Genetics 101:

What does it mean to have an LBSL-specific mutation

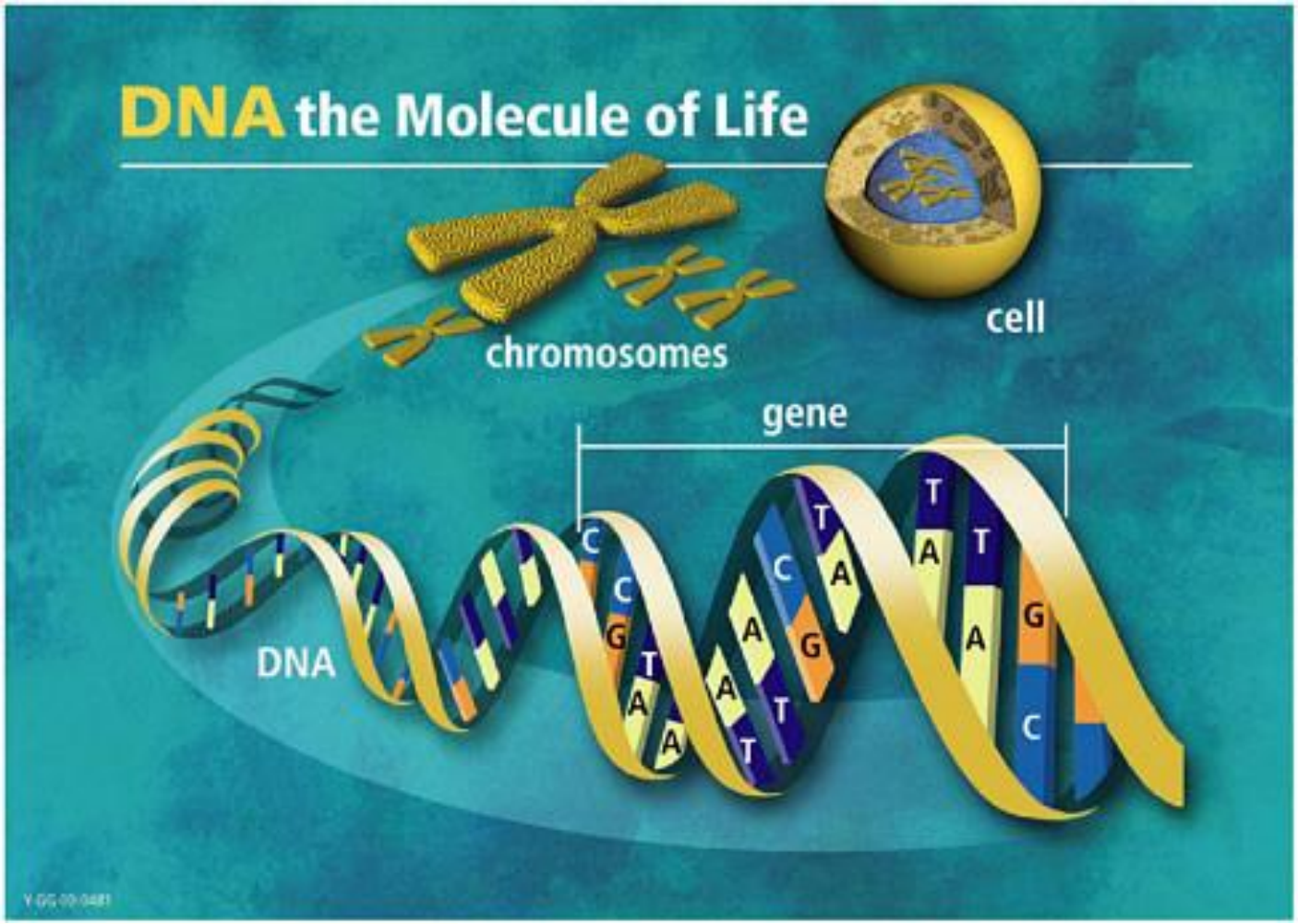
Rebecca McClellan, MGC, CGC
Johns Hopkins – Center for Inherited Heart Disease
Kennedy Krieger – Metabolism Clinic

Kennedy Krieger Institute
UNLOCKING POTENTIAL

JOHNS HOPKINS
MEDICINE

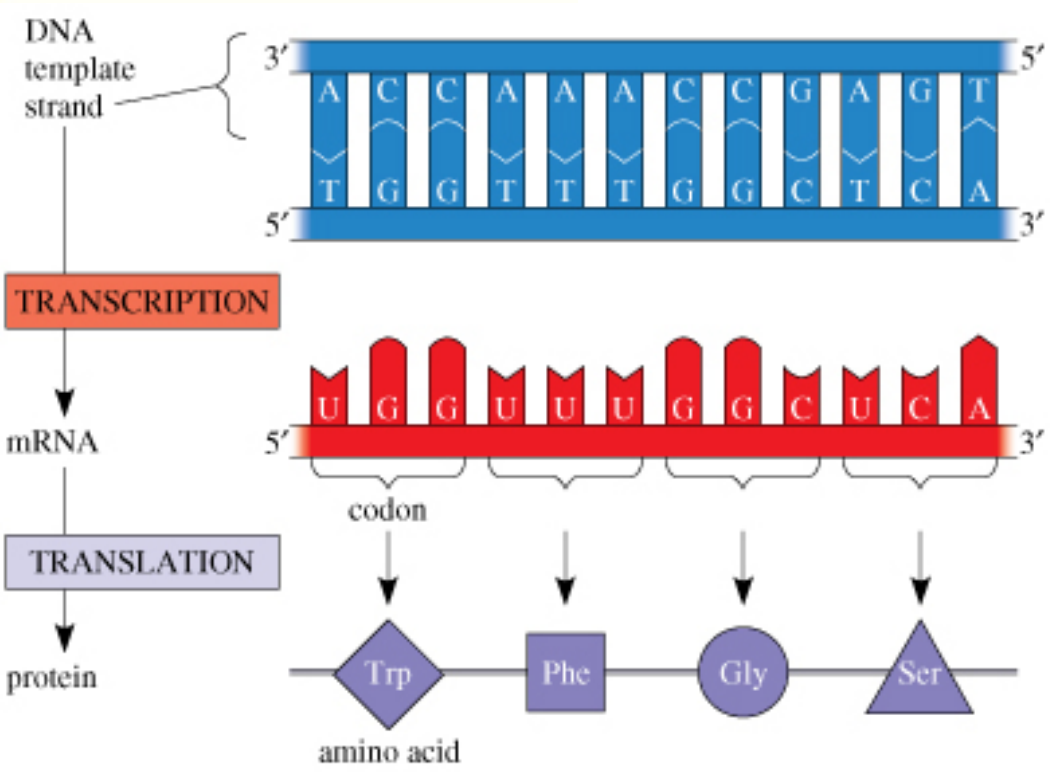
Genetics 101

- A gene is the working unit of DNA
- Genes tell the cell how to make proteins
  - 4 nucleotides (base pairs) make up DNA – A, C, G, & T

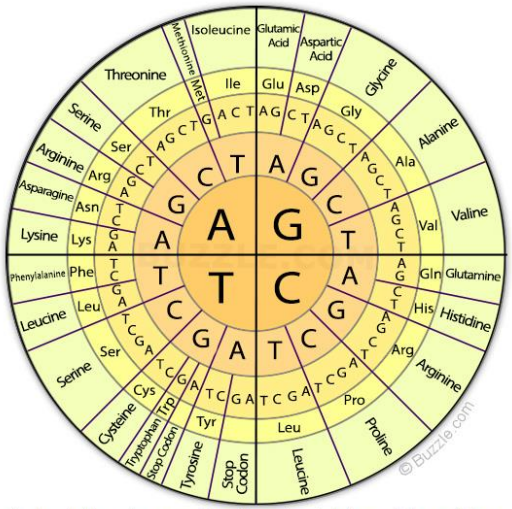


Genetics 101

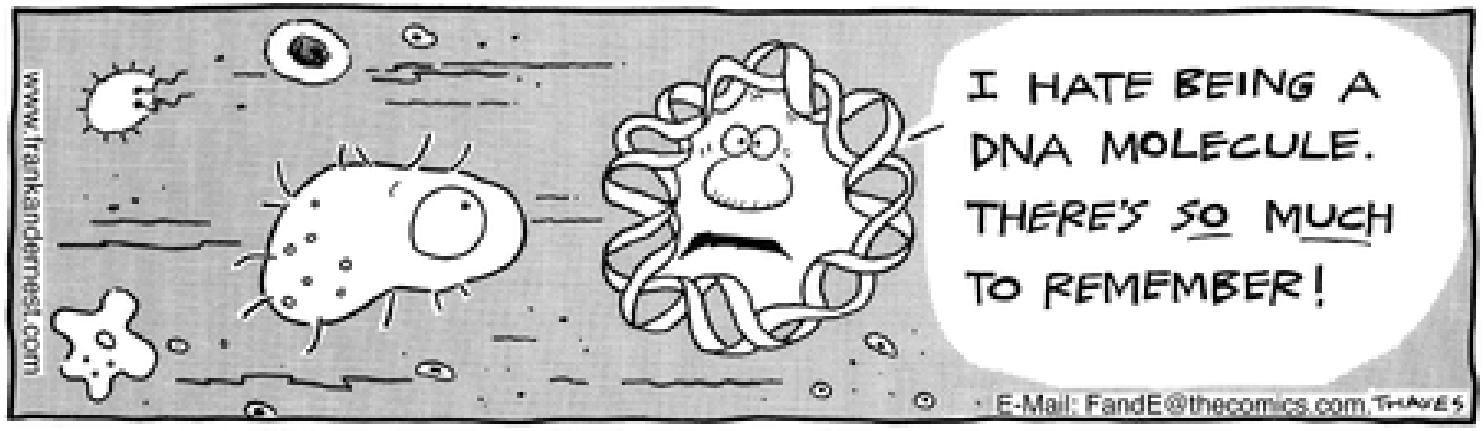
- The nucleotides are the code that defines the amino acids which make up proteins
- Proteins perform all cellular functions



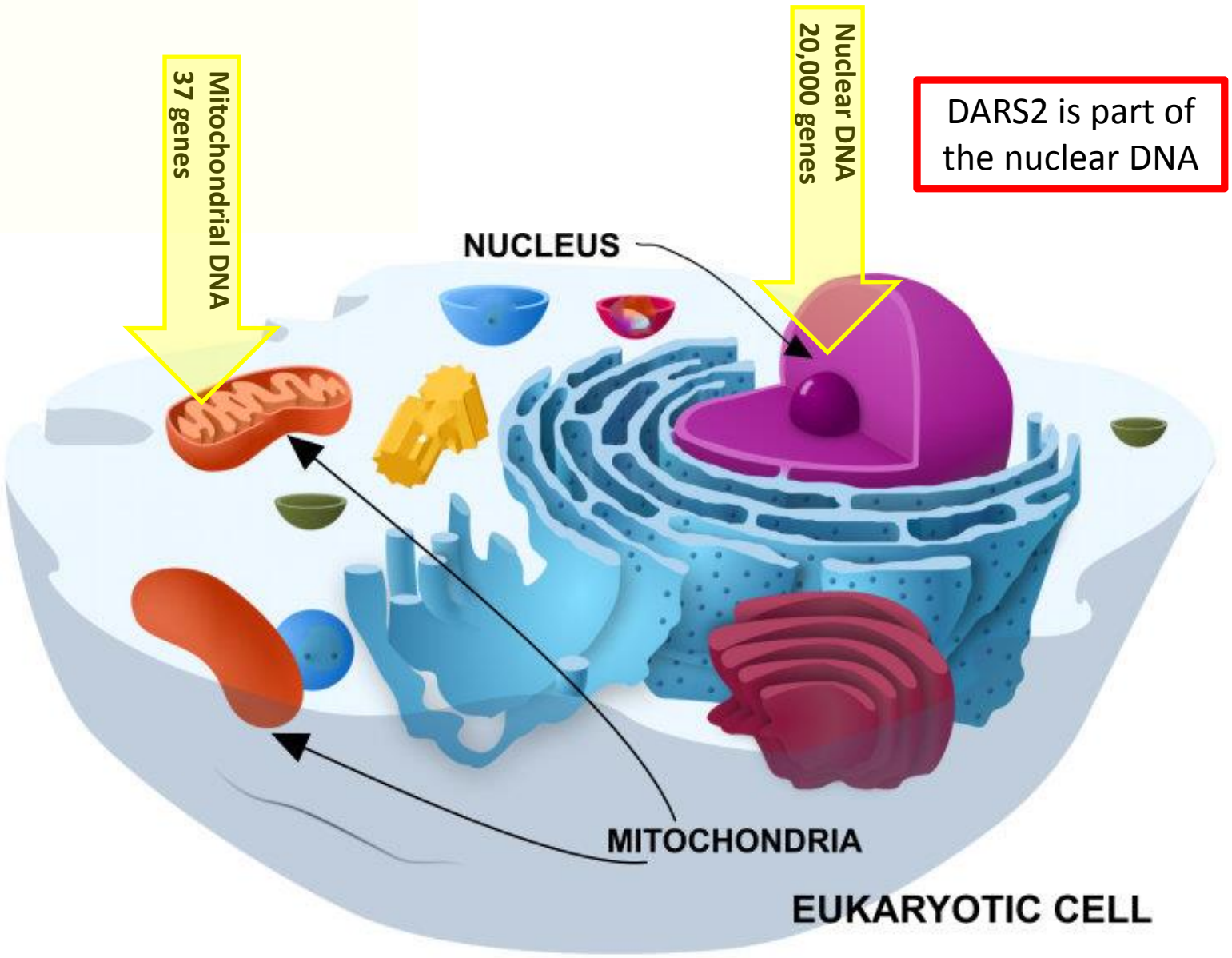
From *Biology* by Campbell and Reece © 2008 Pearson Education, Inc.



Frank and Ernest



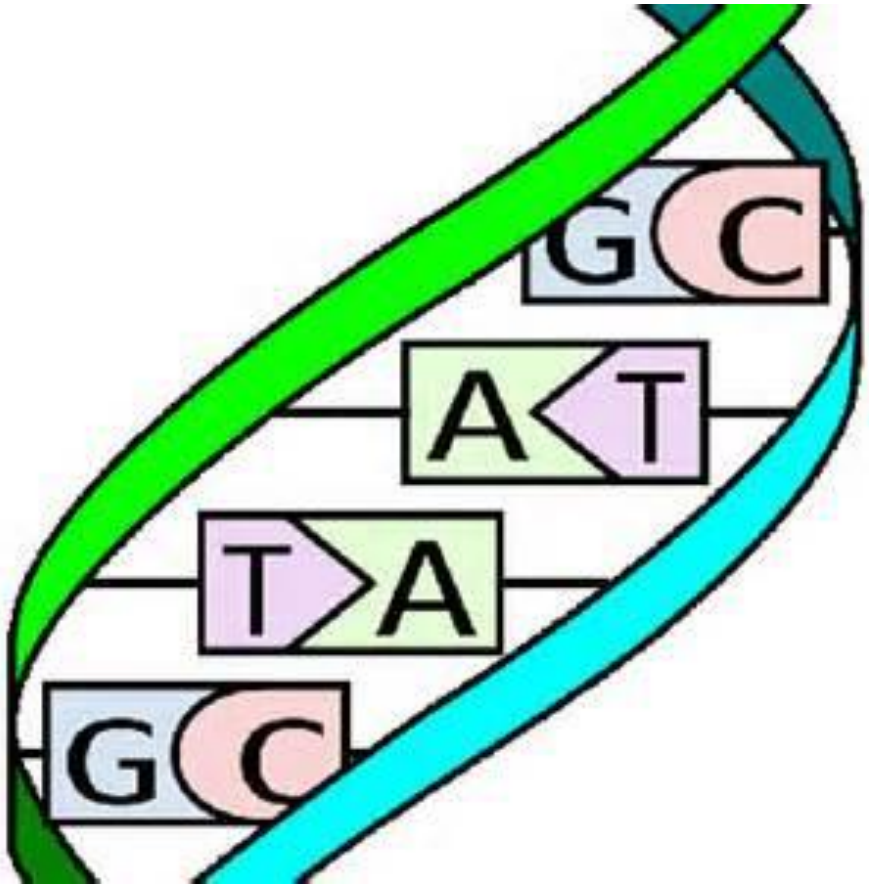
Genetics 101



DARS2 is part of the nuclear DNA

Genetics 101

- A mutation refers to a change in the DNA code
  - Also referred to as a variant
- 3 ways that DNA may be changed in LBSL
  - Misspelling
  - Deletion
  - Duplication

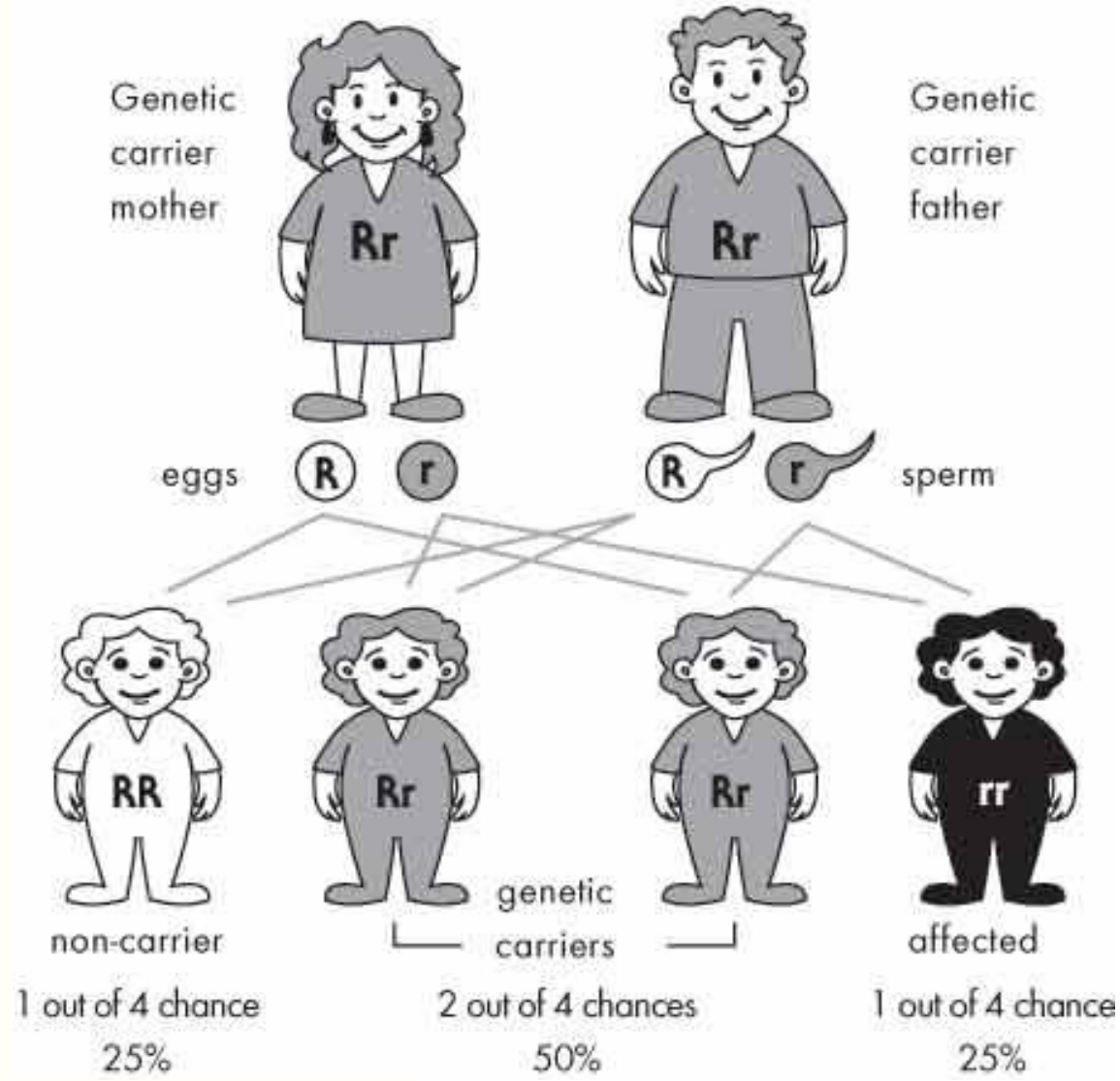


Genetics 101

- Patterns of inheritance:
  - Autosomal Dominant
  - Autosomal Recessive
  - X-linked

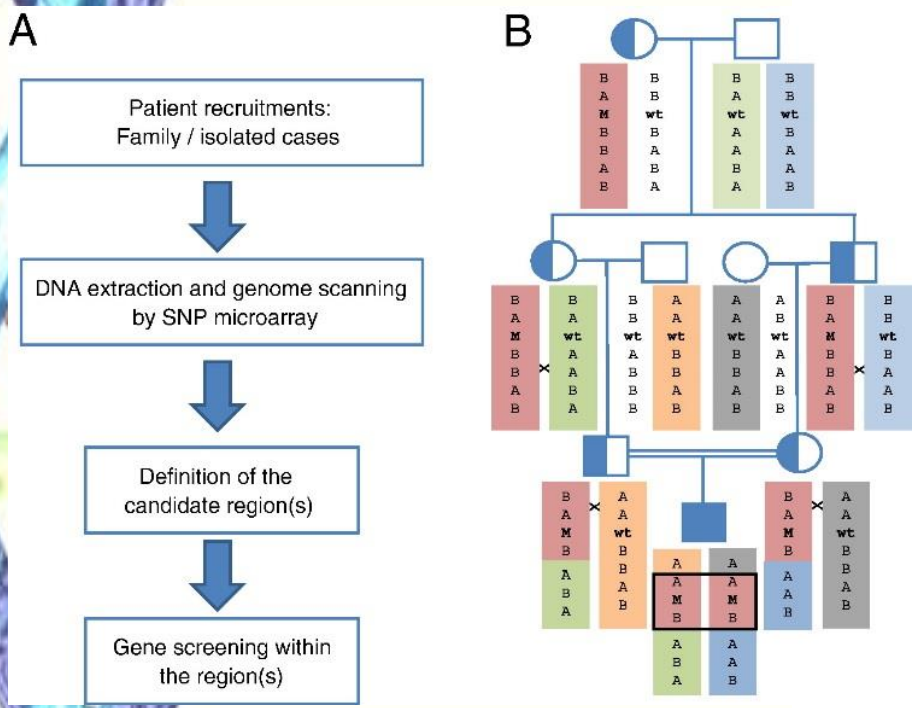
- Genetic disorders are not always inherited
  - Sporadic or “de novo”

Autosomal Recessive Inheritance



LBSL/DARS2

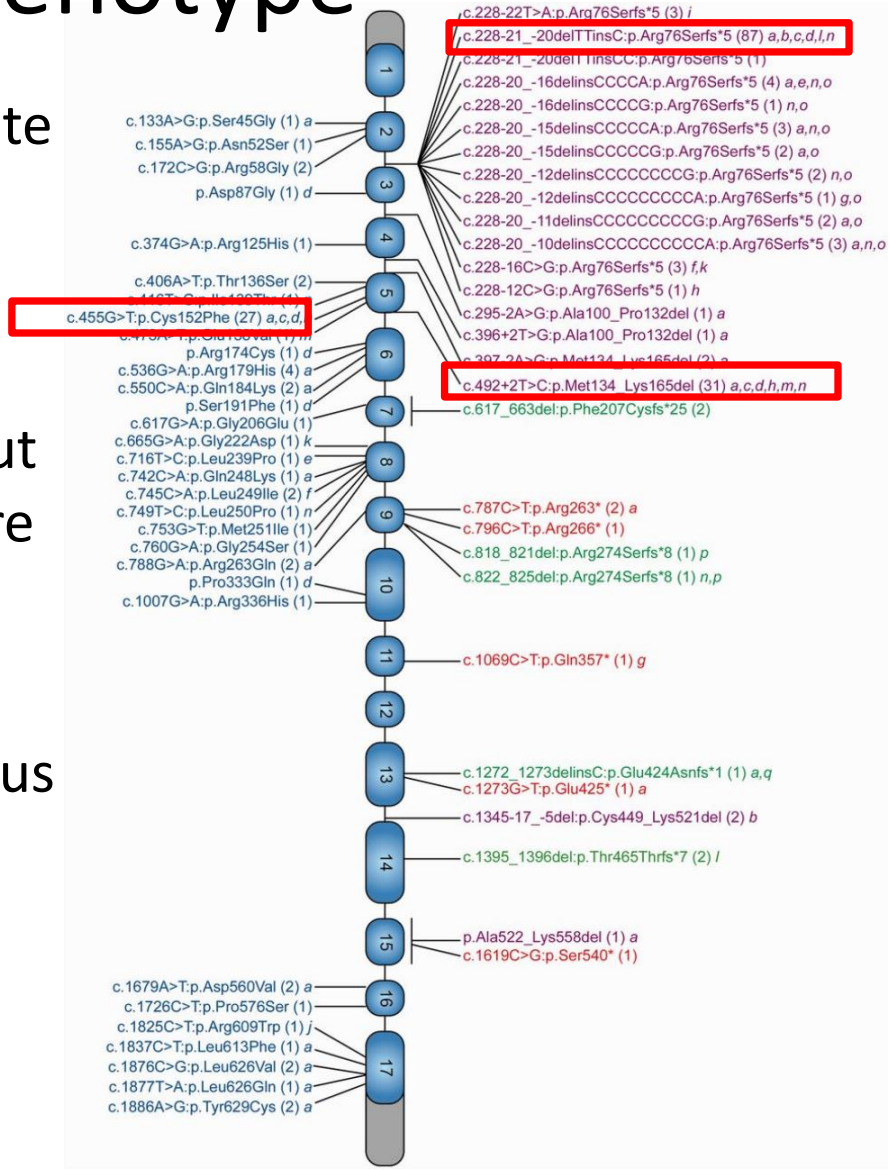
- 2003: LBSL first clinically described in 8 patients (van der Knaap et al)
  - Presented at ULF meeting July 2001
- 2005: Structure of DARS2 gene defined (Bonnefond et al)



- 2007: DARS2 linked to LBSL
  - Linkage studies in 6 affected sib pairs identified two regions
  - Further narrowed chr 1
  - Sequenced 16 candidate genes in region and found mutations in DARS2 in all

Genotype – Phenotype

- Difficult given many private mutations and small #s
- In 45 pts with common c.228-21_-20delTTinsC but different 2nd variants there is spectrum of severity
- c.228-21_-20delTTinsC plus c.455G>T or c.492+2 T>C all had variable onset but mildly progressive neurologically deterioration



From: Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation: clinical and genetic characterization and target for therapy
Brain. 2014;137(4):1019-1029. doi:10.1093/brain/awu026

Genetic Testing – when and what?

- Reasons to test
  - Uncertainty about a diagnosis
  - Confirm diagnosis
    - Treatment decisions
    - Prognosis
  - Provide risk information to family members

- What test do we send?
  - Consider clinical presentation
  - Atypical presentation might suggest broader test
  - Patient comfort level with uncertainty

LBSL Genetic Testing Options

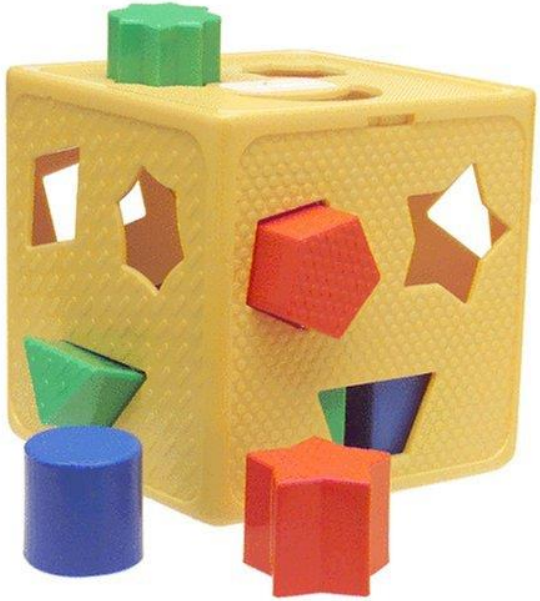
- DARS2 gene sequencing
  - Important to include deletion/duplication
  - TAT 2-6 weeks depending on lab
- Panel testing
  - Nuclear mitochondrial disorder panels
  - TAT 10-14 weeks depending on lab
- Whole Exome Sequencing
  - TAT 8-12 weeks

Genetic Testing Considerations

- Genetic Discrimination
  - GINA provides protection for group health plans and employment
- Insurance coverage
  - Checked individually
  - But most labs offer very good patient assistance if not covered in full
- Possible Results
  - Positive
    - Can use for targeted family screening
  - Negative
    - Might still be genetic, but not LBSL
  - Variant of Uncertain Significance
    - Don’t have enough information to say if benign or disease-causing
    - Can’t use for targeted family screening; might suggest segregation
    - Follow data and revisit periodically

Not all genetic changes impact gene function

- Depends on a number of things:
  - Where the change occurs
    - intron vs. exon, functionally important part etc.
  - How significant is the change
  - How tolerant is that spot in the gene to change



2015 ACMG Classification guidelines

- 5 categories
  - “pathogenic,” “likely pathogenic,” “uncertain significance,” “likely benign,” and “benign”
- Process of classification using typical types of variant evidence (e.g., population data, computational data, functional data, segregation data)

Types of mutations and what they mean

Story of the Sweater Gene

Invented & Designed by Iris Gonzalez, PhD

The Sweater gene provides the instructions to make a sweater; it lists the materials and tells us how to build the sweater

Some changes in DNA are called “synonymous” because they do not change the structure of the final protein product.

Here, a synonymous change in the sweater gene does not alter the structure of the sweater.

Example:
c. 81 T>C= p. Gly27Gly

Missense Variant

Some changes in DNA will change the amino acid produced and cause an alteration of the protein product structure

The change could alter the sweater relatively mildly so you can still use it.

Example:
c. 580 G>A = p. Val194Ile

Missense Variant

Some amino acid substitution mutations severely change the protein structure so it becomes non-functional.

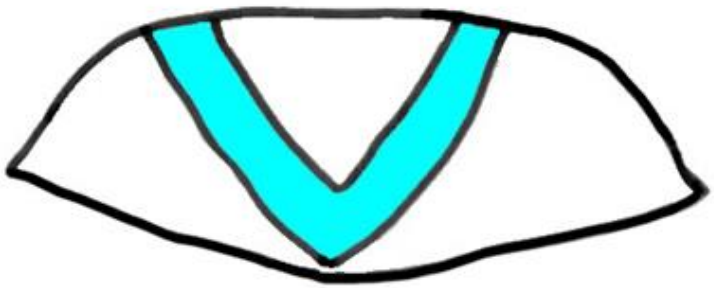
And sometimes the change will impact the sweater in significant way and make it useless:

Example:
c. 455 G>T = p. Cys152Phe

Nonsense variant

Some changes in DNA are called STOP mutations: they cause premature termination of the protein product, meaning that it is shortened, either a lot or a little

Here the sweater gene has a STOP mutation that occurred early, making the product very small and useless



Example:
c. 787 C>T = p.Arg263Ter (exon 9)

A STOP mutation occurring late in the gene:
the product is larger but still incomplete

A STOP mutation late in the sweater gene
still produces a useless sweater

Example:
c. 1273 G>T = p.Glu425Ter (exon 13)

Splice Variants

In splice variants the signals for removing introns (noncoding regions of DNA) are damaged. This leads to incorrect splicing of the exons (the coding regions of DNA) and the product has an abnormal structure.

The sweater gene has a splice mutation so that an additional structure is present which renders the sweater useless

Example:
c. 396+2 T>G
Destroys splice donor site for intron 4 which leaves extra DNA in place

Frameshift Variants

Small insertions or deletions of DNA letters are called “frame shift mutations” because they alter the instructions and the product is different from that point on.

The frame shift mutation at the beginning of the left sleeve led to a complete change of structure from that point on.

Example:
c. 228-21_-20delTTinsC
= p.Arg76Serfs*5

Another way to think about a frameshift variant

[using a text of words as an example]

Text: The teensy weensy spider went up the water spout

If this were DNA, it would have to be read as groups of 3 letters called "codons", each of which specifies an amino acid

We will delete the letter shown in red below, but all the words must still have the same number of letters as before

Compare the "before and after" texts:

- The teensy weensy spider went up the water spout
- The teenyw eensys piderw entu pt hew aters pout_

frameshift from that point on

Questions?

Gratitude to my amazing patients & colleagues

Rebecca McClellan, MGC, CGC
Kennedy Krieger – Metabolism Clinic
Johns Hopkins – Center for Inherited Heart Disease
mcclellan@kennedykrieger.org