Autism is a behaviorally defined lifelong neurodevelopmental disorder, with strong evidence for a complex genetic predisposition.
Prevalence

“The Centers for Disease Control & Prevention (CDC) estimates the prevalence of autism spectrum disorder (ASD) in children is 1 in 68. This new estimate roughly represents a 30% increase from previous estimates reported in 2012 of 1 in 88 children being identified with ASD.” (AAP)

Epidemiology

ASD is almost five times more common among boys than girls: 1 in 42 boys versus 1 in 189 girls. White children are more likely to be identified as having ASD than are black or Hispanic children. (CDC 2014)
The increase is most prominent in the group of children with IQs in the average range. It is notable that less than 1/3 of the entire group of children identified with ASD also had intellectual disabilities. (CDC 2014)

The phenotype of Autism spectrum disorders (ASDs) is extremely heterogeneous, with differences from person to person in a wide range of symptoms and severity as well as differences between the various subtypes of ASDs (e.g., autistic disorder, Asperger syndrome, and pervasive developmental disorder not otherwise specified) (ACMG 2013)
Prenatal factors

- A linked database cohort study of infants born between 1990 and 2002 was performed in Canada (Dodds et al 2011). They found that mothers with prepregnancy weight of 90 kg or more and an 18 kg weight gain during pregnancy were independent risk factors for autism.
- The authors proposed that leptin levels during pregnancy may be involved.
- In addition, women who delivered less than 18 months after a previous delivery and women with no previous deliveries were at increased risk to have a child with autism.
- Teratogens that are known to increase the risk of autism include maternal rubella infection, ethanol, thalidomide, valproic acid, and misoprostol (Arndt et al 2005)
- and an association has been reported between cytomegalovirus infection and autism (Chess 1971; Stubbs 1987; Ivarsson et al 1990).

Immunology

- Significant increases in plasma levels of a number of cytokines, including IL-1beta, IL-6, IL-8, and IL-12p40, have been found in an autism spectrum disorder group compared with typically developing controls.
- It was noted that the increased cytokine levels were predominantly in children who had a regressive form of autism spectrum disorder, and increasing cytokine levels were associated with more impaired communication and aberrant behaviors (Ashwood et al 2011b).
- A study of dynamic adaptive cellular immune function conducted with 66 children with autism spectrum disorder and 73 control subjects showed altered T-cell function in the autism spectrum disorder group.
High fetal testosterone levels have been associated with traits of autism spectrum disorder in toddlers and older children. This supports the extreme male brain theory of autism advanced by Baron-Cohen (Auyeung et al 2010).

Hypothalamic pituitary adrenal dysregulation in persons with autism spectrum disorder have been explored and identified through cortisol, adrenocorticotropic hormone, and dehydroepiandrosterone measurements.

The 11 human studies completed that measured cortisol found few differences in subjects and controls, but the largest study (N= 48) found higher cortisol levels in subjects with severe autistic spectrum disorder indicating a mechanism of possible neurotoxic effect of cortisol (Lam et al 2006)

Recent genome wide studies implicate common and rare variants in genes involved in postsynaptic density, synaptogenesis and neural cell adhesion in susceptibility to autism
Consensus is that autism spectrum disorder is a complex heritable disorder involving multiple genes. Strong evidence supporting genetic factors comes from twin studies.

A 60% concordance for autism was found in monozygotic twin pairs versus 0% in dizygotic twins, with an estimated heritability of over 90%. Ninety-two percent of monozygotic twins were concordant for a broader spectrum of cognitive or social abnormalities versus 10% in the dizygotic twins (Piven 1997).

The microarray-based comparative genomic hybridization test has identified clinically significant copy number variants, such as 16p11.2 and 15q13.3 deletion syndromes, which are associated with intellectual disability and autism spectrum disorder.

1- Studies have reported a higher recurrence risks (3-10%) with single-sibling involvement.
2- The reported risk is 7% of another affected child if the first affected child is female and 4% if the first affected child is male. If multiple children (two or more) have autism, the recurrence risk is on the order of 33–50% for any future pregnancy.
The roles of the clinical geneticist are to determine the etiology of the ASD when possible, to improve care and management, and to provide genetic counseling for the family. “ (ACMG 2013)

In light of the expected benefits, a genetic evaluation should be offered to every person with an ASD (or his or her family).” (ACMG 2013)
The rate of success for identifying a specific etiologic diagnosis in persons with ASDs has been reported as 6–15%. (ACMG 2013)

Continued improvements in cytogenetic approaches have increased the diagnostic yield of conventional cytogenetic studies to approximately 3%.

Numerous cytogenetically detectable deletions and duplications have been associated with an ASD phenotype.
### Selected Genetic “hot spots”

<table>
<thead>
<tr>
<th>Region</th>
<th>Linkage</th>
<th>Cytogenet</th>
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### Selected Genetic “hot spots”

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Chromosomal analysis in the evaluation of persons with ASDs should now be reserved only for certain exceptions such as a clinically suspected chromosome aneuploidy (e.g., Turner, Klinefelter, and Down syndromes) or a family or reproductive history suggestive of chromosomal rearrangements. “ (ACMG)
Chromosomal Microarray

- Arrays use different techniques to scan the genome for copy-number variants (CNVs).

DNA microarray

- Each spot on a microarray contains multiple identical strands of DNA.
- The DNA sequence on each spot is unique. Each spot represents one gene.
- Thousands of spots are arrayed in orderly rows and columns on a solid surface (usually glass).
- The precise location and sequence of each spot is recorded in a computer database.
- Microarrays can be the size of a microscope slide, or even smaller.
Estimates of CNV frequencies in unselected populations of individuals with an ASD are from 8 to 21%.

Some of the loci found on CMA studies (shown in the prior table), can be quite common in an ASD population. Locus 16p11.2 has been reported to have CNVs occurring in 0.5–1% of all individuals with ASDs.
Microarray

- CMA is the first tier of genetic testing in Autism spectrum disorders as well as in individuals with developmental disabilities.

- CMA is diagnostic in close to 30% if the ASDs is complex (individuals with additional clinical findings).
Microarray

- CNV (copy number variants) on a microarray should be interpreted with caution, testing parents is always helpful, as well as detailed family history.
- The expression of CNVs may vary in the same family.

Single gene disorders

- Fragile X syndrome
- Methyl-CPG-binding protein 2 (MECP2) spectrum disorders
- Phosphatase and tensin homolog (PTEN)-related conditions.
Approximately 20% of boys with fragile X syndrome meet diagnostic criteria for ASDs.

It is recommended that all males with unexplained autism be tested for fragile X syndrome.

Routine testing of females with ASDs for fragile X does not meet evidence-based criteria.

Fragile X studies in females with ASDs should be done in patients with clinical parameters such as:
- Phenotype compatible with fragile X
- Family history positive for X-linked neurodevelopmental disorders
- Premature ovarian insufficiency, ataxia, or tremors in close relatives.
Mutations in MECP2 were originally reported as the primary etiology of Rett syndrome. A broad range of other phenotypes were described in conjunction with MECP2 mutations. Idiopathic ASD was one such phenotype.

Aggregate data from nine studies in which MECP2 testing was performed on girls with non-syndromic ASDs identified 16 of 400 (4%) with pathogenic mutations (range: 0–20%). In one of these studies, the only patients identified with a mutation were those with a phenotype of the “preserved speech variant” subtype of Rett syndrome.
In an another study of 313 cases of girls with MECP2 mutations, the authors found that the patients with an initial diagnosis of autism had fewer symptoms of classic Rett syndrome and better functional outcomes.

These girls with an autism diagnosis and MECP2 mutations were associated with two specific sequencing mutations: R306C and T158M.

Routine MECP2 testing in males with autism is not recommended. However, the geneticist should be alert to the features of MECP2 duplications (e.g., drooling, recurrent respiratory infections, hypotonic facies) and consider MECP2 duplication testing in boys with autism and such features.
PTEN

- Collective data from four studies identified 15 of 318 individuals (5%) with ASDs who had pathogenic PTEN mutations.
- Although most of these studies did not select for patients with ASDs with macrocephaly, retrospective analysis showed that macrocephaly was present in all positive cases.
- It is suggested by the ACMG that PTEN testing be reserved for patients with ASDs with a head circumference above the 98th percentile.

X-linked

- "When a family history is consistent with X-linked inheritance and the patient has cognitive impairments, an "X-linked intellectual disability gene panel" is a consideration.
- Several X-linked genes are known to present as either ASD or intellectual disability.
- Another disorder to consider is the X-linked creatine transporter defect (SCL6A8 gene). Patients with this condition have been reported with neurobehavioral changes in the ASD spectrum, along with hypotonia and seizures.
Metabolic disorders

- Metabolic disorders associated with an ASD phenotype are relatively rare, typically autosomal recessive in nature, and present early in life.
- Most metabolic disorders are associated with other clinical symptomatology such as seizures, extrapyramidal signs (movement disorders, dystonia, and parkinsonism), failure to thrive, or neuroregression that is atypical for the autistic patient.

3β-Hydroxycholesterol-7-reductase deficiency (Smith-Lemli-Opitz syndrome)
- 6-N-trimethyllysine dioxygenase deficiency
- Adenylosuccinate lyase deficiency
- Cerebral folate deficiency
- Cytosolic 5’nucleotidase superactivity
- Dihydropyrimidinase deficiency
- Disorders of creatine transport or metabolism
- Disorders of γ-aminobutyric acid metabolism
- Phosphoribosylpyrophosphate synthetase superactivity
- Succinic semialdehyde dehydrogenase deficiency
- Sulfation defects
Clinical symptoms that prompt metabolic or mitochondrial testing in persons with ASDs

- Acid/base or electrolyte disturbances
- Anemia with an elevated mean corpuscular volume
- Cyclic vomiting
- Developmental regression associated with illness or fever
- Gastrointestinal dysfunction, gastroparesis
- Hypotonia/dystonia
- Lactic acidosis
- Lethargy
- Neurodegeneration outside of the typical ASD speech loss at 18–24 months
- Poor growth, microcephaly
- Seizures
- Dermatologic changes: alopecia, hypertrichosis, and pigmented skin eruption
- Multisystem involvement, especially cardiac, hepatic, or renal (physical and/or laboratory evidence)

Metabolic evaluation

- Complete blood count
- Serum metabolic profile
- Serum amino acid and
- Urine screening for glycosaminoglycans
New literature suggests a link between mitochondrial dysfunction and ASDs. This association has been recognized in persons with autistic behaviors and loss of speech after a febrile illness or immunization with subsequent encephalopathy. Constitutional symptoms, hypotonia, repeated regressions after the age of 3 years, and multiple organ dysfunctions are clues to consider mitochondrial disease.

A review of 25 patients with a known mitochondrial disorder who presented with an initial diagnosis of ASD found that all had an abnormal neurologic examination and/or an elevated plasma lactate concentration.
Clinical Genetic diagnostic evaluation

First tier
- Three-generation family history with pedigree analysis
- Initial evaluation to identify known syndromes or associated conditions
- Examination with special attention to dysmorphic features
- If specific syndromic diagnosis is suspected, proceed with targeted testing
- If appropriate clinical indicators present, perform metabolic and/or mitochondrial testing (alternatively, consider a referral to a metabolic specialist)
- Chromosomal microarray: oligonucleotide array-comparative genomic hybridization or single-nucleotide polymorphism array
- DNA testing for fragile X (to be performed routinely for male patients only)

Second tier
- MECP2 sequencing to be performed for all females with ASDs
- MECP2 duplication testing in males, if phenotype is suggestive
- PTEN testing only if the head circumference is >2.5 SD above the mean
- Brain magnetic resonance imaging only in the presence of specific indicators (e.g., microcephaly, regression, seizures, and history of stupor/coma)
For patients in whom a metabolic etiology is suspected, combining magnetic resonance spectroscopy with standard neuroimaging should be considered.

Abnormal functioning of the brain areas that participate in face processing and social cognition has been consistently demonstrated in persons with autism spectrum disorder and reflects hypoactivation in the amygdala and fusiform gyrus (Grelotti et al. 2004).

Approximately 30% of persons with autism spectrum disorder have increased head size and brain volume. The largest study evaluating age-related changes in brain size in subjects from 12 months to 50 years of age showed early brain overgrowth during infancy and the toddler years in children with autism spectrum disorder, followed by an accelerated rate of decline in size and perhaps degeneration with age (Courchesne et al. 2011).

A study evaluated infants using brain magnetic resonance imaging and found that those who later developed autism spectrum disorder (n=10) had significantly greater extra axial fluid at 6 to 9 months, which remained elevated at 18 to 24 months. The amount of extra axial fluid detected as early as 6 months was predictive of more severe autism spectrum disorder symptoms at the time of outcome. These infants also had significantly larger total cerebral volumes.

This study raises the potential for magnetic resonance imaging to help with early detection in individuals at higher risk for autism spectrum disorder (Shen et al. 2013).
**Neuroanatomy**

- Studies suggest that the frontal lobes, amygdala, and cerebellum are involved in the pathogenesis of autism spectrum disorder. However, there is no clear and consistent pathology. In fact, the time course of brain development may be more related than the final structure (Amarai et al 2008).
- A multicenter MRI study was conducted in men with autism spectrum disorder. They found that individuals with autism spectrum disorder had significantly increased gray matter volume in the anterior temporal and dorsolateral prefrontal regions and significant reductions in the occipital and medial parietal regions compared with controls.
- These regional differences were significantly correlated with the severity of specific autistic symptoms (Ecker et al 2012).
- Another study revealed larger brain volume in the frontal lobes, whereas the occipital lobes are smaller in size in individuals with autism (Philip et al 2012).
- Autopsied brain samples from patients with autism spectrum disorder have revealed a loss of Purkinje cells in the hippocampus, amygdala, and cerebellum.

**Neurotransmitters**

- Elevated levels of whole-blood serotonin have been found in 25% to 40% of patients with autism (Anderson 2002). No significant associations have been found between serotonin level and behavioral outcomes, except for an inverse relationship between serotonin level and self-injury (Kolevzon et al 2010).
- Low urine tryptophan levels have been found in children with autism spectrum disorder compared to controls (Kalunza-Czaplinska et al 2010).
- Glutamate, an excitatory neurotransmitter, is important in neuronal plasticity and cognitive functioning. High levels of glutamate can be neurotoxic. Studies have shown that people with autism spectrum disorder have higher levels of plasma glutamate than controls. Because plasma glutamate levels are correlated with CSF glutamate levels, there is potential for a screening or diagnostic tool (Fatemi et al 2002; Shimmura et al 2011).
Genetic diagnostic in ASDs

- CMA (10%)
- Fragile X (1–5%)
- MECP2 (4% of females)
- PTEN (5% of those with head circumferences >2.5 SDs that are tested)
- Karyotype (3%)
- Other (10%), including metabolic disorders, brain anomalies and genetic syndromes

Genetic syndromes and ASDs

- 22q11.2 deletions including velocardiofacial (Shprintzen) syndrome
- Angelman syndrome
- CHARGE syndrome
- de Lange syndrome
- Fragile X syndrome
- MED12 disorders (including Lujan–Fryns syndrome)
- Prader–Willi syndrome
- PTEN-associated disorders (Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome)
- Rett syndrome
- Smith–Lemli–Opitz syndrome
- Smith–Magenis syndrome
- Sotos syndrome
- Tuberous sclerosis
Additional Genetic testing

- CDLK5 testing
- Cholesterol/7 dehydrocholesterol
- Chromosome 15 methylation/UBE3A gene testing
- Methylation/epigenetic testing
- Mitochondrial gene sequencing/oligoarray
- NSD1 testing
- Reduction-oxidation studies
- Screening for disorders of purine/pyrimidine metabolism (serum and urine uric acid)
- Screening for folate-sensitive fragile sites
- Selected neurometabolic screening (mucopolysaccharides, creatinine phosphokinase, amino acids, organic acids, lactate, ammonia, acylcarnitine profile)

Autism and Genetics

Questions?