Approach to the Genetic Diagnosis of Autism

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EASTERN MAINE MEDICAL CENTER

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)

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*

1st Tier, continued

- Woods lamp exam
- Targeted testing if specific diagnosis is considered
  - Rubella titers
    - Rare (<10 cases/yr)
    - Sensorineural deafness (58%)
    - 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 lacate, amino acids, ammonia, acyl-carnitine profile, CK, LFTs.
- Chromosomes and fragile X (if not already performed)

Chromosome abnormalities

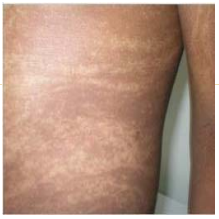
- 46,XY,t(5;16)(p13.2;p13.2)
- 46,XX,inv(2)(p11;2q13)
- 46,XY,t(5;17)(q33;p13)
- 46,XY,t(3;6)(q26.2;q16.2)
- 46,XY,t(3;5)(q26.2;q22)
- 46,XX,t(6;7)(q13;q11.2)
- 46,XY,t(6;9)(q16.2;q13)
- Duplication (13)(q14.1q21.3)
- 46,XY,del (6)(q16.1q21)

- 46,XY,dup(15)(q11q13)
- 46,XY,del(10)(q26.3).ish del(10) (q telomere)(D10S2490-)
- 47,XY,+idic(15)(q13)
- 47,XX,+21 (2)
- 46,XY,?ins(6)(?p23?q13?q21)
- 46,XY,inv(9)(p11q13)(2)
- 47,XXY

Pediatrics, 2010

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

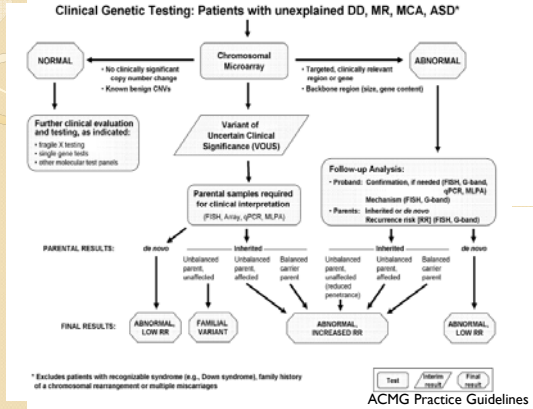
- Identifiable cause in 20-50% depending on study
- 5% high resolution chromosome abnormalities (2.2%)
- 5% Fragile X (0.5%)
- 5% Rett syndrome
- 3% PTEN mutations
- 10% other syndromes (Tuberous sclerosis, etc.)
- 10-20% microdeletion/duplication (18%)

Shen et al, Pediatrics, 2010

Test	Abnormal Result	(%)
Fragile X DNA, *n/N*	*2/852*	*(0.23)*
G-banded karyotype, *n/N*	*19/852*	*(2.2)*
Chromosomal microarray, *n/N*		
Variant (s) identified	204/848	(24.1)
Clinically significant	59/848	(7.0)
Deletions, *n/N (% of abnormal results)*	*37/59*	*(62.7)*
De novo, *n (% of deletions)*	*16*	*(43.2)*
Maternally inherited	5	(13.5)
Paternally inherited	2	(5.4)
Unknown	15	(40.5)
Duplications, *n/N (% of abnormal results)*	*22/59*	*(37.3)*
De novo, *n (% of abnormal duplications)*	*12*	*(54.5)*

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

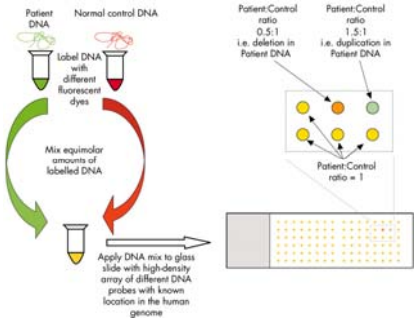


ACMG Practice Guidelines

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.



Sharkey F H et al. Arch Dis Child 2005;90:1264-1269

ADC

Microarray

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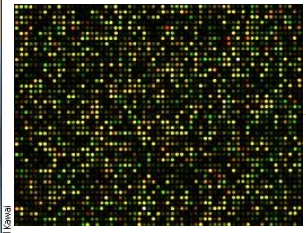
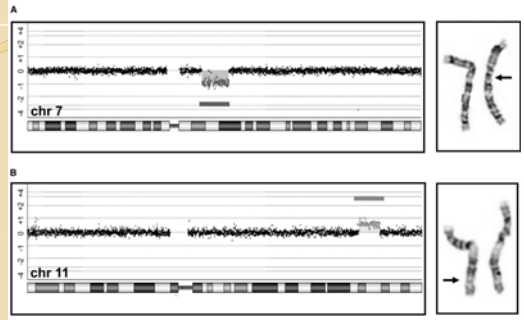
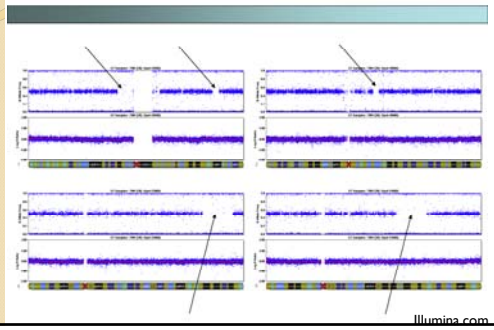


Microarray



Unexpected findings

consanguinity



Illumina.com

Abnormal Microarray Results

1p36.13 Dup	6q16.3 Del	15q11.2 Dup
1q21.1 Del (2)	7q11.22 Del	15q11.2q13.1 Dup
1q43q44 Dup	7q11.23 Dup	15q13.2q13.3 Del (3)
2p16.3 Del	8pq Mosaic Dup	15q14 Del
2p16.3 Del (2)	8q23.3 Del	16p11.2 Del (5)
2p21 Del	8q24.22q24.3 Del	16p11.2 Dup
2q13 Del (2)	9q34.2 Dup	16p13.2 Dup
2q33.1 Del	10q11.21q11.23 Dup	16q23.3 Del
3p22.1 Del	10q26.3 Del	7q12 Del
3q23 Del	12p11.22 Del	18p11.31p11.23 Del
3q29 Dup	12p13.33 Del	19p13.13 Dup
4q23 Del	12q14.2 Dup	Xp22.31 Del
4q35.2 Del	13q12.11 Del (2)	Xp22.31 Dup
6p21.32 Dup	15q11.1 Dup	Xq12 Del
6q16.1q21 Del	15q11.2 Del (2)	Xq27.1 Del

Pediatrics. 2010

Common array abnormalities

- 15q11q13 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 healthy parent		+
Expanded/altered CNV from parent	+	
Identical CNV inherited from affected parent	+	
Similar to CNV in relative		+
Similar to CNV in affected relative	+	
CNV contained within reported genomic imbalance in healthy individuals		+
CNV overlaps region of genomic imbalance assoc 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 a 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
- Corneila de Lange syndrome
- Fetal Alcohol syndrome

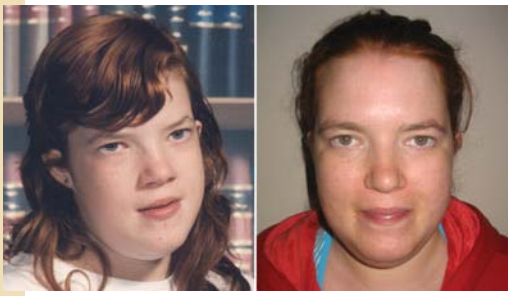
Autism evaluation recommended

- 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

Figure 1: Mild dysmorphic facial features of a woman with 22q11.2 deletion syndrome (left, aged 11 years; right, aged 25 years).



Kapadia R K , and Bassett A S CMAJ 2008;178:391-393

CMAJ·JAMC

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 was 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
    - Duplications (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)

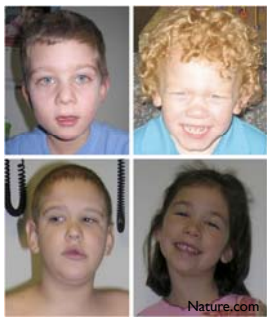
Fragile X full mutation

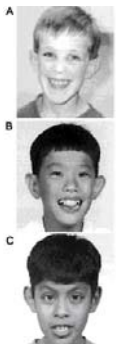
Full mutation (>200 CGG repeats)
- 1 in 3600 males
- 1 in 2700 females

- Premutation (55-200 repeats)
  - 1 in 130-260 females
  - 1 in 300-800 males

Full Mutation (25-80% of children)	Premutation in Children (10-30% of children)	Premutation in Adulthood (20-25% of males, 4-8% of females)
ADHD	ADHD	Anxiety
Autism spectrum disorders	Autism spectrum disorders	Depression
Flat feet	Hyperextensible finger joints	POF
Hand biting	Prominent ears	FXTAS (30-35% of males,
Hand flapping	Shyness	- Tremor
High arched palate	Social Anxiety	- Ataxia
Hyperextensible finger joints		- Neuropathy
Long face		- Muscle pain
Macroorchidism		- Hypothyroidism
Mitral valve prolapse		- Cognitive decline
Mood instability		- Anxiety
Perseverative speech		- Depression
Poor eye contact		- Apathy
Prominent ears		- Dysinhibition
Shyness		
Social anxiety		
Tantrums		

Facial phenotype





med.miami.edu

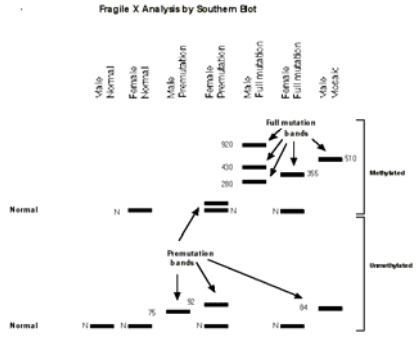
Fragile X and Autism

- 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

Southern Blot



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 FMR1 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, AFF2, AP1S2, ARX, ATRX, BCKDK, BRAF, CACNA1C, CASK, CDKL5, CHD7, CNTNAP2, CREBBP, DHCR7, DMD, EHMT1, FGD1, FMR1, FOLR1, FOXG1, FOXP1, FOXP2, GABRB3, HPRT1, KDM5C, L1CAM, MBD5, MECP2, MED12, MEF2C, MID1, NHS, NIPBL, NLGN3, NLGN4X, NR1I3, NRXN1, NSD1, OPHN1, PAFAH1B1, PCDH19, PHF6, PNKP, PQBP1, PTCHD1, PTEN, PTPN11, RAB39B, RAI1, RELN, SCN1A, SLC2A1, SLC9A6, SMARCB1, SMC1A, TCF4, UBE2A, UBE3A, VPS13B, ZEB2
- Misses mutations in promoter region, deep intronic regions, or other regulatory elements, and does not detect large deletions.
- Whole exome sequencing
  - De novo mutations revealed by whole-exome sequencing are strongly associated with autism, (Sanders et al, Nature 2012)

Genetic Counseling

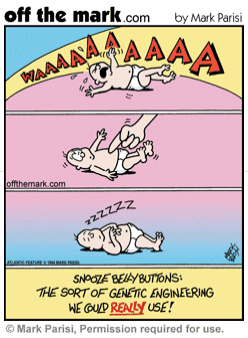
- Recurrence risk for ASD varies by gender for the second child to be affected
- 4% if the first child affected is female and
- 7% if a male
- 25–50% if the second child is also diagnosed with ASD

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



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J Dev Behav Pediatr. 2013 Apr;34(3):147-55. doi: 10.1097/DBP.0b013e318287cd17.A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile x syndrome.Leigh MJ, Nguyen DV, Mu Y, Winarni TI, Schneider A, Chechi T, Polussa J, Doucet P, Tassone F, Rivera SM, Hessl D, Hagerman RJ.

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