Educational Objectives

- Identify the role of genetics in autism from both a clinical and scientific perspective
- Recognize several syndromes, chromosome abnormalities, and genes that are associated with autism.
- Understand the appropriate laboratory evaluations for a child with autism.

Autistic Spectrum Disorders

Clinical definition

Clinically heterogeneous group of complex behavioral disorders that develop before three years of age characterized by:

- Impairments in social interaction
- Impairments in communication
- Repetitive stereotypic behaviors

Why Study Autism?

Prevalence of Autism

- Prior to 1990, most studies estimated a general population prevalence of 4-5/10,000 (Jorde et al 1990, Fombonne 2001).
- Apparent prevalence of all ASDs is increasing worldwide (Includes PDD & Asperger syndrome).
- Earlier age at diagnosis and inclusion of milder cases accounted for the majority of the increase, but the extent to which the continued rise could represent a true increase in the occurrence of autism remains unclear. [Hertz-Picciotto & DeLeache 2009].

Prevalence of Autism

- The most recent study by the Center for Disease Control reported the prevalence of autism spectrum disorders as 1 in 68 in children ages 8 years and 1/42 in boys and 1/189 girls, making it the most common developmental disorder in the general population.
- In Arizona – 1 in 64 children have autism.
  - Increase of 23% over the previous data from 2006.
  - Prevalence is highest in non-Hispanic white children but greatest increase (42%) was seen in non-Hispanic black children.

Center for Disease Control, Morbidity and Mortality Weekly Report (MMWR) 61(SSO3); 1-19, March 30, 2012.
### Environmental Factors

- Maternal medications
  - Valproate, thalidomide, misoprostol
  - Antidepressants
  - Anti-convulsant medications
- Organophosphate – chlorpyrifos
- Prenatal infections
  - Rubella
  - TORCH
- Vitamin D deficiency [Kocovska et al, 2012]
- Hormonal effects
  - Oxytocin
  - Serotonin
  - Sex hormones
  - Melatonin
- Postnatal environment

### Perinatal Factors

- Extreme Prematurity (<26 weeks gestation)
- Gestational diabetes
- Maternal hemorrhage
- Viral infections – trigger immune response, increased IL-6
- Umbilical cord complications
- Fetal presentation
- Fetal distress
- Birth injury/trauma
- Low birth weight
- Low 5 minute Apgar
- Meconium aspiration
- Neonatal anemia and hyperbilirubinemia

### Inflammation/Immune dysfunction

- T-cell dysfunction, autoantibody production, increased activated B cells & NK cells
- Increased proinflammatory cytokines
- Landmark study – microglial & astroglial activation in brain tissue [Vargas et al, 2005]
- Increased CSF proinflammatory and modulatory cytokines

### Classification of Autism

- “Idiopathic” or isolated autism comprises the majority of cases
  - “Essential” autism – lack of physical abnormalities; accounts for 70% of cases
  - “Complex” (syndromic) autism – presence of dysmorphic features and/or microcephaly; accounts for 20-30% of cases
- “Secondary” autism — cases in which either a chromosome abnormality, single-gene disorder, or environmental agent can be identified
Genetic Syndromes associated with Autistic Spectrum Disorder

Dual Diagnoses are Common

- Tuberous Sclerosis
- Fragile X Syndrome
- Neurofibromatosis
- Rett Syndrome
- Angelman Syndrome
- Timothy Syndrome
- CHARGE Syndrome
- Cornelia de Lange Syndrome
- Cohen Syndrome
- Down Syndrome
- Smith-Lemli-Opitz Syndrome
- Joubert Syndrome
- Williams Syndrome
- Russell-Silver Syndrome
- Sotos Syndrome
- Smith-Magenis Syndrome
- Myotonic/Duchenne Muscular Dystrophy
- Phelan-McDermid Syndrome
- Velocardiofacial Syndrome (22q)

Genetic Syndromes associated with Autistic Spectrum Disorder

Dual Diagnoses are Common

- 22q11.2 deletion syndrome (DiGeorge/Velocardiofacial syndrome) - Autism/ASD symptoms in approximately 20%

Fragile X Syndrome

- Prevalence: 1/1000 males, 1/700 females
- Found in all races
- Represents the most common known familial form of intellectual disability
- Accounts for 25-50% of all cases of X-linked intellectual disabilities
- Fragile X syndrome is the most common known single gene cause of autism.
- Accounts for 1-3% of autism cases

Fragile X Syndrome - Females

- Heterozygous for full mutation
- Milder Physical Features
  - Long ears
  - Prominent ears
  - Long, narrow face
  - High arched palate
  - Hyperr flexible joints
  - Double-jointed thumbs
  - Single palmar crease
  - Flat feet
  - Murmur or systolic click
- Milder developmental issues

Fragile X Syndrome - Development/Behavior

- Delayed developmental milestones
  - Sit alone (10 months)
  - Walk (20.6 months)
  - First clear words (20 months)
- Prenatal features
  - Developmental delay, especially speech
  - Abnormal temperament: tantrums, hyperactivity, autism
  - Intellectual disability: IQ 30-50
- Postnatal features
  - Shyness, gaze aversion
  - Tactile defensiveness
  - Poor impulse control, distractibility
- Autistic features - ~25% of these children meet criteria for ASD

Fragile X Syndrome - Physical Findings

- Slightly increased birth weight
- Average height in childhood
- Macrocephaly
- Long face; large ears – not all cases
- Macroorchidism – post pubertal
- Lax joints, velvety skin
- MVP/Aortic dilatation – post pubertal
- Developmental delay/Autism
- Periventricular heterotopia and other neuroradiologic abnormalities
Fragile X Syndrome
Molecular Genetics

X-linked inheritance
FMR1 gene – codes for the FMR1 protein – active in glutamate receptors in the brain
Trinucleotide CGG repeat disorder
- 6-50 = normal allele (30 is peak # in normal population)
- >200 = full mutation (100s to 1000s)

Full mutations are completely methylated, so no FMR1 protein is produced
Diagnostic methods – order Fra X DNA test
- PCR with CGG probe
- Southern blot: digestion with EagI/EcoRI

Some patients are mosaic

Tuberous Sclerosis

Periventricular subependymal nodules
Seizures; MR; SEGA
Cardiac rhabdomyomas
Renal angiomyolipomas
Ashleaf spots/Shagreen patch
Adenoma sebaceum; Subungal fibromas
Retinal lesions

Autism
- 16% to 61% have an ASD
- Accounts for 3% of autism cases
- TS with SEGA have 2X risk of autism

Autosomal dominant – 2/3 new mutations
TSC1 gene – 9q34 (~27%)
TSC2 gene – 16p13.3 (~73%)

Rett Syndrome

Normal development for 6-12 months
Period of regression
Atypical – no regression
Loss of purposeful hand movements
Seizures; microcephaly
Intellectual Disability
Autistic features
- Accounts for 1% of autism cases
Hand-wringing; bruxism
Hyperpnea; heart block
X-linked dominant disorder
MeCP2 mutation/deletion

CDKL5 → atypical Rett
Males → MeCP2 duplication

Rett Syndrome

Neurofibromatosis Type 1

≥ 6 café au lait spots
Axillary/inguinal freckling
Plexiform neurofibroma
Scalp lesions; pseudarthrosis
Lisch nodules; optic glioma
Macrocephaly

ID
Autism - rare
Family History of NF1 – 50%
Autosomal dominant
NF1 gene – 17q

Angelman Syndrome

Developmental delay
Feeding problems/FTT
Microcephaly
Seizures; abnormal EEG
Hypotonia; truncal ataxia
Abnormal gait
Absent speech
Frequent laughter
Sleep disorder
Hypopigmentation

Maternal 15q11-13 del or paternal UPD or UBE3A gene mutation

Phelan-McDermid Syndrome

22q13.3 microdeletion syndrome

Neonatal hypotonia
Normal to accelerated growth
Absent to severely delayed speech
Global developmental delay

Normal head circumference
Minor dysmorphic facial features
- Full brow, flat midface, ptosis, puffy eyelids, long eyelashes, wide nasal bridge, bulbous nose, puffy cheeks, pointed chin, large/prominent ears

Autistic behaviors
Mouthing or chewing non-food items, decreased perception of pain
Microdeletion chr 22q13.3 (80%)
Intragenic deletion of SHANK3
Smith-Lemli-Opitz Syndrome

- Pre/postnatal growth deficiency
- Microcephaly
- Characteristic facies
- Cleft palate
- Hypospadias
- Post-axial polydactyly
- Autosomal recessive
- Deficiency of 7-dehydrocholesterol reductase
  - Low cholesterol
  - High 7DHC

Joubert Syndrome

- Hypotonia
- Ataxia
- Developmental delay
- Episodic hyperpnea/apnea
- Oculomotor apraxia
- "Molar tooth" sign on MRI
- Pigmentary retinopathy
- Cystic renal dysplasia
- Autosomal recessive
- 18 causative genes – a ciliopathy
  - AHI1
  - CEP290
  - TMEM67

Sotos Syndrome

- Pre/postnatal overgrowth
- Macrocephaly
- High forehead; prominent chin
- Large hands/feet
- Ventriculomegaly
- Hypotonia; DD; seizures
- Poor coordination
- Scoliosis; heart defects
- Poor social skills; immature
- Phobias; occasional autism
- Autosomal dominant
- Gene-NSD1 on 5q35

Williams Syndrome

- Growth retardation
- Microcephaly; DD
- Characteristic facies
- Supravalvular aortic stenosis
- Pulmonic stenosis
- Multiple arterial stenoses
- Hypercalcemia
- Hypersociable personality
- Occasional autism
- 7q11.2 deletion (FISH)

Russell-Silver Syndrome

- Pre/postnatal growth retardation
- Blue sclerae; triangular face; small chin
- 5th finger clinodactyly
- Leg length discrepancy
- Post-axial polydactyly
- Growth hormone deficiency
- Hypoglycemia
- Increased risk for DD, learning problems, rarely autism
- Maternal UPD chr.7
- H19/Chr.11p15 imprinting

Complex Autism

Phenylketonuria (PKU)
- Mucopolysaccharidoses
- Sanfilippo syndrome (MPS III)
- Adenylosuccinase deficiency
- Creatine deficiency syndromes
- Mitochondrial disorders – more likely in patients with these features:
  - Lactic acidosis
  - Episodic regression
  - Hypotonia/muscle weakness/cardiomopathy
  - Optic nerve atrophy
  - Diabetes/hearing loss
PTEN Hamartoma Syndrome

- Macrocephaly (OFC +3.7 /+ 9.6 SD) + Autism
- Accounts for 1% of autism cases and 10-20% of Autism + macrocephaly
- PTEN gene – tumor suppressor gene; important role in brain development, neuronal survival and synaptic plasticity
- May also cause:
  - Banayan-Riley-Ruvalcaba syndrome – prenatal overgrowth; multiple lipomas; vascular malformations; pigmented macules
  - Proteus-like syndrome – hemihypertrophy, bony exostoses, macrodactyly, ID
  - Cowden syndrome – mucocutaneous lesions; colon polyps; breast, thyroid; endometrial cancer
- Autosomal dominant with familial cases
- Patients need tumor surveillance in adulthood

Evidence for Hereditability in Autism

- Syndromic causes
- Multiplex families
- Simplex families – ↑ paternal age → de novo mutations
- Twin Studies
  - Concordance for strict autism in monozygotic males 58%/females 60%
  - Concordance for strict autism in dizygotic males  21%/females 27%
  - Concordance for broader ASD in monozygotic males 77%/females 36%
  - Concordance for broader ASD in dizygotic males 31%/females 36%
  - [Hallmayer et al 2011]
- Prevalence of males 4 times that of females
- High frequency of mild traits & other learning/psychiatric disorders in family members
- Only consensus is that it is NOT Mendelian

The search for autism susceptibility genes

- G banding (> 4 Mb)
- FISH [40 to 250 Kb per clone]
- CMA [a collection of FISH-verified clones and average of 20 Oligo/clone]

Essential Autism - Copy number variants

It's important to get the dose right

- 3-5% have chromosome abnormalities on high-resolution chromosomes
- Includes XXY, XYY, 45X
- Recent CMA studies revealed copy number variants in 7-20% of ASD cases
- Review of 33 studies of 22,698 patients by the International Standard Cytogenetic Array consortium – CMA diagnostic yield of 15-20% in patients with ASDs or intellectual disability
- 16p11.2 del/dup most common - accounts for 1.1-1.2% of cases
  - 16p11.2 del/dup
  - 7p11.2 del/dup
  - 15q11.2 del/dup
  - 15p13.3 del/dup
  - 15q11.2 dup
  - 17p11.2 dup
  - 19p13.2 dup
  - 22q13.3 del/dup
Genetic Causes of Autism

Molecular Diagnostic Yield of Chromosomal Microarray Analysis and Whole-Exome Sequencing in Children with Autism Spectrum Disorder

Known and putative Autism Genes

Neuronal Activity/Regulation

- MECP2
- UBE3A
- SEL11
- A3BP1

Neuronal Cell Adhesion and/or Synapse Function

- MAGA
- NUDM1
- SHANK3
- SHANK1
- TNFRSF6
- CD2"R2
- CD164C
- PCDH10
- NRCAM

Other genes

- OR1T
- LAMB1
- RNF8

Neurodevelopmental Genes

- EN2
- HOXA1
- RELN
- WNT17
- AXIN
- DKK1
- PTPN11
- SLIT2

Calcium Channel

- CACNAD1
- CACNAD2
- CACNA1C
- CACNA1H

Neurotransmitter Genes

- GABRB3
- GABRA5
- GABRG3

Mitochondrial Genes

- SLC25A12

Evidence from Genome-wide Association Studies

- De novo copy number variants are more common in simplex cases of ASD and account for >10% [Sanders et al, 2015]

- Small de novo deletions, but not large ones, contain one high ASD-risk gene

- De novo mutations are associated with ASD in individuals with a high IQ

- Oligogenic and complex patterns of inheritance may play a more important role in multiplex families

- Large copy number variants likely contain multiple modest-effect risk genes [Sanders et al, 2015]

- Scientists estimate the total number of ASD copy number variant (CNV) loci at 200 and risk genes at 800 [Sanders et al, 2015]

Gene Clusters found in analysis of De Novo CNV Regions Observed in Autistic Individuals

Autism-related Genes Associated with the Morphogenesis of Dendritic Spines

Rare CNV Variants in Autism Perturb Synaptogenesis

- Study significantly expanded the collection of genes implicated in ASD (433).
- The cluster genes are strongly connected (p=0.013) to proteins of the postsynaptic density in human neocortex.
- Many of these code for either scaffolding proteins, trafficking/signaling proteins, or proteins that modify/interact with the actin filament network.
- CNVs that disrupt these gene functions lead to increased dendritic spine growth/density – consistent with the finding of increased spine density in the cerebral cortex of autistic individuals [Hutsler and Zhang, 2010]
- Analysis supports the hypothesis that autism is primarily a disease of synaptic and neuronal connectivity malfunction.

Genome-wide differential expression of synaptic long noncoding RNAs in autism spectrum disorder

- Found genome-wide differential expression of long noncoding RNAs (lncRNAs) in blood specimens of 25 pairs of children with ASD.
- LncRNAs are a subset of RNA molecules >200 nt in length that are transcribed but not translated. Disregulation of these been implicated in many complex diseases (168).
- LncRNAs function as translational and posttranslational regulators of brain development and differentiation.
- In this study, 2407 were upregulated and 1522 downregulated.
- Results suggest that synaptic vesicle transportation and cycling are important in the delivery of proteins to synapses in ASD.

Autism Epigenetic Factors

- Refers to changes in chromatin state (via DNA methylation/histone methylation/acetylation) that regulate gene expression but do not change the primary DNA sequence
- Rett syndrome – caused by mutations in MeCP2 gene, which binds to methylated DNA and represses transcription of target genes
- Several imprinted regions of the genome linked to autism – Common CNV - maternal 15q13.3 microduplication
- Turner syndrome & autism - Traits correlate with inheritance of parental X
- MTHFR (gene in methylation pathway) – no definitive proof of link to ASD
- Association of autism with ART (Assisted reproductive technology)

Why haven’t we solved the puzzle of autism?
- Wide variability in phenotype
- Broadly heterogeneous GROUP of disorders with multiple genetic causes
- Majority are simplex cases
- Specific mutations manifest variable expressivity and incomplete penetrance, even within the same family.
- The expression of autism may require the accumulation of several hypomorphic variants in a specific pathway or subcellular compartment to exceed a threshold
- Role of environment, perinatal, inflammatory, epigenetic factors must be determined

Why is a genetic evaluation valuable for the child with autism?
Importance of a Genetic diagnosis in autism spectrum disorders

- Families are empowered by knowledge of the underlying cause of their child’s disorder.
- A specific diagnosis may provide prognosis and natural history information.
- A specific diagnosis facilitates genetic counseling for parents and family members to provide recurrence risk.
- Identification of a known syndrome, chromosome or metabolic disorder guides management and treatment.
- Identification of a specific gene mutation facilitates research toward specific pharmacogenetic treatment.

Evaluation of Autism
ACMG Practice Guidelines

- 1st Tier Testing
  Chromosomal microarray analysis (CMA)
  Fragile X (FMR1) DNA
  CMP, Plasma amino acids; serum ammonia
  Urine organic acids and mucopolysaccharides
  Serum lactate, acylcarnitine profile
  TORCH titers – if clinical indicators present

- 2nd Tier Testing
  MECP2 DNA
  PTEN DNA (+ macrocephaly)
  Skin biopsy for chromosome analysis if pigmentary abnormalities
  NLGN3/NLGN4 → consider whole exome sequencing (WES)

- 3rd Tier Testing
  Uric acid → if ↑, HGPRT & PRPP synthetase, if ↓ purines/pyrimidines
  Mitochondrial DNA testing/Muscle biopsy for mitochondrial enzymes
  CSF neurotransmitters

Genetic Counseling in Autism
Recurrence Risks – Complex Autism

- 25% for autosomal recessive – most metabolic disorders
- 50% for autosomal dominant if parent affected
- 50% for male offspring in X-linked disorder if mother is a carrier
- Chromosome disorders
  - Must also test parents
  - Up to 50% risk if parent also mildly affected
- Mitochondrial disorders
  - Up to 100% for maternally inherited genes
  - 25% for autosomal recessive genes

Genetic Counseling in Autism
Recurrence Risks - Essential Autism

- Male siblings of proband have a 7% risk for autism and an additional 7% risk for milder autistic symptoms.
- Female siblings of proband have a 1% risk for autism. Risk for milder symptoms is unknown.
- For families with ≥ 2 affected children, the recurrence risk approaches 35%.

Current Directions in Research in Autism

- Whole exome sequencing
  - Multiple large-scale sequencing projects underway
- Study of Functional Gene/Protein Networks
  - Identification of functional relationships and interactions between various ASD-proteins connected through functional networks will likely identify signaling pathways and subcellular compartments that encompass subgroups of genes common targets amenable to pharmacotherapy
- Prospective Epidemiological Studies of environmental factors, Genetic x Environmental interactions
  - The National Children’s Trial will follow 100,000 children from conception to age 21
  - The Autism Birth Cohort will follow 100,000 children from conception to age 7

Current Treatment Trials for Autism

- Drug Treatment Trials
  - STX209/Arbaclofen – GABA (neurotransmitter) agonist
  - Fragile X syndrome → Deficiency of neurotransmitter GABA.
  - Showing promising results in treating irritability, social withdrawal, social responsiveness in autism. (Erickson CA et al, Autism Res Disord 2014)
  - Minocycline → Antibiotic with anti-inflammatory properties.
  - Studies utilizing it as treatment for regressive type autism.
  - Oxytocin → Intranasal treatment improved mood and social function in autism
Always Unique Totally Interesting Sometimes Mysterious

100% for Children