CdLS Foundation Genetic Testing- How to read your child's test results

April 24, 2019

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

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CdLS Foundation
Cornelia de Lange Syndrome Foundation, Inc.

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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

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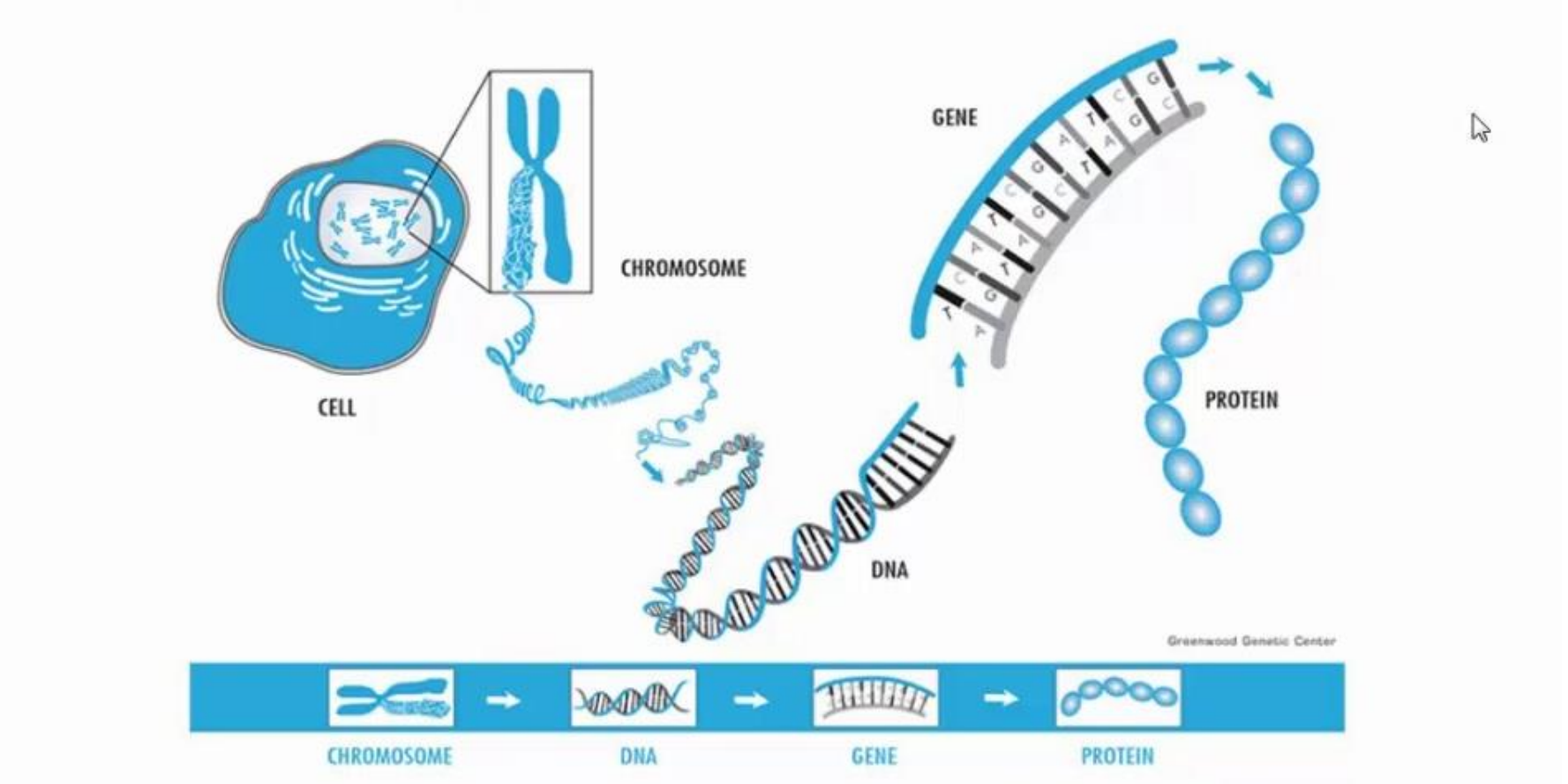
Nuts and Bolts: Chromosomes and Genes



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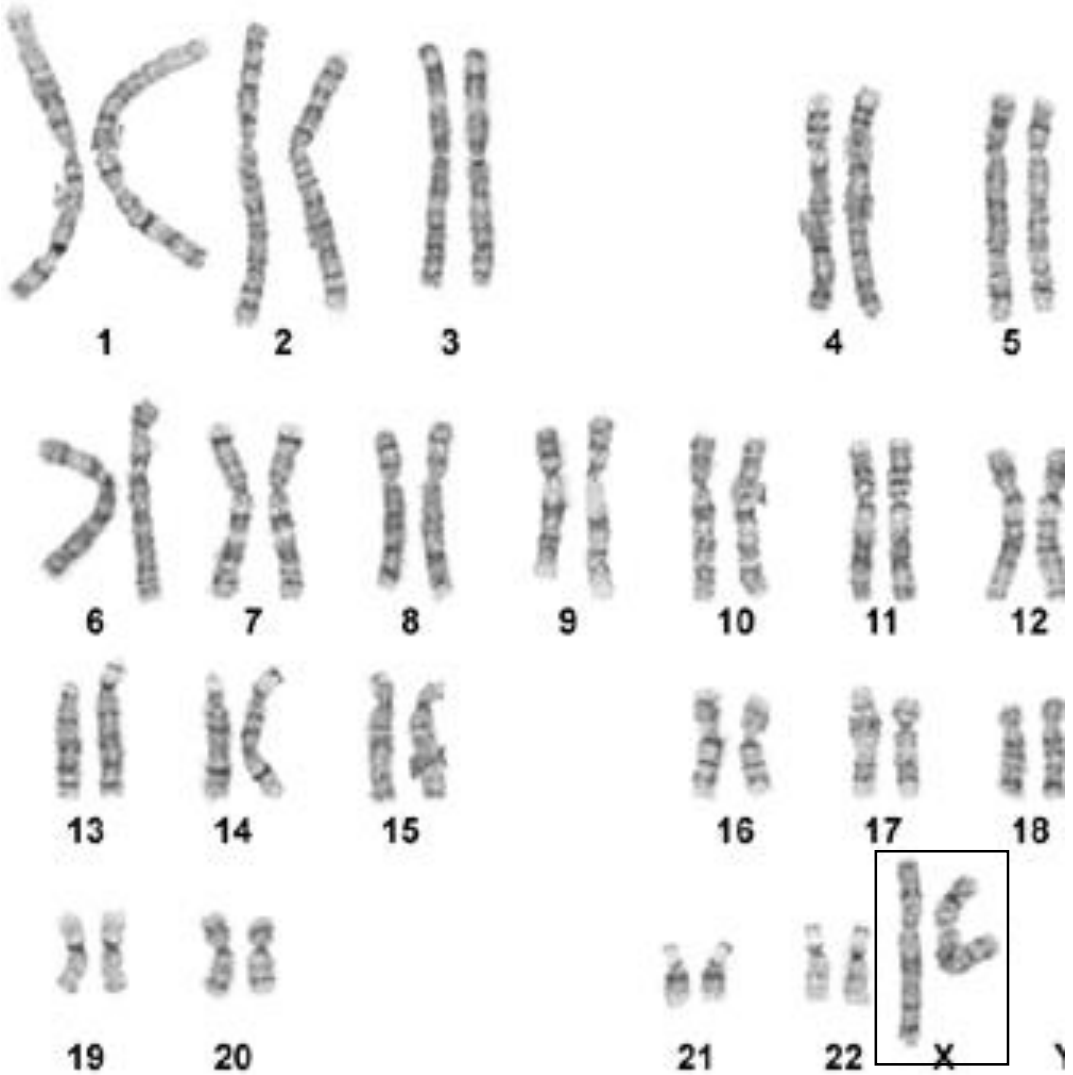
Nuts and Bolts: Chromosomes

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…

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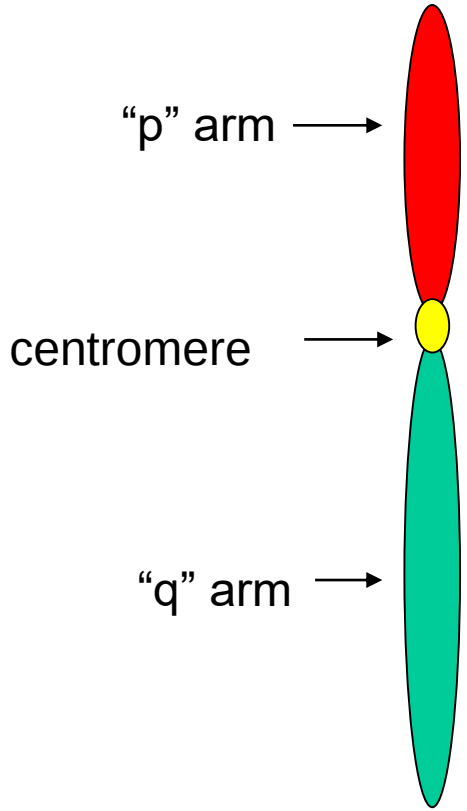
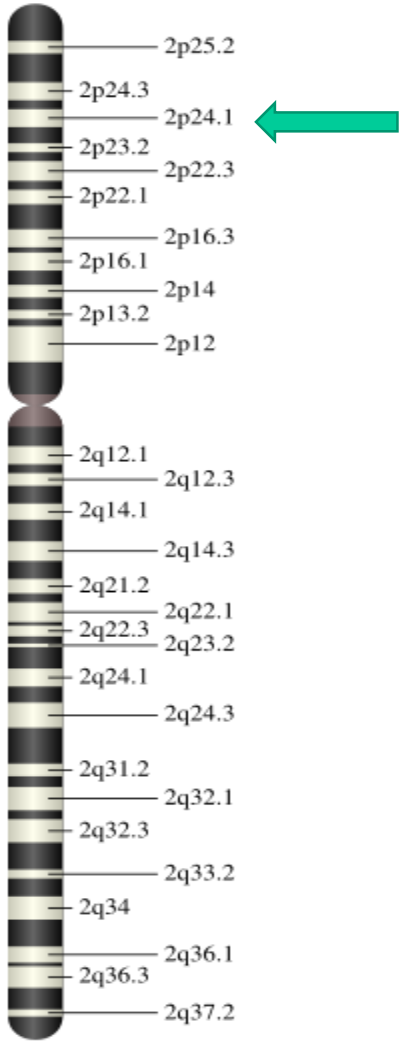
Chromosome nomenclature example:

2p24.1 means

Chromosome 2
P arm
Band 24.1



Chromosome 2



Chromosome 2

Nuts and Bolts: Chromosome Differences

Too many

Ex: Trisomy 21 (Down syndrome)

Too few

Missing pieces (deletions)

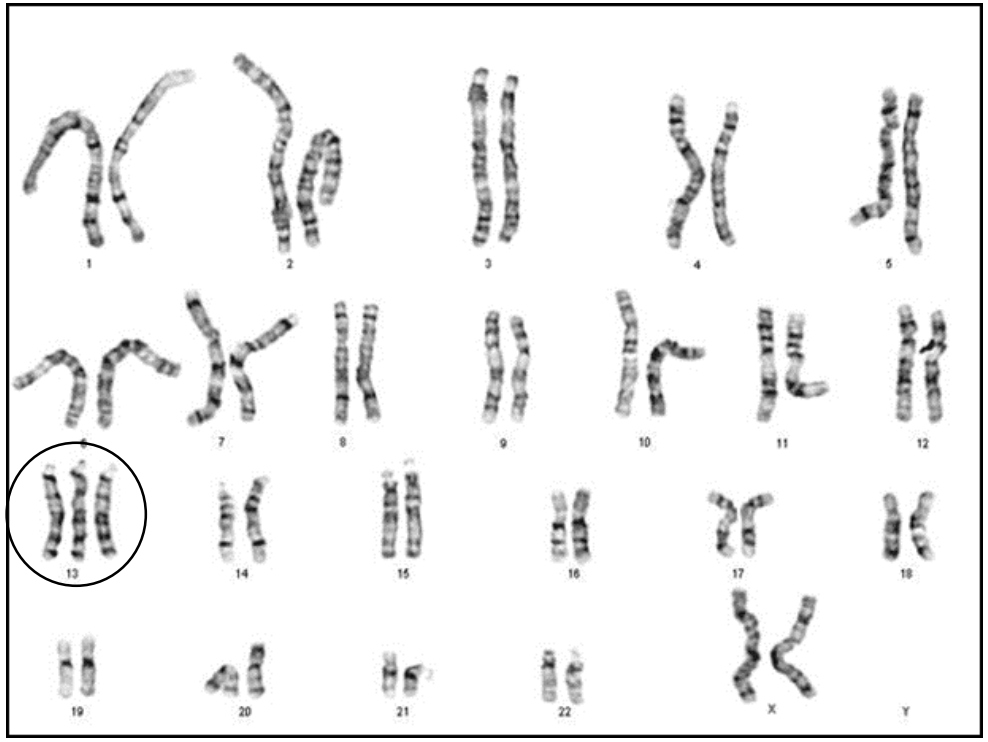
**Ex: 5p13 deletion including a CdLS gene

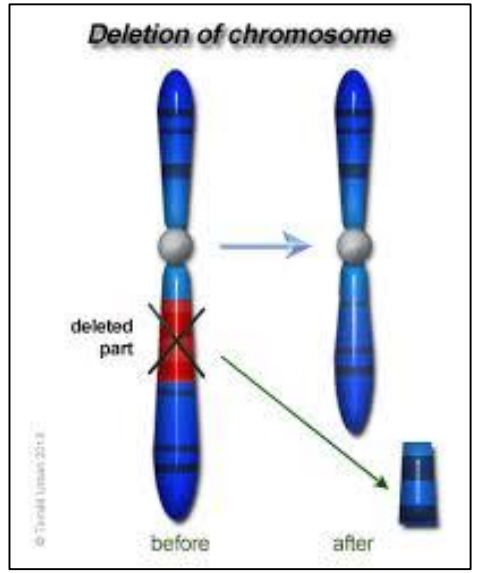
Extra pieces (duplications)

Pieces that switch places (translocations)

Balanced translocation

Unbalanced translocation





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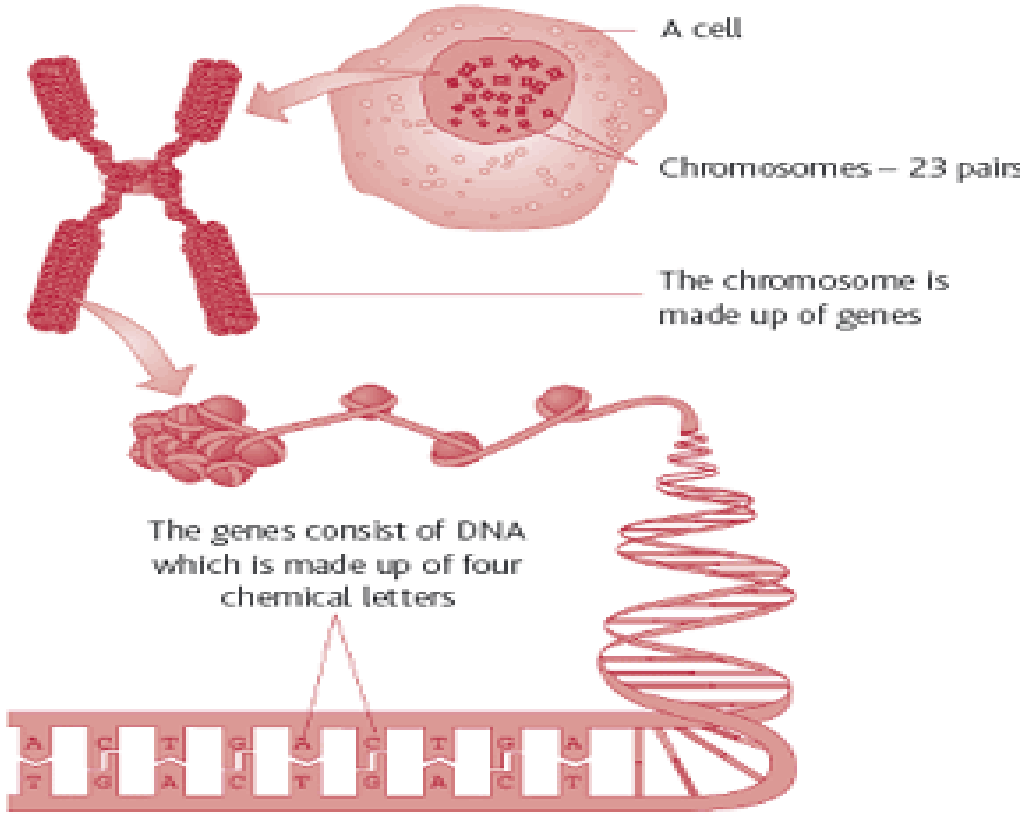
Nuts and Bolts: Genes

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)

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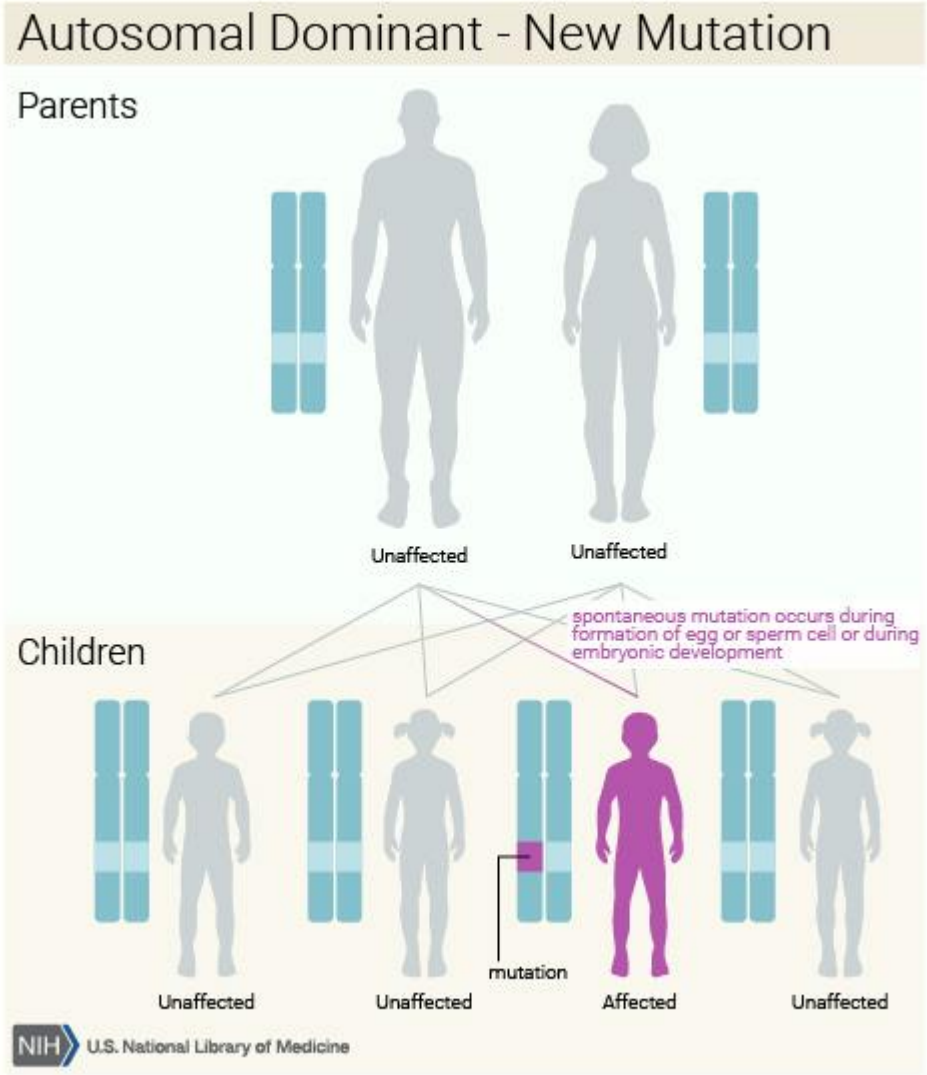
De novo = new

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

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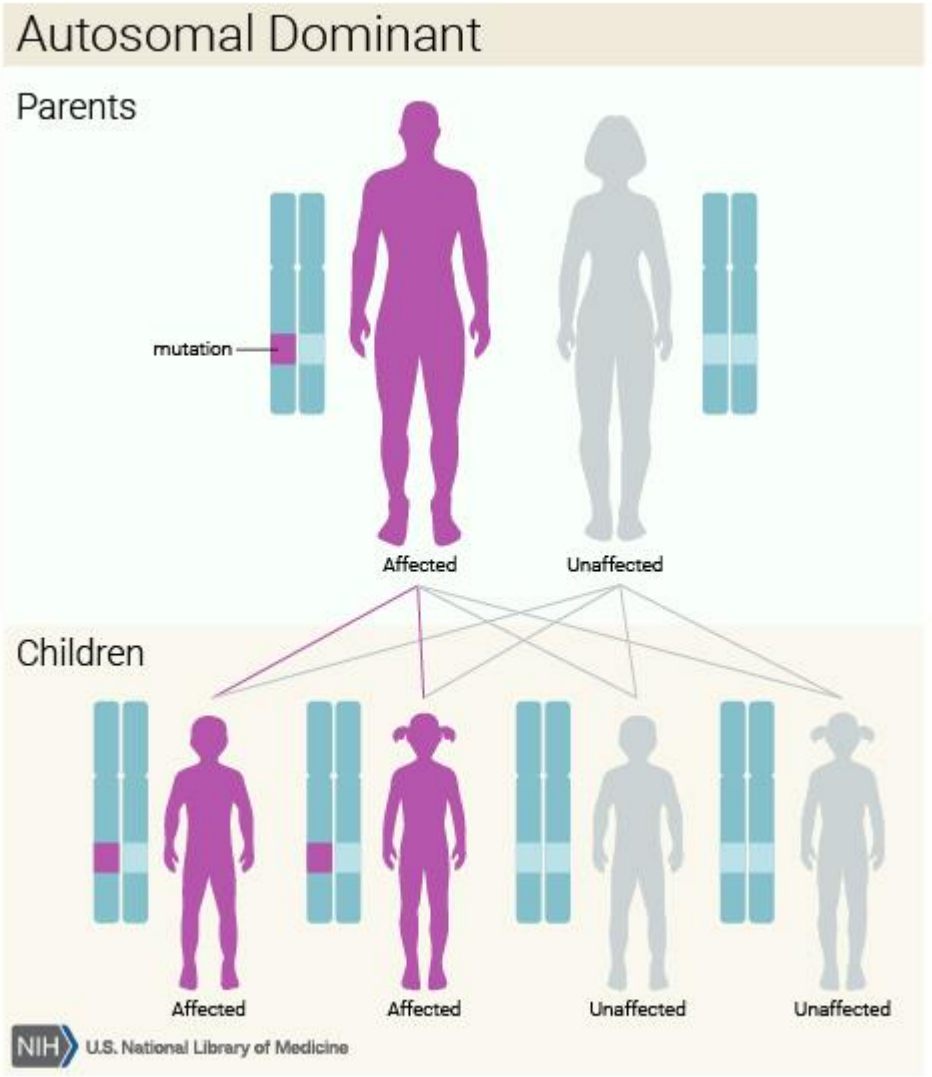
<1% individuals have been found to have genetic change inherited from a parent



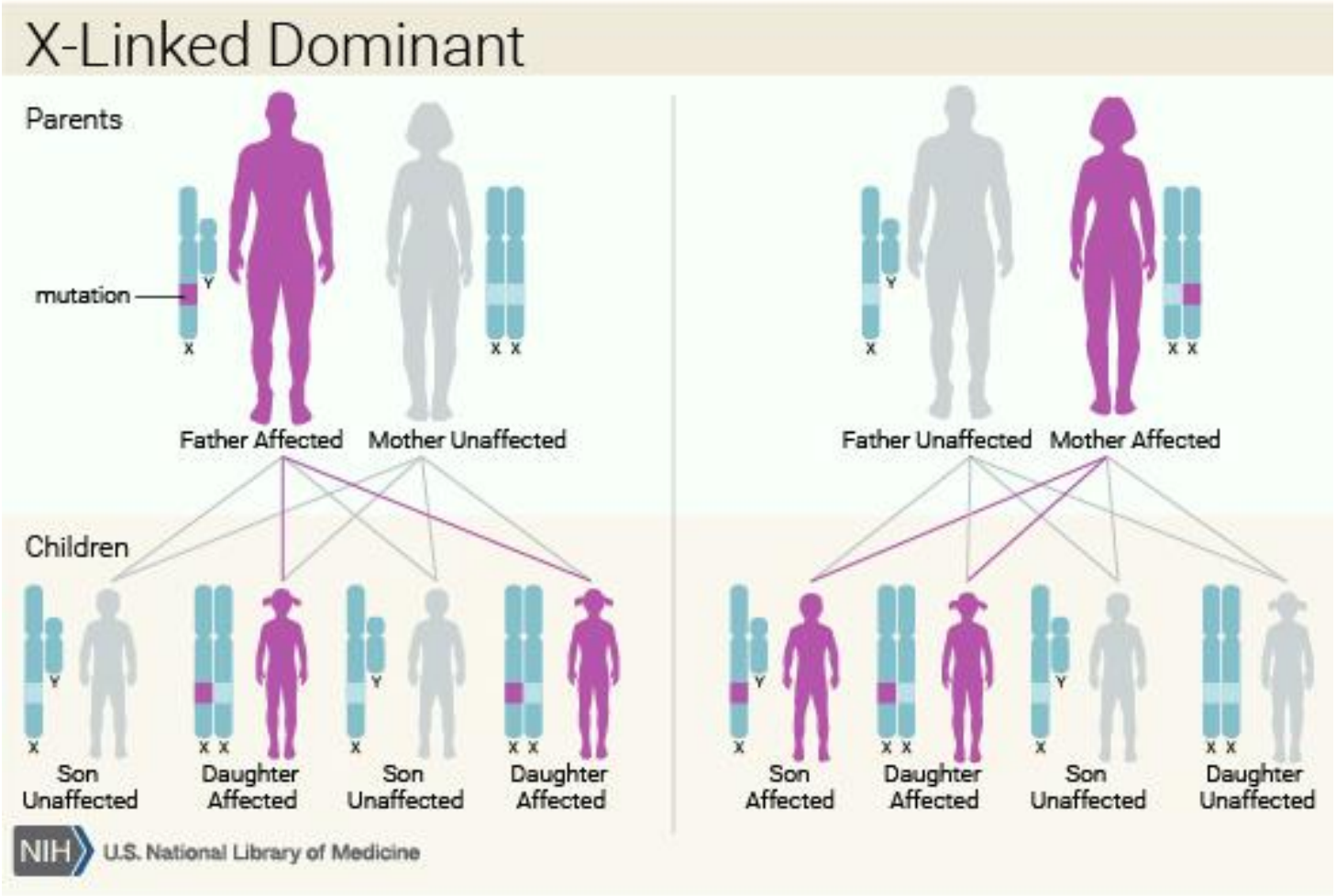
X-Linked:

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.



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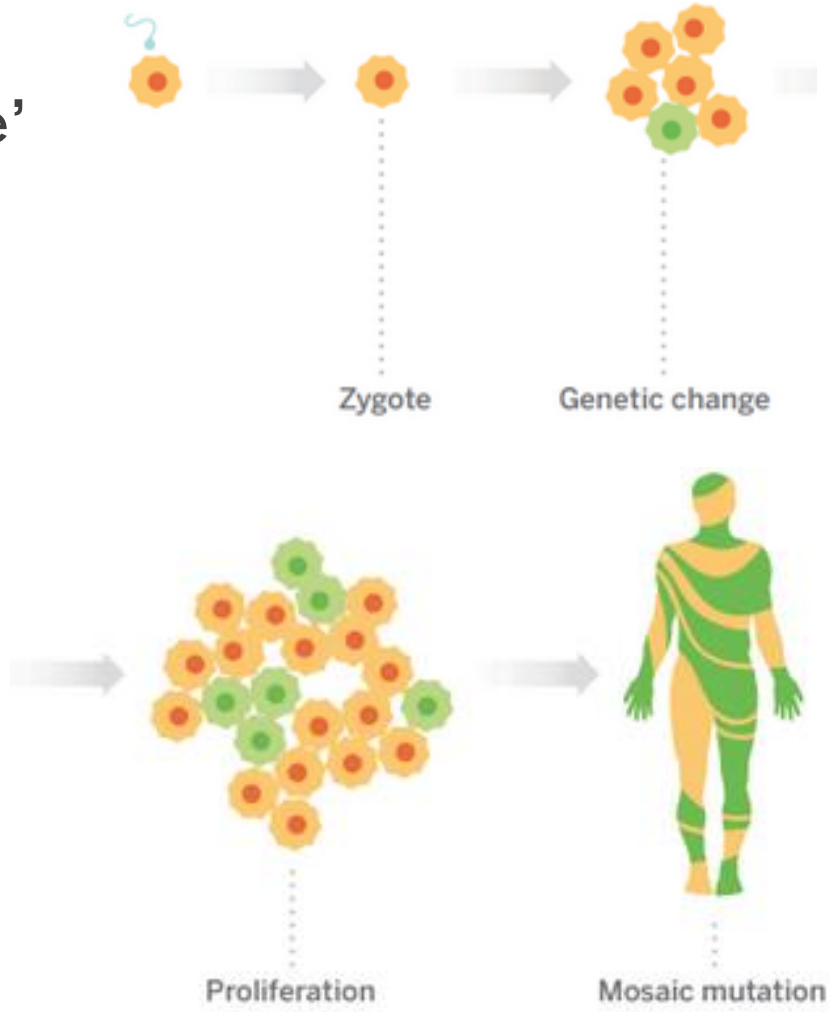
Mosaic Mutation

Few individuals have a ‘mosaic change’

Genetic change in only some cells

Symptoms occur depending on where the mutation is located



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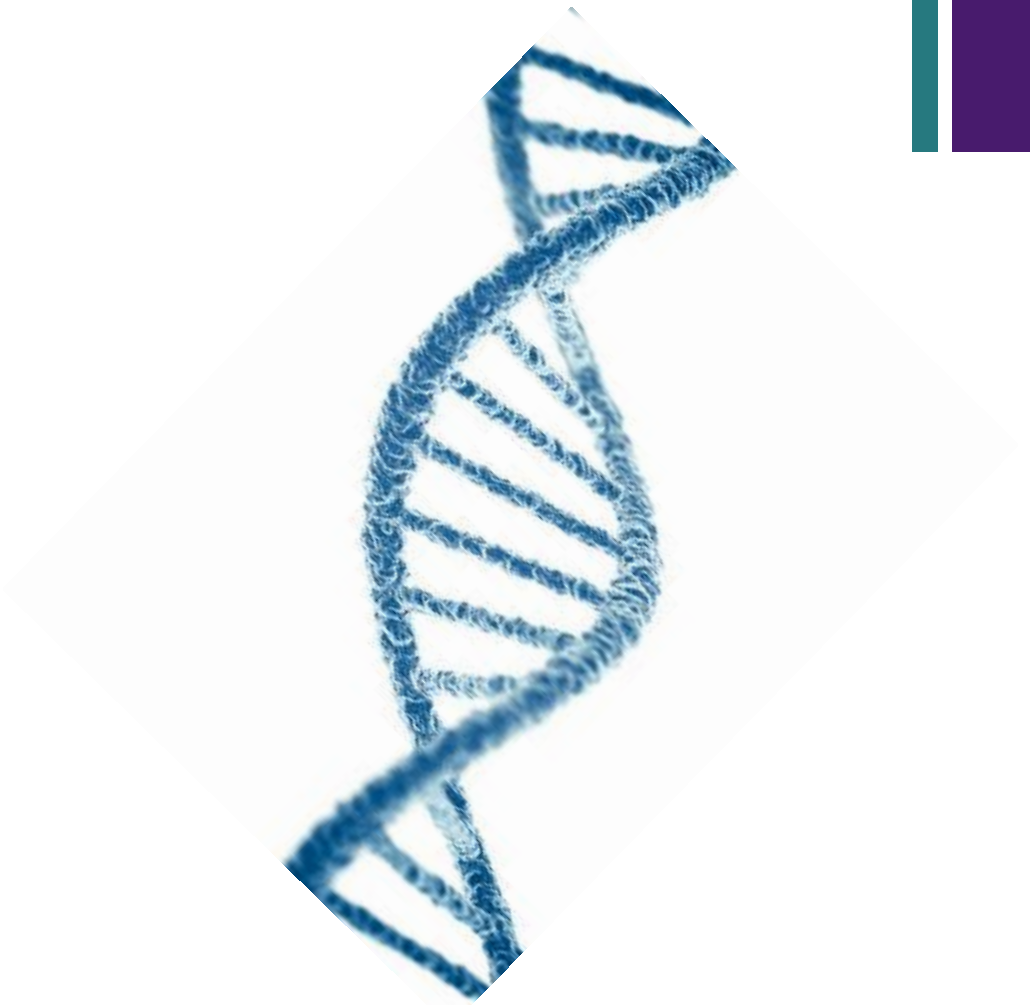
Genes Known For CdLS

Classic:

- *NIPBL*: 60%
- *SMC1A*: 5% (X-Linked)
- *HDAC8*: 4% (X-Linked)
- *SMC3*: 1-2%
- *RAD21*: <1%

Atypical/Overlapping:

- *BRD4*
- *KMT2A*
- *AFF4*
- *ANKRD11*
- *TAF1/6*



Diagnostic Genetic Testing

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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*

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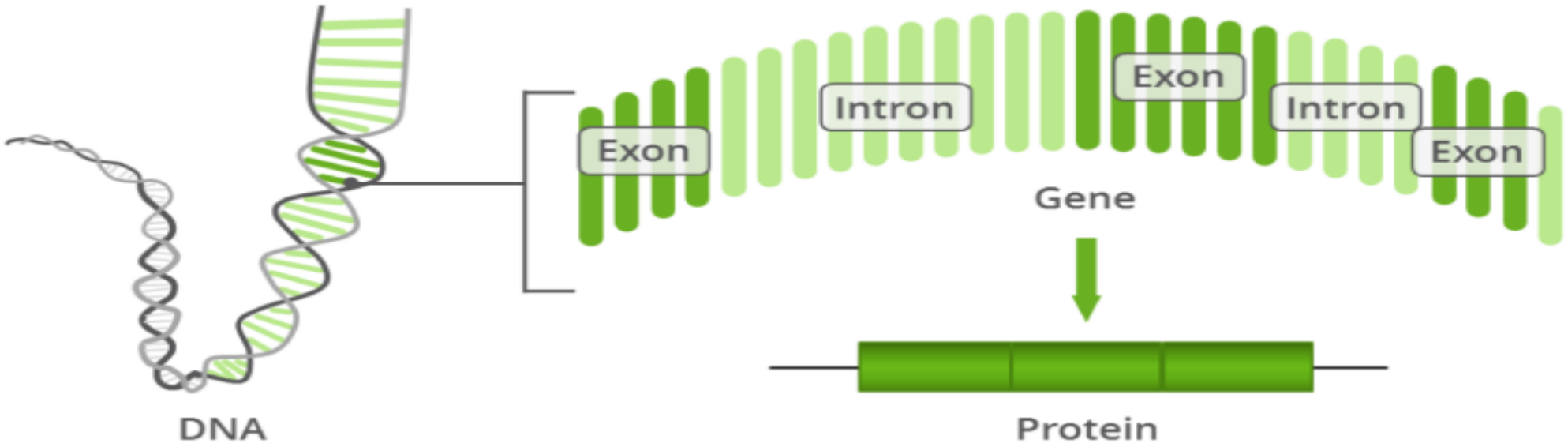
Diagnostic genetic testing: Exome

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/

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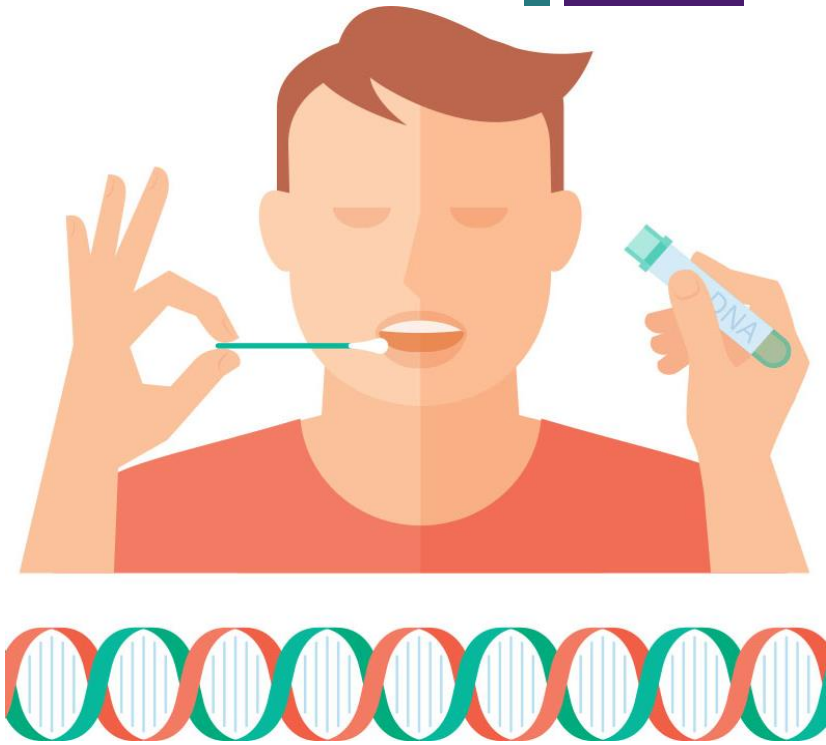
Sample Types

Blood

Saliva



Cheek Swab

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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

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Reading A Genetic Test Report - **POSITIVE**

THE UNIVERSITY OF CHICAGO
GENETIC SERVICES LABORATORY

5841 S. Maryland Ave., Rm. G701, MC 0077
Chicago, Illinois 60637
Toll Free: (888) UC GENES
Local: (773) 834 0555 FAX: (773)702-9130
ucgslabs@genetics.uchicago.edu
www.dnatesting.uchicago.edu
CLIA #: 14D0917593 CAP #: 18827-49

NIPBL mutation analysis

Ref physician: Ian Krantz, M.D. **Phone:** 215-590-2931 **Fax:** 215-590-3850 **cc:** Sarah Noon, CGC Children's Hospital of Philadelphia	**Name:** [redacted]	**Gender:** female
	Sample accession#: 12.2429	[redacted]
	Date [redacted]	**Sample type:** peripheral blood-EDTA
	Received: 11/19/2012	**Collected:** NA
	Indication for testing: other specified anomalies	

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

GENE	NUCLEOTIDE CHANGE	AMINO ACID CHANGE	ZYGOSITY	INTERPRETATION
NIPBL	c.7219C>T	p.Arg2407*	Heterozygous	Mutation

INTERPRETATION: This pathogenic sequence change is the likely cause of this patient's Cornelia de Lange syndrome phenotype.

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Reading A Genetic Test Report - **NEGATIVE**

PREVENTION GENETICS
DISEASE PREVENTION THROUGH GENETIC TESTING

CLIA #: 52D2065132 • CAP #: 7185561
3800 S. Business Park Ave. · Marshfield, WI 54449
Ph: 715-387-0484 · Fax 715-384-3661
www.preventiongenetics.com
Bruce R. Krawisz, M.D.
Clinical Laboratory Director

Patient	Sample Information	Ordering Provider(s)
[redacted]	Type: Whole Blood Collected: April 05, 2016 Received: April 08, 2016 PG ID: 2016-099-027	Paula Goldenberg, MD Massachusetts General Hospital

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), *SMC1A* (NM_006306.3), *SMC3* (NM_005445.3)

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Reading A Genetic Test Report – **INCONCLUSIVE**

The Children's Hospital of Philadelphia®

DEPARTMENT OF PATHOLOGY & LABORATORY MEDICINE

34TH STREET AND CIVIC CENTER BOULEVARD ·PHILADELPHIA, PA 19104 · PHONE: 215-590-2277 · FAX: 215-590-2171

Robert W. Doms, M.D., Ph.D., Chair and Pathologist-in-Chief

Patient:	[redacted]			Order #:	DGD-18-2264
Account#:	[redacted]	LOC:	GEN	Collection D/T:	4/23/2018 2:06 PM
MR#:	[redacted]	ATT DOC:	IAN KRANTZ	Received D/T:	4/23/2018 3:26 PM
Age/Sex:	[redacted]	Req By:	IAN KRANTZ	Date of report:	5/23/2018
DOB:	[redacted]	Other Doc:	SARAH RAIBLE	Sample type:	Blood

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.

Sequence Variant(s)

Gene	Transcript	Variant	Zygosity	Classification	Inheritance	Disease	Position
SMC1A	NM_006306.3	c.1301G>C; p.Arg434Pro	Hem	VOUS	XL	Cornelia de Lange Syndrome 2	ChrX: 53436388

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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

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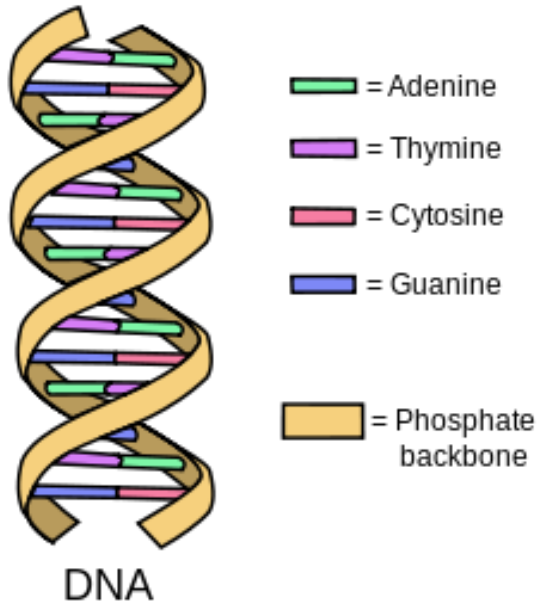
Alphabet of Genetic Results… the c's and p's

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)



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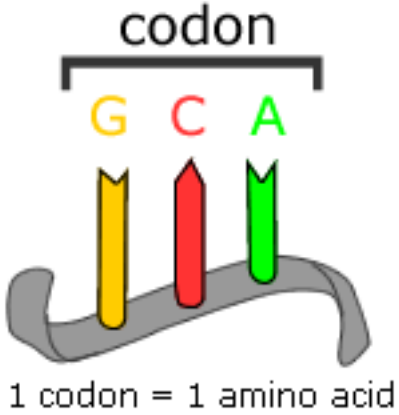
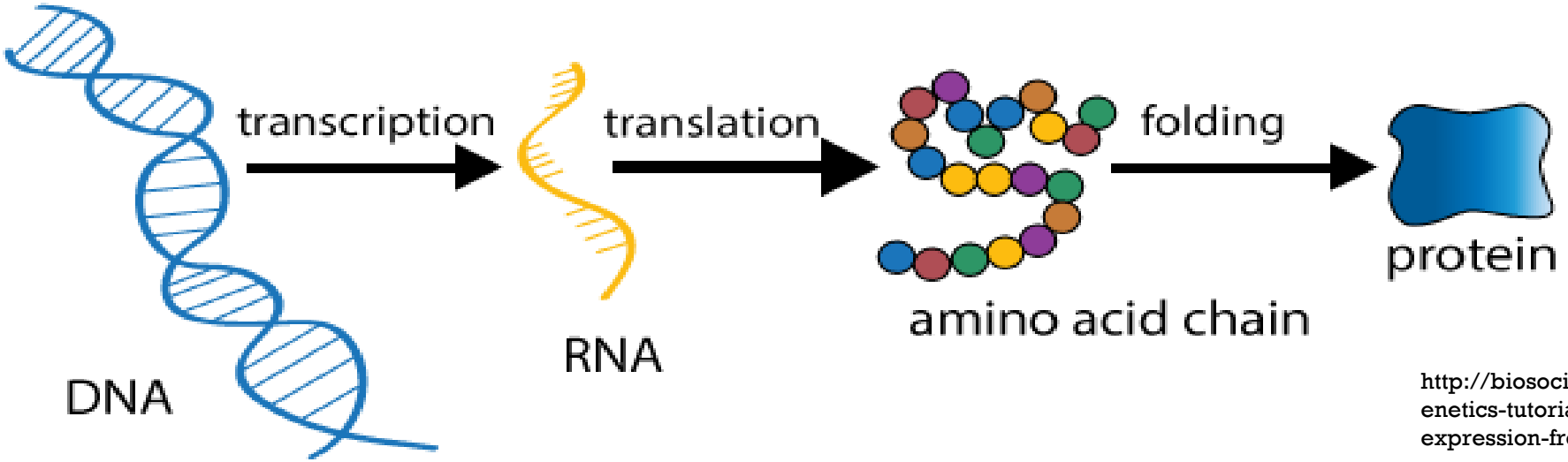
Alphabet of Genetic Results… the c's and p's

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



http://biosocialmethods.isr.umich.edu/epigenetics-tutorial/epigenetics-tutorial-gene-expression-from-dna-to-protein/

Types of Mutations

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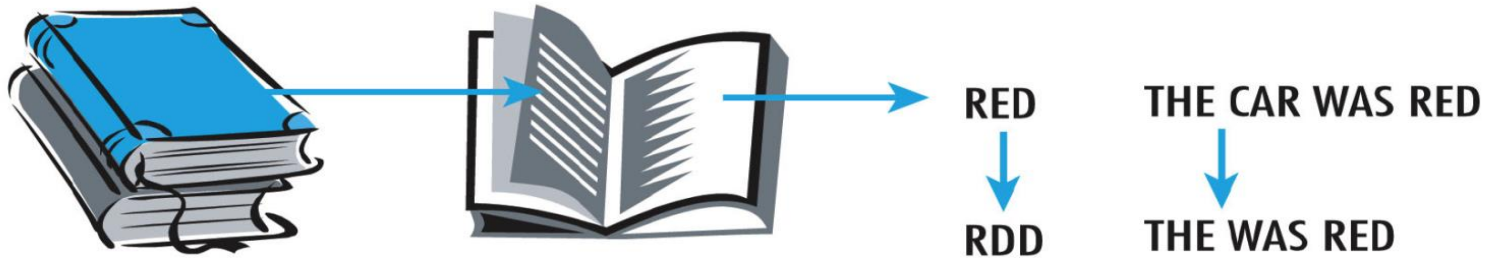
Types of Gene Mutations



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.

MISSENSE MUTATIONS change one word or letter

THE CAR WAS RED → THE CAR WAS HAT
→ THE CAR WAS RDD

INSERTION MUTATIONS add one word or letter

THE CAR WAS RED → THE CAR HAT 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 RWA SRE D

Greenwood Genetic Center

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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)

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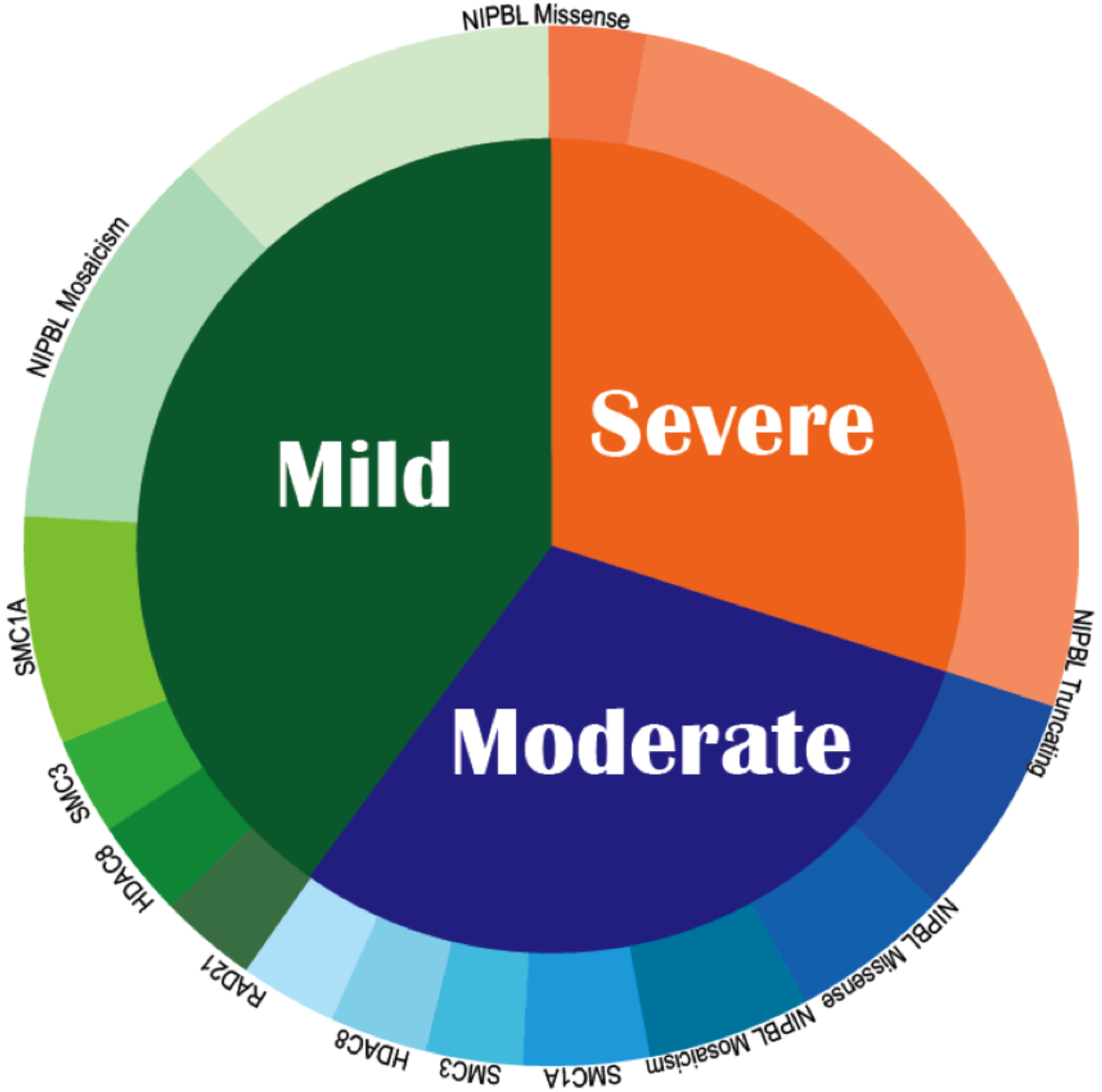
Genotype/Phenotype Correlations



"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

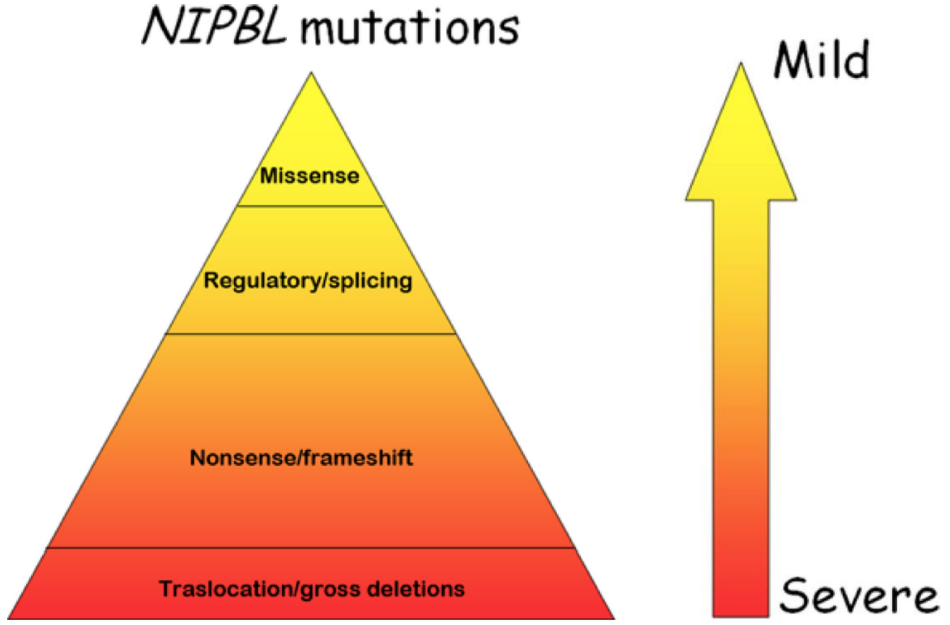


Figure 3.
Genotype–phenotype correlations in CdLS due to *NIPBL* muta

Mannini et al. 2013

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

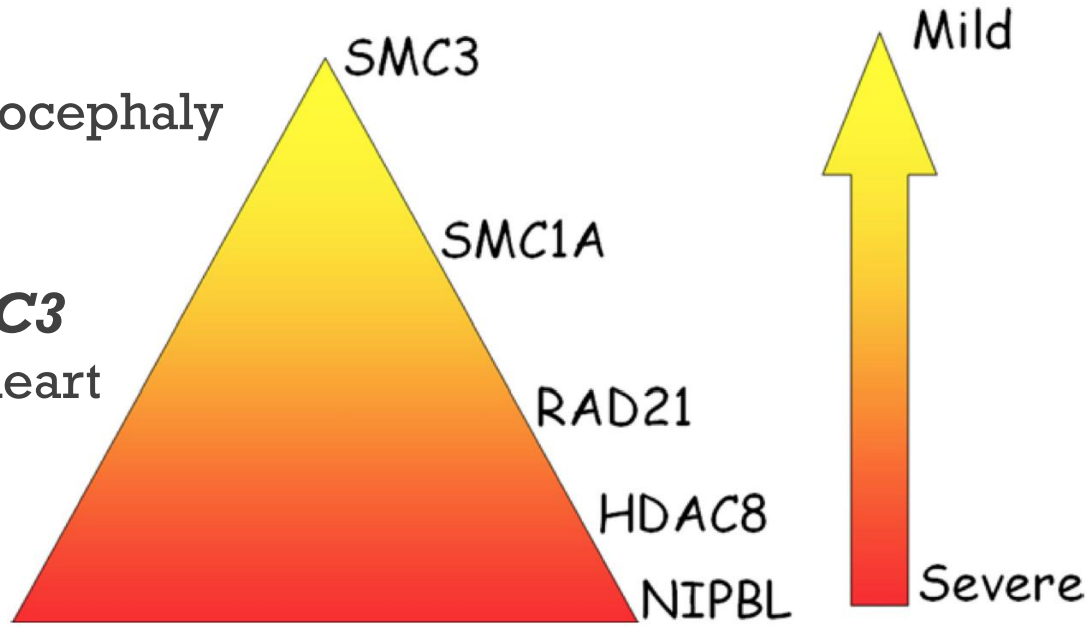


Figure 4.
Diagram representing the correlation genotype–phenotype of the five CdLS causative genes.

Mannini et al. 2013

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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

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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

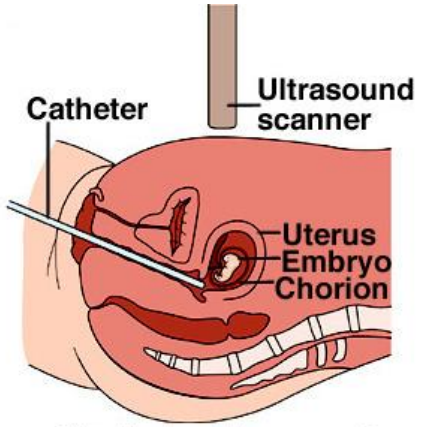
Targeted variant

- Testing for known genetic change in family

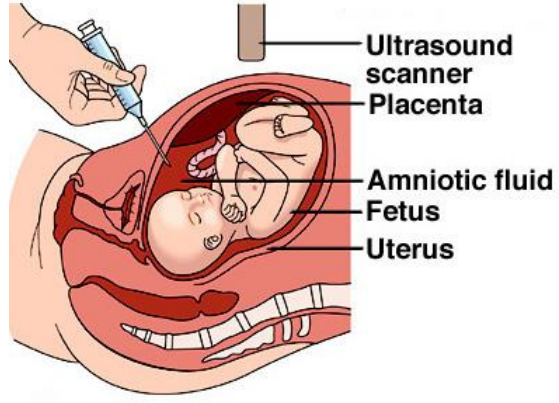
  Through amniocentesis or CVS

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



Chorionic villus sampling



Amniocentesis

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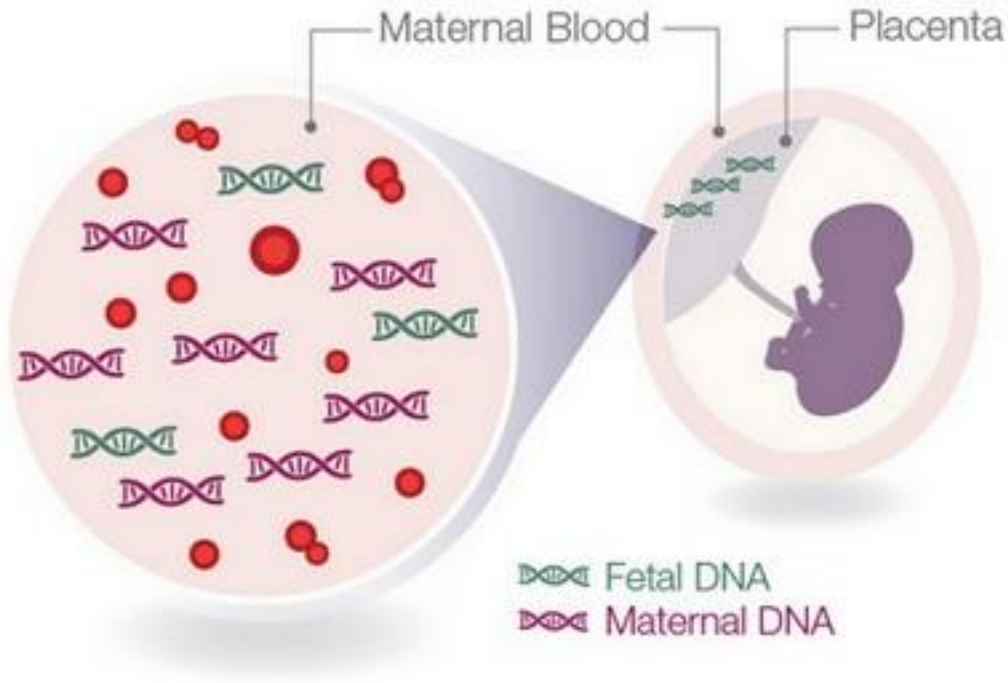
Noninvasive Prenatal Genetic Testing (NIPT)

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



Cell free fetal DNA

Vistara - offered through Natera Lab

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