Approach to Clinical Genetics and Testing: The Old and The New

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Overview of Presentation
• Common reasons children are seen in genetics clinic
• Discuss routine genetic testing and interpretation of results
• Strategies to guide genetic testing
• Advances in genetic testing with next-generation sequencing

Genetics and Pediatrics
• Genetic conditions are prevalent in the pediatric population
• The pediatrician is often the first provider to suspect there may be a syndrome, autism or delays in a child
• There is a shortage of geneticists which account for 0.18% of physicians in the U.S.
  • 36% general genetics
  • 28% pediatric genetics
  • 14% metabolic genetics
• It is helpful to know what studies should be ordered for the initial evaluation and when to consider referral for a genetics consultation

Incidence of Genetic Disorders
• 53/1,000 live-born individuals will have a disease with an important genetic component before the age of 25 years
• 20-30% of all infant deaths are due to genetic disorders
• Chromosome abnormalities 1.8/1,000
• Multifactorial disorders 46/1,000
• Single-gene disorders 3.6/1,000
  • Autosomal dominant 1.4/1,000
  • Autosomal recessive 1.7/1,000
  • X-linked recessive 0.5/1,000

Common Indications for a Pediatric Genetics Referral
• Evaluation of developmental delay or intellectual disability
• Evaluation of dysmorphic features, single or multiple anomalies
• Abnormalities in growth
• Presence of a suspected single-gene disorder
• Presence of a chromosome abnormality
• Person at risk for a genetic condition
• Teratogen exposure
• Evaluation for abnormal newborn screen or possible metabolic disease

Approach to Clinical Genetics
• Diagnosis depends on recognizing the pattern of anomalies
  • Individual defects are nonspecific and even rare anomalies may be found in several conditions (etiologic heterogeneity)
  • A syndromic diagnosis is not made on the basis of a single defect
• Understanding variability and heterogeneity of a condition
• Interpretation of pedigree information
• Understand modes of inheritance and risk determination for other family members
• In many cases an exact diagnosis may not be evident
What to Expect From A Clinical Genetics Evaluation

**Before the visit**
- Request of child’s medical records, lab results, imaging studies for review prior to appointment
- Complete questionnaire, inquire about family history, bring photographs
- Allow adequate time for the consultation

**At the visit**
- Review the history and milestones
- Obtain a family history (pedigree)
- Complete dysmorphology and physical exam
- Take photographs
- Impressions, recommendations and counseling issues discussed

**After the visit**
- Lab studies: blood, urine, sometimes CSF or muscle
- Imaging studies
- Request for other consultations
- Review results, management and counseling

Benefits of Genetic Evaluation

**For patient and parents**
- Provide information about cause of child’s problem and specifics of the condition
- Early recognition may improve outcome
- Discuss associated complications
- Guidance of what to expect
- Treatment if available
- Counseling about recurrence risk for siblings and other relatives
- Provide parents with literature and support group information
- Follow-up even if diagnosis is unknown

**For PCP**
- Clarify etiology, prognosis, treatment options, recurrence risk
- Avoid unnecessary testing
- Treatment or management plan
- Screen for associated complications
- Co-management of appropriate patients

Genetic Evaluation

**Construct a Pedigree**
- Three or four generation family tree
- Miscarriage, stillbirth, neonatal death
- Congenital anomalies
- Sensory impairment
- DD/ID, autism
- Mental illness
- Genetic or metabolic disorders
- Medical conditions
- Consanguinity
- Ethnicity

Dysmorphology

- The study of abnormal physical development
- Dysmorphic feature - describes a body part that has not followed the normal pattern of growth or formation and is often disproportionate when compared normal

Syndrome

- A recognizable pattern of structural defects thought to be due to a particular chromosomal, genetic, teratogenic or unknown cause
- The combination of features is unique but not its parts
- The word syndrome translated from Greek means “running together”
- 1% of newborns have multiple anomalies or a syndrome

Congenital Anomaly

- A structural abnormality that departs from normal and is present at birth
- 3% of liveborn infants have a major congenital anomaly
- By age 2-5 years major anomalies are detected in 5-7% of children
- Two-thirds of anomalies are isolated, affecting a single body site
- Major anomaly is a defect that significantly impairs normal body function or reduces life expectancy. Usually requires medical or surgical intervention.
- Minor anomaly is a feature that is usually of no serious functional, medical or cosmetic significance. Often provide clues to diagnosing a syndrome. Each specific feature occurs in less than 4% of the population.
Frequency of Minor Anomalies

• 15% of newborns have a single minor anomaly
• 0.8% of newborns have two minor anomalies
• 0.5% have three minor anomalies
• 20% of newborns with 3 or more minor anomalies will have a major anomaly and a significant chance of having a syndrome
• 42% of children with DD/ID have 3 or more minor anomalies

Frequency of Chromosome Anomalies

• 0.6% of newborns have a chromosomal abnormality
• 7% of stillborn babies have a chromosomal abnormality
• Chromosomal abnormalities occur in 25% of neonatal deaths with congenital anomalies
• 50% of spontaneous abortions due to chromosome abnormality

Causes of Anomalies

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Incidence</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>35-40%</td>
<td>many syndromes</td>
</tr>
<tr>
<td>Multifactorial</td>
<td>20-30%</td>
<td>neural tube defects, cleft lip, club foot</td>
</tr>
<tr>
<td>Familial Conditions</td>
<td>14%</td>
<td>Non-specific ID</td>
</tr>
<tr>
<td>Chromosome Abnormalities</td>
<td>17%</td>
<td>trisomy 21, Turner syndrome, 18q-, abnormal CMA</td>
</tr>
<tr>
<td>Mendelian Conditions</td>
<td>3-7%</td>
<td>Noonan syndrome, achondroplasia</td>
</tr>
<tr>
<td>Environmental Factors</td>
<td>4-5%</td>
<td>maternal diabetes, fetal alcohol syndrome</td>
</tr>
</tbody>
</table>

Aneuploidy

A numerical disorder with an abnormal number of chromosomes.
• Monosomy: missing a chromosome from a pair
• Trisomy: three copies of a chromosome
• Can also have tetrasomy, hexasomy, triploidy

Structural Chromosome Anomalies

• Deletion/microdeletion-a portion is missing
• Duplication/microduplication-a portion is duplicated
• Translocation-a portion of one chromosome is transferred to another
• Inversion-a portion has broken off, turned upside down and reattached
• Insertion-a portion from one chromosome is deleted from the normal location and is inserted into another chromosome
• Ring-a portion of a chromosome has broken off and forms a circle or ring
• Isochromosome-mirror image copy of a chromosome segment including the centromere

Developmental Delay and Intellectual Disability

• 3% of general population has intellectual disability (IQ <70)
• Cause can be identified in 40% to 60% of cases
• For mild DD/ID cause is found in 24%
• Mild ID occurs 7-10 times more frequently than moderate or severe ID
• Global developmental delay occurs in 1% to 3% of general population under 5 years old
• Developmental disabilities affect 5-10% of children
**Etiology**

- Syndromic causes
- Timothée syndrome
- Metabolic tuberous sclerosis

**Causes of Autism and Genetic Contribution**

- <5% cytogenetically visible chromosome abnormality
- CMA detects relevant de novo genomic imbalances in 7-20% of individuals with autism of unknown cause with higher yield in syndromic autism
- CNVs (copy number variants) are found in 5-8% of cases with essential high-functioning autism
- Single-gene disorders account for 5-7%
  - Fragile X in 1-3% of cases
  - 1% each for PTEN macrocephaly syndrome, tuberous sclerosis and Rett syndrome
  - Timothy syndrome, Joubert, SHANK3 and NRXN1 mutations are rare causes
- Metabolic etiology accounts for <5%

**Autism and Genetics**

- Strong genetic basis
- Male predominance (M:F = 4:1)
- Concordance rates of 88-95% in monozygotic twins of 10-31% in dizygotic twins
- Risk for siblings of 5-10% for autism and 10-15% for milder conditions in the spectrum
- Genetics of ASD is complex
- Likely 100-300 genes involved, each accounting for a small percent of cases
- An underlying etiology can be identified in 20-25% of cases

**Clinical Findings or Lab Abnormalities Suggestive of a Metabolic Disorder**

| Failure of appropriate growth | SIDS
| Recurrent unexplained illness | Unusual odor (maple syrup, musty)
| Seizures | Metabolic or lactic acidosis
| Ataxia | Hyperuricemia
| Loss of psychomotor skills | Hyperammonemia
| Hypotonia | Low cholesterol
| Coarse appearance | Structural hair abnormalities
| Eye abnormalities (cataracts, ophthalmoplegia, corneal clouding, retinal abnormality) | Unexplained deafness
| Recurrent somnolence/coma | Bone abnormalities (dysostosis, occipital horns, punctate calcifications
| Arachnodactyly | Skin abnormalities (angiokeratoma, "orange-peel" skin, ichthyosis
| Hepatosplenomegaly | Multiple organ failure
| Multiple organ failure |

**Other Findings in Autism**

- Essential autism occurs in 70-80% of cases and has absence of dysmorphic features, higher M:F ratio of 6:1, higher sibling recurrence risk of up to 35%
- Complex autism or syndromic autism occurs in 20-30% of cases and has dysmorphic features, M:F ratio of 3.5:1 and lower sibling recurrence risk of 4-6%
- Dysmorphic features in 15-20%
- Structural brain abnormality in 40%
- Seizures in 25%
- Intellectual disability in 45%
- Microcephaly in 5-15%
- Macrocephaly in 30%

**Fragile X Syndrome**

- Most common inherited cause of DD/ID
- Autism occurs in 25%
- Children with a premutation (55-200 repeats) may exhibit autism
- Testing should be done in males and females with unexplained ID especially with
  - A positive family history of ID
  - Phenotypic features of large ears, long jaw, high forehead, macroorchidism
- Hypertensive joints and soft skin
- Shyness, poor eye contact
What Tests Should Be Ordered?

- Chromosomes first observed in plant cells in 1843
- 1953 Watson and Crick DNA structure is a double helix
- 1955 humans have 46 chromosomes
- 1958 landmark discovery by Dr. Jerome Lejeune
  - Down syndrome is caused by trisomy 21
- 1963 cri du chat caused by 5p−1968 hbd idld
dh
- 1968 chromosome banding techniques developed
- 1977 Sanger sequencing
- 1980s high resolution chromosomes, PCR and FISH
- 1990 to 2003 Human Genome Project completed ahead of schedule
- Late 1990s-molecular cytogenetics with array CGH
- Recent developments with next generation sequencing:
  - multi-gene panels, whole exome and whole genome sequencing

We’ve Come Along Way, Baby!
From Chromosomes to the Whole Genome!

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Chromosome Analysis

- Standard staining
- G-banded
- Each chromosome has a unique banding pattern.
  - A karyotype is a complete set of chromosomes in an individual.
  - Typically performed on cultured T lymphocytes
  - Arranged in a standard format by size and centromere location
  - Identifies total number, large missing or duplicated regions and rearrangements

Chromosome Banding Pattern and Nomenclature

Example:

- Xp22.3 = X chromosome, short arm region 2, band 2, sub-band 3
- Band is read as Xp two-two point three not Xp twenty-two point three

Level of Resolution

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<thead>
<tr>
<th>Lower Resolution</th>
<th>Highest Resolution</th>
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<tr>
<td>G banding [≥5 Mb]</td>
<td>DNA sequence [1 bp]</td>
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</table>

Indications for Routine Chromosomes

- Suspected aneuploidy or other chromosome disorder
- Down syndrome, trisomy 13 or 18, etc.
- Cri du chat
- Suspected sex chromosome anomaly
- Turner, XXY, XYY
- Newborn with ambiguous genitalia
- Balanced translocation or family history of known rearrangement
- Low-level mosaicism of <10%
- Couples with recurrent pregnancy loss
- Complex microarray results
Molecular Cytogenetics

- FISH or fluorescence in situ hybridization developed in 1980 for metaphase and interphase cells
- Array CGH or comparative genomic hybridization developed in late 1990’s for cancer genetics and in 2004 for constitutional chromosomal abnormalities

Fluorescence in situ hybridization

- Locus-specific DNA probes for microdeletion and microduplication syndromes
- Only provides result for the specific region requested based on phenotype
- Does not delineate exact size or genes involved
- Duplications may be difficult to detect
- Can identify origin of structurally abnormal chromosomes
- Results often available within a few days

Chromosomal Microarray Analysis (CMA)

- CMA detects copy number variations (CNVs) by comparing two samples (patient and control), i.e. comparative genomic hybridization (CGH)
- CNVs are segments of deletion or duplication in the genome that may be either rare or common benign variants, pathologic variants or variants of unknown significance (VOUS)
- A single test that detects large and small deletions (losses) and duplications (gains) throughout the genome
- Much greater sensitivity than standard or high resolution chromosome studies but does NOT detect balanced rearrangements

Process for Oligo Array

- Fluorescently tag DNA from a patient sample with one color
- Combine it with a control sample tagged in a different color
- The two samples are mixed together and added to the array chip and hybridize with the DNA fragments on the chip
- By comparing the test sample versus the control, computer analysis can determine where genetic material has been deleted or duplicated in the patient
- A green signal indicates a gain and a red signal represents a loss in the patient’s genetic information

Types of Arrays

- Oligo array - utilizes oligonucleotides consisting of single sequences of about 60 bp
- SNP array - utilizes pairs of probes that each contain one of the two possible bases in the sequence and can detect uniparental disomy, consanguinity and low-level mosaicism
- Combined oligo + SNP based arrays available at several labs
- BAC arrays have largely been replaced and use fewer and larger DNA fragments

Single Nucleotide Polymorphism Arrays

- A SNP is a single base difference between two DNA sequences either in different individuals or between paired chromosomes in an individual
  - example: GCTCGAGGCCTAGCTTC
  - GCTCGAGGCCTAGCTTC
- 10 million SNPs in the human genome
- Each individual usually inherits one copy of each SNP position from each parent and the genotype at each SNP is either homozygous AA, BB, or heterozygous AB.
- Runs of homozygosity (absence of heterozygosity) occurring on a single chromosome may indicate an area of uniparental disomy (UPD) or consanguinity if present on many chromosomes.
- Disadvantage of SNP only array is that probe distribution is restricted by the non-uniform availability of informative SNPs throughout the genome.
Comparing Array Platforms

- Both SNP and oligo arrays are effective in detecting CNVs over several hundred kb in size.
- Oligo arrays can not detect copy number neutral regions of AOH.
- SNP arrays detect AOH and have higher sensitivity to detect low level mosaicism but have limited ability to detect very small CNVs <10 kb.
- Combined oligo + SNP arrays optimize diagnostic capability offering simultaneous detection of small CNVs and AOH > 10 Mb.
- Currently have a combined diagnostic yield of 19% with pathogenic CNVs in 14% and AOH in 5%.
- Can detect very small intragenic, single exon CNVs (500 bp to <1 kb).
- SNP coverage is less robust than on SNP-only array.

Specific Array Platforms

- Oligo array with 180,000 oligos has maximum sensitivity to detect gains and losses at a 30 kb resolution and includes exon by exon coverage of 1,700 genes, 700 microRNAs and entire mitochondrial genome. Does not detect AOH.
- Oligo+SNP array detects copy number changes and AOH with 400,000 probes targeting almost 5,000 genes at the exon level as small as < 1 kb. Includes 60,000 SNPs to detect regions of AOH >10 Mb.
- SNP array uses >1 million SNPs to detect copy number changes of all well-known microdeletion and duplication syndromes, novel variants >300 kb and AOH. Does not detect small copy number changes outside well-known regions.

SNP Arrays Detect Consanguinity and Incest

- **CAUTION** - with a SNP array it is imperative to counsel the family that consanguinity and incest can be detected before ordering the test.
- Multiple regions of AOH are present on chromosomal segments that are identical by descent in related parents.
- AOH is seen in 1/4 of genome in 1st degree relatives, 1/8 in 2nd degree relatives and 1/16 in 3rd degree relatives.
- The results may have legal or ethical consequences if the mother is a minor or if a parent had been adopted or conceived by artificial technology.

Classification of CNVs

- Abnormal/Pathogenic
  - Aneuploidy
  - Known microdeletion/duplication syndromes
  - Copy number changes involving pathogenic single genes
  - Genomic imbalances > 2 Mb
- Benign - if CNV is polymorphic in the normal population (seen in >1% of population), over 99% are inherited
- VOUS-variants of unknown significance
- CNVs smaller than 2 Mb that have not been correlated with a clinical phenotype

Assessment of Pathogenicity of a CNV

- Frequently a CMA result may identify a VOUS which presents challenges to the lab, physician and family.
- Parental studies may be requested at a charge or no charge depending on the lab.
- Likely pathogenic if CNV is
  - De novo or inherited from an affected parent
  - Size > 2.3 Mb
  - Loss > gain
  - Gene content
    - OMIM reference sequence genes
    - Gene rich region

Assessing the Significance of a CNV

[Table showing criteria for assessing the significance of a CNV]
**CMA as First-Tier Diagnostic Test**

- For over 35 years chromosome analysis has been the 1st tier test for developmental delay/intellectual disability, autistic spectrum disorders and multiple congenital anomalies
- Based on current evidence it is recommended that CMA be ordered as the 1st tier genetic test in place of a karyotype for patients with unexplained DD/ID, ASD or MCA

**Chromosomes vs. CMA**

**Chromosome Analysis**

- Detects imbalances in 5-10 Mb range
- 3-5% yield for patients with DD/ID
- 5-6% yield for congenital anomalies
- <5% yield for autism
- Routine study of 20 cells can detect mosaicism at a level of 14%

**CMA**

- Detects imbalances ~100-200 kb and <1 kb in some regions
- 15-20% yield for DD/ID, autistic spectrum and multiple congenital anomalies
- Can detect mosaicism at 20-30% level by oligo and at ≤5% level by SNP array
- SNP array allows detection of AOH

**Advantages of CMA**

- CMA detects subtle chromosome abnormalities not detected by other techniques
- Detects all known microdeletion/duplication syndromes, subtelomeric abnormalities and mosaicism
- Many new microdeletion/duplication syndromes have been recognized
- Utilizes genomic DNA from whole blood containing multiple cell lineages and not only T lymphocytes
- Readily identifies origin of marker or ring chromosomes
- Detects deletions and duplications simultaneously
- Diagnoses difficult to suspect phenotypes such as del 1p36, Smith-Magenis syndrome
- SNP arrays also detect long regions of homozygosity that can occur in uniparental disomy and consanguinity

**Limitations of CMA**

- Does not detect balanced rearrangements
- reciprocal translocations
- insertions
- Does not distinguish cause of a gain whether due to translocation, tandem duplication or marker chromosome
- Difficulty with clinical interpretation of new or rare variants
- Distinguishing between benign variants from disease causing gains or losses can be challenging
- Parental studies may be requested but parent(s) may not be available
- Confirmation of abnormality by FISH is recommended
- Routine chromosomes may be needed to clarify a complex CMA result

**Identification of New Disorders by CMA**

- Contiguous gene deletion syndromes have been recognized for many years such as Williams, Prader-Willi, DiGeorge, Miller-Dieker, etc.
- With CMA many novel microdeletion and microduplication syndromes have been described with some listed below:

  **Microdeletions**
  - 1q41q42
  - 3q29
  - 9q22.3
  - 9q34.4
  - 15q13.3
  - 15q24
  - 16p11.2
  - 17q21.31

  **Microduplications**
  - 1q21.1
  - 3q29
  - 7q11.23
  - 16p11.2
  - 17p11.2
  - 17p13.3
  - 22q11.2
  - MECP2 duplication

**Support Information for Chromosome Disorders**
Microarray in Neonates with Anomalies

Overall, 17% of neonates with anomalies were identified with clinically significant CNVs.

16p11.2 CNVs and Autism

- Deletions and duplications reported in 0.76% of individuals with ASD
- DD/ID involving language and cognitive function more than motor delay
- Usually mild range to normal IQ
- Expressive language is more affected than receptive
- Increased risk for obesity with deletion
- Minor dysmorphic features in some
- 16p11.2 CNVs also observed in ADHD, dyslexia, seizures, bipolar disorder and schizophrenia

Opposing Microdeletion and Microduplication Phenotypes

- Williams Syndrome caused by a deletion 7q11.23
  - Hypersociability, overfriendliness
  - Empathy
- Williams-Beuren Region duplication of 7q11.2
  - Language deficits
  - Decreased social interaction, ASD
- Maternal duplication or paternal deletion of 11p15
  - Decreased expression of IGF2
  - Impaired growth, Russell-Silver syndrome
- Paternal duplication of 11p15
  - Increased expression of IGF2
  - Overgrowth, Beckwith-Wiedemann syndrome

Reasons to Consider Molecular Testing

- Common questions: What is the diagnosis? What caused it? Is there a treatment? Can it happen again? Who else in the family could get it?
- Establish a diagnosis in a symptomatic individual
- Prognosis
- Inheritance pattern
- Surveillance and treatment options
- Predictive testing for a relative at risk for a treatable condition
- Predictive testing for a relative at risk for an untreatable condition
- Carrier testing
- Prenatal testing

Caveats with Molecular Testing

- No mutation may be detected
- Distinguish a true negative from a false negative
- Is the correct gene being tested?
- There may be locus heterogeneity
- A mutation may be present but not detectable by current technology
- Indeterminate result with a VOUS
- Detection of only one mutation for an autosomal recessive condition does not confirm a diagnosis
- An affected individual must be tested first to identify the mutation before testing can be offered to at risk relatives

Molecular Testing

- Single gene
- Sequence analysis for missense, nonsense, splice site mutations, small intragenic deletions/insertions
- Deletion/duplication testing for exonic or whole gene del/dup
- Phenotype first approach-clinician uses detailed phenotypic features to determine genes most likely to be mutated
- Multi-gene panels
- Clinician identifies a broad phenotype
- Genes included in panel vary by lab
- Tackles locus heterogeneity
How to Locate a Genetic Test

<table>
<thead>
<tr>
<th>Name</th>
<th>URL</th>
<th>Information</th>
<th>Test Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCBI</td>
<td><a href="https://www.ncbi.nlm.nih.gov">Link</a></td>
<td>Detailed information on genes and diseases</td>
<td>Database of Genes and Genomics</td>
</tr>
<tr>
<td>BGI</td>
<td><a href="https://www.bgiusa.com">Link</a></td>
<td>Broad range of genetic testing services</td>
<td>Next Generation Sequencing</td>
</tr>
<tr>
<td>Myriad</td>
<td><a href="https://www.myriad.com">Link</a></td>
<td>Comprehensive genetic testing solutions</td>
<td>Molecular diagnostics</td>
</tr>
</tbody>
</table>

Autism and PTEN Related Disorders

- Includes Cowden Syndrome, BRRS (Bannayan-Riley-Ruvalcaba syndrome) and Proteus
- Autism with significant macrocephaly, average OFC +5.4 SD
- Found in 1% of those with autism
- Autosomal dominant inheritance
- Increased lifetime risk for tumors/cancer
- Hamartomatous intestinal polyps
- Breast
- Thyroid
- Endometrial

Tumor screening for thyroid cancer begins at age of diagnosis (revised recommendation)

Summary of Routine Genetic Studies

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomes</td>
<td>Suspected aneuploidy or other chromosome disorder, sex chromosome abnormality, balanced rearrangement</td>
</tr>
<tr>
<td>CMA</td>
<td>First tier test for DD/ID, autism and multiple congenital anomalies</td>
</tr>
<tr>
<td>DNA fragile X</td>
<td>Male or female with DD/ID and especially with positive family history ID</td>
</tr>
<tr>
<td>Metabolic studies</td>
<td>Targeted based on symptoms in ID or autism</td>
</tr>
<tr>
<td>Molecular studies</td>
<td>Over 2,500 clinically available tests, testing usually based on phenotype</td>
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Next Generation Sequencing

- Encompasses a variety of technologies that permit rapid sequencing of large numbers of segments of DNA, including entire genomes by massively parallel sequencing
- Differs from the initial approach of sequencing one strand of DNA at a time
Next Generation Sequencing (NGS)

- Whole genome sequencing - WGS
  - Determines most of the 3.1 billion DNA base-pair sequence comprising the genome of an individual.
- Whole exome sequencing - WES
  - Determines the DNA sequence of most of the exons or the protein encoding regions of the genome. The exome represents ~1.5% of the genome which contains ~85% of disease causing mutations.
- Multi-gene panels offer complete coverage of all clinically relevant genes for a particular phenotype with a reduction in calls of VOUS and incidental findings. Useful in genetically heterogeneous disorders that have overlapping and non-specific features.

Multi-Gene Panel and Noonan Syndrome

- Includes several syndromes with overlapping phenotypes: Noonan, LEOPARD, Costello, Cardio-Facio-Cutaneous
- Disorders are related at the molecular level due to mutations in various gene involved in the RAS/mitogen activated protein kinase (MAPK) signaling pathway
- Clinical gene panels currently test up to 11 genes
- Overall yield is 67-80%

Whole Exome Sequencing (WES)

- The exome is the coding (exonic) region of the genome.
- Comprises about 1.5% of the genome with ~180,000 exons and ~20,000 genes
- Most likely regions to cause mutations that lead to a clinical phenotype
- 92% of the exome can be analyzed by current techniques
- The role of genes in human disease has only been determined in about 20% of the human exome (~4,000 genes)
- The exons are captured and sequenced using massively parallel sequencing. The patient’s consensus sequence is compared to published normal reference sequences and to other individuals in the family.
- Commercially available from 6 labs in US: Ambry, ARUP, Baylor, Emory, GeneDx and UCLA

Multi-Gene Panels

<table>
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<tbody>
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<td>• Overall RAS Clinical molecular disorders</td>
</tr>
<tr>
<td>• Includes overlapping Noonan, Disorders</td>
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<tr>
<td>• Determined by genes in human disease has only been ~92%</td>
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Whole Genome Sequencing (WGS)

- Determination of the sequence of most of the DNA content comprising the entire genome of an individual
- Identifies approximately 4 million variants per individual sequenced
- Human Genome Project cost $2.7 billion over 13 years. In 2008 a human genome was sequenced for $1.5 million in 5 months. In 2011 WGS can be done in a few days at a cost of <$10,000 and the $1,000 genome is anticipated this year.
- Research studies such as NIH rare disease program
- Currently limited availability in routine clinical practice

Indications for WGS/WES

- A genetic disorder is suspected based on clinical findings and there is limited or no targeted testing/panel available
- A patient has a likely genetic disorder but prior testing has not arrived at a diagnosis
- The patient’s clinical presentation is unclear/atypical and there are multiple genetic conditions in the differential diagnosis
- A patient has a defined genetic disorder associated with significant genetic heterogeneity so that analysis of multiple genes by WES is a more practical approach
- A novel gene is suspected, but has yet to be discovered
### Genetic Counseling and WES
- Genetic counseling and obtaining informed consent are essential components of the process.
- Parents need to decide about whether they wish to be informed about incidental findings (can opt out).
- Biological parental samples needed to facilitate interpretation of the child’s result but a separate parental report is not issued.
- Non-paternity.
- Reporting results back to the family and implications for relatives.
- Protection of privacy - DNA is a powerful personal identifier.

### WES Results
- Causal gene variants have been detected in 30-50% of cases.
- On average, 20,000+ DNA variants are detected in exons per patient and the vast majority (over 95%) are polymorphisms in human populations.
- On average 2% of the single nucleotide variants (SNVs) are novel in an individual.
- Potential disease-causing variants are identified by filtering the data.
- Confirm results with and established method of sequencing before report is issued.
- Option to reanalyze the data at a future date or if new symptoms arise for an additional charge.

### Reporting Results
- Deleterious mutations in disease genes related to clinical phenotype.
- VOUS in genes related to phenotype.
- Deleterious mutations in disease genes unrelated to the phenotype with either childhood or adult onset (incidental or secondary findings).
- VOUS in disease genes unrelated to the phenotype.
- Carrier status for recessive Mendelian conditions.
- Variants in genes involved in drug metabolism.
- Deleterious mutation in genes with no currently known disease association.
- Immediately medically actionable deleterious mutations.

### Medically Actionable
- Finding with direct clinical utility based on established guidelines and/or medical literature.
- Availability of treatment or established guidelines for disease prevention.
- Unrecognized secondary diagnosis: Marfan, NF1, NF2.
- Preventable disease: HNPCC, BRCA1/2.

### Benefits of Whole Exome Sequencing
- Obtaining a diagnostic result.
- Potential for early treatment or preventive care through identification of a medically actionable condition.
- Improved understanding and expanded phenotypic spectrum of many disorders.
- Many novel discoveries.

### Challenges of Whole Exome Sequencing
- Lack of a definitive diagnosis.
- Expense of testing.
- Overwhelming amount of data obtained.
- Interpretation of results.
- Counseling issues and reporting of incidental findings.

### Conclusions
- Genetic conditions and anomalies are prevalent in the pediatric population.
- The ability to establish and confirm a diagnosis has increased with current technology.
- The cause of many syndromes and rare conditions is being discovered by next generation sequencing.
- The discovery of a genetic etiology leads to the potential for prevention and therapeutic interventions.