Evolution of Genetic Testing

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Genetic Testing

• Chromosomal analysis
• Flourescent in situ hybridization (FISH)
• Chromosome microarray
• Next Generation Sequencing
• Whole Exome Sequencing
• Whole Genome Sequencing
Chromosomal analysis

- Congenital anomaly
- Developmental Delay/ Intellectual Disability
- Dysmorphic Features
- IUGR, Growth disturbance
- Suspected Chromosome Diagnosis
- Balanced Translocation
- Family history of chromosomal rearrangement
Frequency of Chromosome Abnormalities

- Livebirths 0.6%
  - Congenital anomaly with MR 23.0%
  - Congenital heart disease 13.0%
  - Institutionalized individual with MR 12.0%

- Couples with multiple spontaneous abortions 5.0%

- Stillbirths and perinatal deaths 6.0%

- Spontaneous abortions (first trimester) 60.0%
Down Syndrome
Chromosomal Analysis

Best for:
• Trisomy/ Monosomy
• Balanced translocation
• Confirmation of some prenatal results
• Easy to obtain, less expensive

Would miss:
• Small deletions and duplications
• Single gene disorder
FISH

• Must know what you are looking for
• Suspected microduplication or microdeletion syndrome
• Family History of above
• Confirmation of microarray results in other family members
FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Step 1
DNA probe specific for region of interest
DNA probe hybridizes to complementary sequences on the chromosomes.

Step 2
Fluorescent antibodies recognize the DNA probe
Antibodies attach to DNA probe on the chromosomes.

Step 3
Fluorescent dye stains the chromosomes
Signals from the probe are examined through a special microscope.
FISH Analysis of a Deletion Syndrome

NORMAL

DELETION
Triplication
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Williams</td>
<td>7q11.23</td>
</tr>
<tr>
<td>Langer-Giedion</td>
<td>8q24</td>
</tr>
<tr>
<td>Wilms tumor-aniridia (WAGR)</td>
<td>11p13</td>
</tr>
<tr>
<td>Beckwith-Wiedemann</td>
<td>11p15 (dup)</td>
</tr>
<tr>
<td>Prader-Willi</td>
<td>15q11-13</td>
</tr>
<tr>
<td>Angelman</td>
<td>15q11-13</td>
</tr>
<tr>
<td>Smith-Magenis</td>
<td>17p11.2</td>
</tr>
<tr>
<td>Miller-Dieker</td>
<td>17p13.3</td>
</tr>
<tr>
<td>VCF/DiGeorge</td>
<td>22q11.2</td>
</tr>
</tbody>
</table>
FISH

Benefits:
• Fast results and inexpensive

Limitations:
• Specific disorders only (need probes)
Chromosome Microarray: indications

• “Molecular Karyotype”
• Array based genomic copy number analysis
• Higher resolution than chromosomes
• Diagnostic yield is 15-20% in unexplained developmental disability/intellectual disability, Autism, multiple congenital anomalies

• First tier test (insurance issues)
Chromosome Microarray

Would miss:

• Balanced translocations
• low level mosaicism
• Single gene disorder
Array CGH: The Complete Process

**Steps 1-3** Patient and control DNA are labeled with fluorescent dyes and applied to the microarray.

**Step 4** Patient and control DNA compete to attach, or hybridize, to the microarray.

**Step 5** The microarray scanner measures fluorescent signal intensity.

**Step 6** Computer software gathers the data and generates a plot.

- **Step 5**
  - Computer Software
  - DNA dosage loss

- **Step 6**
  - Data Plot
  - (Chromosome 7)
Regions of gain or loss can be found anywhere in the genome.
Case 1

- Infantile spasms, myoclonic seizures
- Global developmental delay
- Nephrocalcinosis
- Strabismus
- FTT (Ht, Wt <5%tile, HC at 5%tile)
- Dysmorphic features
Results

Microdeletion 9q34.1

- 1.8 Mb deletion
  - Multiple genes missing
    - STXBP1
      - Infantile epileptic encephalopathy
    - ENG
      - Hereditary Hemorrhagic Telangiectasia
Follow-up

- EEG improving
- Seizures under good control
- Intellectual Disability
- Recurrent UTIs
- Renal stones s/p surgery

- AVM in lungs s/p embolization
- Small Atrial Septal Defect
- Normal brain MRI: No cerebral AVM
- Normal abdominal MRI: No AVMs
Chromosome Microarray

50-80% Patients with abnormal results have medical recommendations based on results

- Specialty consults (37.7%)
- Imaging studies (21.3%)
- Lab testing (12%)
- Surveillance Protocols (15%)
- Family Investigations (14%)

NGS

What is “next-generation” sequencing?

Massively Parallel:

-- first-generation sequencers: --
Sanger sequencer: 384 samples per single batch

-- next-generation sequencers: --
Illumina, SOLiD sequencer: billions per single batch, ~3 million fold increase in throughput!
DNA Sequencing

- **Single gene testing** for specific genetic disorder
- **Syndrome Panel Testing**: for genetic syndromes with more than one gene
- **Symptom Panel Testing**: for symptoms associated with multiple Diagnoses and genes
Case 2

- 32 wk twin, IUGR, 2lb 9oz / 4lb 7oz
  - 47d (15d) NICU: ROP, PFO/PDA/ VSD
- Wolf Parkinson White
- Poor overall growth (3-5 SD below)
- Global developmental delay
- Pale optic nerve on right at age 3
- Normal MRI of brain
• Neuro-ophthalmology:
  – **Right Optic neuropathy**
  – MRI orbits: Mild R optic nerve atrophy; abnormal signal with enlargement of the tectum - suspected Glioma

• Oncology:
  – Brain MRI: *symmetric signal abnormality* tectum, tegmentum, cerebellar peduncle.

• Neurology:
  – Extensive metabolic work-up normal
  – EEG: slow, **diffuse encephalopathy**
Genetics

• Twin normal stature and development
• Petite, triangular face
• Exam fairly non-dysmorphic
• Suspected mitochondrial disorder (ND5)
  – Normal lactate, repeat high
  – Microarray normal
Results

NGS: Mito DNA Panel

ND1 mut (100%) – LHON
ATP8 mut (70%) - ?
Leber Hereditary Optic Neuropathy

• Bilateral painless subacute visual failure in young adults (teens, 20s)
• Males 4X > females
• Neurologic: tremor, peripheral neuropathy, movement disorder
• Idebenone seems promising
• Family testing
ATP8

- Mutations not previously reported
- Likely pathogenic (frameshift)
- Managing as a patient with primary mitochondrial disease
- Need to find other patients with mutation in this gene
Whole Exome Sequencing (WES)

1) Collect blood
2) Extract and fragment DNA
3) Capture exome DNA with probes
4) Recover only exome DNA fragments
5) Sequence on next-generation platform
Whole Exome Sequencing

- Sequence the expressed genes in the genome (exons)
- 1.5% of the human genome: 180,000 exons, 30 million bps, 22,000 genes
- Often done as a triad (can do as single)
- Mutations in known genes or “candidate genes”
- Variants of unknown significance
- Secondary targets
- Non-paternity
WES Indications

• Complex patients
• Overall yield about 29%
• Highest yield (>30%):
  – Hearing, vision, myopathy, skeletal dysplasia, multiple congenital anomalies, skin, CNS (ID/ DD)
  – Cardiac, Metabolic (26-28%)
  – Autism (20%)
  – Some patients with 2 or 3 diagnoses
  – 6% had secondary target

The 30-10 rule of exome sequencing

Results of diagnostic exome sequencing

- not solved
- diagnosis
- treatment change

Exome sequencing in neurodevelopmental disorders
30% of patients can be diagnosed
10% of patient will have a significant change in treatment/management

Case 3

- Apneic episode at 2.5 months – reflux
- 3 months admitted with seizures
- EEG: diffuse encephalopathy
- MRI: brain atrophy
- Non-dysmorphic, microcephaly
- Normal birth history
- Normal Newborn Screen
Results

Normal
• Lactate, pyruvate
• Ammonia
• Anion gap
• CBC (no anemia)
• Acylcarnitines
• CSF Amino Acids
• CSF Neurotransmitters
• Folate
• Blood Methylmalonic acid

Abnormal
• Plasma Amino Acids
  – High Homocysteine
  – Low methionine
• Urine Organic Acids
  – High Methylmalonic acid
• Urine Amino Acids
  – High Homocysteine
• Low B12
Cobalamin Defect

- Valine
- Isoleucine
- Threonine
- Methionine
- Odd chain fatty acids
- Cholesterol

- Propionyl-CoA
- L-methylmalonyl-CoA
- Adenosylcobalamin
- Succinyl-CoA
- Krebs cycle
- Methylmalonyl-CoA mutase
- CblA (MMASA)
- CblB (MMASB)
- CblC (MMACHC)
- CblD (MMACDH)
- Methylcobalamin
- Methionine synthase (MTR)
- Methionine synthase reductase (MTRR)
- CBS (cystathionine beta synthase)
- CblE

Indicates increased plasma concentration
Indicates decreased plasma concentration

Cbl = cobalamin
Cbl III = 3+ state (oxidized) cobalamin
Cbl II = reduced cobalamin
CBS = cystathionine beta synthase
MMA = methylmalonic acid
Diagnosis

- WES triad: Mom, Dad, Sibling
- Common mutation in Cbl F
- Common mutation in Cbl G
- Confirmed 2 mutations on NBS card

- Digenic inheritance?
Case 4

- Microcephaly, Short stature
- VSD, bicuspid AV, mild AR enlargement
- Mod/severe bilateral hearing loss
- Astigmatism, myopia
- Hypospadias, Hernias
- Normal development
- Pectus, bifid uvula, hypermobility
Patient at 10 years

Facial view

Aplastic 5th toenails
Work-up

Initially seen at age 4:
- Chromosomes – normal
- FISH for William syndrome – negative
- Microarray – normal
- PTPN11 (Noonan) – negative
- Whole Exome Sequencing
Results

- Variant of unknown significance in KDM3A
- Compound heterozygote (2 mutations)
- Mutations predicted to cause loss of function
- Candidate gene?
- Need to see other patients reported
KDM3A

• Associated with decreased function of cilia.
• Mutations found in 3 males with azoospermia/oligospermia
• Plays role in cell division, expression of genes in response to heat shock, brown fat utilization and obesity resistance.
• Pathways involved in multiple neoplasias including breast and gastric cancers, as well as Ewing Sarcoma
Tips for DNA Testing

• Find the right test & lab (GeneTests)
• NYS approval
• Pre-authorization for billing
• Informed Consent
  – Variant of unknown significance
  – Non-paternity
  – Secondary target
Whole Genome Sequencing
Whole Genome Sequencing

• Complete DNA sequence
• Mostly research: regulatory regions, copy number variants
• More difficult to interpret
• Limited availability clinically
• Most Expensive