Approach to the Genetic Diagnosis of Autism
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Why Genetics?
- 1:2500 (1980s)
- 1:88 to 1:100 (current)
- >500% increase over the last 20 years
- Declared “epidemic”
- 4-5 times more prevalent in boys
- Present in all racial, ethnic, social groups
- High heritability
- MZ twins 60-90% concordance
- DZ twins 0-10% concordance

Why Genetics?
- Examine and evaluate patient and family members
- Determine etiology
- Definitive diagnosis helps patient acquire services
- Provide information on prognosis
- Screen and potentially prevent morbidity
- Counsel on recurrence risk
- Targeted therapies (metabolic disorders, FXS)
- Empower the family by knowledge of underlying cause
**Evaluation Scheme (ACMG)**

- **Pre-evaluation**
  - Accurate ASD diagnosis
  - Sensory screening: complete audiogram
  - Cognitive testing
  - EEG (if seizures suspected)
  - Verify newborn screening results
  - Prenatal history (GA, Wt, parental ages, exposures)
  - Karyotype
  - Fragile X (AAP recommendations)

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**Tier 1 - Physical Exam**

- Evaluation for known syndromes or associated conditions
- Intellectual disability (ID) (75%)
- Dysmorphic features and epilepsy (25%)
- MRI and EEG abnormalities (less common)
- Microcephaly (10%)
- Macrocephaly (20–40%)
- Congenital anomaly (6% vs. 3% in gen pop)
  - Congenital anomalies double risk of autism (0.4% vs. 0.2% in gen pop)
  - Brain and eye more likely to be associated with autism
- Majority are **nondysmorphic with no other medical features suggestive of a syndrome**

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**1st Tier, continued**

- Woods lamp exam
- Targeted testing if specific diagnosis is considered
  - Rubella titers
  - Rare (<10 cases/yr)
  - Sensorineural deafness (50%)
  - Eye abnormalities—retinopathy, cataract and microphthalmia (43%)
  - Congenital heart disease (50%)
  - “Standard” metabolic screening
    - clinical indicators present
    - suspected condition not screened for on NB screening
    - Urine mucopolysaccharides and organic acids
    - Serum lactate, amino acids, ammonia, acyl-carnitine profile, CK, LFTs
  - Chromosomes and fragile X (if not already performed)
**Chromosome abnormalities**
- 46,XY,t(5;16)(p13.2;p13.2)
- 46,XY,inv(2)(p11.2;q13)
- 46,XY,t(5;17)(q33;p13)
- 46,XY,t(3;6)(q26.2;q16.2)
- 46,XY,t(3;5)(q26.2;q22)
- 46,XX,t(6;7)(q13;q11.2)
- 46,XYt(6;9)(q16.2;q13)
- Duplication (13)(q14.1q21.3)
- 46,XY,del(6)(q16.1q21)
- 46,XY,dup(15)(q11q13)
- 46,XY,del(10)(q26.3).isol(del(10)(q)telomere)(D10S2490)
- 47,XY,+dic(15)(q13)
- 47,XX,+21 (2)
- 46,XY,ins(6)(p23?q13?q21)
- 46,XY,inv(9)(p11q13)(2)
- 47,XXX

**Evaluation Scheme- 2nd Tier**
- Fibroblast karyotype if clonal pigmentary abnormalities present
- Chromosomal microarray
- MECP2 gene testing (females)
- MECP2 duplication analysis (males)
- PTEN testing (HC >2 SD above mean)

**Evaluation Scheme- 3rd Tier**
- Brain MRI
- Serum and urine uric acid (inborn errors of purine and pyrimidine metabolism)
- Consider adenylosuccinase deficiency testing
Inborn Errors of Metabolism

- Up to 5% of ASDs
- PKU
- Purine metabolism errors
  - Adenylosuccinase deficiency
  - Adenosine deaminase deficiency
- Creatine deficiency syndromes
- Smith-Lemli-Opitz syndrome
- Biotinidase deficiency
- Histidinemia

Most common etiologies

- Identifiable cause in 20-50% depending on study
- 5% high resolution chromosome abnormalities (2.2%)
- 5% Fragile X (0.5%)
- 5% Rett syndrome
- 3% PTEN mutations
- 10% other syndromes (Tuberous sclerosis, etc.)
- 10-20% microdeletion/duplication (18%)
Microarray

- AKA CGH arrays, CMAs, SNP arrays
- Detects gains and losses of DNA
  - deletions/duplications
  - Smaller than can be seen on karyotypes <5MB
- Hybridize patient’s DNA with thousands of areas on chip
- Confirmed with FISH

Clinical Genetic Testing: Patients with unexplained DD, MR, MCA, ASD

Microarray

- Uses
  - Congenital anomalies
  - Developmental delay
  - Autism
- Limitations
  - Balanced chromosomal rearrangements
  - Gene sequence alterations, single base pair changes
  - Genes and loci not included in chip
  - Mosaicism
  - Findings of unknown significance/parental samples not available
Array-CGH analysis: a test and a normal control DNA are labelled with different fluorochromes (in the figure, test is green, normal is red) and co-hybridised to a microarray containing DNA clones whose position in the human genome is known.


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Microarray

- Image of a microarray chip showing gene expression levels.

Microarray

- Diagram of a microarray experiment with DNA hybridisation and signal detection.

Microarray

- Graphs representing gene expression data from different samples.
Unexpected findings

- consanguinity

Abnormal Microarray Results

<table>
<thead>
<tr>
<th>Abnormality</th>
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<tr>
<td>1q36.3 Dup</td>
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<td>Xq27.1 Del</td>
</tr>
</tbody>
</table>

Common array abnormalities

- 15q1 1q13 del or dup (maternal) 5%
  - candidate genes including UBE3A, GABRA5, and GABRB3
- 16p11.2 del or dup
- 17q12 del
- 22q13 del
- 22q11.2 del
  - incidence 1:2000
  - Social deficits can be misdiagnosed as autism (20-50%)
- 7q22-7q33 deletions
  - FOXP2 gene involved in language
Benign vs. Pathogenic CNVs

Primary Criteria | Pathogenic | Benign
---|---|---
CNV inherited from a healthy parent | + | 
Expanded/shifted CNV from parent | + | 
Identical CNV inherited from affected parent | + | 
Similar to CNV in a relative | + | 
Similar to CNV in an affected relative | + | 
CNV contained within a reported genomic imbalance in a healthy individual | + | 
CNV overlaps a region of genomic imbalance associated with ID/DD/Autism/MCA | + | 
CNV overlaps genomic coordinates of known deletion/duplication syndrome | + | 
CNV contains morbid OMIM genes | + | 

Benign vs. Pathogenic CNVs

Primary Criteria | Pathogenic | Benign
---|---|---
CNV is gene rich | + | 
CNV is gene poor | + | 
CNV is a deletion | + | 
CNV is a homozygous deletion | + | 
CNV is a duplication (no known dosage sensitive genes) | + | 
CNV is an amplification (>1 copy gain) | + | 
CNV is devoid of known regulatory elements | + | 

-Databases for variants of unknown significance (VOUS)
-dbVAR (NIH)
-DECIPHER
-ISCA Consortium

EMMC patients

- Dup 1q21.1
- Dup 19p13.3p13.2
- Dup 9p22.32
- Dup 6p22.1
- Dup 22q11.23q12.1
- Dup 9p13.1p12
- Del 15q11.2
- Dup 15q25.3
- Dup 16p11.2
- Del 20p13
- Del 22q11.2
Syndromes associated with Autism

- Fragile X
- Rett syndrome
- Angelman syndrome
- Prader-Willi syndrome
- Smith-Lemli-Opitz
- Smith-Magenis syndrome
- Tuberous sclerosis
- PTEN (Cowden, Bannayan-Riley-Ruvalcaba syndrome)
- 22q11.2 deletions
- Sotos syndrome
- CHARGE syndrome
- Hypomelanosis of Ito
- Lujan-Fryns syndrome
- Cornelia de Lange syndrome
- Fetal Alcohol syndrome
- 22q11.2DS

Autism evaluation recommended

- Apert syndrome
- Williams syndrome
- Joubert syndrome
- Down syndrome
- Noonan syndrome
- Turner syndrome
- Neurofibromatosis 1
- Myotonic dystrophy
- Duchenne muscular dystrophy
- Moebius syndrome
- Cohen syndrome
- Oculo-auriculo-vertebral spectrum

22q11.2DS Clinical Features

- Congenital heart disease (conotruncal) (74%)
- Palatal abnormalities (69%)
- Characteristic facial features
- Learning difficulties (70%-90%)
- Immune deficiency (77%)
- Hypocalcemia (50%)
- Significant feeding/swallowing problems
- Renal anomalies (31%)
- Hearing loss
- Laryngotracheoesophageal anomalies
- Growth hormone deficiency
- Seizures
- CNS
- Ophthalmologic abnormalities
22q11.2DS and Autism

- Reported in Time Magazine
- 29 children with 22q11.2DS
- ADOS, SCQ, BASC-2 PRS
- Previous data- 10-40% prevalence of ASD
- BASC-2 PRS
  - 4/5 with elevated ADOS showed elevated anxiety and/or somatization scores
- Abnormal SCQ scores 2/29
- Abnormal ADOS- 5/29
  - 1/5 of these were above autism cut off
  - 4/5 above ASD cut off below Autism cut off
  - Strengths-social interaction
  - Weakness-imagination and insight
  - Elevated ADOS scores associated with communication weakness
- None had both elevated SCQ and ADOS scores

22q11.2 DS and Autism

- False elevations in ASD rates
  - Comorbid conditions
    - Anxiety, ADHD
    - Reciprocal social interaction, Communication, and Repetitive/Restricted Behavior/Interests
    - Developmental delay, conceptual immaturity, borderline cognitive abilities (FSIQ 75), reduced social competence
    - Concrete language/thinking characteristic of 22q11.2DS
    - Perseverative and repetitive behaviors
  - Limitations
    - Age range (7-14 yrs)
    - ABA may not be appropriate
    - Highly structured/repetitive
    - May lead to rigid and/or oppositional behaviors
    - ASD subgroup symptoms
    - Higher risk of psychosis/schizophrenia
Single Gene Disorders

- Single-gene conditions (<20%)
  - 10% or less
    - Tuberous sclerosis
    - Fragile X syndrome
      - 2-6% of autism caused by FXS
  - MECP2 Xq28
    - Deletions
    - Duplication (male)
      - Infantile hypotonia, severe to profound ID, autism or autistic features, poor speech development, recurrent infections, epilepsy, progressive spasticity, developmental regression (less common)
    - Most maternally inherited
      - Mothers may have mild neuropsychiatric symptoms

Fragile X

- Full mutation expansions (>200 CGG repeats)
  - Generally silenced
  - Resulting in absence of the FMR1 protein and fragile X syndrome.
- Smaller expansions in the premutation range (55-200 CGG repeats)
  - Resulting in excess gene activity and RNA toxicity
  - Neurodegenerative disorder fragile X-associated tremor/ataxia syndrome (FXTAS)
Fragile X full mutation

- Full mutation (>200 CGG repeats)
  - 1 in 3600 males
  - 1 in 2700 females
- Premutation (55-200 repeats)
  - 1 in 130-260 females
  - 1 in 300-800 males

<table>
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<tr>
<th>Full Mutation (&gt;200 CGG repeats)</th>
<th>Premutation (55-200 CGG repeats)</th>
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<td>ADHD</td>
<td>ADHD Depression</td>
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<td>Autism spectrum disorders</td>
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<td>Flat foot</td>
<td>Hypopenstal digging</td>
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<td>Head turning</td>
<td>Prominent ears</td>
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<tr>
<td>Head flopping</td>
<td>Hyperextensible finger joints</td>
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<tr>
<td>High arched palate</td>
<td>Social Anxiety</td>
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Facial phenotype

A, B, C: Images of children with Fragile X syndrome.
Fragile X and Autism

- 15-30% of patients also have autism
  - Autism as high as 30%
  - PDD as high as 20%
- More common in boys with FXS
- More severe ID in FXS plus autism

Fragile X testing

- Southern blot analysis test determines
  - full mutation
  - Approximate repeat size
  - whether the gene has been methylated
  - mosaicism of the gene
  - Used to confirm diagnosis after expanded repeats found by PCR
- Polymerase chain reaction (PCR) analysis determines
  - Actual number of CGG repeats
  - PCR has been not the test of choice to diagnose a full mutation
  - accurate in determining premutation and normal gene repeat numbers.
  - less expensive and quicker than Southern blot
  - Used to screen pregnant women
- Rare mutations unrelated to CGG repeats will be missed
Fragile X- ?Future therapies

- Mavoglurant (AFQ056)
  - mGluR5 antagonist
  - Targets excessive mGluR in fragile X brain cells
  - Early studies show can correct some deficits (Doering, 2013)
  - Restores social behavior in PMR1 KO mice
  - Open clinical trial
- Minocycline
  - Decreases levels of matrix metalloproteinase-9 (MMP-9)
  - Synaptic transmission and plasticity
  - Clinical Global Impression Scale-Improvement
  - Improvement in anxiety and mood symptoms
  - No side effects (Leigh, 2013)

Autism Gene Sequencing

- Next generation sequencing
  - ADSL, APLP2, ARX, ATAX, BCSD1, BRAT, CADN, CASK, CEBU, CHD7, CNTPN3, CR5BBR1, CTHRC1, DUSP11, DYN, EDE1, FAM, FAS, FCN1, FOXP3, GABRB3, HIP3T, KIAA1968, MCP2, MESP1, MEF2C, MEF2D, NEMO, NIPBL, NLGN3/NLGN4X, NUN1, NEDD4, OPRL1, PAR1, PAR2, PCDH19, PHF6, PNKD, PQBP1, PTCHD1, PTEN, PPARG, RAD50, RAF, RB1, ROR1, SCN2A, SLC2A5, SLC34A2, SMARCB1, TSC2, UBE3A, UBE2A, VPS13B, ZEB2

- Whole exome sequencing
  - De novo mutations revealed by whole-exome sequencing are strongly associated with autism, (Sanders et al, Nature 2012)

- Misses mutations in promoter region, deep intronic regions, or other regulatory elements, and does not detect large deletions.

Genetic Counseling

- Recurrence risk for ASD varies by gender for the second child to be affected
  - 4% if the first child affected is female and
  - 7% if a male
  - 25–50% if the second child is also diagnosed with ASD
Pitfalls for primary care

- Genetic testing
  - What test to order/what lab to use
  - Who to test
  - Pretest counseling
  - Insurance coverage
  - Interpretation of results
  - Time limitations

Conclusions

- Consider a genetics evaluation for all children with autism
- Physical exam is important
- Testing
  - Chromosomes and fragile X
  - Microarray
  - Single gene disorders
- Consider MRI brain, EEG
References


Recent publications

- AFF2 gene
- Associated with Fragile XE/ FMR2
  - Different fragile site than FMR1
- Form of nonsyndromic ID
- Sequenced 202 male ASD probands and found that 2.5% of males sequenced had missense mutations at highly conserved evolutionary sites. (Mondal et all, 2012)
The Future: Whole Exome Sequencing

- De novo mutations revealed by whole-exome sequencing are strongly associated with autism, Sanders et al, Nature 2012

The Future: Autism Genome Project

- Launched 2004
- 50 research and academic centers, NIH, Autism Speaks, and Autism Genetic Resource Exchange (AGRE)
- Phase 1 – 1200 patient samples analyzed with CMA
- Phase 2 – study susceptibility genes with high throughput sequencing
- NRXN1, SHANK2, SYNGAP1, DLGAP2, PTCHD1 (x-linked) identified