Genetic Testing in Autism: EGL’s Tiered Approach
Affects ~ 1 in 100 children

Spectrum includes Asperger Syndrome, Autism Disorder, and PDD-NOS

Major domains affected: social interaction, language, and repetitive behaviors

4x more prevalent in males

60-92% concordance rate in monozygotic twins

5-10% concordance rate in siblings
Autism Testing Available at Emory Genetics Laboratory

**Autism Panel Complete Tier 1***
Cytogenetic, Molecular, and Biochemical

**Autism Panel Tier 1: Cytogenetics & Molecular**
*EmArray Cyto & Fragile X CGG repeat analysis*
Sample Needed: Whole Blood (Purple AND Green top tubes)

**Autism Panel Tier 1: Biochemical**
*Urine organic acid, Plasma amino acid, SLO, & LSD screen*
Sample Needed: Urine & Plasma (Green top tube)

**Autism Panel Tier 2**
*61-gene NGS Panel*
*61-gene-targeted array CGH*
*FRAXE CGG repeat analysis*
Sample Needed: Whole Blood (Purple top tube)
Pre-evaluation
Confirmation of diagnosis of autism by trained professional using objective criteria and tools
Sensory screening (complete audiogram)
Electroencephalogram—if clinical suspicion of seizures
Cognitive testing
Verify results of newborn screening

First tier
Initial evaluation to identify known syndromes or associated conditions
   Examination with special attention to dysmorphic features
      Should include Woods lamp evaluation
   If specific diagnosis is suspected, proceed with targeted testing
      Rubella titers—if clinical indicators present
      “Standard” metabolic screening—if clinical indicators present and if suspected condition was not assessed by newborn screening
      Urine mucopolysaccharides and organic acids
      Serum lactate, amino acids, ammonia, and acyl-carnitine profile
Comparative genomic hybridization (chromosomal microarray)
DNA for Fragile X—if not already performed

Second tier
Fibroblast karyotype if leukocyte karyotype is normal and clonal pigmentary abnormalities are noted
MECP2 gene testing (females only)
PTEN gene testing (if the head circumference is 2.5 SD greater than the mean)

Third tier
Brain magnetic resonance imaging
Serum and urine uric acid
   If elevated, Hypoxanthine-guanine phosphoribosyl transferase (HgPRT) and Phosphoribosylpyrophosphate (PRPP) synthetase superactivity testing
   If low, purine/pyrimidine panel (uracil excretion, xanthine, hypoxanthine)
ACMG Practice Guidelines:

First tier
Initial evaluation to identify known syndromes or associated conditions:

- Examination with special attention to dysmorphic features
  - Should include Woods lamp evaluation

If specific diagnosis is suspected, proceed with targeted testing
- Rubella titers—if clinical indicators present

“Standard” metabolic screening—if clinical indicators present and if suspected condition was not assessed by newborn screening
- Urine mucopolysaccharides and organic acids
  - Serum lactate, amino acids, ammonia, and acyl-carnitine profile

Comparative genomic hybridization (chromosomal microarray)

DNA for Fragile X—if not already performed

Chromosomal Microarray:
- CGH arrays have identified clinically relevant genomic imbalances in 7 – 20% of individuals with autism
- The yield is higher in those with “syndromic autism”

ACMG Practice Guidelines:

Fragile X:
- Approximately 2-3% of children ascertained on the basis of autism diagnosis can be shown to have fragile X syndrome
- Up to 25% of individuals with fragile X also have autistic features

First tier
Initial evaluation to identify known syndromes or associated conditions:

- Examination with special attention to dysmorphic features
  Should include Woods lamp evaluation

- If specific diagnosis is suspected, proceed with targeted testing
  Rubella titers—if clinical indicators present

- “Standard” metabolic screening—if clinical indicators present and if suspected condition was not assessed by newborn screening

- Urine mucopolysaccharides and organic acids
  Serum lactate, amino acids, ammonia, and acyl-carnitine profile

- Comparative genomic hybridization (chromosomal microarray)

- DNA for Fragile X—if not already performed
<table>
<thead>
<tr>
<th>Disorder</th>
<th>Onset</th>
<th>Clinical Characteristics</th>
<th>Diagnostic Tests</th>
<th>Potential Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylketonuria</td>
<td>Neonatal</td>
<td>Autism, severe mental retardation, seizures (in untreated patient)</td>
<td>Quantitative plasma amino acids analysis</td>
<td>Restriction diet + amino acids integration</td>
</tr>
<tr>
<td>Adenylosuccinase deficit</td>
<td>First year</td>
<td>Autistic phenotype, psychomotor retardation, epilepsy</td>
<td>Presence in urine and cerebrospinal fluid of succinyl aminomimidazole, carboxamide ribose, and succinyl adenosine</td>
<td></td>
</tr>
<tr>
<td>Creatine deficiency (guanidinoacetate methyltransferase deficiency, arginine-glycine amidinotransferase deficiency, transmembrane creatine transport deficiency)</td>
<td>3 mo-2 y</td>
<td>Autistic phenotype, mental retardation, speech delay, epilepsy, extrapyramidal symptoms and signs</td>
<td>Brain magnetic resonance spectroscopy, blood and urinary concentration on creatine and guanidinoacetate, ratio creatine/creatinine; gene mutations</td>
<td>Oral creatine supplementation</td>
</tr>
<tr>
<td>Inborn errors of cholesterol biosynthesis (variant of Smith-Lemli-Opitz syndrome)</td>
<td>After infancy</td>
<td>Autism, psychomotor retardation, poor expressive language, behavioral abnormalities</td>
<td>Abnormal sterol pattern</td>
<td>Cholesterol replacement therapy</td>
</tr>
<tr>
<td>Biotinidase deficiency</td>
<td>3-12 mo</td>
<td>Autism, ataxia, seizure, hypotonia, skin rash, alopecia, vision problems, hearing loss, organic academia</td>
<td>Serum biotinidase deficiency $\rightarrow$ sequence of gene</td>
<td>Cofactor biotin therapy</td>
</tr>
<tr>
<td>Infantile ceroid lipofuscinosin</td>
<td>≥ 2 y</td>
<td>Autistic phenotype; regressed milestones; seizures; myoclonus; choreiform movements; visual loss; ataxia</td>
<td>Histopathologic examination shows inclusion on lymphocytes and brain</td>
<td>None</td>
</tr>
<tr>
<td>Sanfilippo syndrome</td>
<td>During first y</td>
<td>Autistic phenotype; severe mental retardation; hyperactivity</td>
<td>Quantitative urinary glycosaminoglycan analysis; mutation analysis</td>
<td>Only palliative, nonspecific</td>
</tr>
<tr>
<td>Histidinemia$^{16,17}$</td>
<td>Birth</td>
<td>Risk factor for the development of autism. Mental retardation, speech disturbances, many asymptomatic patients</td>
<td>High blood histidine, elevated urinary excretion of histidine and its transamination products</td>
<td>Low histidine formula or restriction diet</td>
</tr>
<tr>
<td>Succinic semialdehyde dehydrogenase deficiency$^{18}$</td>
<td>≥ 3 mo</td>
<td>Autism or pervasive developmental disorder, delayed motor, intellectual; speech and language deafment. Nonspecific phenotypic presentation</td>
<td>$\gamma$-Hydroxybutyrate accumulations in urine, serum, and cerebrospinal fluid; enzymatic deficiency in lymphocytes and cultured lymphoblasts</td>
<td>Vigabatrin</td>
</tr>
<tr>
<td>Dihydropyrimidine dehydrogenase deficiency$^{19,20}$</td>
<td>≤ 1 y</td>
<td>Autism; seizures; motor and mental retardation; ocular abnormalities; growth retardation; microcephaly; asymptomatic individuals</td>
<td>Gas chromatography-mass spectrometry analysis of urine organic acids († uracil + thymine) $\rightarrow$ other investigations</td>
<td></td>
</tr>
</tbody>
</table>
**Second tier**

Fibroblast karyotype if leukocyte karyotype is normal and clonal pigmentary abnormalities are noted

*MECP2* gene testing (females only)

*PTEN* gene testing (if the head circumference is 2.5 SD greater than the mean)

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**Rett Syndrome:**

- *MECP2* mutations have been reported in approximately 1% of children diagnosed with autism

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**Second tier**
Fibroblast karyotype if leukocyte karyotype is normal and clonal pigmentary abnormalities are noted
*MECP2* gene testing (females only)
*PTEN* gene testing (if the head circumference is 2.5 SD greater than the mean)

**PTEN Macrocephaly Syndrome:**

- Frequency of *PTEN* mutations as a cause of ASD is unclear
- Results from studies of children ascertained through autism and macrocephaly range from 1% to 8.3% to 17%
- Children with ASD who are found to have a *PTEN* mutation generally have extreme macrocephaly ranging from +3.7 to +9.6 SD (average: +5.6 SD)

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Autism Panel Tier 2
61-gene NGS Panel
61-gene-targeted array CGH
FRA X CCG repeat analysis
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For individuals with a diagnosis of ASD, approximately 10% will have clinically relevant genomic imbalances by cytogenetic array.

For individuals with a diagnosis of ASD, 2-3% will have fragile X syndrome. CGG repeat expansion testing will indicate full mutation and premutation status.

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- **Autism Panel Tier 1: Cytogenetics & Molecular**
  - EmArray Cyto
  - Fragile X CGG repeat analysis
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Expanded phenotypes for some metabolic conditions can include ASDs
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Sample Needed: Whole Blood (Purple top tube)
Contains 61 genes which have ASDs or autistic features as part of the clinical profile, genes that have been associated with non-syndromic ASDs, and genes associated with conditions involved in the differential diagnosis of Rett syndrome and/or Angelman syndrome.
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**FRAXE (AFF2/FMR2) CCG repeat analysis**
- This testing indicates CCG repeat size and methylation status
## Tier 2 Autism NGS Panel – 61 genes

<table>
<thead>
<tr>
<th>Gene</th>
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<tr>
<td>ADSL</td>
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<td>FOXP2</td>
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<td>GABRB3</td>
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<td>GLUT1/SLC2A1</td>
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</table>
Thank You!