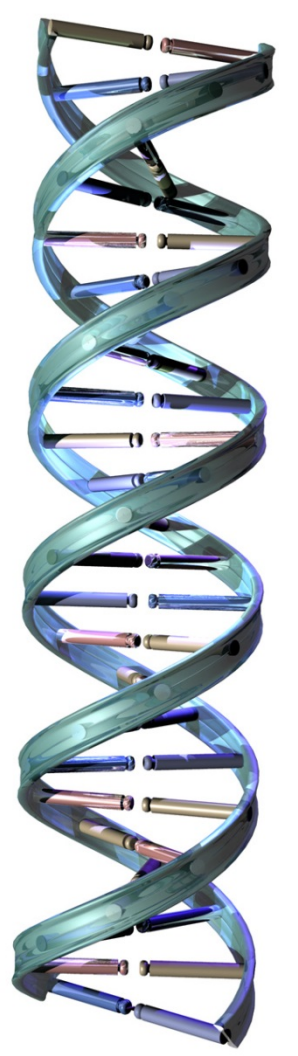
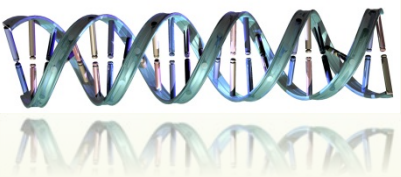
National Human Genome Research Institute



Understanding the Fundamentals: The Language of Genetics

Genetics Webinar Series for Blue Plans

Bob Wildin, MD
Chief, Genomic Healthcare Branch
Division of Policy Communications and Education
NHGRI



Agenda

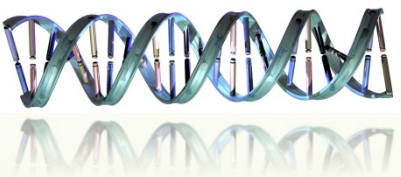
I. Case Study

II. Genetic Terminology

III. Types of Genetic Alterations

IV. Inheritance

V. Case Study Discussion



I. Case Study: Roger

- **Patient history**

A 6 y/o boy is brought by his mother because he is struggling in first grade. His growth has fallen off and he is the shortest in his class (3rd %ile). He has had one seizure. His head circumference is normal, 95%ile.

- **Family history**

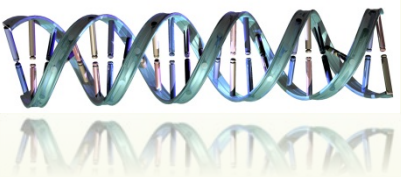
Mother and father have normal intelligence, but father is unemployed due to generalized weakness and pain. Mother is average stature, father is 5'4" tall, and stocky. Mother is pregnant.

- **Lab tests and Differential Diagnosis**

Tests for Thyroid and Growth Hormone deficiency are normal.

Pediatrician wonders if he has an intellectual disability "syndrome" even though his appearance is normal.

Genetics consultant detects mild brachydactyly and borderline upper/lower segment and armspan to height ratios, indicating mild limb shortness and suspects a skeletal dysplasia.

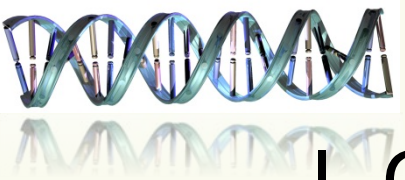


I. Case Study: Roger

- **Genetic testing**

FGFR3 gene sequencing is ordered. The ordered test sequences only exon 13; it is targeted to detect two variants, c.1620C>**A** and c.1620C>**G**. It does not examine other FGFR3 exons, including exon 10, where the fully penetrant pathogenic variant responsible for Achondroplasia is located.

The gene test result confirms a heterozygous p.Asn540Lys mutation and the diagnosis of Hypochondroplasia.



I. Case Study Discussion: Preview

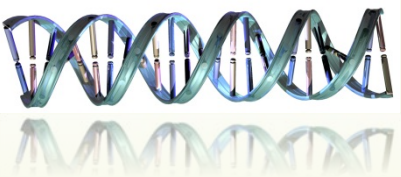
1. Why do the **c**DNA variants **c**.1620C>A and **c**.1620C>G both result in **p**rotein variant **p**.Asn540Lys?

2. How many copies of the hypochondroplasia variant allele were found? Is this a dominant or recessive disorder?

3. How can Roger's diagnosis possibly help his father?

4. Only some persons with hypochondroplasia have intellectual disability. What two phenomena explain this?

5. The doctor could have ordered a complete radiographic survey including skull, pelvis, AP and lateral spine, legs, arms, and hands, instead of a genetic test, to diagnose hypochondroplasia. Give three reasons why she might have chosen the genetic test over the radiographic diagnostic approach. What did she risk by choosing the genetic test?



II. Genetic Terminology: DNA

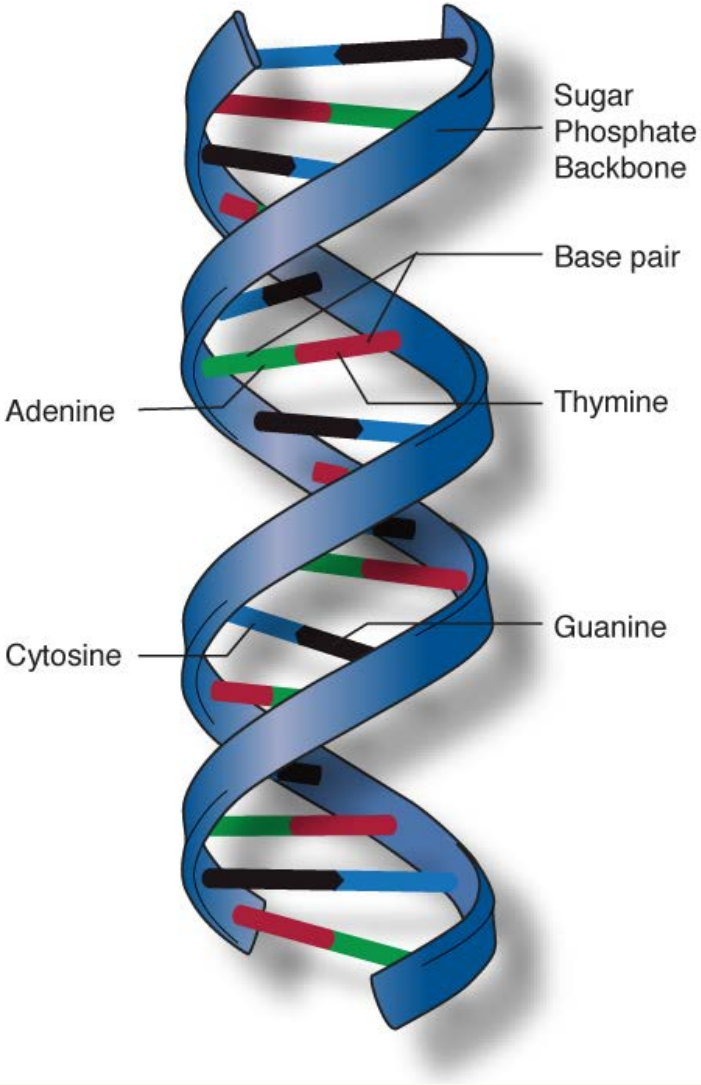
All the genetic material in the nucleus, plus the mitochondrial genome

Molecules of DNA that contain the coded instructions for how to build, maintain, and replicate a human being

Is not identical in anyone but identical twins

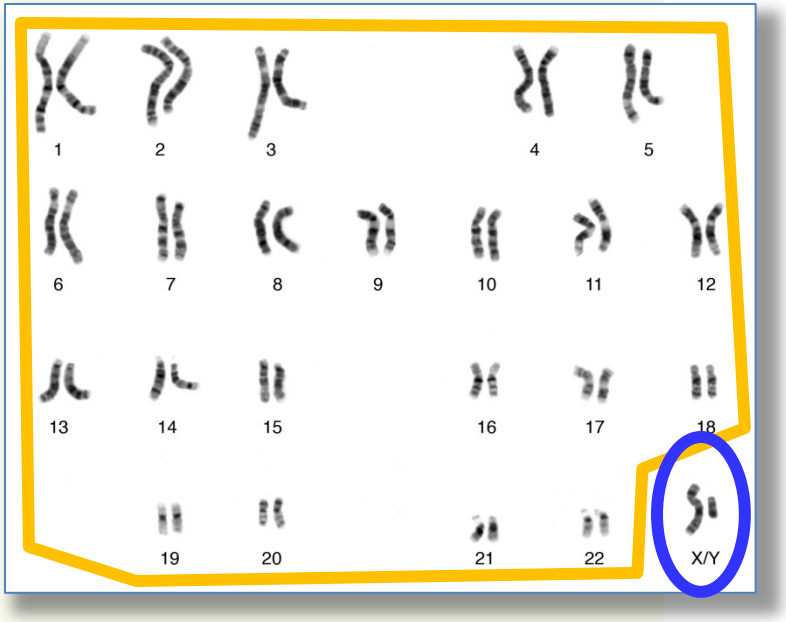
Always contains both benign variation and variation that can cause or contribute to disease(s)

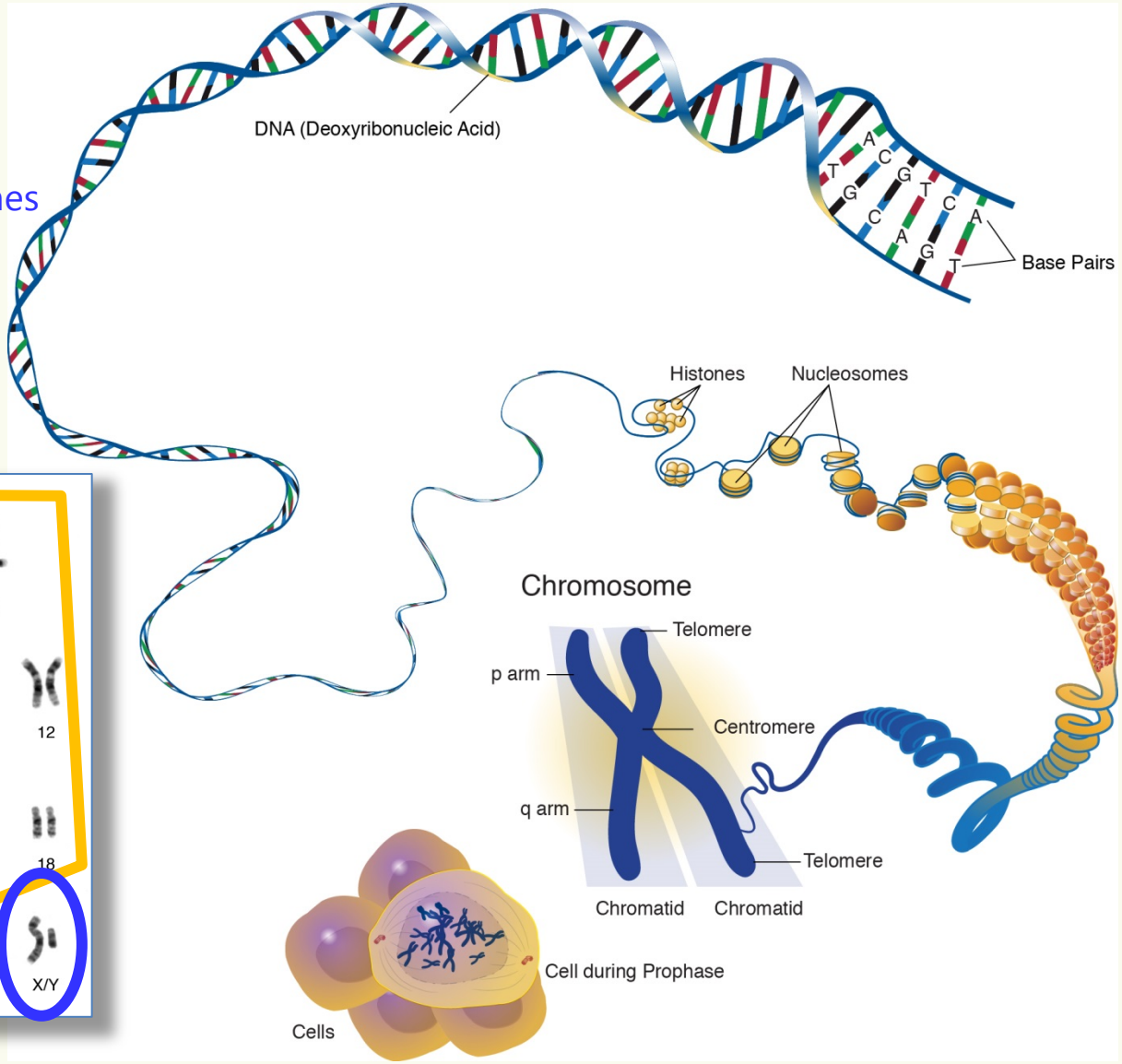
It's big! 3,300,000,000 base pairs

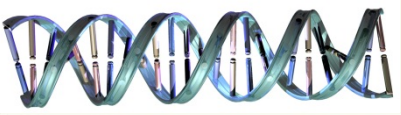


II. Genetic Terminology: Chromosomes

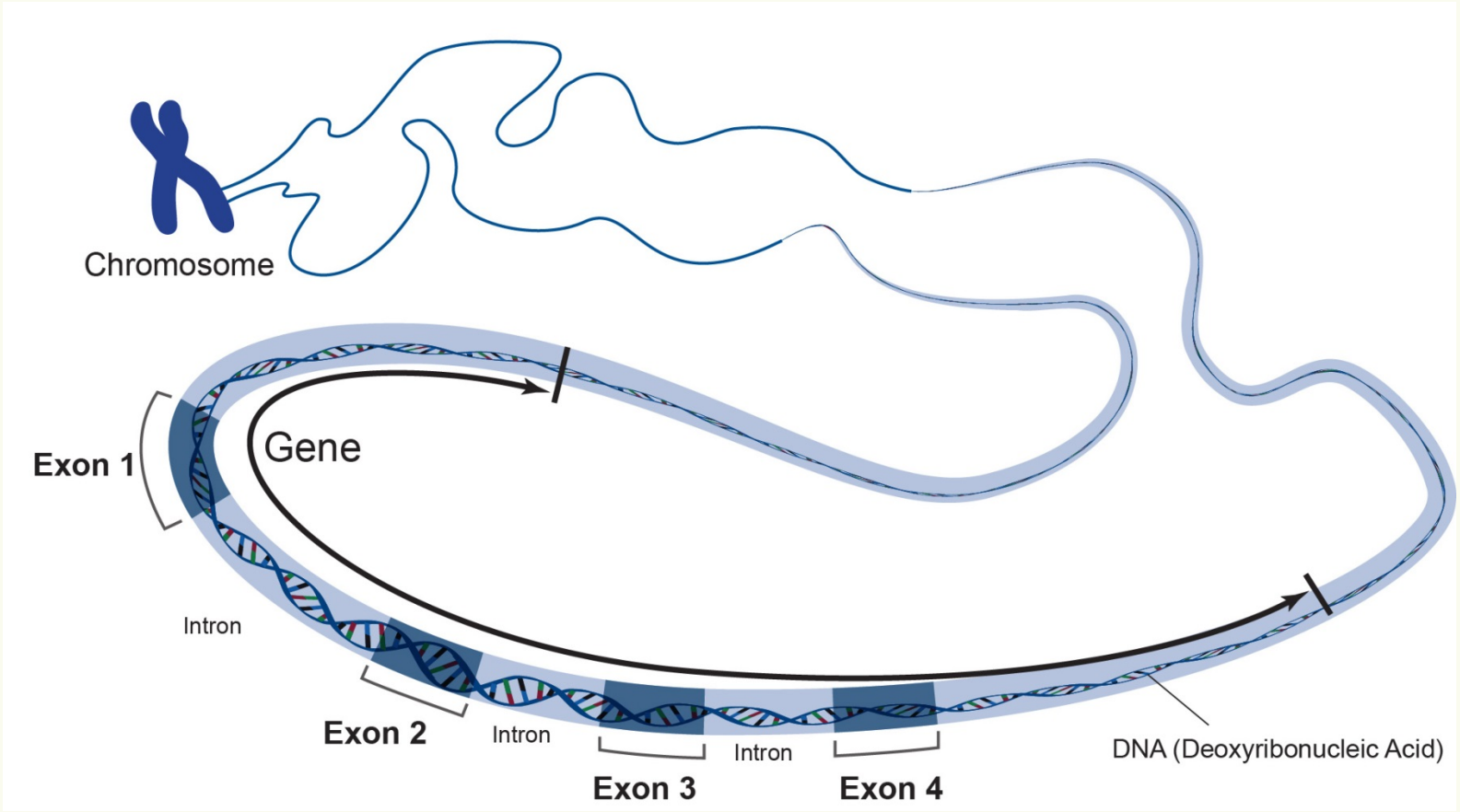
- 23 pairs (pairs!)
  - 22 pairs of autosomes
  - 1 pair of sex chromosomes
- Packages of DNA
- Consistent structure





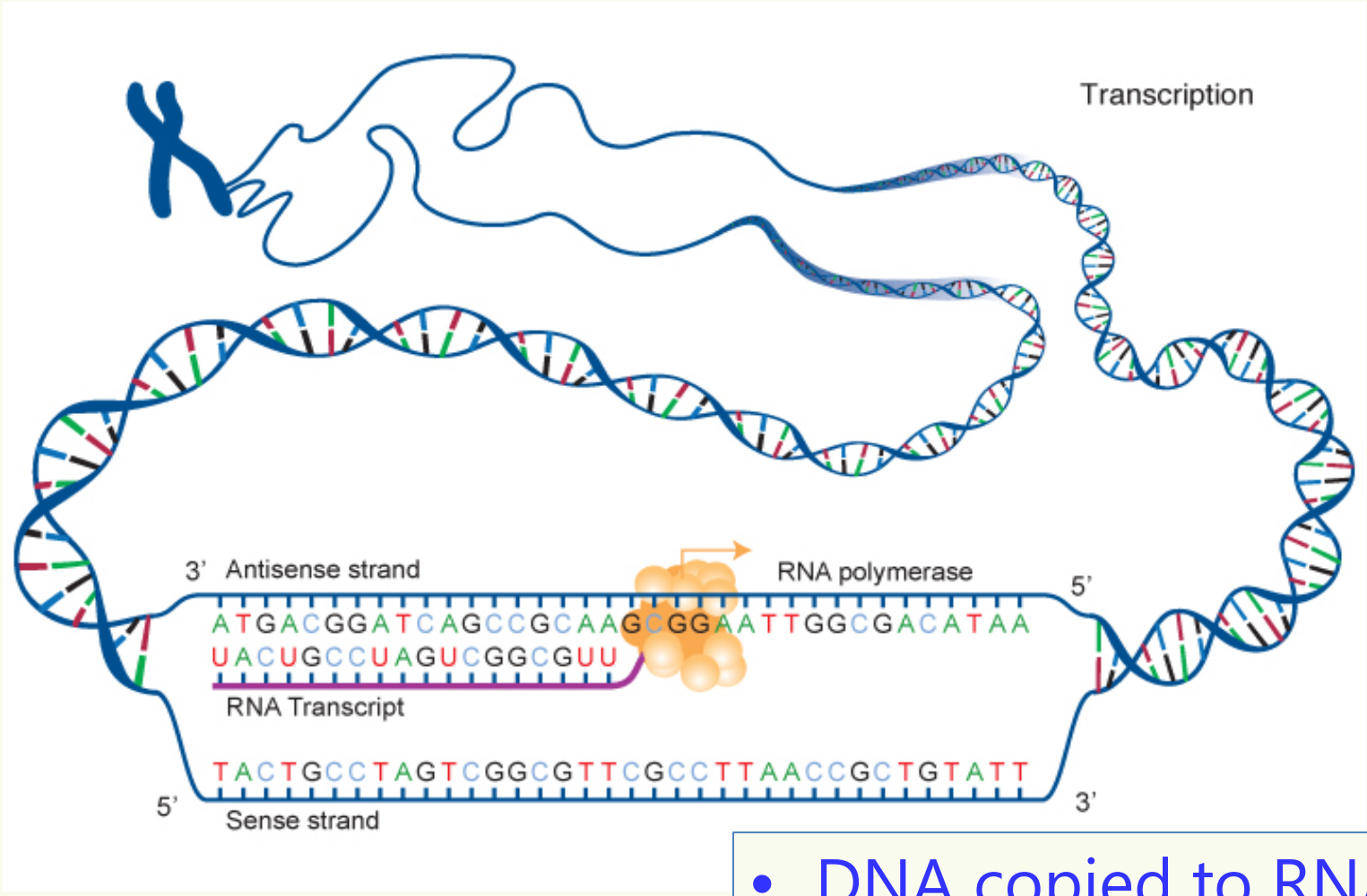


II. Genetic Terminology: Structure



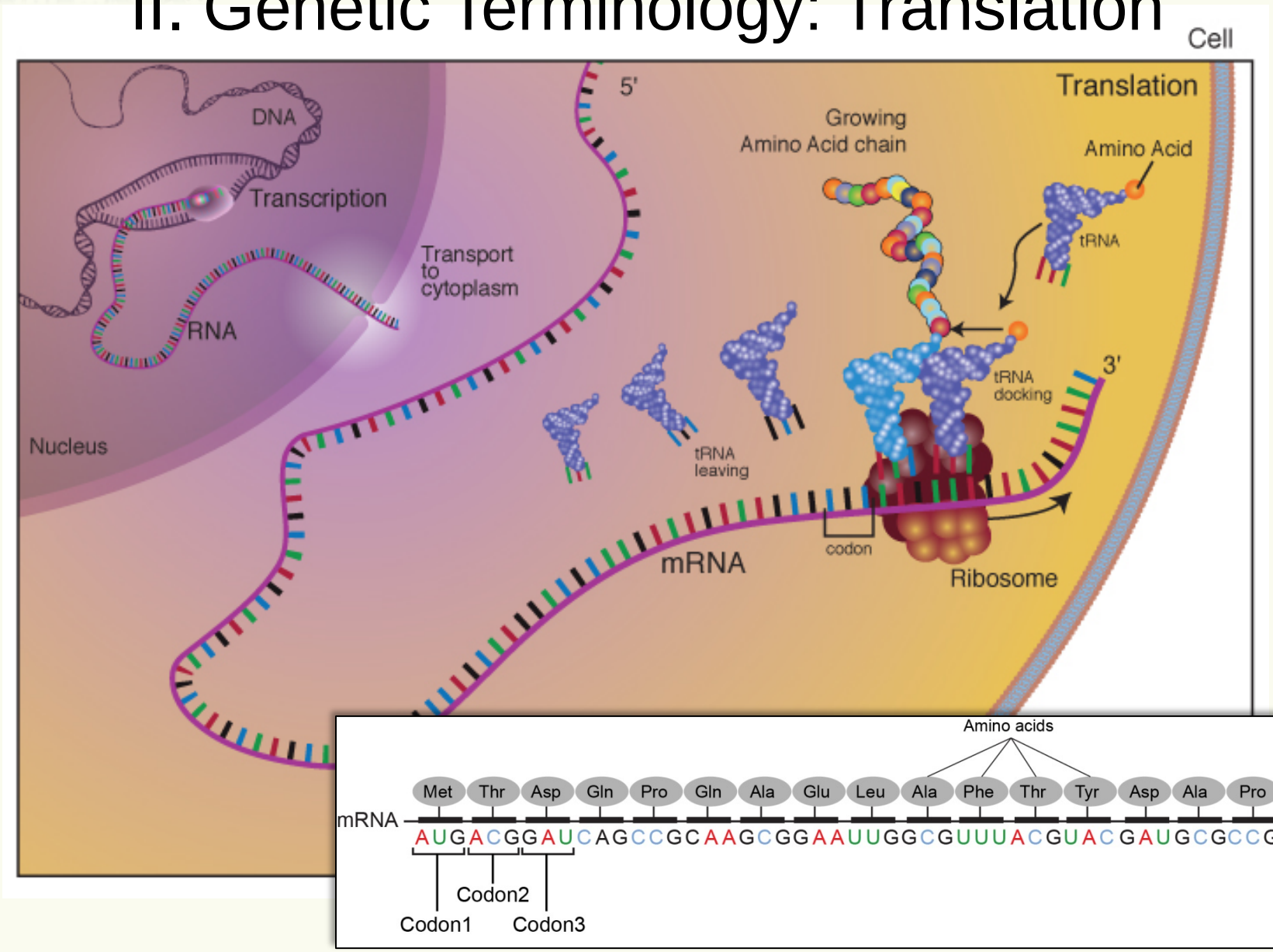
- Exons are segments of genes that contain code for proteins
- Introns are spacers that get cut out after transcription
- Gene coding regions are about 1% of the genome

II. Genetic Terminology: Transcription



- DNA copied to RNA
- "Sense" strand

II. Genetic Terminology: Translation



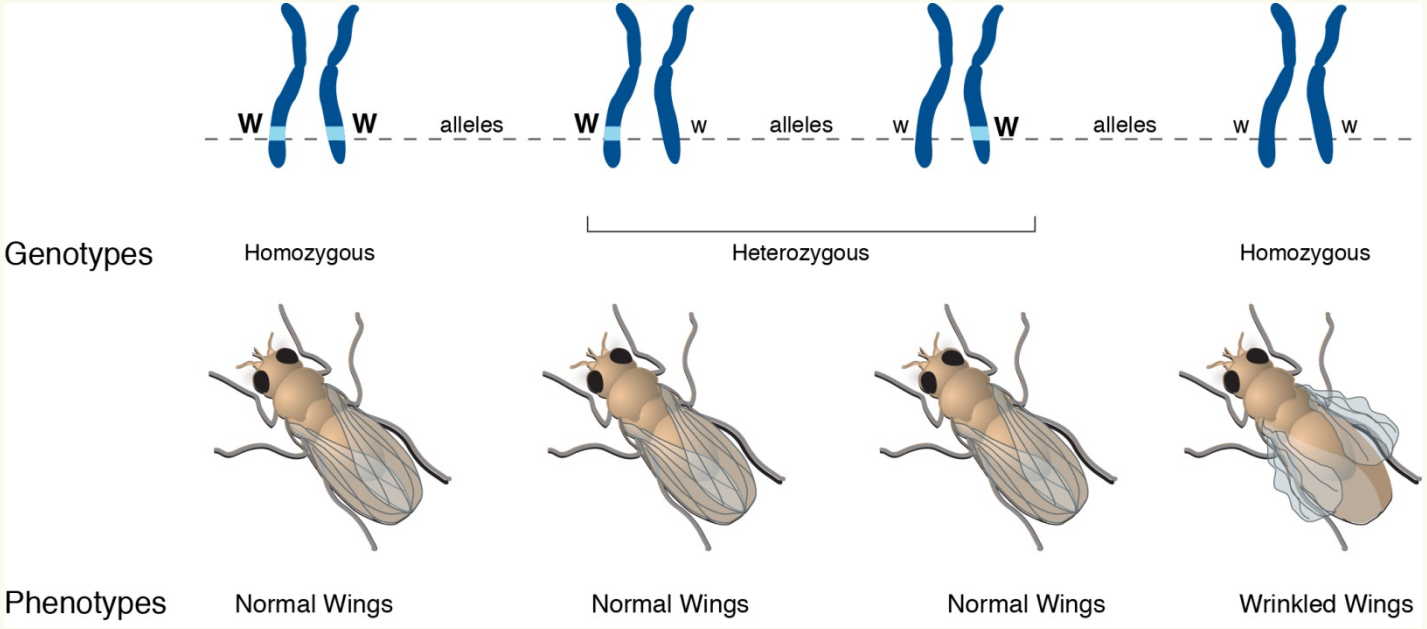
II. Genetic Terminology: Genotype and Phenotype

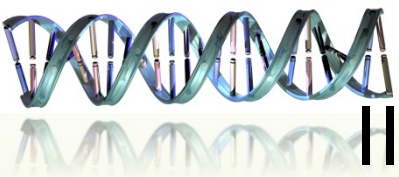
Genotype

- The *genetic code* describing an individual

Phenotype

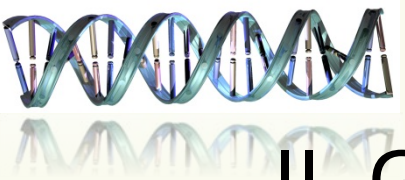
- The *physical manifestations* of genotype in an individual





II. Genetic Terminology: Genetic Heterogeneity

Allelic Heterogeneity	• Disease results from **different variants in the same gene**
Locus Heterogeneity	• Disease results from **variants in different genes**
Phenotypic Heterogeneity	• Disease manifestations are **different in different people**



II. Genetic Terminology: Expressivity

Disease Expression

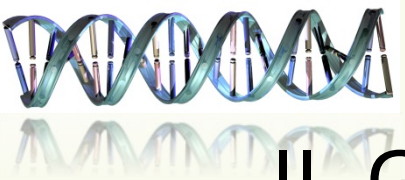
- What the *detectable disease manifestations* in an affected individual are
  - Phenotype
  - Molecular

Variable Expressivity

- Affected persons *show different features or different combinations of features*
- "Pleiotropy"

Patterns

- Within families ➔ unknown factors despite gene identity
- Among families ➔ genotype-phenotype correlations

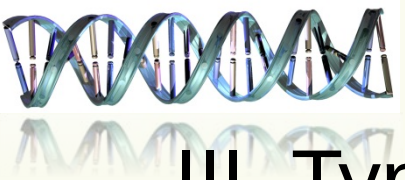


II. Genetic Terminology : Penetrance

- **Complete Penetrance** — Everyone with pathogenic genotype expresses the disease
- **Incomplete Penetrance** — Some, but not all, will express the disease
  - Lifelong
  - Age-related
  - Environment-dependent

"Because of evidence that the height range in **hypochondroplasia** may overlap that of the normal population, individuals with **hypochondroplasia** may not be recognized as having a skeletal dysplasia unless an astute physician recognizes their disproportionate short stature. However, there have been no reports of individuals with an *FGFR3* mutation without demonstrable radiographic changes compatible with **hypochondroplasia** or one of the other phenotypes known to be associated with mutations in this gene (see Genetically Related Disorders)."

-- GeneReviews.org



III. Types of Genetic Alterations: Structure

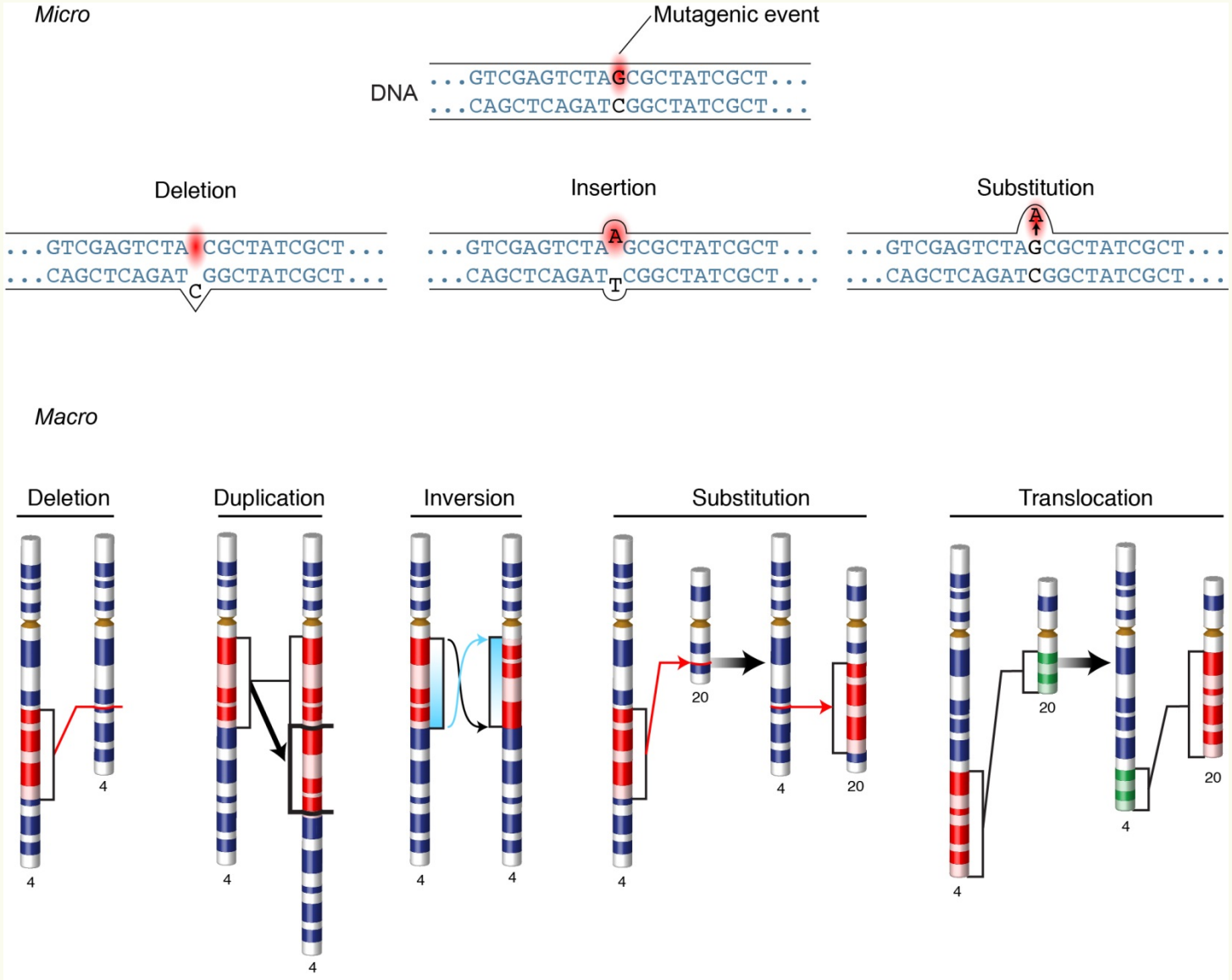
RNA codon table

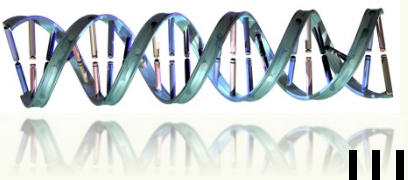
1st position	2nd position U	2nd position C	2nd position A	2nd position G	3rd position
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	stop	stop	A
	Leu	Ser	stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G
	Amino Acids				

Ala: Alanine
Arg: Arginine
Asn: Asparagine
Asp:Aspartic acid
Cys:Cysteine
Gln: Glutamine
Glu: Glutamic acid
Gly: Glycine
His: Histidine
Ile: Isoleucine
Leu: Leucine
Lys: Lysine
Met: Methionine
Phe: Phenylalanine
Pro: Proline
Ser: Serine
Thr: Threonine
Trp: Tryptophane
Tyr: Tyrosisne
Val: Valine

- Universal
- Three bases => 1 amino acid, or termination
- Degenerate
  - some base changes don't result in amino acid changes, they are **synonymous**
- Translation is reading-frame dependent
  - Insert/delete can shift triplet frame → translated differently

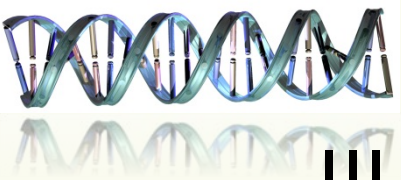
III. Types of Genetic Alterations: Mutation





III. Types of Alterations: Variation

- Base Substitution – one base replaces another
- Copy Number
  - Deletion (copy loss)
  - Duplication, triplication, etc. (copy gain)
- Repeat Number
  - *Location*: Tandem, flanking
  - *Orientation*: Direct, inverted
  - *Size*: Large, Trinucleotide, mononucleotide
- Structural
  - Rearrangement (sections of DNA moved around)
  - Translocation (sections moved to a different chromosome)
- **Different lab technologies detect different types of variation!**



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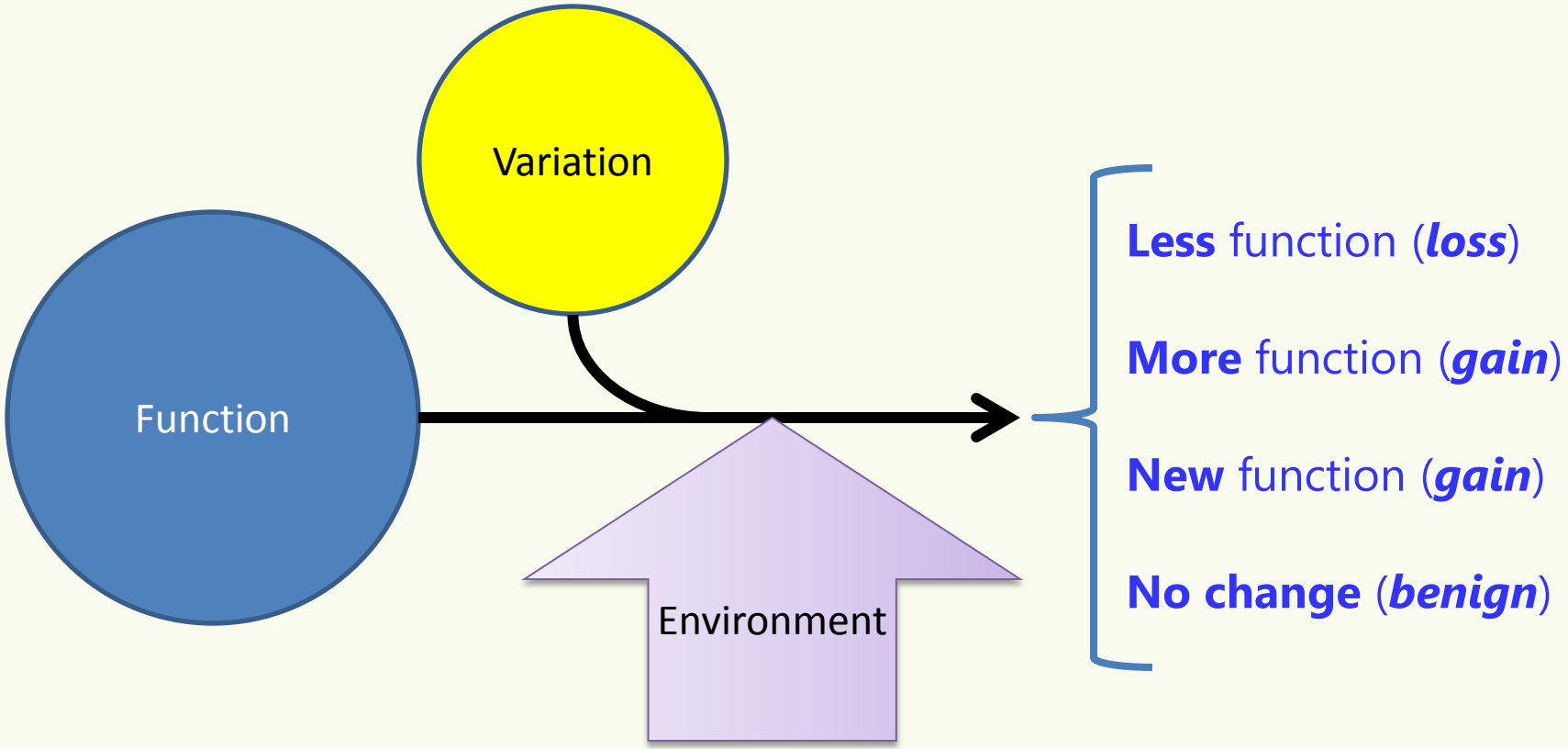

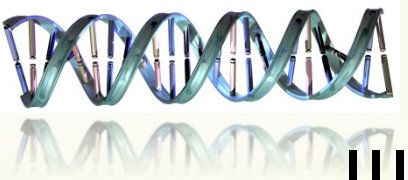

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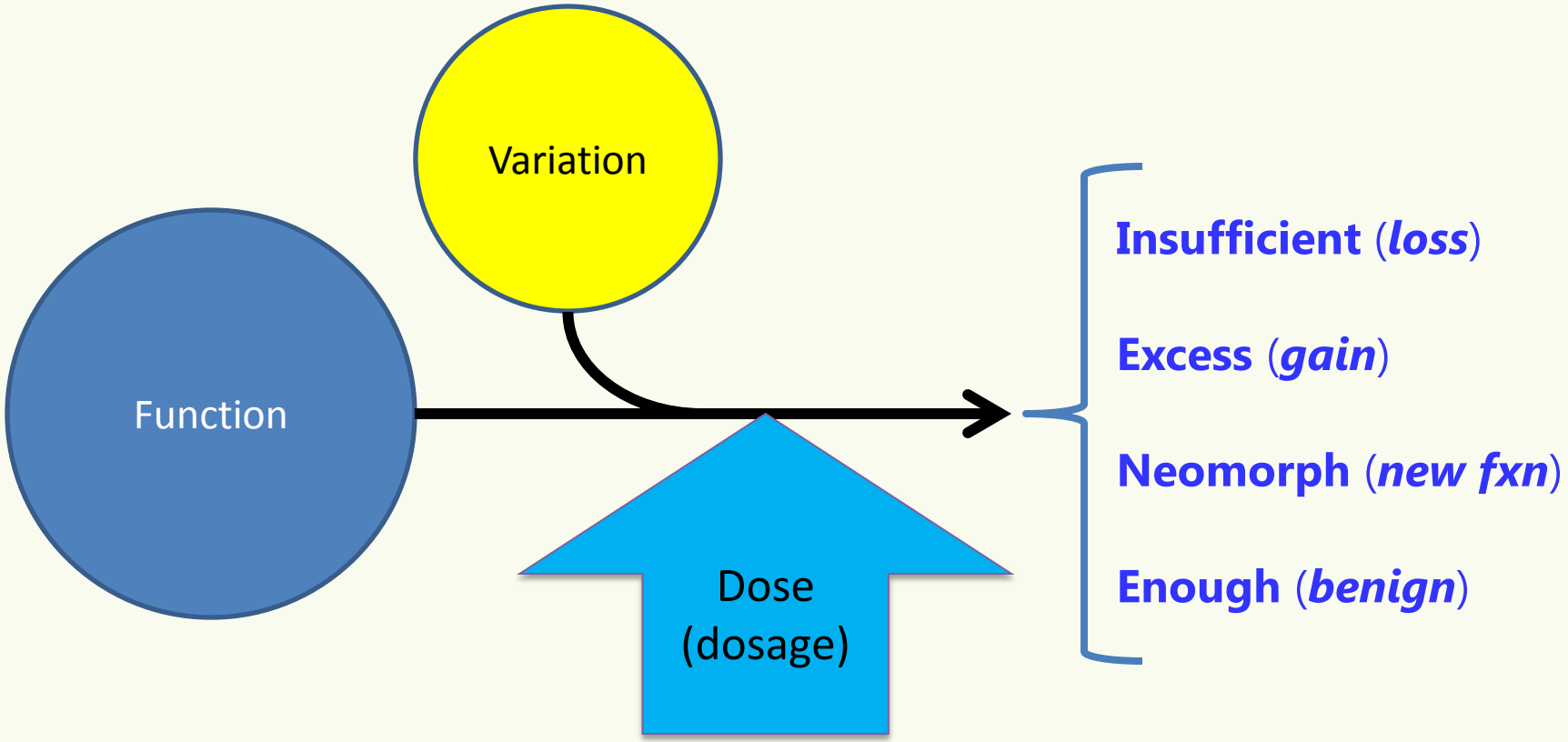
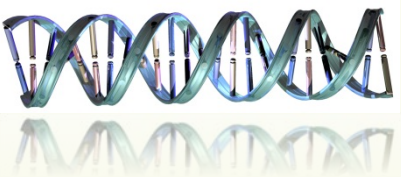
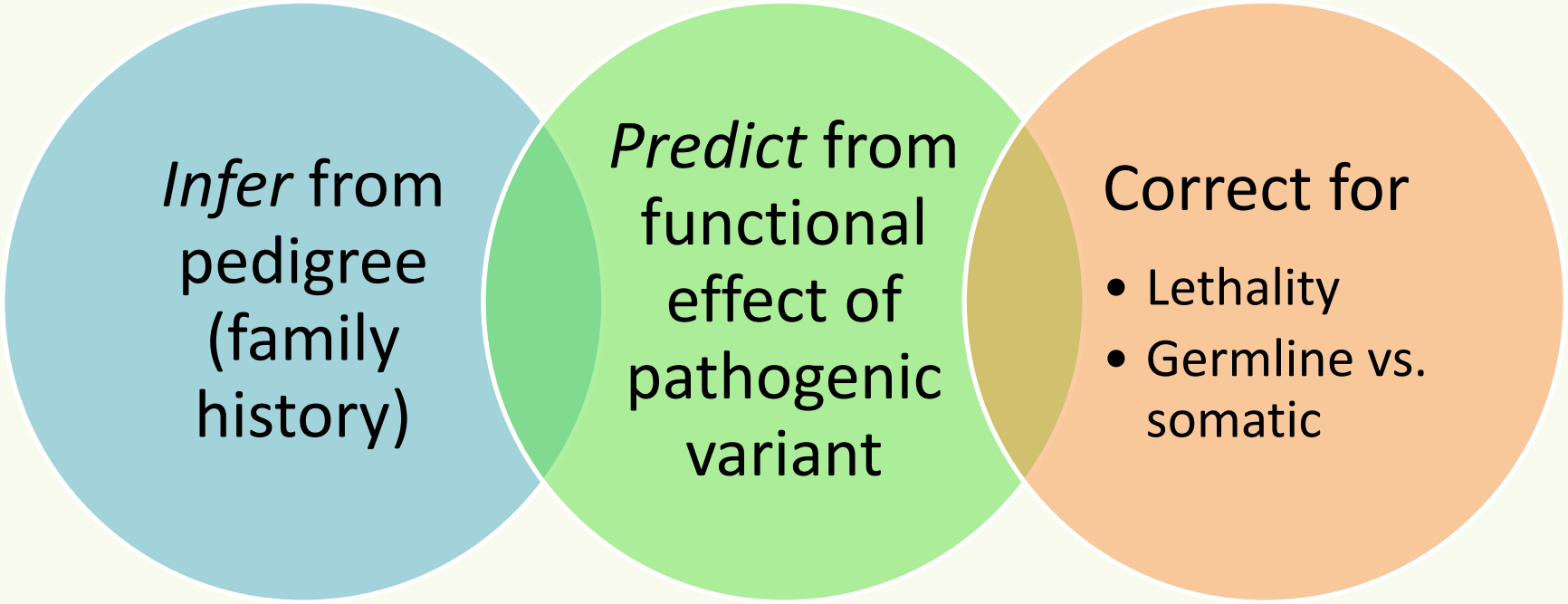


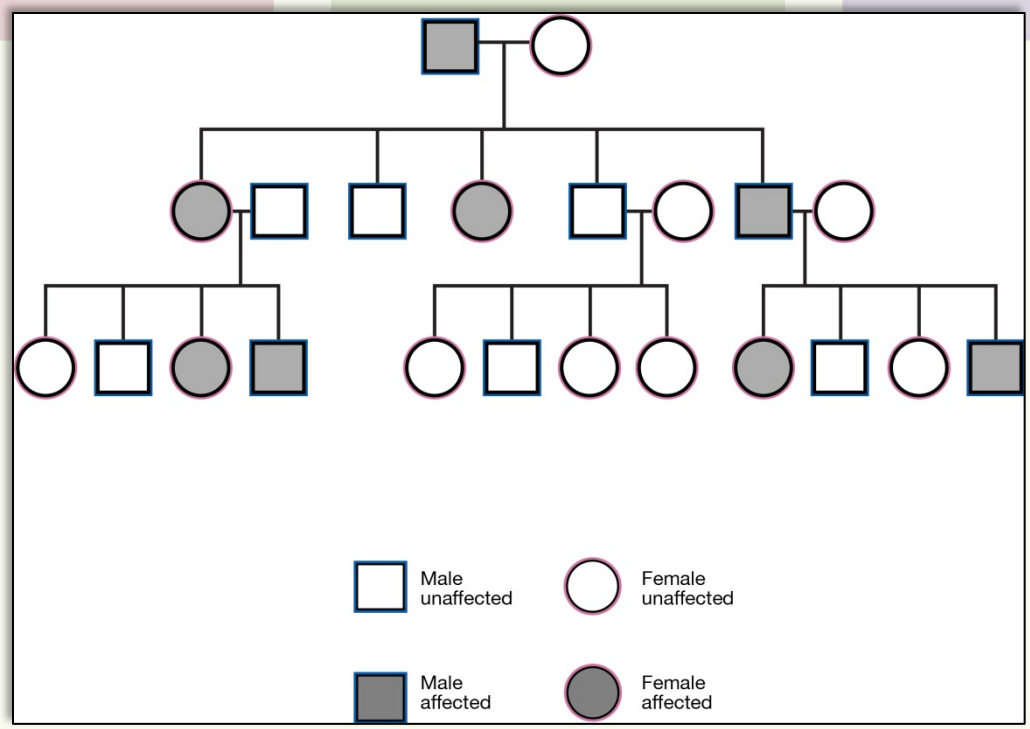


IV. Inheritance



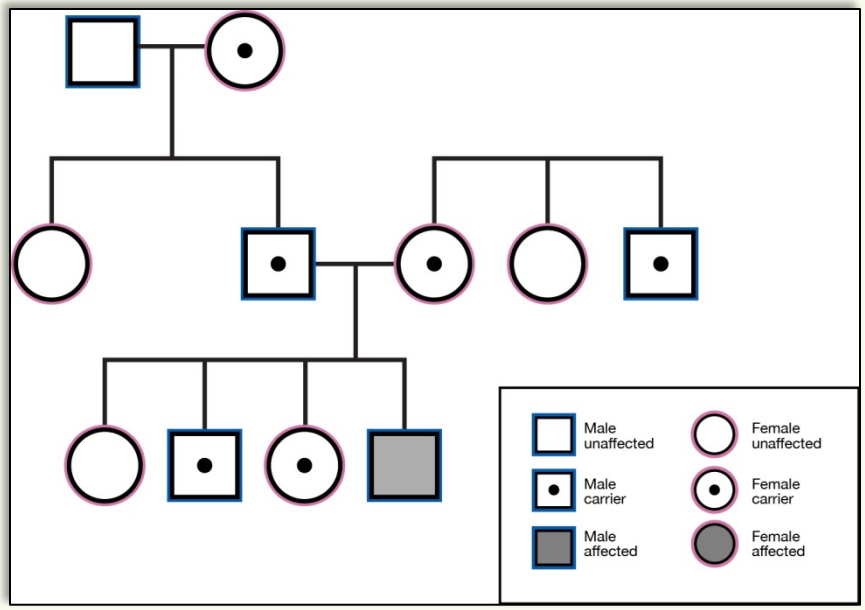
IV. Inheritance: Dominant

Affected	Unaffected	Vertical pattern
• both sexes • one of two alleles	• no disease allele • no transmission	• multiple generations • 50-50 chance of transmission



IV. Inheritance: Autosomal Recessive

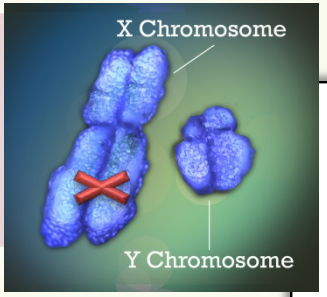
Affected	Unaffected	"Carrier"	*Both parents* of an affected *are carriers* (or affected)
• No normal copy	• At least one normal copy	• Unaffected • Transmits 50-50	• An affected parent creates pseudo-dominant inheritance



IV. Inheritance: X-linked Recessive

Affected

- No normal copy
- Males
- All daughters are carriers
- All sons are unaffected
- Rare females



Unaffected

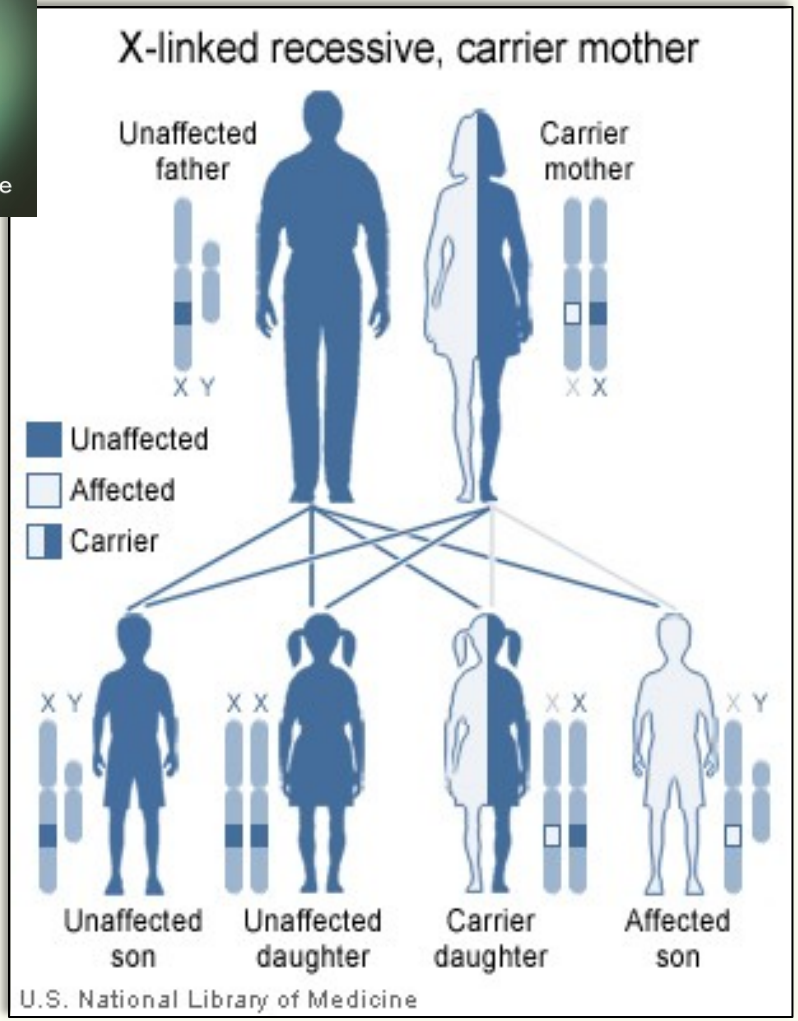
- At least one normal copy
- Non-carrier males
- Most females

"Carrier"

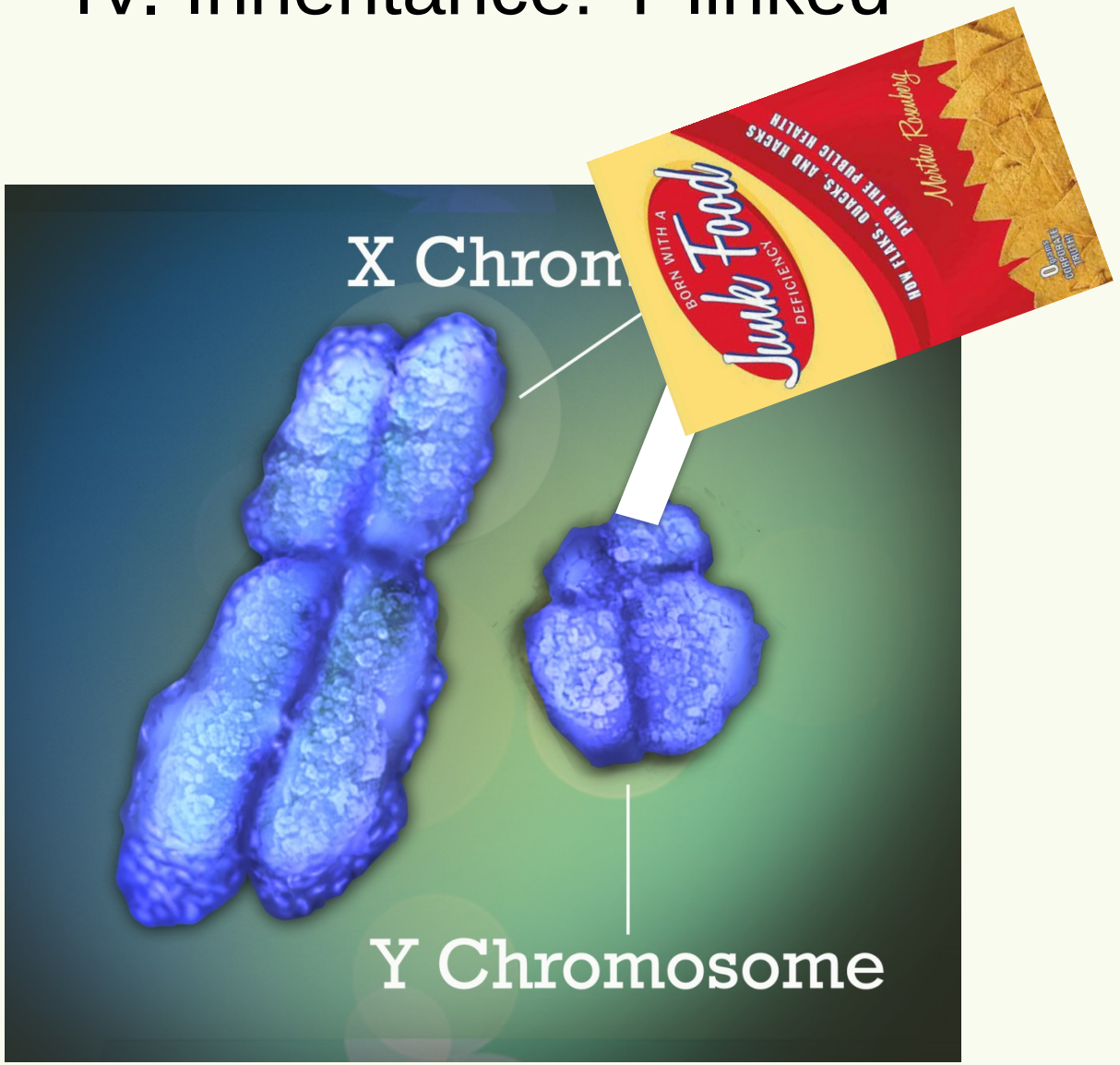
- Unaffected
- Females (and XXY males)
- Transmits 50-50

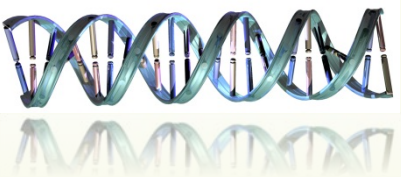
Mother **of an affected** ***is a carrier***

- Always - for benign condition
- 2 out of 3 - when affected males can't reproduce
  - 1 out of 3 is de novo



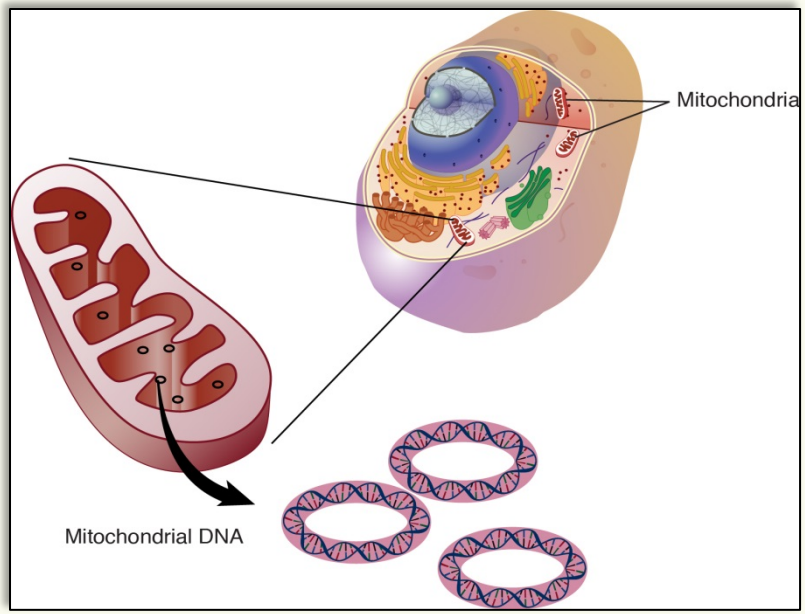
IV. Inheritance: Y-linked



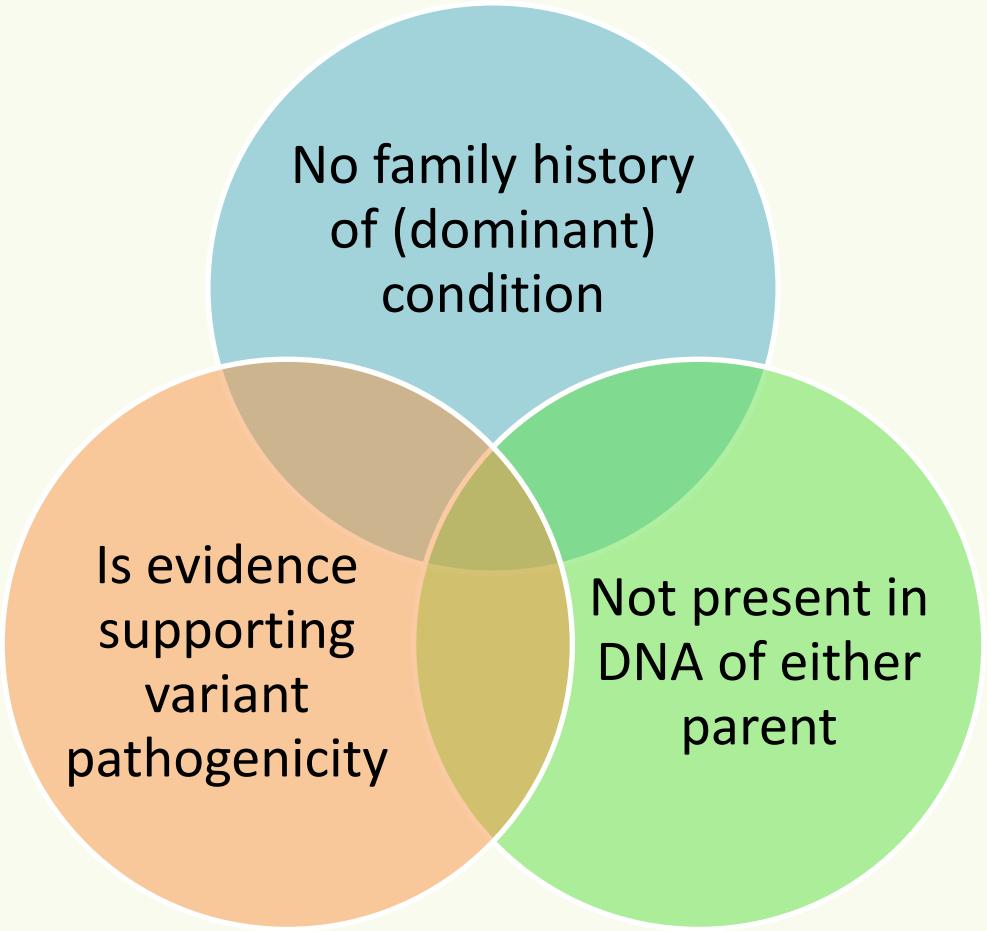


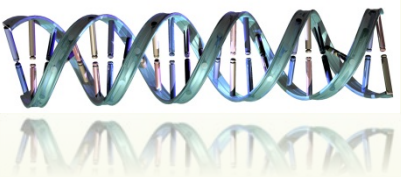
IV. Inheritance: Mitochondrial

Both sexes affected	Vertical transmission	Energy-intensive organs
• Variable expression	• Variable chance • *Maternal lineage* only • *No* transmission from males	• Brain • Muscle • Liver • …



IV. Inheritance: *De Novo* (New Mutation)





V. Case Study: Roger

- **Patient history**

A 6 y/o boy is brought by his mother because he is struggling in first grade. His growth has fallen off and he is the shortest in his class (3rd %ile). He has had one seizure. His head circumference is normal, 95%ile.

- **Family history**

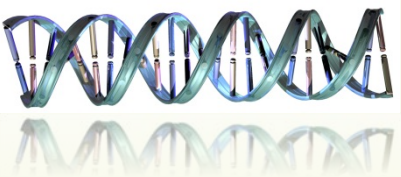
Mother and father have normal intelligence, but father is unemployed due to generalized weakness and pain. Mother is average stature, father is 5'4" tall, and stocky. Mother is pregnant.

- **Lab tests and Differential Diagnosis**

Tests for Thyroid and Growth Hormone deficiency are normal.

Pediatrician wonders if he has an intellectual disability "syndrome" even though his appearance is normal.

Genetics consultant detects mild brachydactyly and borderline upper/lower segment and armspan to height ratios, indicating mild limb shortness and suspects a skeletal dysplasia.



V. Case Study: Roger

- **Genetic testing**

FGFR3 gene sequencing is ordered. The ordered test sequences only exon 13; it is targeted to detect two variants, c.1620C>**A** and c.1620C>**G**. It does not examine other FGFR3 exons, including exon 10, where the fully penetrant pathogenic variant responsible for Achondroplasia is located.

The gene test result confirms a heterozygous p.Asn540Lys mutation and the diagnosis of Hypochondroplasia.

V. Case Study: Discussion Questions

1. Why do the **c**DNA variants **c**.1620C>A and **c**.1620C>G both result in **p**rotein variant **p**.Asn540Lys?

2. How many copies of the hypochondroplasia variant allele were found? Is this a dominant or recessive disorder?

3. How can Roger's diagnosis possibly help his father?

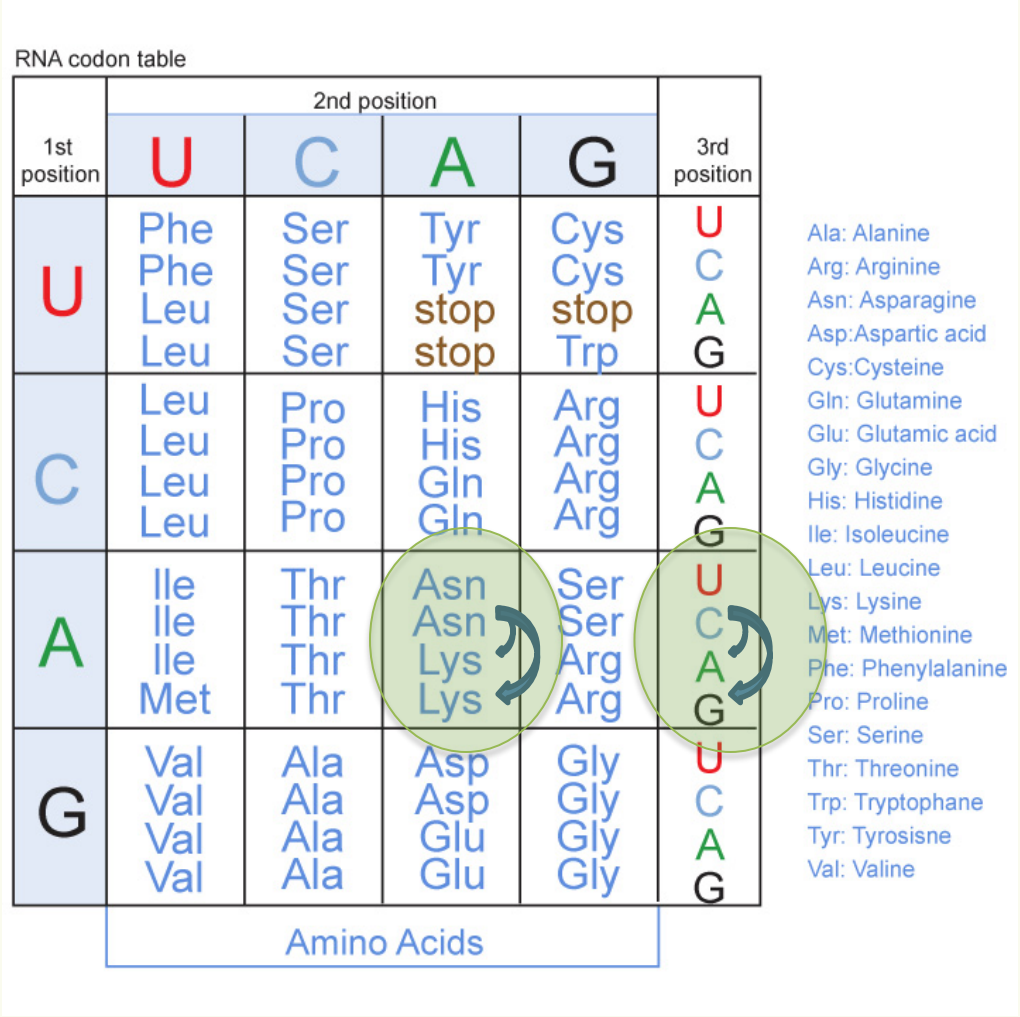
4. Only some persons with hypochondroplasia have intellectual disability. What two phenomena explain this?

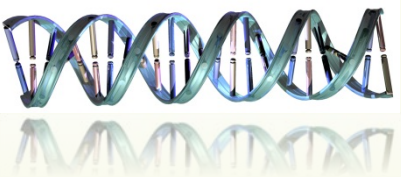
5. The doctor could have ordered a complete radiographic survey including skull, pelvis, AP and lateral spine, legs, arms, and hands, instead of a genetic test, to diagnose hypochondroplasia. Give three reasons why she might have chosen the genetic test over the radiographic diagnostic approach. What did she risk by choosing the genetic test?

V. Case Study: Answers

Q. Why do the **c**DNA variants **c**.1620C>A and **c**.1620C>G both result in **p**rotein variant **p**.Asn540Lys?

A. Degenerate Codons for Lysine amino acid





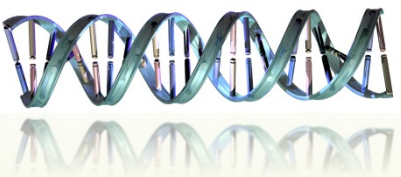
V. Case Study: Answers

Q. How many copies of the hypochondroplasia variant allele were found? Is this a dominant or recessive disorder?

A. *One variant allele and one normal allele were identified in the ATP-binding segment of the FGFR3 tyrosine kinase domain.*

The test result was heterozygous for the disease-associated variant (compared with a normal reference sequence).

Hypochondroplasia is a dominant disorder, both by inference from pedigrees, and by biologic basis, which is constitutive activation of the receptor tyrosine kinase, a "gain of function."



V. Case Study: Answers

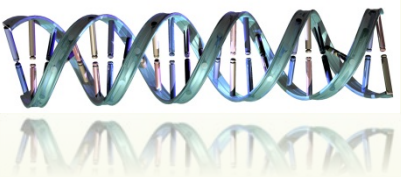
Q. How can Roger's diagnosis possibly help his father?

A. Father's short stature and stocky build suggest Roger may have inherited Hypochondroplasia from him.

A significantly increased incidence of spinal stenosis and bony compression occurs in this disorder.

Roger's diagnosis might lead to diagnosis in father, and detection of and surgery for spinal stenosis.

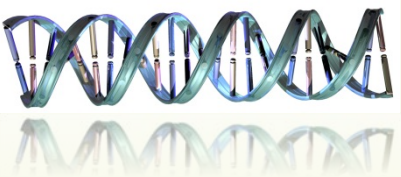
Roger's father might recover from pain and disability.



V. Case Study: Answers

Q. Only some persons with hypochondroplasia have intellectual disability. What two phenomena explain this?

A. Variable expressivity.
Genotype-phenotype correlation.



V. Case Study: Answers

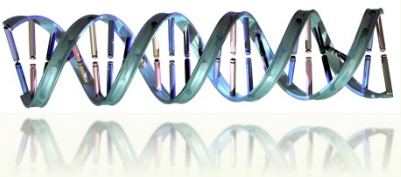
Q. The doctor could have ordered a complete radiographic survey including skull, pelvis, AP and lateral spine, legs, arms, and hands, instead of a genetic test, to diagnose hypochondroplasia. Give three reasons why she might have chosen the genetic test over the radiographic diagnostic approach. What did she risk by choosing the genetic test?

A. The complete radiologic survey is necessary to diagnose hypochondroplasia, and radiation exposure is significant. Even then, radiologic diagnosis can be difficult and the criteria are controversial.

The gene test is less expensive ($200-300 for a single exon)

The tested-for variant is associated with higher incidence of intellectual disability. The positive test result will likely stop further etiologic testing for intellectual disability.

13% to 42% of Hypochondroplasia is due to other pathogenic variants in FGFR3 (allelic heterogeneity), or have no detectable FGFR3 mutation (locus heterogeneity). If the test result had been normal, she could have pursued whole gene sequencing, or radiologic survey for diagnosis, or for detection of other, clinically overlapping, skeletal dysplasias.



Thank you