Cross-tissue integration of genetic and epigenetic data offers insight into autism spectrum disorder

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What can we learn by integrating ASD genetic and epigenetic information?

**Background:**
- Epigenetic variation contributes to gene regulation/expression
- Epigenetic variation is tissue and timing dependent
- Epigenetic variation is in part controlled by genetic variation

![Diagram showing the relationship between genotype (SNP), epigenotype, and DNA methylation (DNAm). The term “meQTL” is used to indicate a genetic variant affecting gene expression through epigenetic mechanisms.](image-url)
What can we learn by integrating ASD genetic and epigenetic information?

**Background:**

- Epigenetic variation contributes to gene regulation/expression
- Epigenetic variation is tissue and timing dependent
- Epigenetic variation is in part controlled by genetic variation
  - Genetic-epigenetic “maps” can be created, by tissue

meQTL “target”
meQTL “Maps” Across Tissues

• From joint genotype and methylation data of
  • Peripheral blood (discovery in SEED, 2-5 yo)
  • Cord blood (discovery in EARLI, birth)
  • Fetal Brain (Mill, published list)

Child blood
Genotype ➔ Epigenotype

Cord (infant) blood
Genotype ➔ Epigenotype

Fetal brain tissue
Genotype ➔ Epigenotype
What can we learn by integrating ASD genetic and epigenetic information?

What can we learn using meQTL information?
1. Are ASD-associated SNPs enriched for meQTLs for particular tissues including blood?
2. Do ASD-associated SNP meQTL targets (CpGs) point to particular biology?
3. Do ASD-associated SNP meQTL targets point to genes not previously implicated?
What can we learn using meQTL information?

1. Are ASD-associated SNPs enriched for meQTLs for particular tissues including blood?
What can we learn using meQTL information?

1. Are ASD-associated SNPs enriched for meQTLs for particular tissues including blood?  
   ✓ YES

<table>
<thead>
<tr>
<th>Tissue</th>
<th>meQTL FDR = 10%</th>
<th>meQTL FDR = 5%</th>
<th>meQTL FDR = 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal brain</td>
<td>1.70 (&lt;0.001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>1.22 (&lt;0.001)</td>
<td>1.20 (&lt;0.001)</td>
<td>1.23 (&lt;0.001)</td>
</tr>
<tr>
<td>Cord blood</td>
<td>1.14 (0.032)</td>
<td>1.21 (0.011)</td>
<td>1.20 (0.023)</td>
</tr>
<tr>
<td>Lung</td>
<td>—</td>
<td>1.09 (0.343)</td>
<td>—</td>
</tr>
</tbody>
</table>

Enrichment fold statistics and P values based on 1000 permutations.  
aLD pruning performed with 1000 Genomes CEU samples  
bLD pruning performed with the study-specific genotype data. See Methods for additional details.
What can we learn in autism using meQTL information?
1. Are ASD-associated SNPs enriched for meQTLs for particular tissues including blood?
   ✓ YES

Table 2: Enrichment statistics for meQTLs derived from 4 tissue types in ASD GWAS SNPs

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>ASD P value = 1e-03</th>
<th>ASD P value = 1e-04</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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Enrichment fold statistics and P values based on 1000 permutations.
PD pruning performed with 1000 Genomes CEU samples.
PD pruning performed with the study-specific genotype data. See Methods for additional details.
What can we learn using meQTL information?

✓ Are ASD-associated SNPs enriched for meQTLs for particular tissues including blood? YES

2. Do ASD-associated SNP meQTL targets (CpGs) point to particular biology?
Table 3 Gene Ontology terms significantly enriched in multiple tissue types in comparison of ASD-related meQTL targets to meQTL targets generally

<table>
<thead>
<tr>
<th>Term</th>
<th>Peripheral blood scaled rank&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cord blood scaled rank&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fetal brain scaled rank&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response to interferon-gamma</td>
<td>0.14</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Positive regulation of relaxation of cardiac muscle</td>
<td>0.20</td>
<td>0.46</td>
<td>0.30</td>
</tr>
<tr>
<td>Production of molecular mediator of immune response</td>
<td>0.65</td>
<td>0.22</td>
<td>0.28</td>
</tr>
<tr>
<td>Cellular response to interferon-gamma</td>
<td>NA</td>
<td>0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>Detection of bacterium</td>
<td>NA</td>
<td>0.18</td>
<td>0.06</td>
</tr>
<tr>
<td>Detection of biotic stimulus</td>
<td>NA</td>
<td>0.26</td>
<td>0.04</td>
</tr>
<tr>
<td>T-helper 1 type immune response</td>
<td>NA</td>
<td>0.08</td>
<td>0.34</td>
</tr>
<tr>
<td>Regulation of interleukin-10 secretion</td>
<td>NA</td>
<td>0.09</td>
<td>0.43</td>
</tr>
<tr>
<td>Interferon-gamma production</td>
<td>NA</td>
<td>0.57</td>
<td>0.19</td>
</tr>
<tr>
<td>Regulation of interleukin-4 production</td>
<td>NA</td>
<td>0.24</td>
<td>0.62</td>
</tr>
<tr>
<td>Interleukin-4 production</td>
<td>NA</td>
<td>0.29</td>
<td>0.60</td>
</tr>
<tr>
<td>Interleukin-10 production</td>
<td>NA</td>
<td>0.25</td>
<td>0.74</td>
</tr>
<tr>
<td>Tongue development</td>
<td>NA</td>
<td>0.68</td>
<td>0.32</td>
</tr>
<tr>
<td>Inflammatory response to antigenic stimulus</td>
<td>NA</td>
<td>0.32</td>
<td>0.81</td>
</tr>
<tr>
<td>Endochondral bone growth</td>
<td>NA</td>
<td>0.71</td>
<td>0.53</td>
</tr>
<tr>
<td>Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II</td>
<td>0.01</td>
<td>0.05</td>
<td>NA</td>
</tr>
<tr>
<td>T-cell costimulation</td>
<td>0.05</td>
<td>0.01</td>
<td>NA</td>
</tr>
<tr>
<td>Positive regulation of hormone secretion</td>
<td>0.09</td>
<td>0.04</td>
<td>NA</td>
</tr>
<tr>
<td>Antigen receptor-mediated signaling pathway</td>
<td>0.08</td>
<td>0.13</td>
<td>NA</td>
</tr>
<tr>
<td>Immunoglobulin production involved in immunoglobulin mediated immune response</td>
<td>0.24</td>
<td>0.03</td>
<td>NA</td>
</tr>
<tr>
<td>Single organism cell-cell adhesion</td>
<td>0.23</td>
<td>0.12</td>
<td>NA</td>
</tr>
<tr>
<td>Single organism cell adhesion</td>
<td>0.34</td>
<td>0.16</td>
<td>NA</td>
</tr>
<tr>
<td>Negative regulation of nonmotile primary cilium assembly</td>
<td>0.16</td>
<td>0.39</td>
<td>NA</td>
</tr>
<tr>
<td>Antigen processing and presentation of polysaccharide antigen via MHC class II</td>
<td>0.02</td>
<td>0.58</td>
<td>NA</td>
</tr>
</tbody>
</table>

<sup>a</sup> Scaled rank is calculated using a statistical method to compare enrichment of GO terms in different tissue types.
What can we learn using meQTL information?

☑ Are ASD-associated SNPs enriched for meQTLs for particular tissues including blood? YES

2. Do ASD-associated SNP meQTL targets (CpGs) point to particular biology?
   ☑ YES - Blood, Cord blood, and Fetal Brain ASD meQTL targets implicate the immune system
     ▪ Consistent with ASD findings to date:
       ▪ Genetic variation does not (generally) point to immune system
       ▪ Expression (and now methylation) results do, as well as many epidemiologic findings
Immune System Implicated by Expression and Methylation

**Brain Studies**

Transcriptomic analysis reveals dysregulation of innate immune response genes and neuronal activity-dependent genes in autism

Irina Voineagu, Xinchen Wang, Patrick Johnston, Jennifer K. Lowe, Yuan Tian, Steve Horvath, Jonathan Miller, Rita M. Cantor, Benjamin J. Blencowe, & Daniel H. Geschwind

**Blood Studies**

Peripheral blood gene expression signature differentiates children with autism from unaffected siblings


Transcriptome Profiling of Peripheral Blood in 22q11.2 Deletion Syndrome Reveals Functional Pathways Related to Psychosis and Autism Spectrum Disorder

Maria Jalbrzikowski, Maria T. Lazaro, Fuying Gao, Alden Huang, Carolyn Chow, Daniel H. Geschwind, Giovanni Coppola, Carrie E. Bearden

DNA methylation analysis of the autistic brain reveals multiple dysregulated biological pathways

S. Nardone, D. Sharan Sams, E. Reuveni, D. Getsele, O. Oron, M. Karpuj, and E. Elliott
What can we learn using meQTL information?

✓ Are ASD-associated SNPs enriched for meQTLs for particular tissues including blood? YES
✓ Do ASD-associated SNP meQTL targets (CpGs) point to particular biology? Immune system
3. Do ASD-associated SNP meQTL targets point to genes not previously implicated?
What can we learn using meQTL information?

✓ Are ASD-associated SNPs enriched for meQTLs for particular tissues including blood? **YES**
✓ Do ASD-associated SNP meQTL targets (CpGs) point to particular biology? Immune system

3. Do ASD-associated SNP meQTL targets point to genes not previously implicated?
Fig. 1 'Expansion' of ASD loci through meQTL mapping in peripheral blood, cord blood, and fetal brain. Each tissue-specific panel presents, from bottom to top: genomic location, gene annotations, SNP locations, SNP-CpG associations, CpG locations. Light gray meQTL association lines denote all SNP to CpG associations in that tissue type; Dark meQTL association lines denote SNP-CpG associations for ASD-associated SNPs in PGC (P value ≤ 1e-04). a Locus at chr8. b Locus at chr19. Data are presented for meQTL maps for fetal brain (top); cord blood meQTLs (middle), and peripheral blood meQTLs (bottom). Please note locus coordinates differ from those in Supplementary Data 6 because in this context they encompass the locations of meQTL target CpG sites.
What can we learn using meQTL information?

✓ ASD-associated SNPs are enriched for meQTLs for particular tissues including blood?
✓ ASD-associated SNP meQTL targets (CpGs) point to particular biology
✓ ASD-associated SNP meQTL targets point to genes not previously implicated

➢ Blood–based meQTL information pointed to similar conclusions!
△ Some important limitations regarding meQTL lists and ASD SNP list
# Summary of SEED I “Omic“ Data

## SEED 1 Genotype Data

<table>
<thead>
<tr>
<th>Platform</th>
<th># SNPs</th>
<th># SEED 1 – child</th>
<th># SEED 1 – mom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omni-Quad</td>
<td>&gt;1M</td>
<td>419</td>
<td>0</td>
</tr>
<tr>
<td>Affy axiom KP</td>
<td>&gt;700K</td>
<td>173</td>
<td>0</td>
</tr>
<tr>
<td>Omni-5M+ exome</td>
<td>&gt;4.5M</td>
<td>13</td>
<td>301</td>
</tr>
<tr>
<td>Illumina MEGA</td>
<td>&gt;1.4M</td>
<td>0</td>
<td>1269***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Platform</th>
<th># CpGs</th>
<th># SEED 1 – child</th>
<th># SEED 1 – mom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illumina 450K</td>
<td>455,664</td>
<td>455</td>
<td>0</td>
</tr>
</tbody>
</table>

* measured. Imputed SNP ~ 8M

** includes possible low functioning cases (n=6)

*** genotype cleaning in-progress; numbers could change slightly
Collaborative Projects To Date

3 ASD GWAS contributions (meta-analysis and replication)
3 ASD EWAS contributions (meta-analysis)
2 non-ASD EWAS contributions (meta-analysis, PACE)
2 multi-omic collaborative projects

SEED-only methods contributions (not ASD focused):
2 EWAS / meQTL methodologic contributions
1 smoking environmental biomarker paper
Research Group

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Homay Farzadegan (PI)

JHU IGM:
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