Genetic Issues in Hypoxic-Ischemic Encephalopathy Cases

Barbara K. Burton, MD
Professor of Pediatrics
Northwestern University Feinberg School of Medicine
Division of Genetics, Genomics and Metabolism
Ann & Robert H. Lurie Children's Hospital of Chicago

Overview of Presentation

- Why genetic issues arise in HIE cases
- “Red flags” that may indicate a genetic disorder is present in potential HIE cases
- Review of basic genetics and types of genetic disorders
- Types of genetic tests and anticipated results

Why Do Genetic Issues Increasing Arise in HIE Cases?

- HIE is not the only cause of a neonatal encephalopathy. Inherited metabolic disorders can also present in this way

- The sequelae of HIE are non-specific. Cerebral palsy, seizures, developmental delay and intellectual disability have many different causes including many genetic causes

- There is an ever-increasing literature on genetic causes of CP, autism and other neurodevelopmental disabilities

Examples of Literature Fueling the Trend

- Molecular Diagnostic Yield of Exome Sequencing in Patients With **Cerebral Palsy**.Moreno-De-Luca A, Millan F, Pesacreta DR, Elloumi HZ, Oetjens MT, Teigen C, Wain KE, Scuffins J, Myers SM, Torene RI, Gainullin VG, Arvai K, Kirchner HL, Ledbetter DH, Retterer K, Martin CL.JAMA. 2021 Feb 2;325(5):467-475.

Reported that 32.7% of patients who had samples sent for whole exome sequencing with a diagnosis of CP had pathogenic or likely pathogenic variants.

- **Genetic testing** in individuals with **cerebral palsy**.May HJ, Fasheun JA, Bain JM, Baugh EH, Bier LE, Revah-Politi A; New York Presbyterian Hospital/Columbia University Irving Medical Center Genomics Team; Roye DP Jr, Goldstein DB, Carmel JB. Dev Med Child

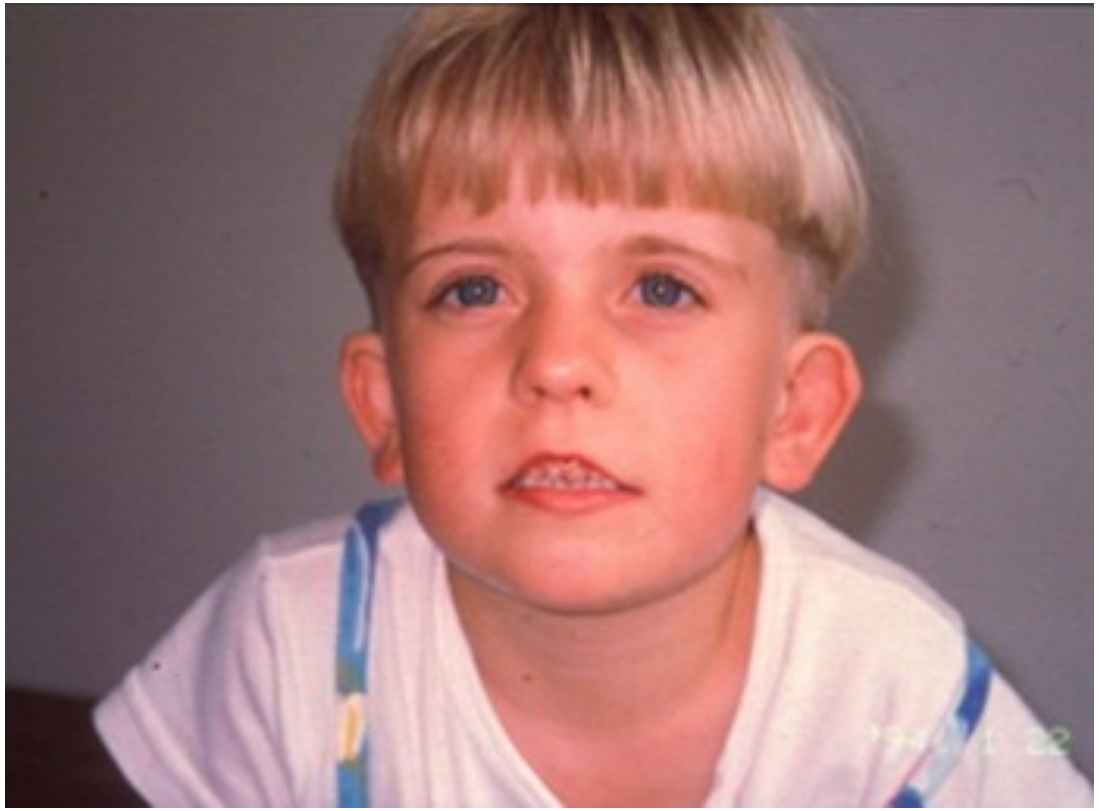
Neurol 2021; 63:1448-1455.

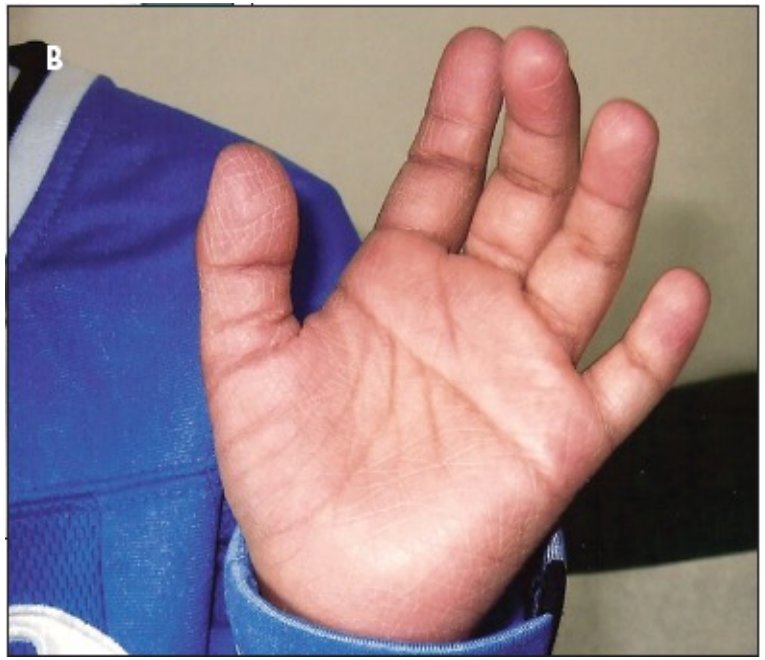
In a small cohort of patients with CP, reported that 9% had a causative genetic variant with no difference between those with “risk factors” and those without

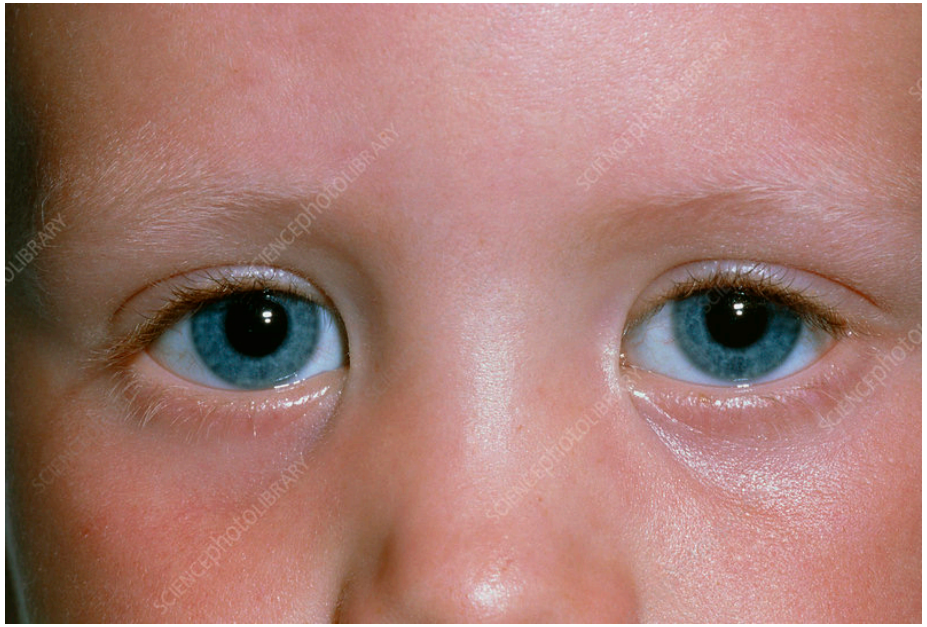
Red Flags Suggesting There Could Be An Underlying Genetic Disorder

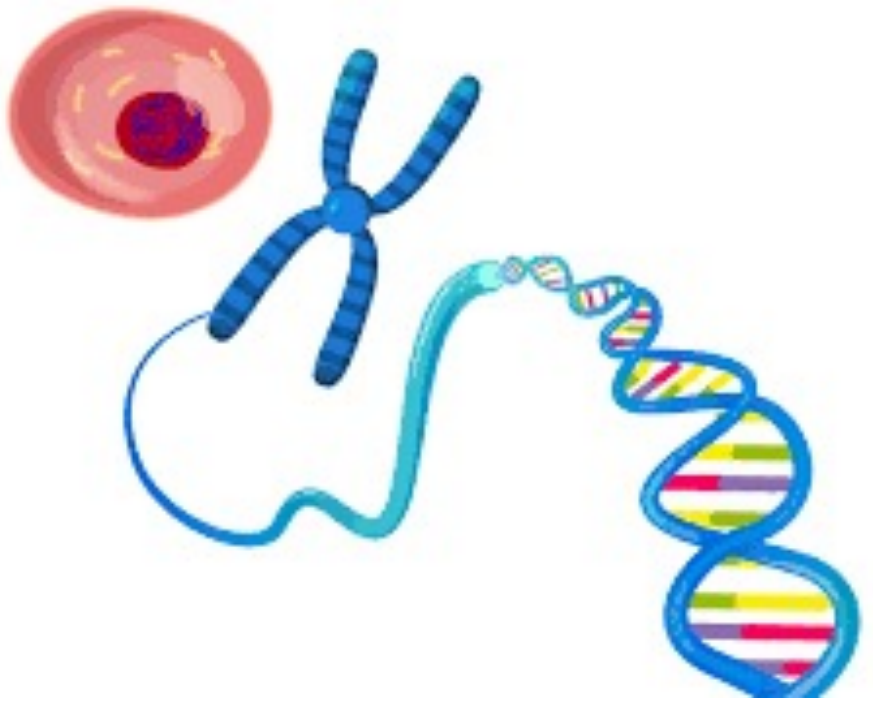
- Birth defects (esp. multiple birth defects) or dysmorphic features
- Developmental abnormalities of the brain
- Family history of cerebral palsy, spastic paraplegia, seizures, autism or other neurologic disorders
- Parents are blood relatives
- Progressive neurologic dysfunction or recurrent episodes of encephalopathy
- Normal Apgars, encephalopathy with onset on day 2 or beyond

Dysmorphic Features





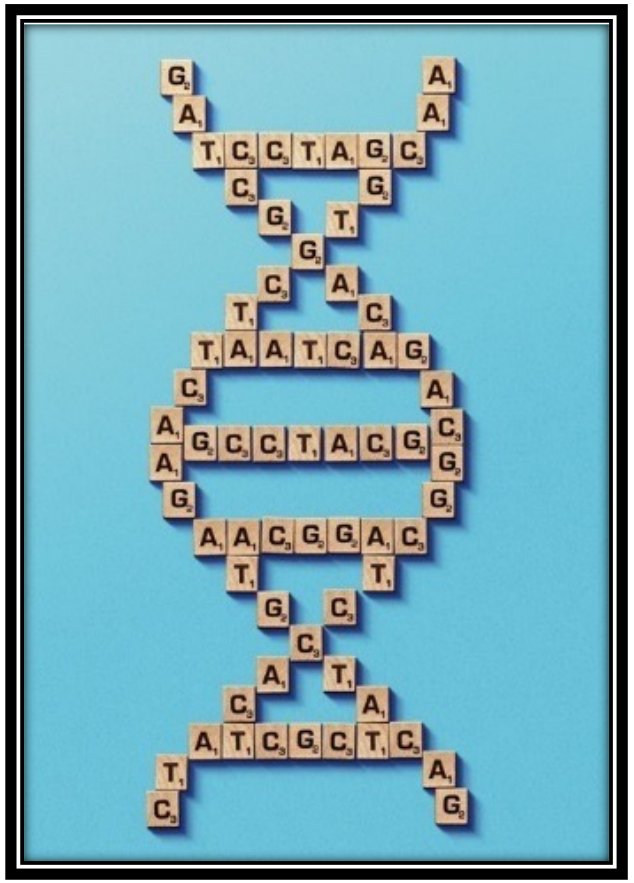


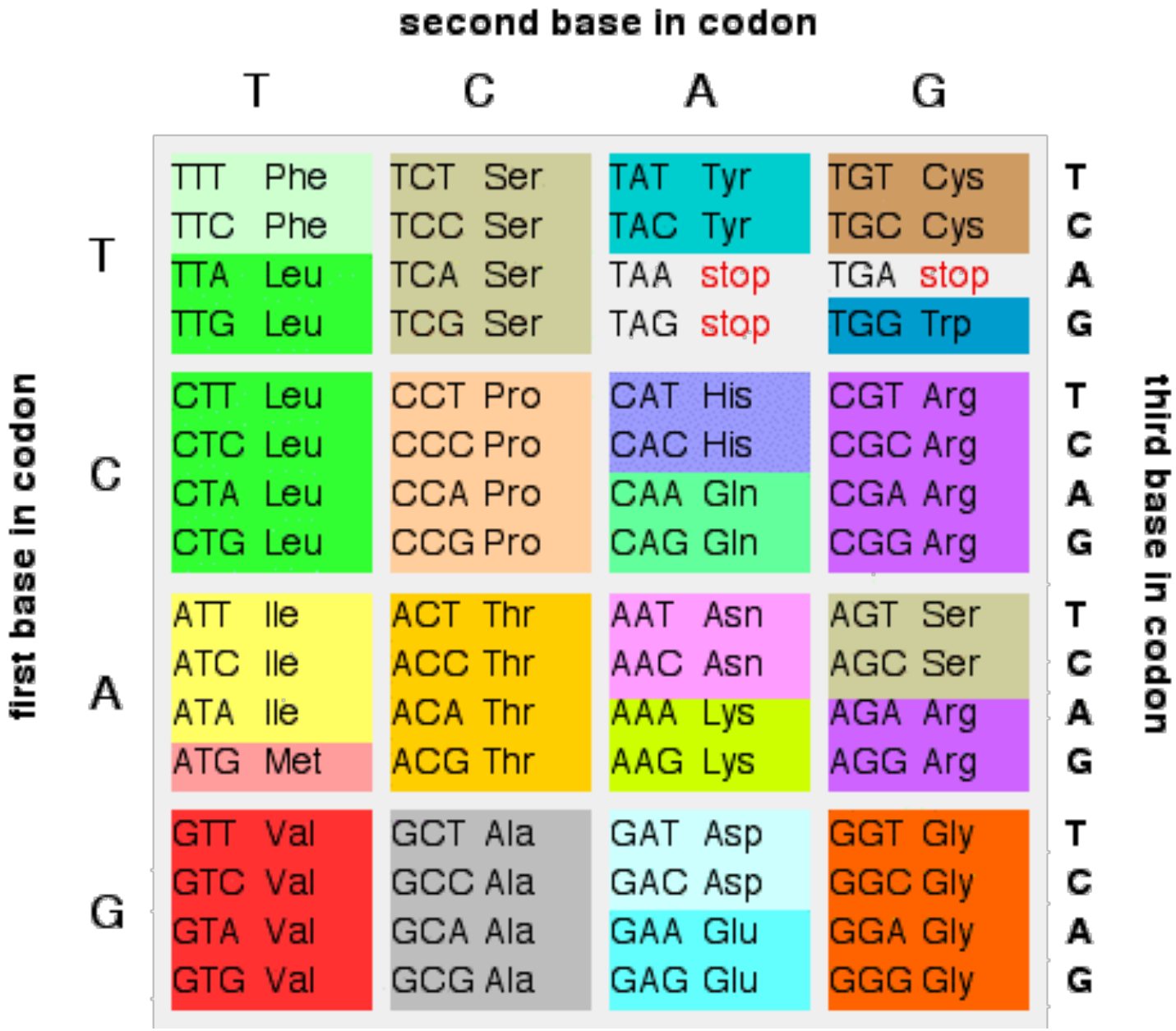


Basic Genetic Concepts

DNA

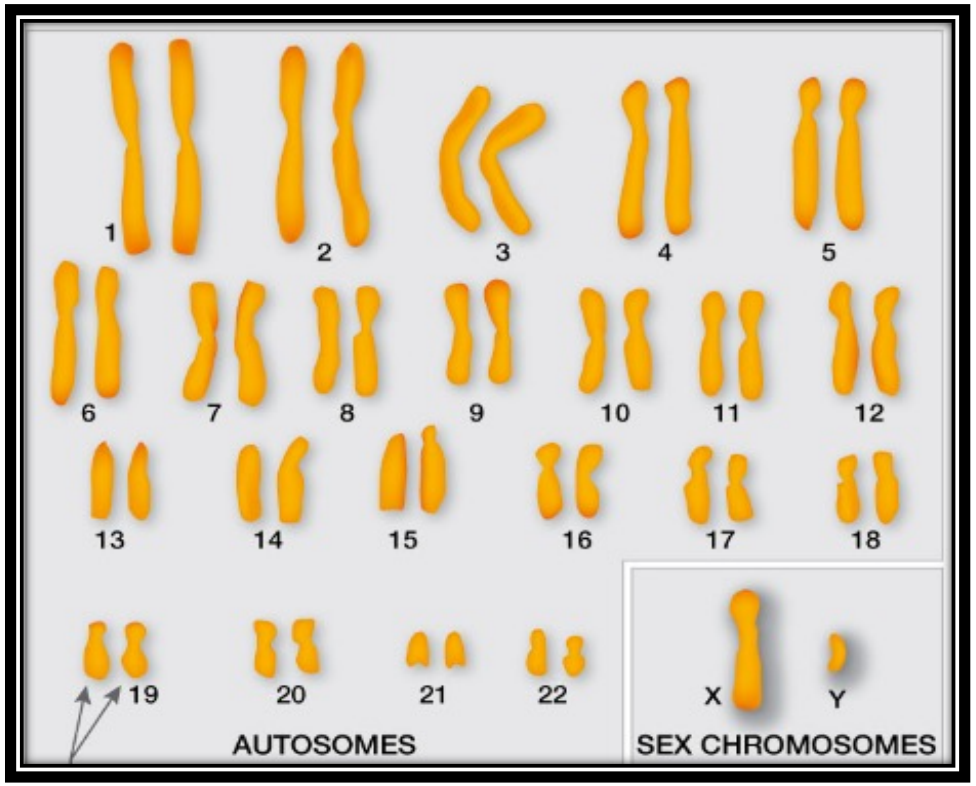
Three billion letters (nucleotides), arranged into over 20,000 words (genes) that make up a <u>blueprint</u> or <u>instruction manual</u> for our body




second base in codon
T
C
A
G
first base in codon
third base in codon
T
C
A
G
TTT Phe
TTC Phe
TTA Leu
TTG Leu
TCT Ser
TCC Ser
TCA Ser
TCG Ser
TAT Tyr
TAC Tyr
TAA stop
TAG stop
TGT Cys
TGC Cys
TGA stop
TGG Trp
CTT Leu
CTC Leu
CTA Leu
CTG Leu
CCT Pro
CCC Pro
CCA Pro
CCG Pro
CAT His
CAC His
CAA Gln
CAG Gln
CGT Arg
CGC Arg
CGA Arg
CGG Arg
ATT Ile
ATC Ile
ATA Ile
ATG Met
ACT Thr
ACC Thr
ACA Thr
ACG Thr
AAT Asn
AAC Asn
AAA Lys
AAG Lys
AGT Ser
AGC Ser
AGA Arg
AGG Arg
GTT Val
GTC Val
GTA Val
GTG Val
GCT Ala
GCC Ala
GCA Ala
GCG Ala
GAT Asp
GAC Asp
GAA Glu
GAG Glu
GGT Gly
GGC Gly
GGA Gly
GGG Gly
T
C
A
G
T
C
A
G
T
C
A
G
T
C
A
G

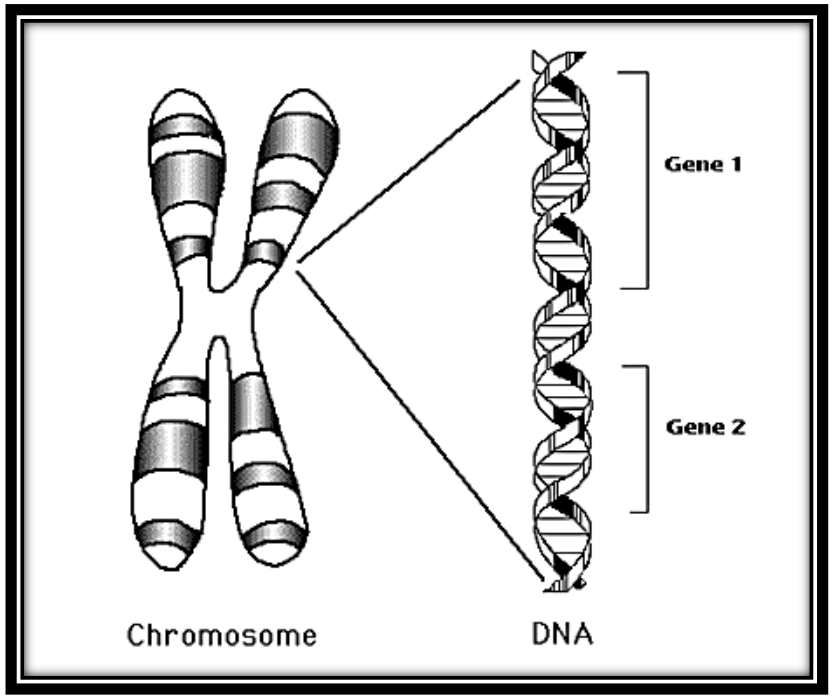
Chromosomes

- Structures that contain and organize the very long strands of DNA in the nucleus of every cell
- We all have 23 pairs of chromosomes:
  - 22 autosome pairs
  - 1 pair of sex chromosomes
- One copy of each chromosome is inherited from the mother and one from the father

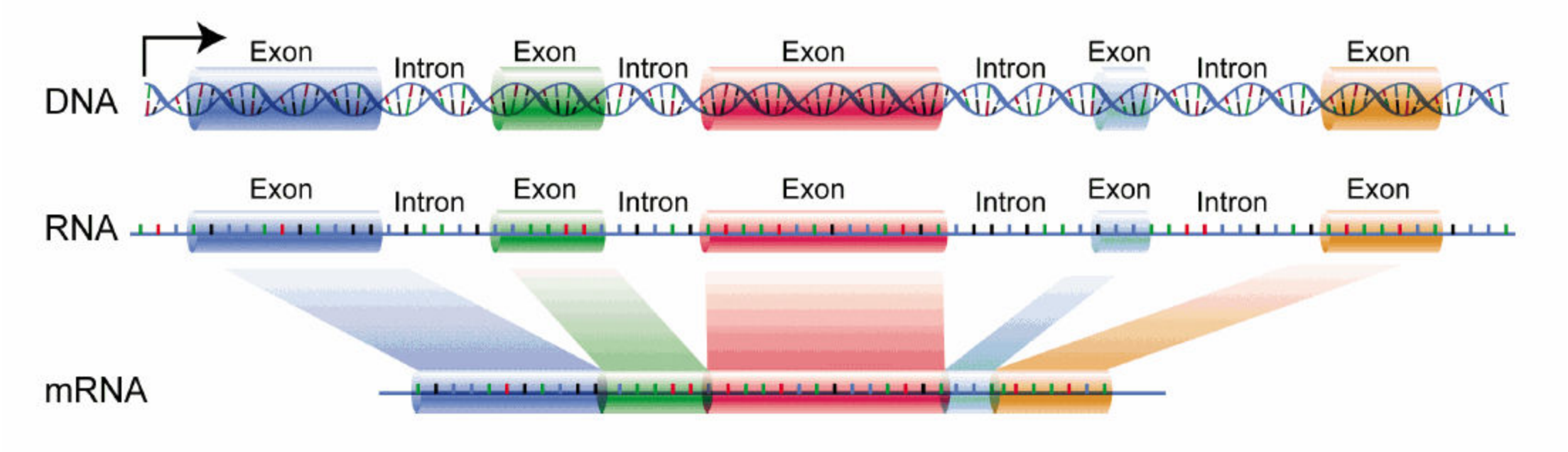


Genes

- Small sections of DNA located on chromosomes
- Give our body instructions on how to grow and develop
- Pass down familial traits, hair color, eye color, physical characteristics etc. Abnormalities in single genes can lead to disease
- ~20,000+ genes in the human body



Structure of Genes



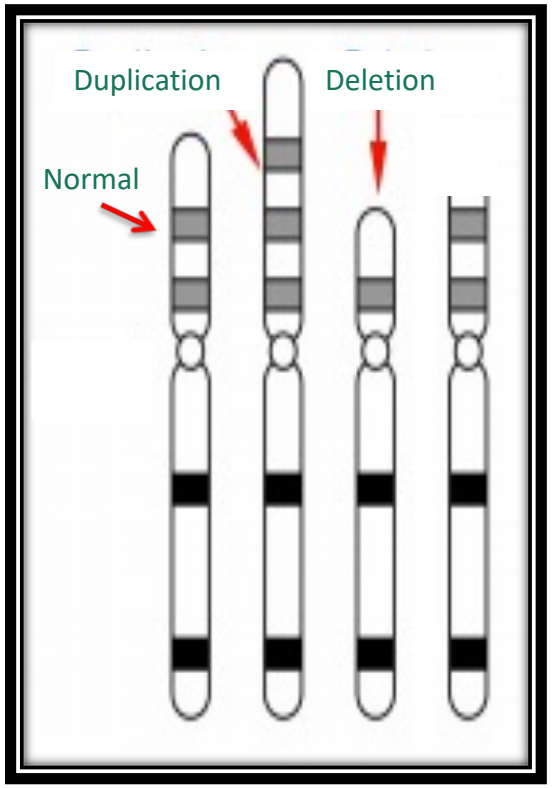
Types of Variants/Mutations

- **Spelling Mistake**: A change in a letter of the DNA code (a typo). This is usually a missense variant but could also be a nonsense variant

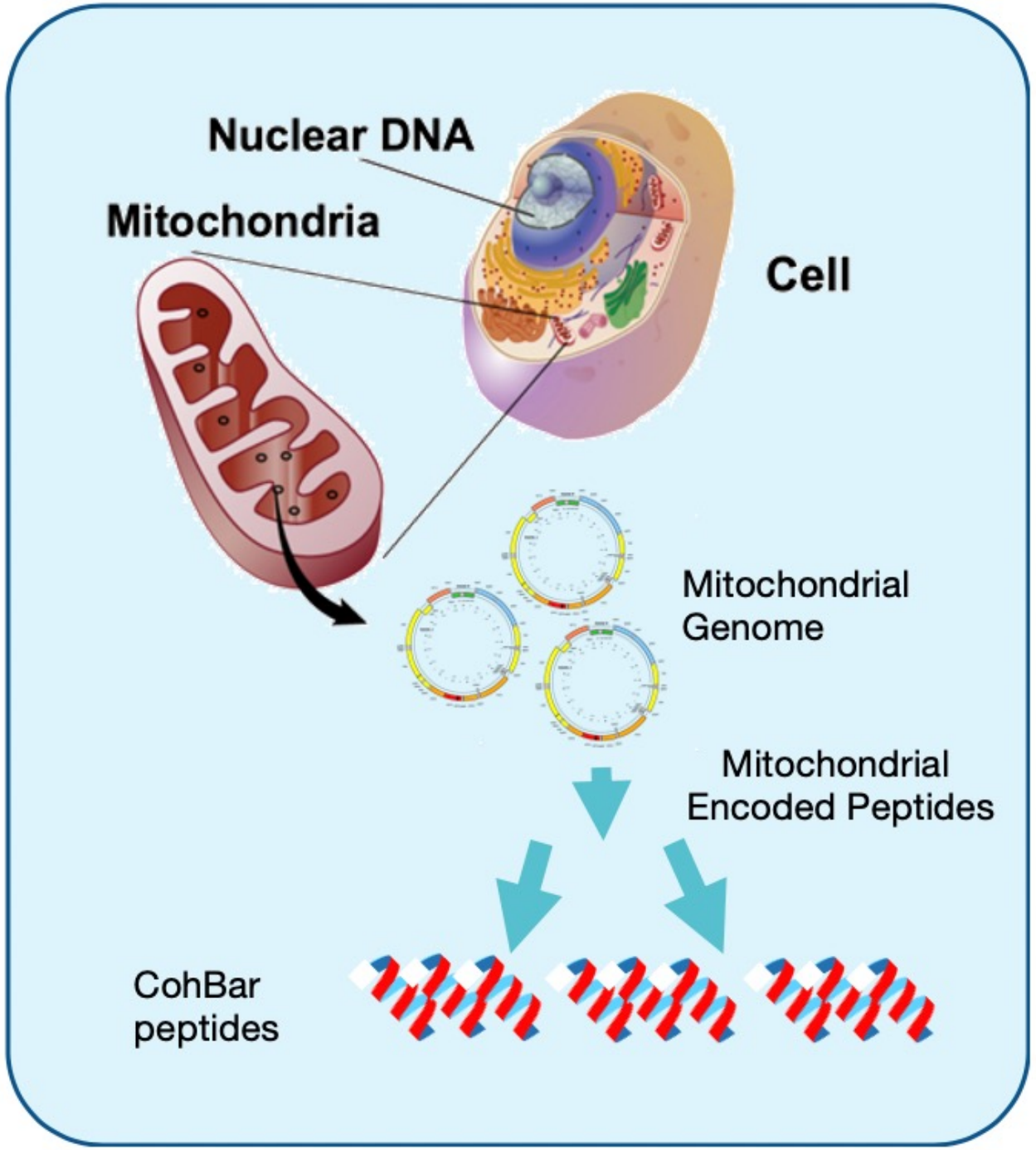
- **Deletion**: Loss of a chunk of DNA (may involve one gene, multiple genes or parts of genes)

- **Duplication**: Gain of an extra chunk of DNA (also may involve one gene, multiple genes or parts of genes)

- Variants in genes can be pathogenic (disease-causing), benign or of undetermined significance



- While most of our DNA is contained on the chromosomes in the nucleus of the cell, there is a small circular piece of DNA, which contains some genes, in the mitochondria of the cell
- Mitochondrial DNA in inherited only from the mother



ACMG Classification of Gene Variants

- Pathogenic
- Likely pathogenic
- Variant of undetermined significance (VUS)
- Likely benign
- Benign

- Richards S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405-24.

How Do Variants (Mutations) Happen?

Genetic changes can either be:

Inherited (passed down) through families, or

Brand new (*de novo*) in the individual with the genetic disease

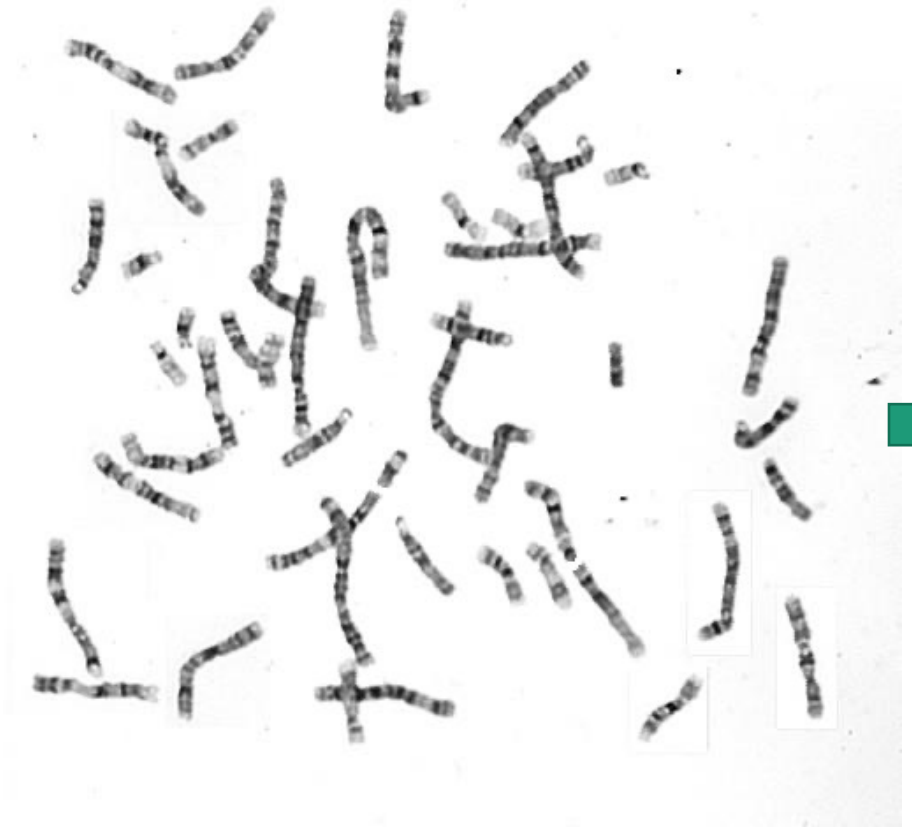
NOTE: A change in the DNA is permanent and cannot be reversed or fixed

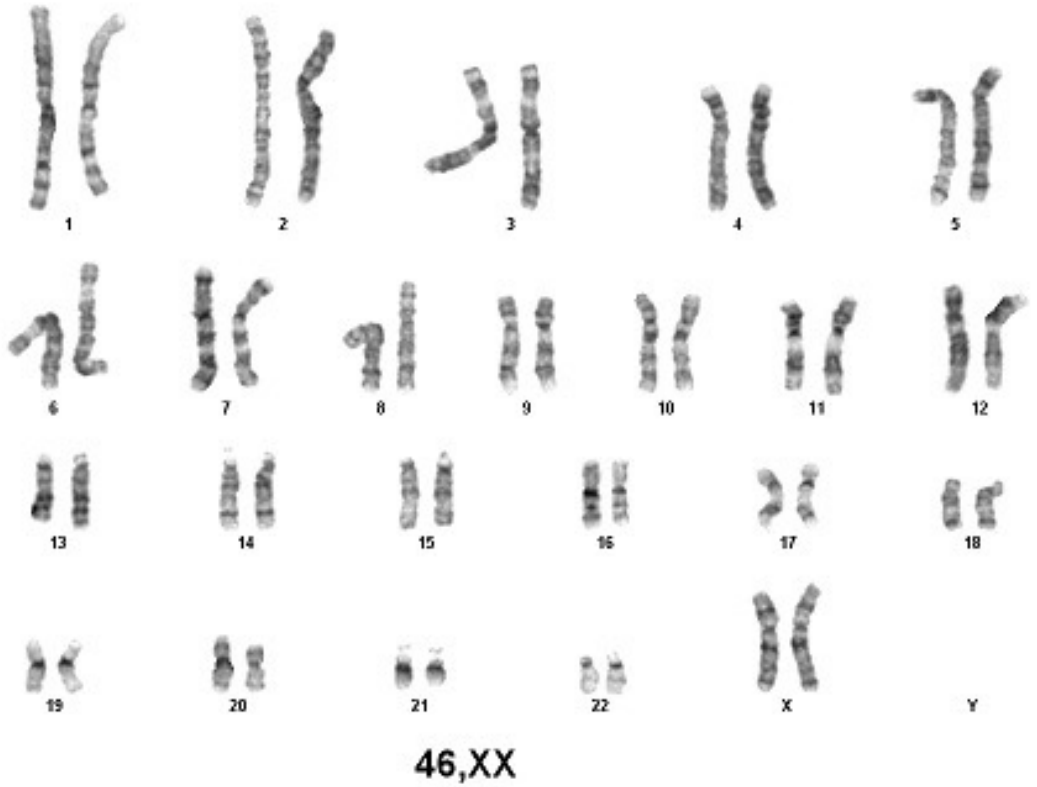
Types of Genetic Testing

Types of Genetic Testing

- Chromosome analysis (karyotype)
- Microarray (comparative genomic hydbridization- CGH)
- Single gene test
- Panel testing of multiple genes- epilepsy panel, intellectual disability panel, etc
- Mitochondrial DNA sequencing
- Whole exome or whole genome sequencing

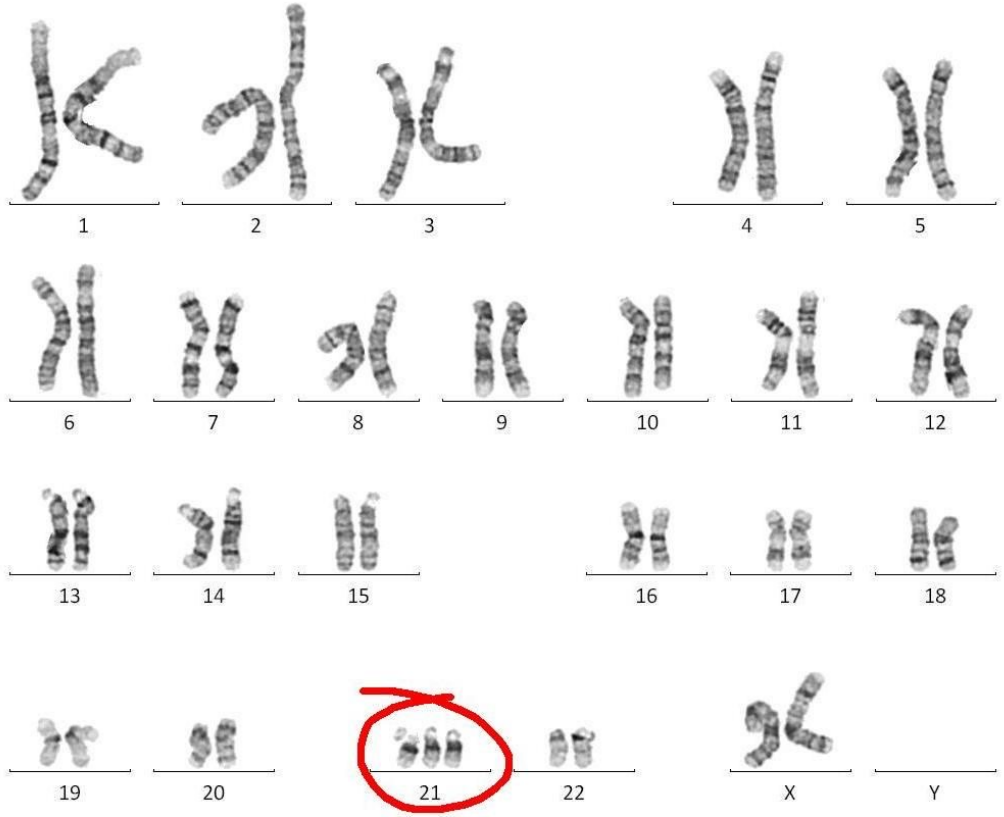
Chromosome Analysis

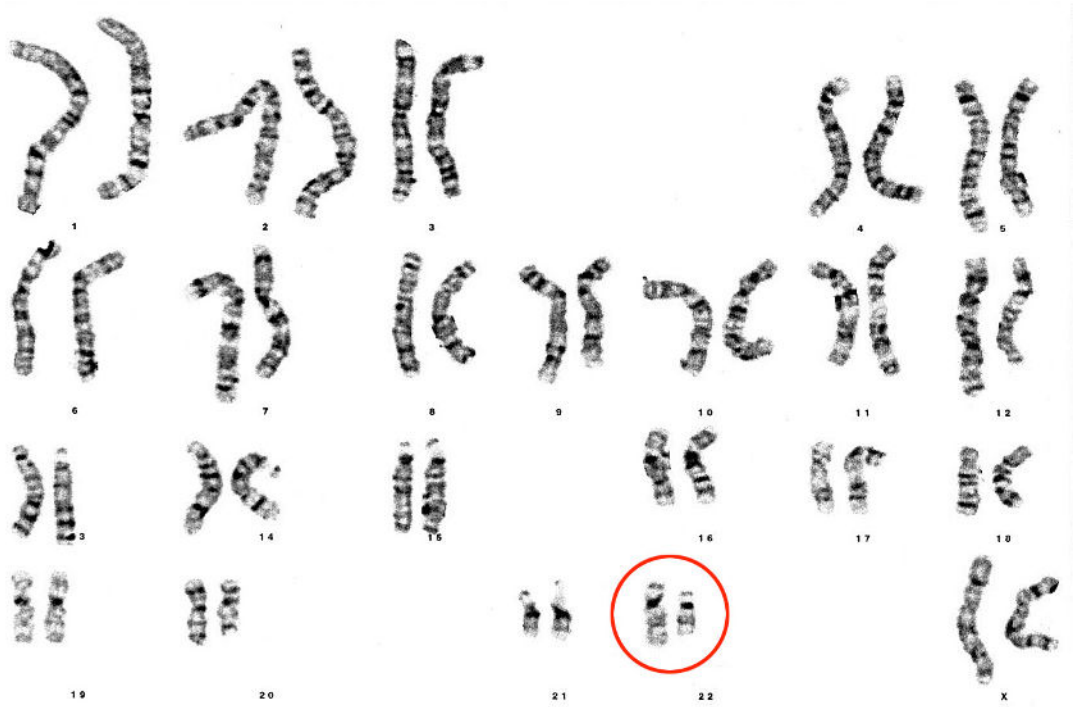




Chromosome Analysis detects changes visible under microscope

Down syndrome karyotype





Microarray

- Uses “probes” to quantitate the amount of DNA all along the lengths of all chromosomes
- Detects chromosome changes too small to be seen under microscope((as well as the larger ones)
- Changes detected as referred to as copy number variations (CNV)
  - Duplications (an extra copy)
  - Deletions (missing copy)
- Two methods- oligonucleotide and SNP

Indications for Microarray

- Developmental delay/intellectual disability/cognitive impairment
- Autism
- Multiple congenital anomalies or dysmorphic features

What Types of Results Might I Get from Microarray?

Positive

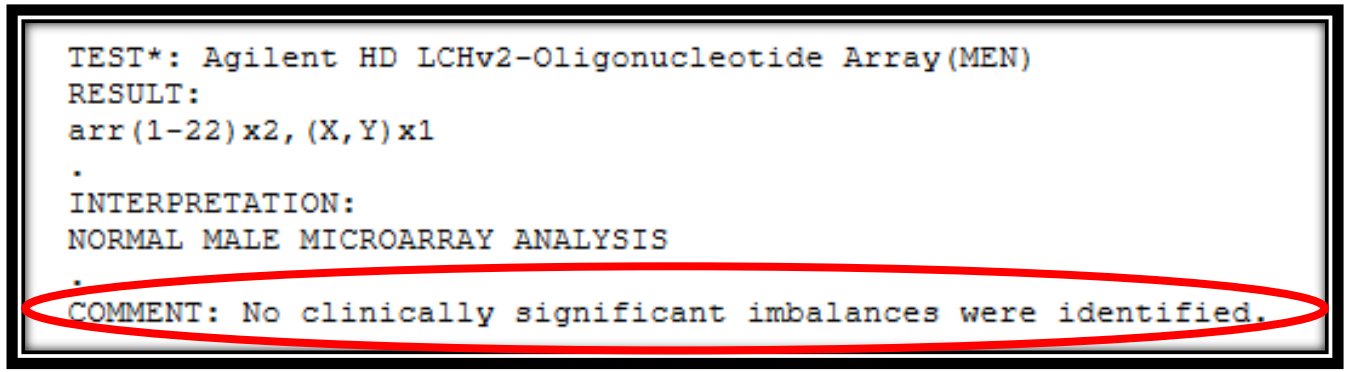
Negative

Variant of Uncertain Significance

Negative Results

Rules out:

- Clinically significant imbalances in chromosomal material
- Imbalances that are benign (or likely benign) based on current, available literature, are not reported



TEST*: Agilent HD LCHv2-Oligonucleotide Array(MEN)
RESULT:
arr(1-22)x2,(X,Y)x1
.
INTERPRETATION:
NORMAL MALE MICROARRAY ANALYSIS
.
COMMENT: No clinically significant imbalances were identified.

Clinical interpretations are based off current knowledge of the human genome at the time of reporting and may change as more information becomes available

- Positive results as seen on the microarray report

Microarray CGH, Whole Genome(O

MR#:
.
REASON FOR REFERRAL: Developmental delay
Dysmorphic features
SAMPLE TYPE: DNA
DATE SAMPLE COLLECTED:
DATE SAMPLE RCVD:
FINAL REPORT DATE:
.
REFERRING PHYS:
, APN
.
TEST*: Agilent HD LCHv2-Oligonucleotide Array
RESULT:
arr[GRCh37] 15q11.2q13.1(23478611_28539828)x1

INTERPRETATION:
ABNORMAL MALE MICROARRY ANALYSIS
.
COMMENT: An approximately 5.1 Mb loss was observed on chromosome 15q11.2q13.1 (from 23,478,611 bp to 28,539,828 bp). The deleted region contains approximately 152 known genes including SNRPN and UBE3A. The phenotype of this abnormality is variable depending on the parent of origin of the deleted chromosome. Deletions of the paternally inherited chromosome 15 are consistent with Prader-Willi syndrome whereas maternal deletions result in Angelman syndrome. Clinical correlation or methylation-specific MLPA is necessary in order to predict the consequence of this abnormality. Genetic counseling is warranted.
.
Array Comparative Genomic Hybridization (arr cgh) analysis was performed with an oligonucleotide array designed to detect copy number imbalances (losses or gains) of specific chromosomal regions and across the genome. Copy number imbalances deemed to be benign or likely benign based on current, available literature are not reported. Quality indicators and sex chromosome internal controls were within acceptable normal ranges indicative of a successful hybridization.
If a G-banded karyotype analysis has not been completed previously, this test should be considered to rule out other

REASON FOR REFERRAL: Developmental delay
Eye anomaly
Dysmorphic features
SAMPLE TYPE: peripheral blood
DATE SAMPLE COLLECTED: 03/30/2018
DATE SAMPLE RCVD: 04/04/2018
FINAL REPORT DATE: 4/24/2018

REFERRING PHYS:
Dr. Barbara Burton

TEST*: Agilent HD LCHv2-Oligonucleotide Array(MEN)
RESULT:
arr[GRCh37] 6q25.2q26(153701699_161066719)
x3,Xq28(153671707_154184072)x3

INTERPRETATION:
MALE WITH VARIANT OF UNKNOWN SIGNIFICANCE

COMMENT: An approximately 7.36Mb gain (size range: 7.31-7.42Mb) was observed on chromosome 6q25.2q26 from 153,701,699 bp to 161,066,719 bp [breakpoint range:(153,686,367 to 153,717,030)_(161,026,367 to 161,107,070)]. This region contains 57 known genes. Duplications of this region have not been described as associated with any known duplication syndrome. Although the clinical significance of this copy number change is unknown, large unbalanced chromosomal rearrangements are usually associated with multiple congenital anomalies and are more likely pathogenic. Parental FISH analysis is recommended to determine mechanism of imbalance and to determine if these copy number changes are inherited or de novo. An additional blood sample from this patient (collected in Na-Heparin) will be needed in order to interpret the parental studies. In addition, an approximately 512kb gain (size range: 511-514 kb) was observed on chromosome Xq28 from 153,671,707 bp to 154,184,072 bp [breakpoint range:(153,671,658 to 153,671,758)_(154,182,719 to 154,185,424)]. This region contains 36 known genes, but does not contain the candidate gene GDI1 that is involved in the Xq28 microduplication syndrome [OMIM#300815]. Therefore the clinical

Fast facts About a VUS on Microarray:

- Missing or extra piece of genetic material was found
- Classified as VUS because the change has never been seen before, or seen only in a few people
- Found in about 12% of patients undergoing testing
- Many times missing or extra pieces are harmless and are found to be inherited from an unaffected parent (89% of time in one series)
- De novo changes are more likely to be clinically significant

Amended Report

cgh) analysis was performed with an oligonucleotide array designed to detect copy number imbalances (losses or gains) of specific

Addendum: Subsequent parental MLPA studies (17-0769 and 17-0770, 06/24/2017), have revealed that the copy number change noted in this patient was maternally inherited. Copy number changes inherited from an unaffected parent are most likely benign. However, variable penetrance or expressivity cannot be excluded. Clinical correlation and genetic counseling are recommended.

contains high density coverage for clinically relevant deletion/duplication syndromes as well as telomeric and pericentromeric regions. In addition, it provides genome-wide coverage with an average probe spacing of approximately 20kb to detect gains or losses at a minimum of 500kb or smaller for regions of clinical interest. The array was built using a commercial platform (Agilent Technologies) and contains approximately 180,000 oligonucleotides (60mers) that represent coding and noncoding human sequences in the genome (content sourced from the UCSC hg19 human genome: NCBI build 37, Feb 2009). A pool of at least 5 normal DNA samples is used as same

Whole Exome or Whole Genome Sequencing

Whole Exome vs. Whole Genome Sequencing

- Both analyze the sequence (alphabet) of every known gene- whole exome includes the exons only (coding regions) while whole genome includes introns and material between genes
- Whole exome is currently the standard of care for patients in whom such extensive testing is indicated but whole genome sequencing is gaining ground
- Whole genome only recently became commercially available and is not yet covered by all payers
- mtDNA testing often but not always done in conjunction with WES or WGS
- Testing for copy number variants is now also done, so microarray is not necessary

Whole Exome Sequencing (WES) or Whole Genome Sequencing (WGS)

- Testing can be done from blood sample or cheek swab
- Parental samples are typically sent simultaneously when done clinically, if parents available, and are analyzed if VUS are detected
- Produces a huge volume of data
- Analysis is limited to genes that are known to have a relationship to clinical findings reported or could potentially have a relationship
- Variants are also reported in a set of “actionable genes” unrelated to clinical findings if requested (ACMG genes). Also referred to as secondary findings

Components of WES or WGS Test Report

- Variants in Disease Genes associated with Reported Phenotype
- Variants in Genes possibly Associated with Reported Phenotype
- ACMG Secondary Findings

- Benign or likely benign variants are not usually reported
- Pathogenic and likely pathogenic variants are typically reported along with variants of unknown significance

Yield of WES/WGS

- In patients who have testing done clinically because of suspicion of an underlying genetic disorder, the test is informative (identifies a disease-causing variant) in 30-40% of cases

- This number is often misused or misquoted in litigation with the suggestion that it means the test detects only 30-40% of genetic disorders which is completely incorrect

- A recent study reported that of over 1300 patients with cerebral palsy who had samples sent for WES, a positive diagnosis was found 32.7% of pediatric subjects and 10.5% of adult subjects. It was more likely if the patient also had intellectual disability, epilepsy and/ or autism.

  (Moreno-De-Luca A, et al. JAMA 2021; 325: 467-75).

How are The Data from WES/WGS Analyzed

- They are compared to a reference genome, presumably assembled from normal individuals
- The original reference genome was compiled from 13 donors in Buffalo NY but not all contributed equally
- The reference genome has been refined over the years but the DNA from a single male individual still accounts for close to 70% of the reference sequence
- In 2008, the DNA from James Watson was sequenced and compared to the reference genome- 3.3 million single nucleotide differences were detected

Wheeler DA, et al. The complete genome of an individual by massively parallel DNA sequencing. Nature 2008; 452: 872-76.

If Testing is Done On Your Client How Likely Is It That You Will Get An Abnormal Report?

- If your case for HIE is strong AND it explains adequately explains all of the findings in the child, it is very UNLIKELY that a variant will be detected that can be definitely stated to be causative of the findings.

- It is VERY LIKELY that one of more variants of undetermined significance (VUS) will be detected. In such cases, you are likely to benefit from having parental testing since many such variants are going to be normal variation in DNA, inherited from one of the parents. If the variant is de novo, it is more likely to be disease-causing but this cannot be stated with certainty in any case.

Variant of Uncertain Significance (VUS)

- A change was found in the patient's DNA, *but*
- it is unclear whether this causes a genetic syndrome or disease

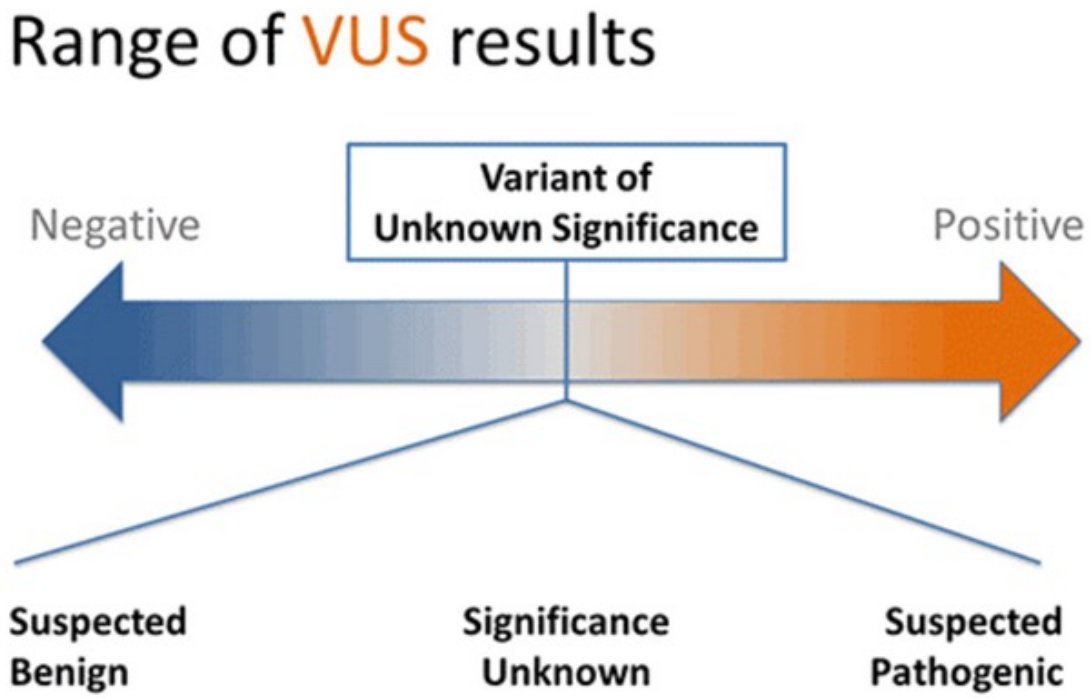


Image Credit: NCHPEG.org

Can a VUS Ever Be Determined to Be Causative of Disease

- Yes, in some cases, but rarely in the setting of HIE

- There are circumstances where the patient's phenotype (clinical findings) precisely match a specific genetic disorder and a VUS is detected in the gene for that disorder- in such a case, a geneticist may conclude that this is the causative mutation depending on a number of factors

- Because the findings related to brain injury are non-specific, it would be virtually impossible to definitely link a VUS to any aspect of the patient's findings- although some may try and the very discussion of this is very confusing to a jury.

Conclusion

- Genetic disorders may cause cerebral palsy-like findings, intellectual disability or autistic features and are increasingly suggested as an alternative cause for neurologic disabilities in children who have suffered brain injury from HIE.

- Genetic testing is used with increased frequency in patients with seizures, cerebral palsy and neurodevelopmental disabilities – even when there is a well-documented non-genetic cause of such disabilities. This may be done clinically, by agreement of the parties or by court order.

- It is a very powerful technology that had advanced patient care but it is complicated and can easily be misinterpreted or misused.