<td></td>
<td>48 ≤ Age ≤ 71</td>
<td>58 (38.7)</td>
<td>59 (39.3)</td>
</tr>
<tr>
<td></td>
<td>Age ≥72</td>
<td>49 (32.7)</td>
<td>50 (33.3)</td>
</tr>
</tbody>
</table>

* Race was missing for 2 controls

M/F Ratio = 6
In the univariate analysis using General Linear Models (GLM), we found significant associations between ASD in children and maternal & paternal age.

In the Multivariate GLM, when adjusted for parity, gestational age, and parental education levels, the joint effects of parental ages remained statistically significant.

Multivariate GLM approach reduces the effect of multicollinearity potentially caused by a high correlation \((r = 0.57)\) between the age of parents.

### Associations between parental age and case status using MGLM based on 68 matched pairs

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Paternal Age (^a) Adjusted Mean</th>
<th>Paternal Mean Age difference (95% CI)</th>
<th>Maternal Age (^a) Adjusted Mean</th>
<th>Maternal Mean Age difference (95% CI)</th>
<th>(P)-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case status, Pairs (^b)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>35.7</td>
<td>4.0 (1.2, 6.8)</td>
<td>30.4</td>
<td>4.8 (2.5, 7.2)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Control</td>
<td>31.7</td>
<td></td>
<td>25.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Case status, Pairs, Gestational Age, Parity, Parental levels of education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>36.4</td>
<td>5.9 (2.6, 9.1)</td>
<td>31.2</td>
<td>6.5 (4.0, 8.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Control</td>
<td>30.5</td>
<td></td>
<td>24.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Parental age at child’s birth. \(^b\) A set of 67 dummy variables that represent 68 matched pairs.

* \(P\)-values are based on the overall F-test of MGLM (Wilks’ Lambda).
The Trace Metals Lab at Michigan Department of Community Health (MDCH) (certified by the CDC) conducted analysis of trace metals.

The whole venous blood samples were assayed for lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), and manganese (Mn).

MDCH followed a fully validated protocol for analyzing Pb, Cd, and Hg in the samples with detection limits of 0.3μg/dL, 0.2μg/L, and 0.3μg/L, respectively.

Mn and As are analyzed through a screening method with no limit of detection.
Children living in Jamaica have higher blood mercury concentrations compared with those of children in developed countries including the US and Canada.

In the univariable analysis, parental education levels, maternal age, and levels of seafood consumption are significantly associated with both ASD status and having elevated blood mercury concentrations.

In the multivariable logistic regression model, after adjusting for parental education, maternal age, and frequency of seafood consumption, we did not find a significant association between ASD and blood mercury concentrations.


---

Unadjusted and adjusted mean blood mercury concentrations for 65 cases and their matched controls based on General Linear Model (GLM)

<table>
<thead>
<tr>
<th></th>
<th>Mean Hg&lt;sup&gt;b&lt;/sup&gt; Cases (μg/L)</th>
<th>Mean Hg&lt;sup&gt;b&lt;/sup&gt; Controls (μg/L)</th>
<th>P-value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>0.67</td>
<td>0.99</td>
<td>0.02</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75</td>
<td>0.82</td>
<td>0.61</td>
</tr>
</tbody>
</table>

<sup>a</sup> Factors adjusted for include: parental education levels, age of mother, and frequency of seafood consumption per week.

<sup>b</sup> Mean Hg indicates the geometric mean = Exp. [Mean (lnHg)].

<sup>c</sup> P-values are based on the log-transformed blood mercury concentrations.
Children living in Jamaica have higher blood arsenic concentrations than children in some other countries, depending on the sources and levels of exposure to arsenic.

In the final multivariable model that was developed to identify factors associated with blood arsenic concentrations, only three variables maintained their statistical significance: source of drinking water, eating avocado, and eating leafy vegetables (callaloo, broccoli, or pak choi).

Based on univariable analysis, we observed a significant difference between ASD cases and controls (4.03µg/L for cases vs. 4.48µg/L for controls, P < 0.01).

After adjusting for potential confounding variables in a multivariable conditional logistic regression model, we did not find a significant association between ASD and blood arsenic concentrations.

---


<table>
<thead>
<tr>
<th>Unadjusted and adjusted mean blood arsenic concentrations for 65 cases and their matched controls based on General Linear Model (GLM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean As</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>**Cases (µg/L)</td>
</tr>
<tr>
<td>Unadjusted</td>
</tr>
<tr>
<td><strong>Adjusted</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup> Factors adjusted for in the GLM include: car ownership, maternal age, parental education levels, source of drinking water, consumption of “yam, sweet potato, or dasheen”, “carrot or pumpkin”, “callaloo, broccoli, or pak choi”, “cabbage”, “avocado”, and the frequency of seafood consumption per week.

<sup>b</sup> Mean AS indicates the geometric mean = Exp. [Mean (lnAS)].

<sup>c</sup> P-values are based on the log-transformed blood arsenic concentrations.
Children living in Jamaica have geometric mean blood cadmium concentrations similar to the children in developed countries.

Our results show that 37.7% of children in our study had a geometric mean blood cadmium concentration above the limit of detection, 0.20µg/L.

Jamaican children who ate cabbage had higher geometric mean blood cadmium concentrations than those who did not (0.16µg/L vs. 0.11µg/L, \( P = 0.02 \)).

In the univariable, we did not find a significant association between ASD and blood cadmium concentrations (\( P = 0.21 \)). After adjusting for potential confounding variables in a multivariable conditional logistic regression model, our results were consistent with the univariable analysis (\( P = 0.68 \)).


### Unadjusted and adjusted mean blood cadmium concentrations for 65 cases and their matched controls based on General Linear Model (GLM)

<table>
<thead>
<tr>
<th></th>
<th>Mean Cd ( b ) Cases (µg/L)</th>
<th>Mean Cd ( b ) Controls (µg/L)</th>
<th>( P )-value ( c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>0.14</td>
<td>0.16</td>
<td>0.21</td>
</tr>
<tr>
<td>Adjusted ( a )</td>
<td>0.16</td>
<td>0.15</td>
<td>0.68</td>
</tr>
</tbody>
</table>

\( a \) Factors adjusted for include: parental education level, birth place in Manchester or surrounding parishes, and consumption of cabbage, “yam, sweet potato, dasheen,” and whole wheat bread.

Mean Cd indicates the geometric mean = \( \exp[\text{Mean (lnCd)}] \).

\( P \)-values are based on the log-transformed blood cadmium concentrations.
Overall, 4.6% of children in our sample had elevated blood manganese concentrations (i.e., >15μg/L)

Mean blood manganese concentrations for children who ate fried plantains was significantly higher than that of children who did not eat this food, (11.3 vs. 8.6μg/L; \( P=0.05 \))

Mean blood manganese concentrations for children who ate shellfish was significantly lower than that of children who did not eat this seafood, (6.2 vs. 11.0μg/L; \( P=0.04 \))

In univariable analysis, we did not find a significant association between blood manganese concentrations and ASD

After adjusting for potential confounding variables in a multivariable conditional logistic regression model, we did not find a significant association between ASD and blood manganese concentrations


### Unadjusted and adjusted mean blood manganese concentrations for 65 cases and their matched controls based on General Linear Model (GLM)

<table>
<thead>
<tr>
<th></th>
<th>Mean Mn Cases (μg/L)</th>
<th>Mean Mn Controls (μg/L)</th>
<th>( P)-value $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>10.8</td>
<td>10.8</td>
<td>0.95</td>
</tr>
<tr>
<td>Adjusted $^a$</td>
<td>11.0</td>
<td>10.6</td>
<td>0.61</td>
</tr>
</tbody>
</table>

$^a$ Factors adjusted for include: consumption of tuna, shellfish, fried plantains, ackee, and whole wheat
Our results do not support an association between total blood lead concentrations and ASD in Jamaican children 2-8 years of age.

We did not find any significant associations between the GST genotypes either with the blood lead concentrations or the ASD status.

Overall, 3.4% of all children living in or near Kingston area had an elevated blood lead concentrations (≥10μg/dL).

After adjusting for potential confounding variables, we did not find a significant association between ASD and blood lead concentrations.


Unadjusted and adjusted mean blood lead concentrations for 59 cases and their matched controls based on General Linear Model (GLM)

<table>
<thead>
<tr>
<th></th>
<th>Mean Pb $^a$ Cases (μg/dL)</th>
<th>Mean Pb $^a$ Controls (μg/dL)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>2.47</td>
<td>2.70</td>
<td>0.46</td>
</tr>
<tr>
<td>Adjusted $^a$</td>
<td>2.84</td>
<td>2.81</td>
<td>0.94</td>
</tr>
</tbody>
</table>

$^a$ Factors include; Living in a house with paint peeling or chipping off, using Teflon pots, eating Shellfish (Lobsters, Crabs), and GSTT1.

$^b$ Mean lead indicates the geometric mean = Exp. [Mean (lnPb)].
There were no significant differences between case and control groups with respect to the distribution of genotypes of GSTM1, GSTP1, and GSTT1 (all $P > 0.66$).

When adjusted for ASD status, there was no significant association between the blood lead concentrations and the GST genotypes, except for GSTT1 that had a marginally significant association ($P = 0.10$).

<table>
<thead>
<tr>
<th>Genes</th>
<th>Genotypes</th>
<th>N (%)</th>
<th>Geometric Mean Pb(μg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Case</td>
<td>Control</td>
</tr>
<tr>
<td>GSTP1</td>
<td>AA</td>
<td>14 (23.7)</td>
<td>13 (22.0)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>34 (57.6)</td>
<td>34 (57.6)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>11 (18.7)</td>
<td>12 (20.4)</td>
</tr>
<tr>
<td>GSTM1</td>
<td>DD</td>
<td>20 (33.9)</td>
<td>19 (32.2)</td>
</tr>
<tr>
<td></td>
<td>I*</td>
<td>39 (66.1)</td>
<td>40 (67.8)</td>
</tr>
<tr>
<td>GSTT1</td>
<td>DD</td>
<td>15 (25.4)</td>
<td>13 (22.0)</td>
</tr>
<tr>
<td></td>
<td>I*</td>
<td>44 (74.6)</td>
<td>46 (78.0)</td>
</tr>
</tbody>
</table>

GSTP1, glutathione S-transferase pi 1; GSTM1, glutathione S-transferase mu 1; GSTT1, glutathione S-transferase theta 1

There are three different GSTP1 genotypes (AA, AG, and GG); the A allele is the wild-type allele and the G allele is the variant allele.

The asterisk (*) means the individual is a carrier of the gene insertion, but the assay cannot detect if they are a homozygote (I/I) or a heterozygote (I/D).

GSTM1-DD and GSTT1-DD are null alleles.

Mean lead indicates the geometric mean = Exp. [Mean (lnPb)]
Challenges

- Transportation of specimens to the US:
  - A license from the Jamaican Government is required to ship specimens outside Jamaica
  - Jamaican government does not allow dry ice in exported shipments
  - CryoPort containers had to be used to keep specimens frozen without the use of dry ice
  - Potential inspection delays by the FDA increase the risk of samples thawing before reaching UTHealth

- Difficulty to obtain hair samples from Jamaican boys due to their short hair
Future Research Directions

- We submitted our R01 proposal to NIEHS in January 2012
- FIC and NICHD are also involved
- R01 extends our effort to investigate other chemicals (PCBs and OC pesticides)
- Our proposed research builds upon Jamaica (JA) Kids Birth Cohort
  - JA Kids study enrolled about 10,000 mothers in the 3rd trimester
    - April 1, 2011 to June 31, 2011
    - July 1, 2011 to September 30, 2011 (about 10,000 Newborns)
- Our R01 will provide an enormous opportunity to expand our collaboration with the UWI for many years to come
Thank you! Any questions?