CdLS Foundation
Genetic Testing-
How to read your child’s test results
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Please note: Knowledge in this presentation is based on current knowledge as of April 2019. Genetics changes over time so after this point we recommend speaking to geneticist/genetic counselor team for any further updates about the topic.
Outline

What is genetic testing

Types of testing available in 2019
  Postnatal for children/adults
  Prenatal testing
  Research

Reading a genetic test report
  What does it mean?

Pros/cons of genetic testing
Nuts and Bolts: Chromosomes and Genes
Nuts and Bolts: Chromosomes

23 pairs, 46 total, in every cell of the body

Both males and females have pairs #1-22

Sex chromosomes: XX and XY

Almost all genes packaged onto chromosomes
Chromosomes: a closer look…

Chromosome nomenclature example:

2p24.1 means

Chromosome 2
P arm
Band 24.1
Nuts and Bolts: Chromosome Differences

**Too many**
- Ex: Trisomy 21 (Down syndrome)

**Too few**
- Missing pieces (deletions)
  - **Ex: 5p13 deletion including a CdLS gene**

**Extra pieces** (duplications)

**Pieces that switch places** (translocations)
- Balanced translocation
- Unbalanced translocation
Nuts and Bolts: Genes

Approximately 25,000 in the human genome

- About 5% of the human genome contains known genes
- Function of much of the genome is unknown

Mistakes happen → mutations

- Deletions, duplications, expansions, point mutations
Autosomal Dominant: New Mutation (de novo)

De novo = new

99% individuals with CdLS have a genetic change inherited in this way

Can be on chromosomes pairs 1-22 (called autosomal dominant)

OR

chromosomes X (X-linked dominant)
Autosomal Dominant: Inherited

<1% individuals have been found to have genetic change inherited from a parent.
X-Linked:

Both males and females with **SMC1A-** and **HDAC8**-related CdLS

In females with HDAC8 changes, there is variability due to **X-inactivation**
- Randomly shut off one copy of the X chromosome

Though **SMC1A** is also located on the X chromosome this X-inactivation process does not apply to the **SMC1A** gene.
Mosaic Mutation

Few individuals have a ‘mosaic change’

Genetic change in only some cells

Symptoms occur depending on where the mutation is located
Genes Known For CdLS

Classic:
- \textit{NIPBL}: 60%
- \textit{SMC1A}: 5\% (X-Linked)
- \textit{HDAC8}: 4\% (X-Linked)
- \textit{SMC3}: 1-2\%
- \textit{RAD21}: <1\%

Atypical/Overlapping:
- \textit{BRD4}
- \textit{KMT2A}
- \textit{AFF4}
- \textit{ANKRD11}
- \textit{TAF1/6}
Diagnostic Genetic Testing

**Single gene:**
- typically start with most common gene (NIPBL)

**Panel:**
- 2 or more CdLS genes at once

**Exome Sequencing:**
- sequencing all genes

Type of testing based on symptoms. Can send testing for one (single gene) or more genes (panel).

- NIPBL
- RAD21
- SMC1A
- KMT2A
- HDAC8
- AFF4
- SMC3
- ANKRD11
Diagnostic genetic testing: Exome

Possibly CdLS based on symptoms, but not sure enough so can have larger/broad scale test to evaluate for any genetic answer.

Evaluate functional parts of almost all 20,000+ genes looking for unexpected changes

https://dnalabsindia.com/blog/what-is-clinical-exome-sequencing/
Sample Types

Blood

Saliva

Cheek Swab
Possible Interpretation of genetic test results

Positive
  Disease causing genetic change

Negative
  No genetic change found in genes associated with CdLS
  Does not mean there is not a genetic change in individuals' genes, just could not be found with current testing modality

Variant of Uncertain Significance
  Genetic change identified
  Not enough evidence to know if benign variation (normal) or affects the gene so that it does not work
NIPBL mutation analysis

<table>
<thead>
<tr>
<th>Name: [redacted]</th>
<th>Gender: female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample accession#: 12.2429</td>
<td></td>
</tr>
<tr>
<td>Date: [redacted]</td>
<td>Sample type: peripheral blood-EDTA</td>
</tr>
<tr>
<td>Received: 11/19/2012</td>
<td>Collected: NA</td>
</tr>
</tbody>
</table>

**RESULT:** c.7219C>T (p.Arg2407*) pathogenic sequence change identified in the NIPBL gene in this patient.

<table>
<thead>
<tr>
<th>GENE</th>
<th>NUCLEOTIDE CHANGE</th>
<th>AMINO ACID CHANGE</th>
<th>ZYGOSITY</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIPBL</td>
<td>c.7219C&gt;T</td>
<td>p.Arg2407*</td>
<td>Heterozygous</td>
<td>Mutation</td>
</tr>
</tbody>
</table>

**INTERPRETATION:** This pathogenic sequence change is the likely cause of this patient's Cornelia de Lange syndrome phenotype.
MOLECULAR GENETICS REPORT:
Cornelia de Lange Syndrome NextGen Sequencing Panel

SUMMARY OF RESULTS: NEGATIVE

RESULTS AND INTERPRETATIONS: In this patient, for the relevant genes, we found no sequence variants that are likely to be a primary cause of disease.

These results should be interpreted in context of clinical findings, family history and other laboratory data. All genetic tests have limitations. Please see limitations and other information for this test on pages 3 - 5.

NOTES: Deletion and duplication testing is in progress and results will be reported separately. Genetic counseling is recommended.

GENES SEQUENCED (Transcript Numbers): HDAC8 (NM_018486.2), NIPBL (NM_133433.3), RAD21 (NM_006265.2), SMCTA1 (NM_006306.3), SMC3 (NM_005445.3)
Reading A Genetic Test Report – INCONCLUSIVE

Cornelia de Lange Seq + Del/Dup Panel

CLINICAL INDICATION
Microcephaly, failure to thrive, global developmental delays

RESULTS SUMMARY
INCONCLUSIVE: An established cause of the reported phenotype was NOT identified.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Transcript</th>
<th>Variant</th>
<th>Zygosity</th>
<th>Classification</th>
<th>Inheritance</th>
<th>Disease</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMCGA</td>
<td>NH_004606.3</td>
<td>c.1301G&gt;C; p.Arg434Pro</td>
<td>Mem</td>
<td>VUS</td>
<td>XL</td>
<td>Cornelia de Lange Syndrome</td>
<td>5q34-35</td>
</tr>
</tbody>
</table>
What Do We Do With A Variant of Unknown Significant (VUS)?

• Interpretation of the variant can change over time if new evidence is learned.

Examples of new evidence:

Another individual with CdLS also has the same genetic change
A study looks at how the change affects mice and it leads to symptoms like CdLS

• Evaluate additional genes if not all genes for CdLS have been tested
Alphabet of Genetic Results... the c’s and p’s

**c.1345 A>G (p.Phe448Tyr)**

- Genes are written in sequence of letters that stand for ‘nucleotides’: A, T, C, G

- “c.” number (position) along gene where there is letter change (in this example, A to G)
Alphabet of Genetic Results... the c’s and p’s

c.1345 A>G (p.Phe448Tyr)

Every three letters code (‘codons’) for amino acid, which all together make up proteins of body

“p.” normal amino acid, codon position in the gene, followed by the new amino acid with the letter change

Types of Mutations

**Chromosomes** are like encyclopedias; one set is from the mother, one is from the father.

**Genes** are like pages of descriptions.

**Mutations** are like misspelled words or the disruption of a sentence.

**Types of Gene Mutations**

**MISSENSE MUTATIONS** change one word or letter
- THE CAR WAS RED → THE CAR WAS HAT
- THE CAR WAS RED → THE CAR WAS RDD

**INSERTION MUTATIONS** add one word or letter
- THE CAR WAS RED → THE CAR HAT WAS RED
- THE CAR WAS RED → THE CAR ESW ASR ED

**NONSENSE MUTATIONS** end the instructions too soon
- THE CAR WAS RED → THE CAR

**DELETION MUTATIONS**
- THE CAR WAS RED → THE WAS RED
- THE CAR WAS RED → THE RWA SRE D
Types of Mutations (aka variants)

**Missense**
- Change in letter changes single amino acid
- Protein made but may be incorrect since wrong amino acid

**Nonsense**
- Change in letter leads to “stop” instruction codon
- No protein or a very shortened protein is made

**Frameshift: insertion/deletion**
- Affects pattern of ‘3 letters=1 codon’
- Change in letter affects multiple amino acids
- Protein may or may not be made, possibly wrong shape

**Splice site**
- Changes part of gene that affects how gene is processed into instruction to make protein
- Without correct instruction, protein not made correctly or at all
Other Terminology

**Heterozygous**
Genetic change only found on one of the two copies of the gene

**Hemizygous**
Genetic change found on the X chromosome in a male. Males only have one X chromosome so term is –hemi versus –hetero

**Paternal or maternal:** if genetic variant was inherited from father (paternal) or mother (maternal)
Genotype/Phenotype Correlations

"Genotype-Phenotype Correlation"

Association between certain mutation in a specific gene (genotype) +
Resulting presence, absence or severity of symptoms (phenotype)
Genotype/Phenotype Correlations

**NIPBL**
- Characteristic facial features
- More commonly have structural differences (i.e. limb differences)
- Severity depends on type of mutation and where in the gene
  - Truncating tends to have a more significant effect on the gene that can ultimately block protein production

**RAD21**
- Typically do not have major structural differences
- Milder learning disabilities
- Small size, minor skeletal differences, and overlapping facial features

Mannini et al. 2013

Figure 3. Genotype–phenotype correlations in CdLS due to NIPBL mutations.
Genotype/Phenotype Correlations

**HDAC8 (XL)**
- Some differences compared to “typical” CdLS facial features
  - Delayed closure of the anterior fontanelle
- Varying pattern of skin pigmentation
- Less growth restriction and a lower frequency of microcephaly
- In females, severity affected by X-inactivation

**SMC1A (XL but not affected by X inactivation) & SMC3**
- Fewer structural differences, (i.e. limb difference or heart difference)
- Less significant impact on growth still have learning difficulties
- Missense vs Truncating SMC1A
  - Missense: CdLS
  - Truncating: seizure and intellectual disability presentation

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*Mannini et al. 2013*
Benefits of Testing

Confirm a diagnosis – psychological benefit

Recurrence Risk
- Prenatal diagnosis - future pregnancies

Therapeutics
- Possibility of drug interactions with known gene mutation (future study)
  - If it appears everyone with CdLS who has variant in a specific gene all has similar reaction to a medication, positive or negative, associations can be made and better recommendations created

Research opportunities - to better understand CdLS
- Expanding “genotype/phenotype” correlations
  - i.e. SMC1A population:
    - frameshift mutation: seizures and other symptoms
    - most missense mutation: typical CdLS presentation

Impact on medical management

https://www.mindtools.com/pages/article/newTED_05.htm
Limitations of Testing

Testing is not perfect:
• Detection less than 100% which leaves possibility for uncertainty of unknown

Variants of Unknown Significance (VUS)
• Leaves individual and family with uncertainty

Cost
• Expensive and not always covered by insurance

Sample collection difficult
Prenatal Genetic Testing

Targeted variant

- Testing for known genetic change in family
  Through amniocentesis or CVS

DNA panel

- Testing for group of genes related to just CdLS or large group of genes that includes those for CdLS
  - Usually recommended when there are ultrasound findings suggesting CdLS through amniocentesis or CVS
Noninvasive Prenatal Genetic Testing (NIPT)

Use Mom’s blood, which contains baby’s blood, to check for genetic change

• Fetal DNA in maternal circulation
• Results from breakdown of fetal cells
• Primarily placental in origin
• Clears from maternal system within hours after delivery

Estimated to be 10-15% of cell-free fetal DNA in maternal system

Vistara - offered through Natera Lab

Cell free fetal DNA

Non-invasive Prenatal “Testing”
Research Genetic Testing

Cost may be covered by research laboratory

Follow-up testing in clinical laboratory
  • Research labs have fewer standard requirements vs. clinical labs

Results
  • Types of results (positive, negative, VUS) are the same
  • NOT all research groups give results
  • Some may report positive results and not the VUS information

If participating in a research study, you can ask if this information will be given
THANK YOU