The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

Aminoacyl-tRNA Synthetases: what are they and what is their connection to human diseases?

Christopher Francklyn, Ph.D.
Department of Biochemistry
University of Vermont

Outline of the talk

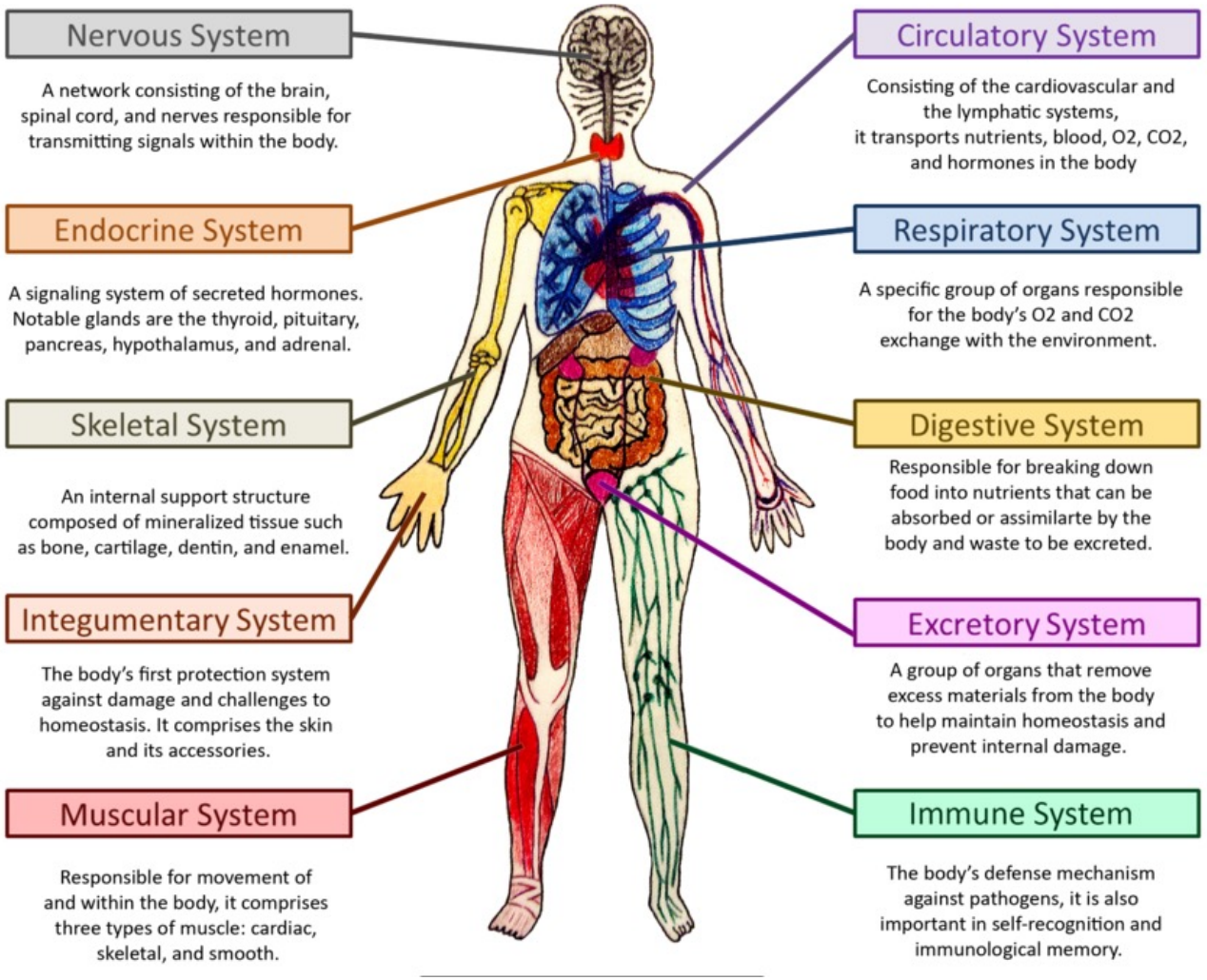
- PART ONE: Introduction to the mechanisms of gene expression
- PART TWO: A sensorineural disease mystery with a happy ending (?)
- PART THREE: Peripheral Neuropathies and autosomal dominance
- PART FOUR: Recessive Diseases: Impacts on Brain Development
- PART FIVE: Introduction to Mitochondrial Disease

The Robert Larner, M.D. College of Medicine

THE UNIVERSITY OF VERMONT

Part One
Introduction to the mechanisms of gene expression

The form and function of cells depends on the proteins that they make

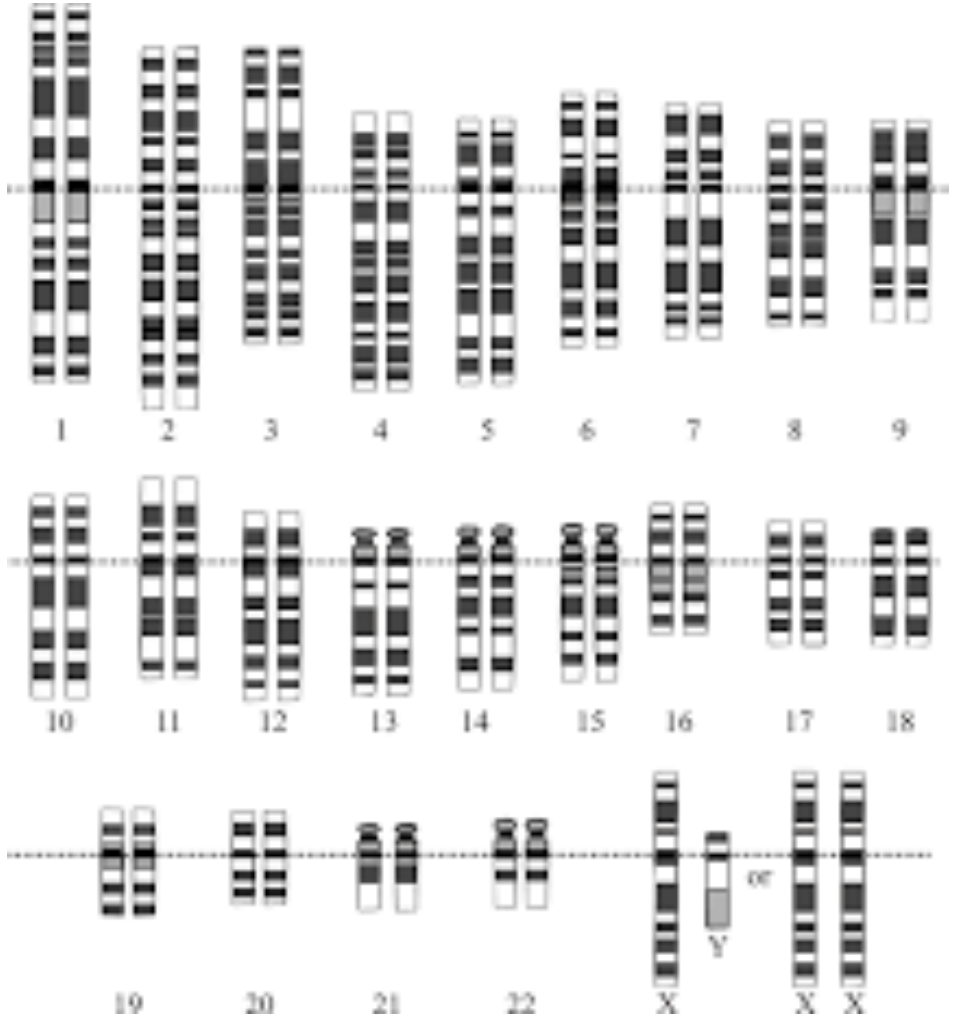


- All cells in a person's body contain a complete copy of their genome.
- Only a portion of that genome is accessed in a particular cell type.
- Particular genes → cell specific proteome→tissue specific features→ organs→ organ system

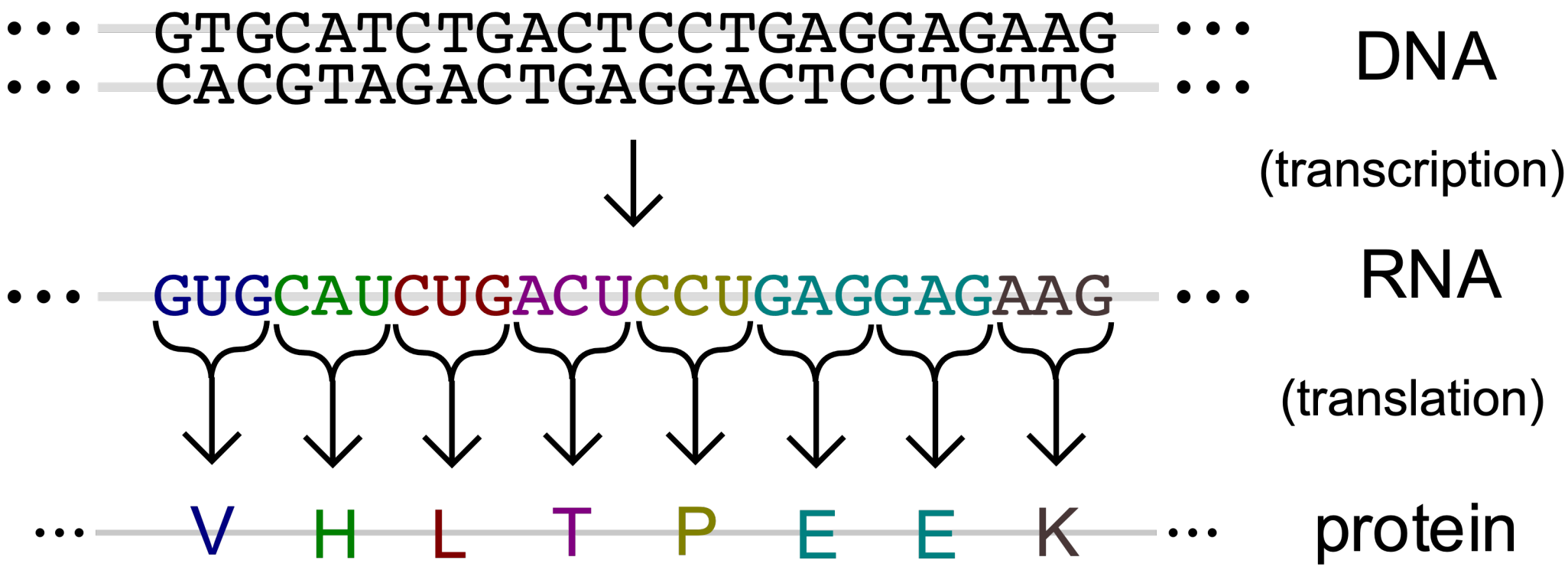
All cells in our body have the same DNA genome physically contained on 46 chromosomes

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

- Consists 23 pairs of Chromosomes comprising 3 billion letters (nucleotides) plus sex chromosomes (XX= female, XY=Male)
- Gene expression mechanisms have to be at work to restrict the pool of genes that are transcribed in a particular cell.
- These choices are a reflection of both pre-organized developmental programs that create specific body structures, and of environmental factors (nutrition, genome modifying agents) that can influence which genes are expressed, and how much they are expressed.

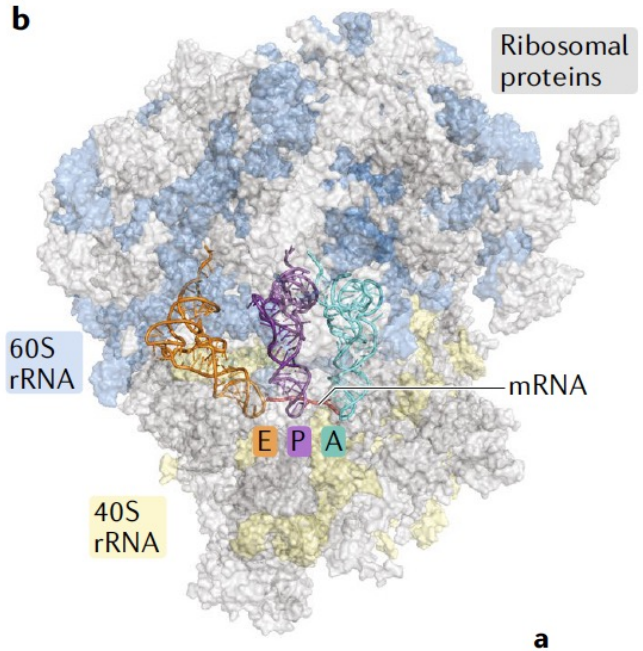


Protein expression depends on translating nucleic acid language into protein language

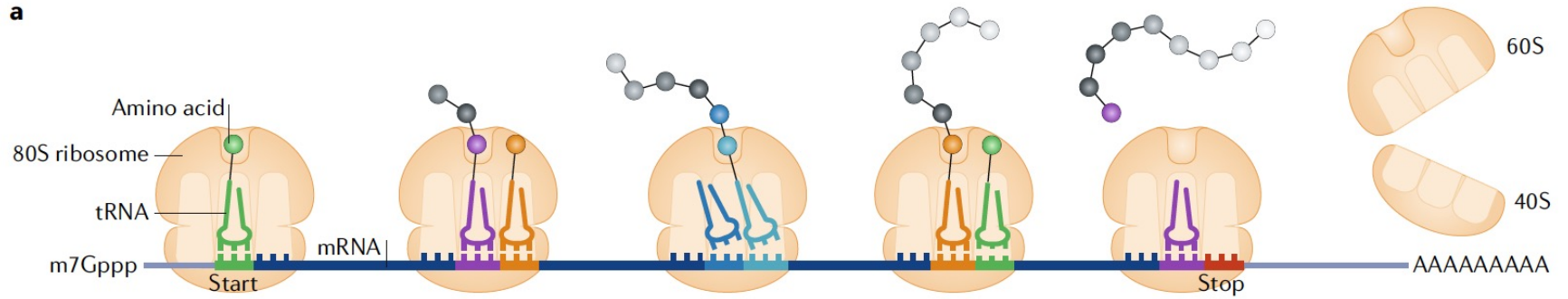


- *Here we are particularly concerned with the translation step.*

The machinery of protein synthesis

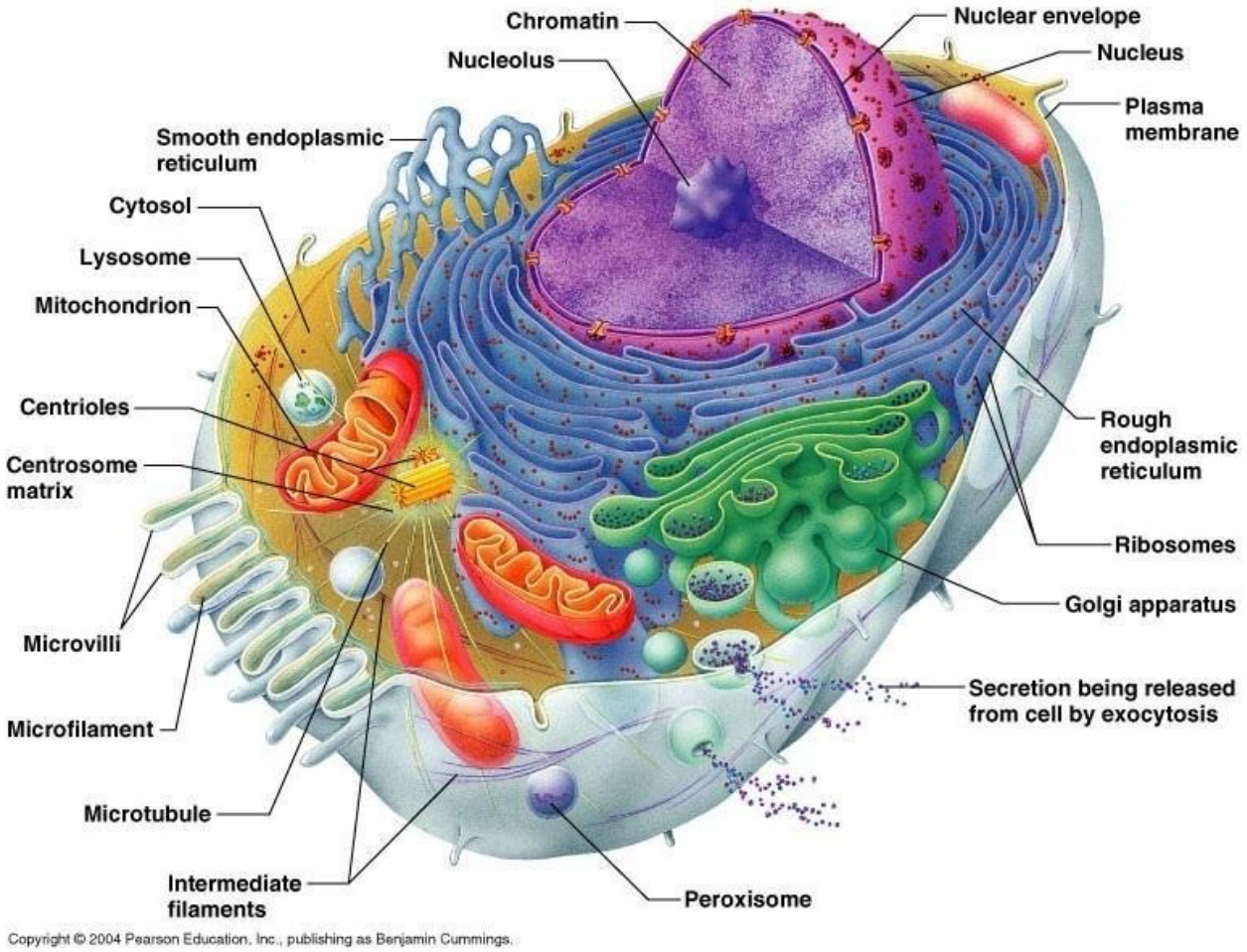


- Ribosome machinery is highly complex, consisting of an mRNA “message”, the ribosome, a huge protein-RNA complex through which the message is fed, the tRNA adaptors, and a large of additional protein factors



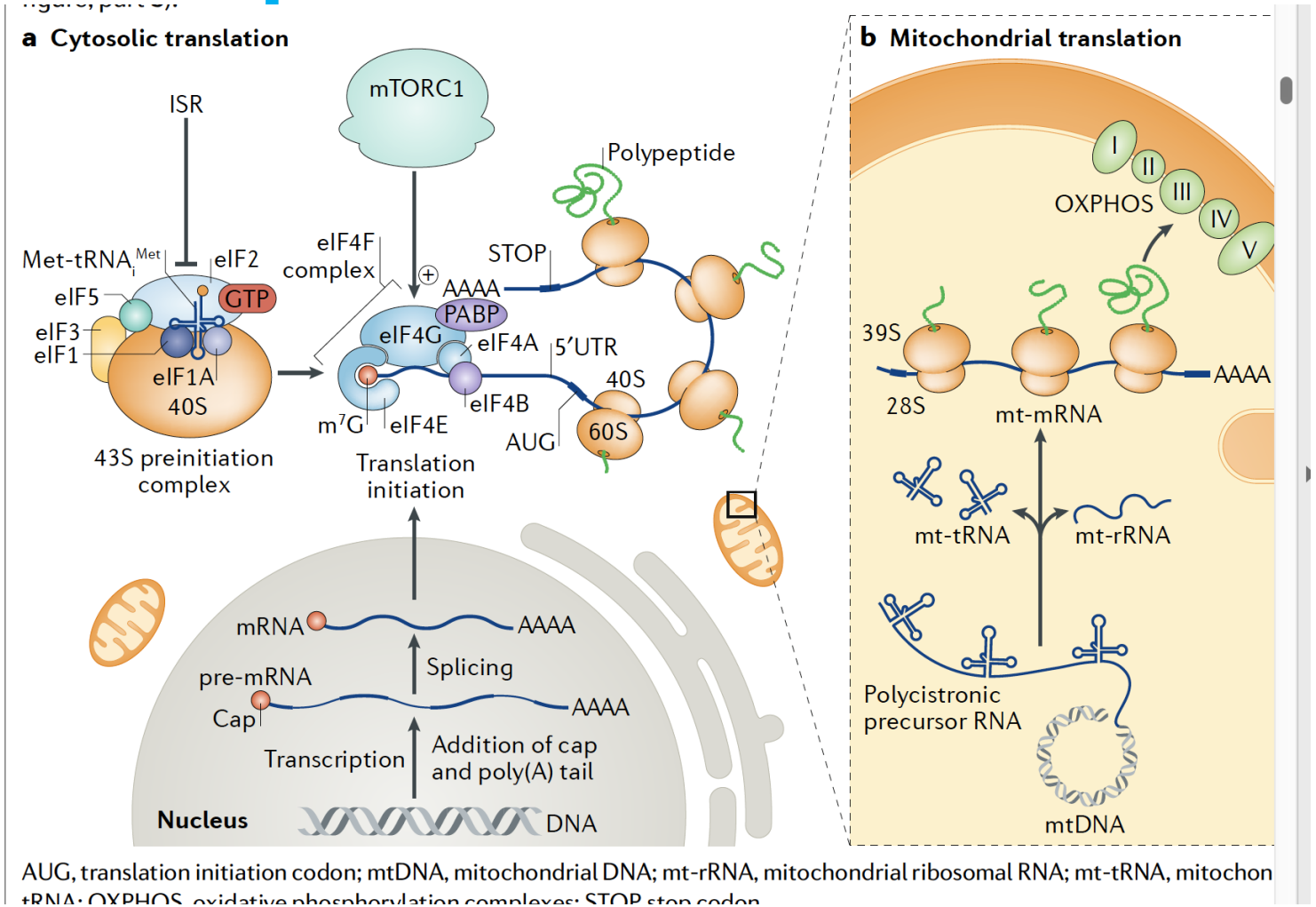
Phase:	Initiation	Elongation	Termination	Recycling
Factors:	eIFs Met-tRNA^{iMet}	eEFs aa-tRNAs	eRFs	ABCE1

Human cells contain two different protein synthesizing systems in two compartments



- A typical human cell is organized into compartments; thesr are typically membrane enclosed structures.
- The nucleus is a special compartment that houses and maintains the genome. Following transcription, mRNA moves into the cytoplasm. Here, it can be translated into proteins
- Translation also occurs inside mitochondria, which are specialized compartments for making ATP.

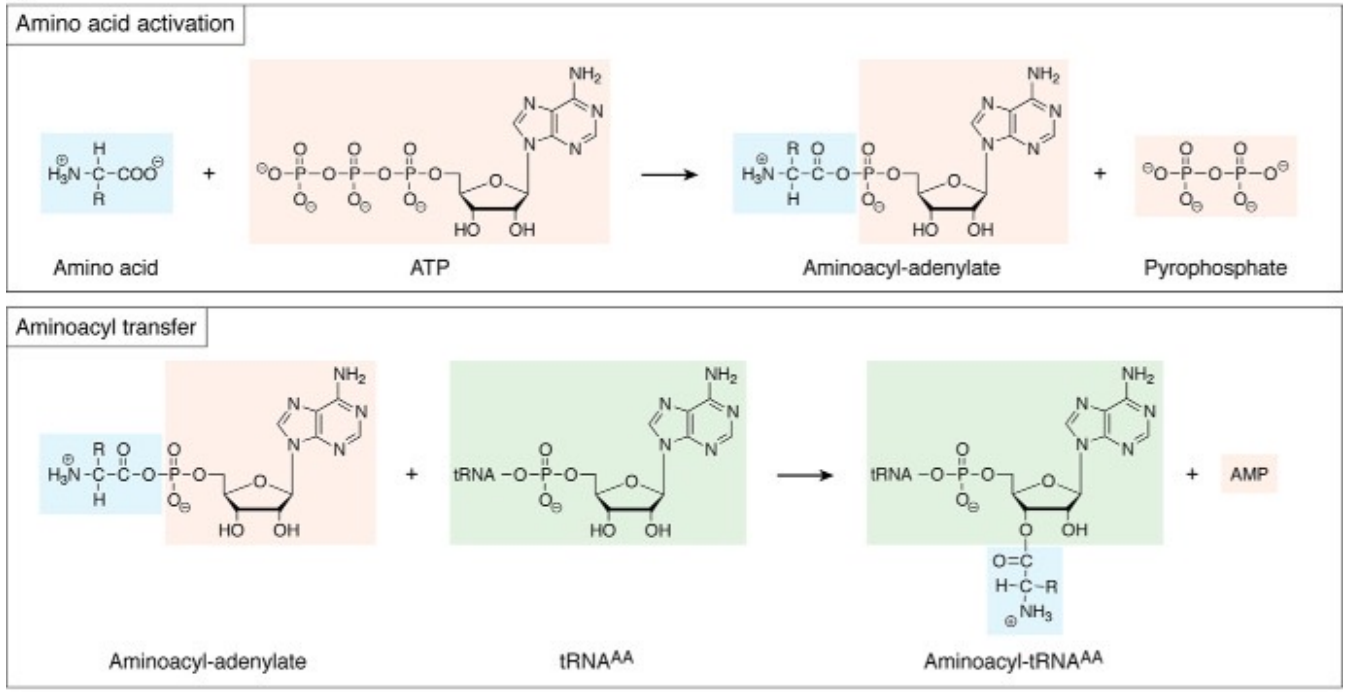
Ribosomes are located in two compartments



AUG, translation initiation codon; mtDNA, mitochondrial DNA; mt-rRNA, mitochondrial ribosomal RNA; mt-tRNA, mitochon
tRNA; OXPHOS, oxidative phosphorylation complexes; STOP, stop codon

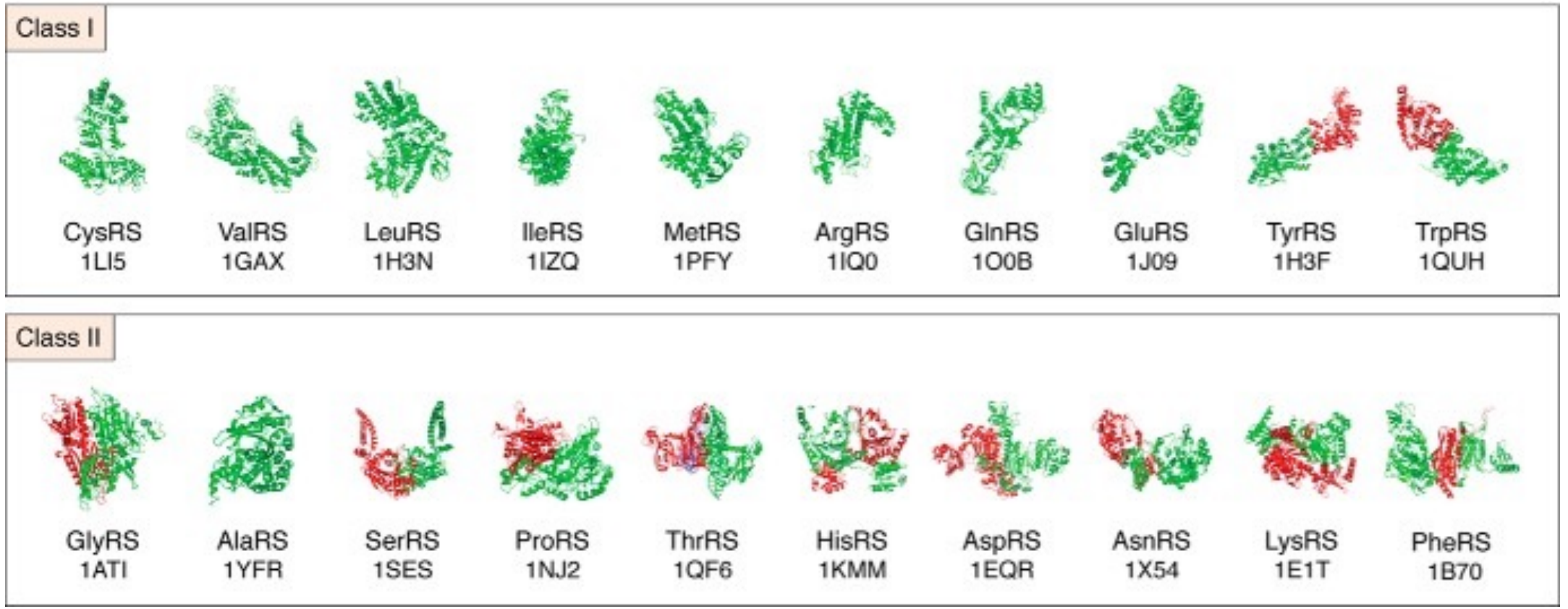
- Cytosolic and mitochondrial translation feature different machineries.
- Cytosolic translation features production of mRNA in the nucleus, processing and then export to the cytoplasm. There, machinery is assembled to translate proteins which can remain in the cytoplasm, be transported into the mitochondria, or be exported out of the cell.
- Mitochondrial protein synthesis involves a transcript produced from the mtDNA. The message can be processed to generate transfer RNAs and rRNAs, and then translated into proteins.

One key element of the coding machinery: the aminoacyl-tRNA synthetase (AARS) family



- The reaction they catalyze brings together amino acids (constituents of the proteins we consume in our diet) with ATP and the tRNA adaptors necessary for genetic coding

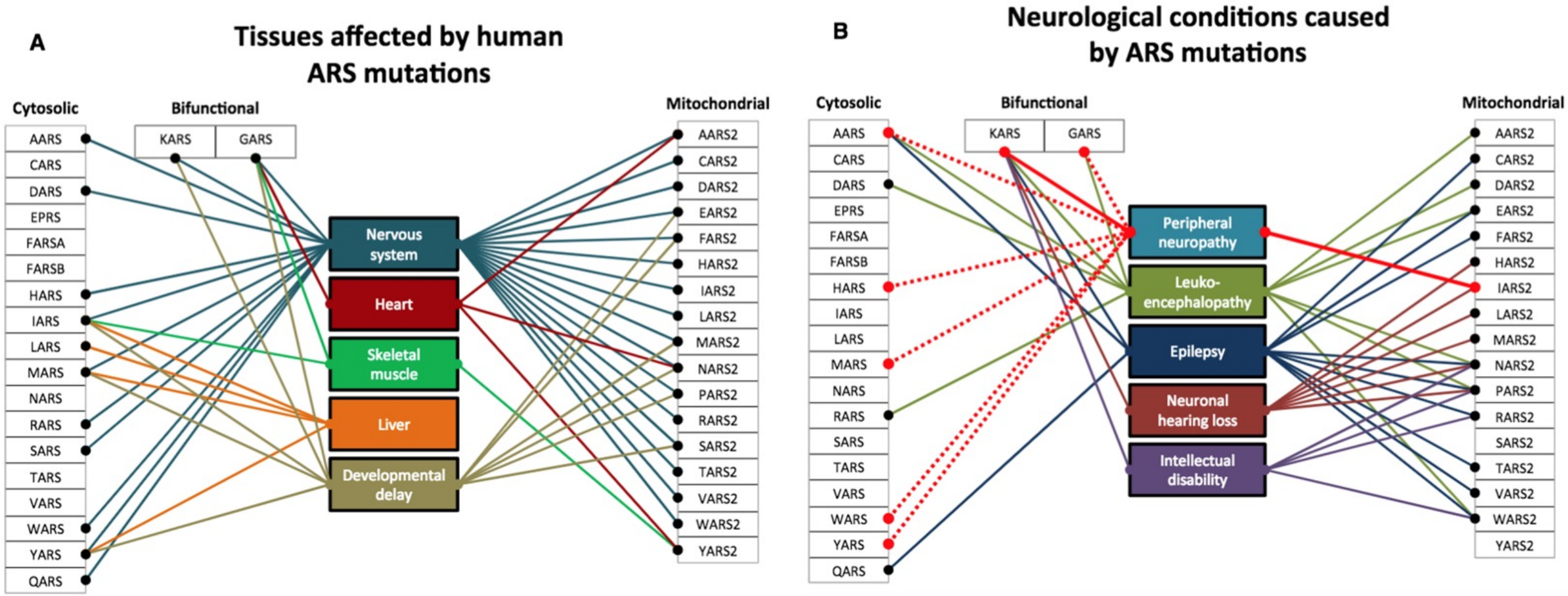
the aminoacyl-tRNA synthetases (AARS) family comprise 20 different enzymes



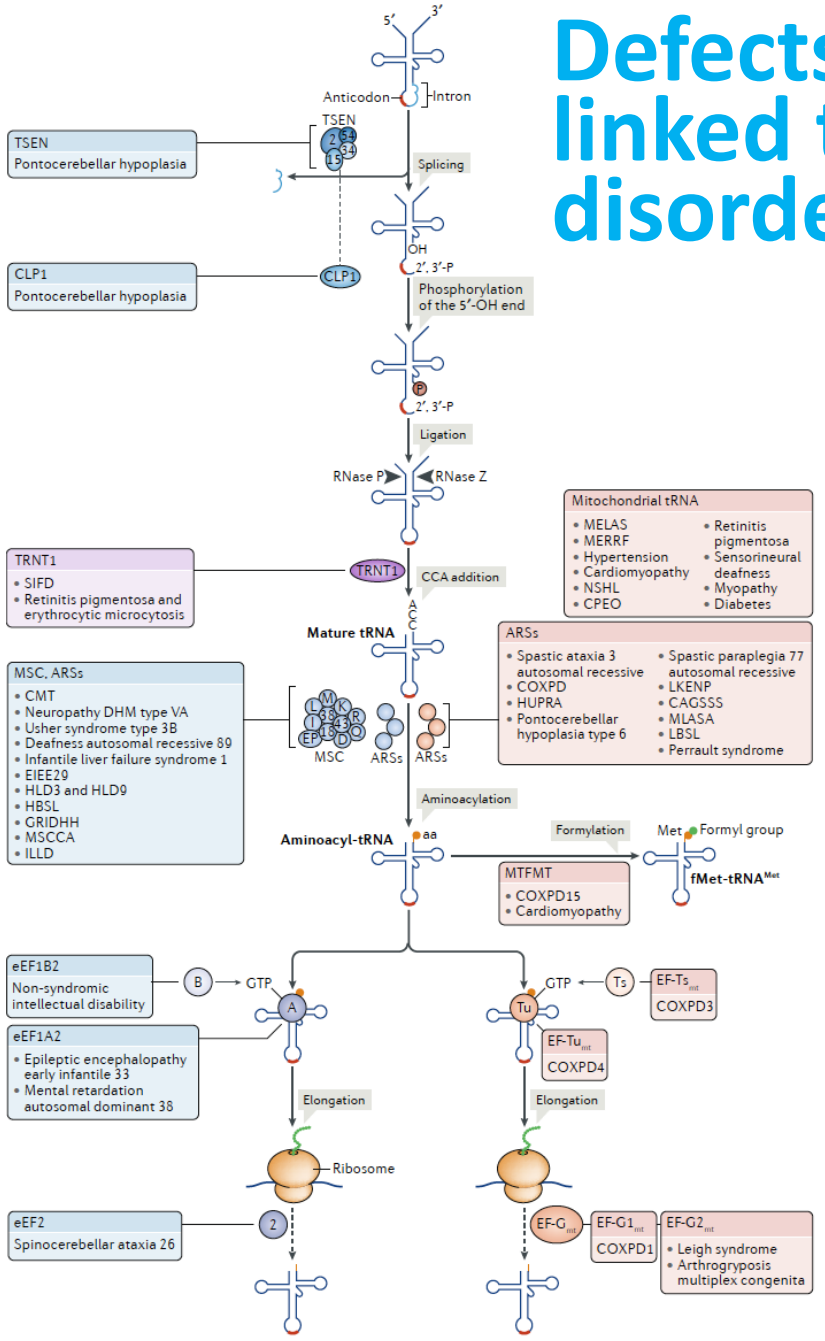
- Oh joy

Defects in AARS and protein synthesis machinery affect multiple human physiological systems

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT



Defects in tRNA biology are linked to nervous system disorders

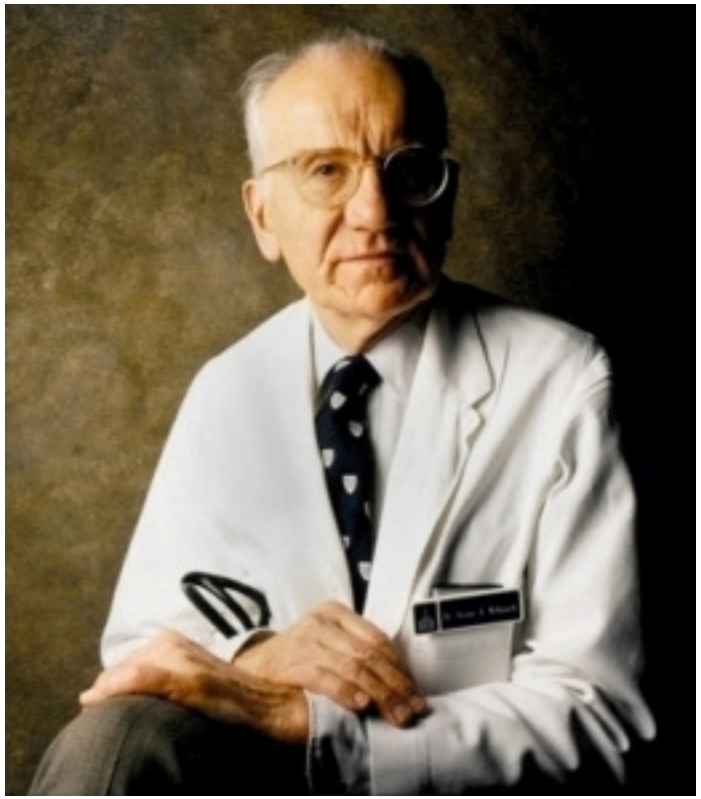


The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

- Transfer RNA undergoes a long complex pathway from its initial synthesis to its utilization by the ribosome.
- While my lab focuses only at one stage in the path- aminoacylation, mutations in genes providing machinery for other other steps are also linked to disease.
- The big question: why do mutations in genes that encode the protein synthesis machinery seem to attack the nervous system in particular?
- We are familiar with common diseases: cancer, heart disease, diabetes, and neurodegenerative diseases like Parkinson's or Alzheimer's.
- But our finding an answer requires that we search for much rarer diseases, that are not so common as the condition listed above.

Part Two
A sensorineural disease mystery with a happy ending

Where is a place to find rare *inherited* human diseases?



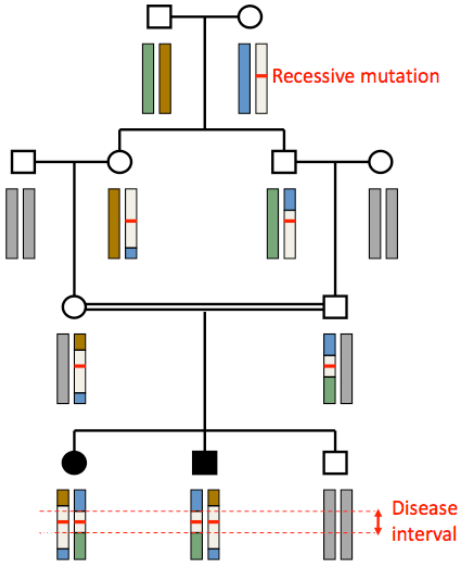
Victor McKusick, M.D.

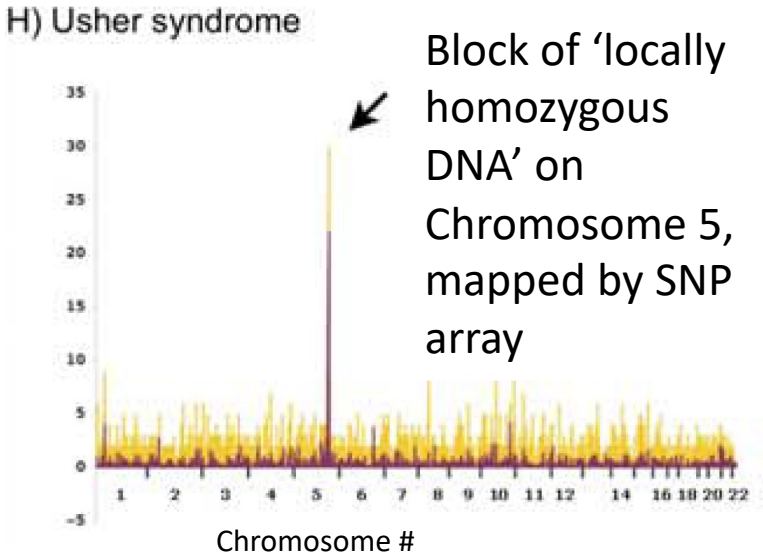
The Clinic for Special Children
Strasberg, PA

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

The clinic discovers a mutation involving our our enzyme affecting a group of Amish Children

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

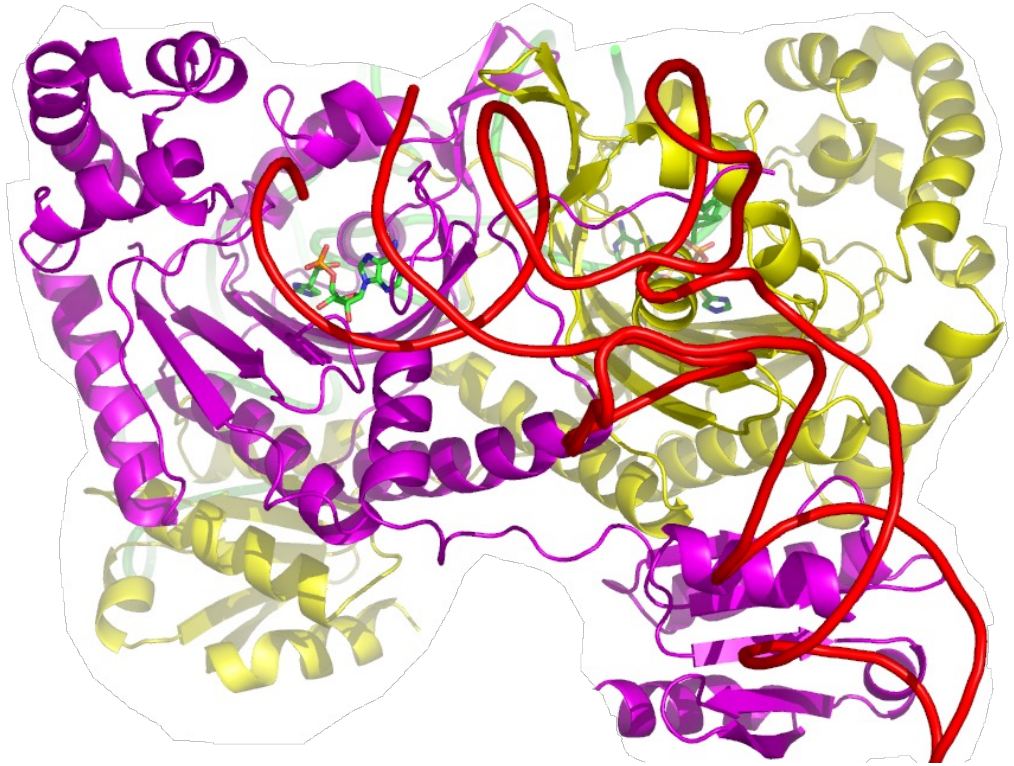




- 80 potential homozygous variants in the mapped interval on Chromosome 5
- Only novel mutation: HARS c.1361A>C (encoded Y454S)
- ***One of the first homozygous recessive ARS diseases affecting a cytoplasmic enzyme***

- The Original mutation thought to originate with Swiss founders of the Amish/Mennonite community. recessive allele is highly enriched in the population due to intermarriage.
- Associated with Usher Syndrome, an inherited sensorineural disease that affect the auditory and visual system. normal infant growth and development, but hearing deteriorates at age 5
- Visual impairment during early childhood; Infectious diseases provoke vivid visual hallucinations

Determining the mechanism of HARS disease

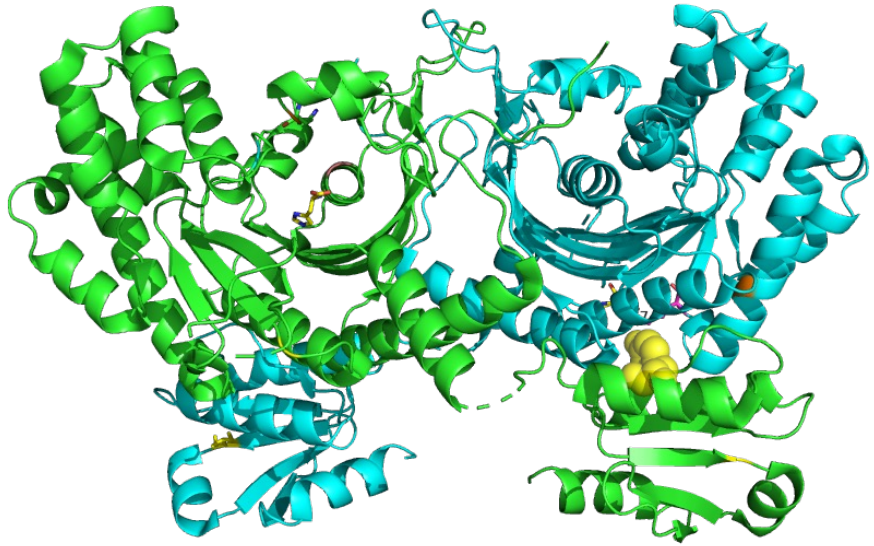


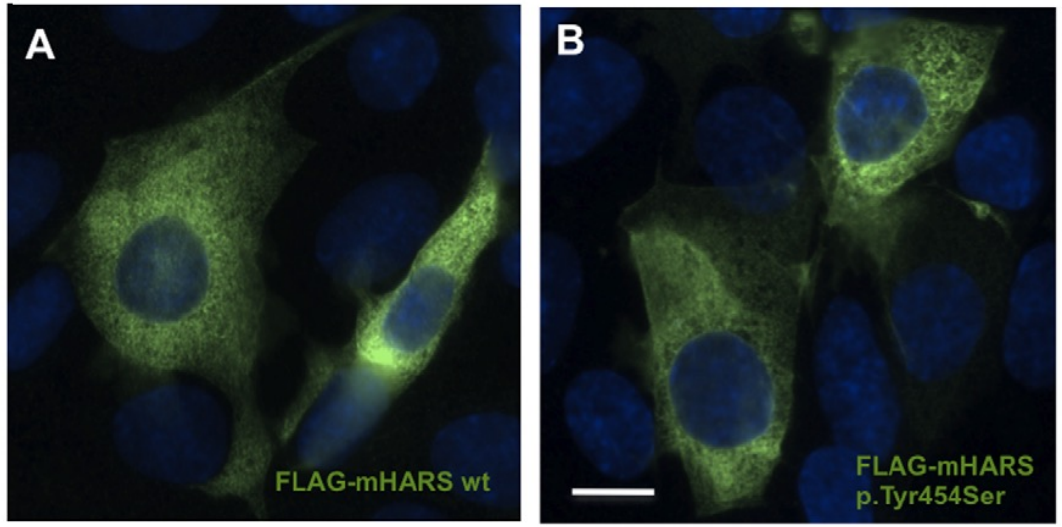
Three Dimensional Structure of the complex between histidyl-tRNA synthetase (HisRS) the aminoacyl-adenylate, and its cognate transfer RNA (tRNAHis)

- *What we would like to know:*
- Does the mutation significantly affect the structure of the enzyme?
- Does the mutation significantly alter the structure of the enzyme?
- Could the mutation be working through a previously unknown mechanism?

Initial assessments of impacts on HisRS structure and function

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

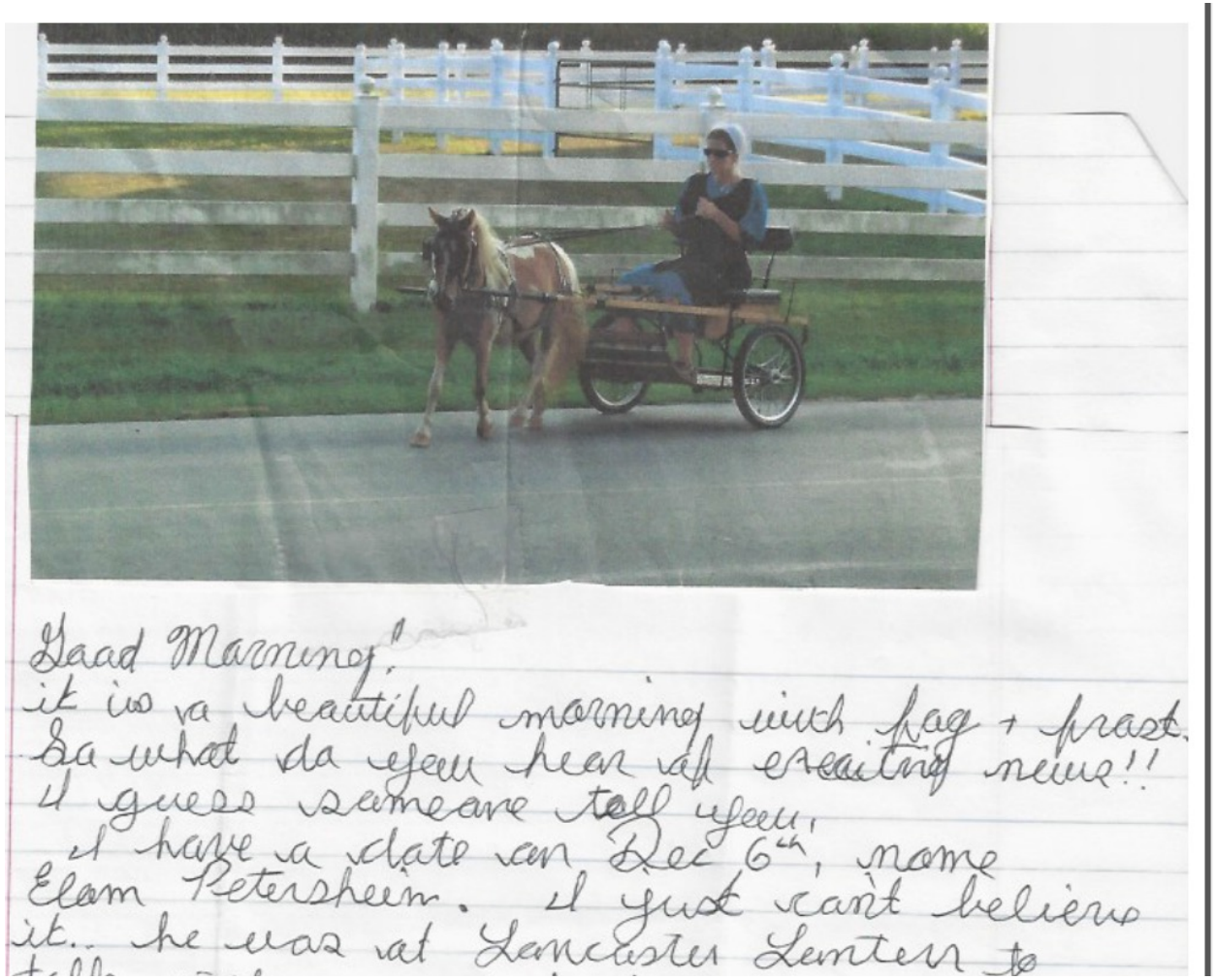




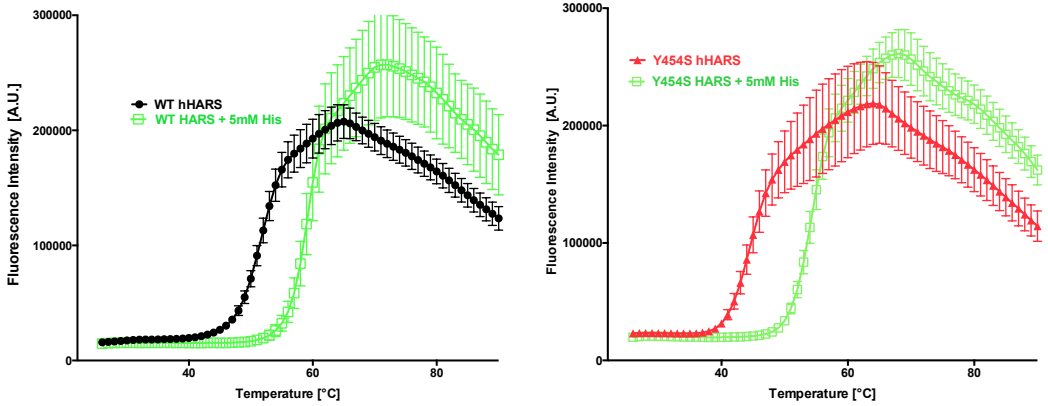
- the mutation changes an amino acid that is situated between two distinct domains on the enzyme.
- When DNA constructs expressing either the wild type or mutant version of the enzyme were expressed in an animal cell cell line, we did not see any difference: mutant protein appears to be stable.

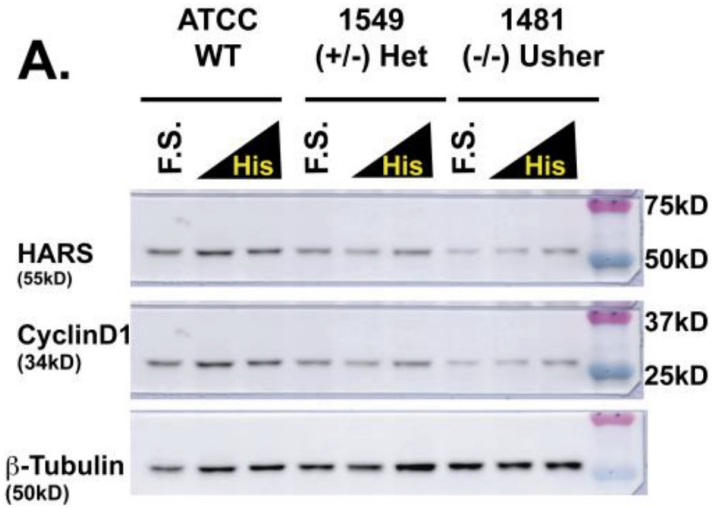
A critical piece of clinical data unlocks the puzzle: the story of Esther

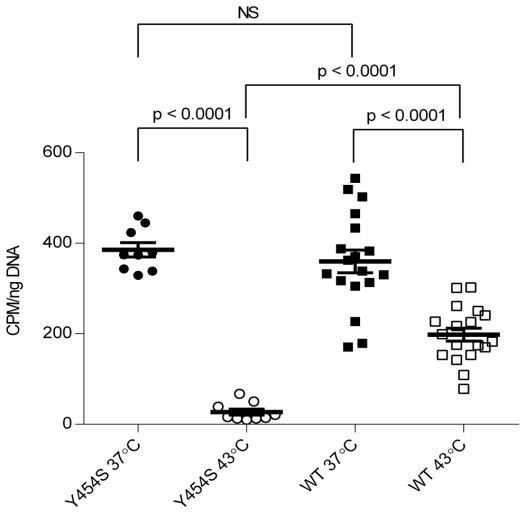
- Holmes Morton contacted us about an HARS Usher patient who was experiencing a fever episode that was accompanied by rapid loss of vision. This led to her hospitalization.
- While in the clinic, Dr. Morton contacted us regarding the possible consequences of treatment with **high dose histidine**.



Could the Usher HisRS mutation be a classic temperature sensitive mutation?







- A melting point analysis comparing wild type and mutant protein showed that the mutant protein melts at 6 degrees lower than the wild type protein. Histidine stabilizes both proteins, raises their melting point by 8-9 degrees
- A comparison of wild type type cells, cells from a parent with one allele, and a child with both alleles showed that there is less of HisRS in the affected child. Levels recover slightly with histidine.
- Protein synthesis in the mutant cells is not significant different at 37 C, but definitely decreased at 43 C.
- Mutant protein is best described as a temperature sensitive mutation.

…ultimately leading to a clinical trial to help children with the disease

- We subsequently teamed with a large Canadian group who provide care to a large Amish community with a high incidence of Usher Type III cases. They are currently a clinical trial to examine the efficacy of high dose histidine to slow the loss in visual and auditory function. Results are ongoing….
- This observation has been substantiated by other investigators (Kok et al. *Genet. Med.* 2021) who observed that amino acid supplementation may be effective in ARS related diseases where there is a clear loss of function phenotype.

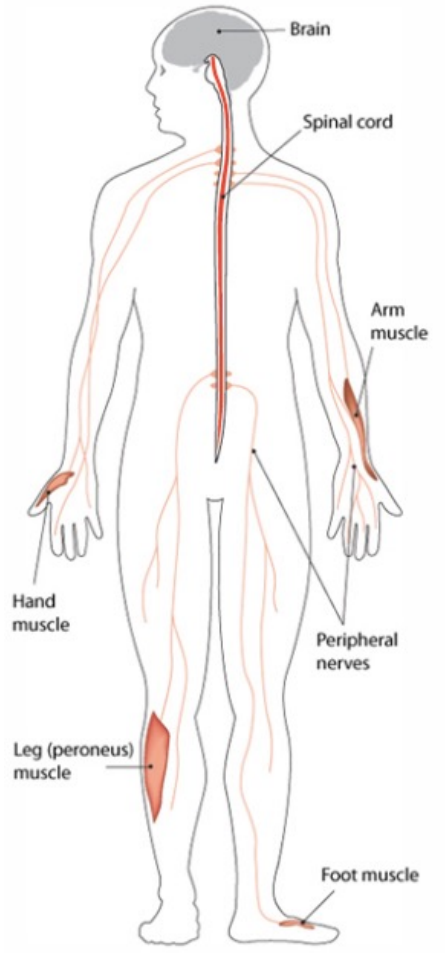
Does this explain all AARS linked diseases; to mutations histidyl-tRNA synthetase?

- These observations show how a temperature sensitive mutation in the gene encoding histidyl-tRNA synthetase (HisRS) leads to a specific compromise of the sensory system, and not a generalized loss of protein synthesis throughout the body.
- How general are such results? Are these observations true of other mutations in the HisRS gene?
- Are they generalizable to other AARSs?
- What do these results indicate about the general impact of translation factor alterations on the nervous system?

Part Three
A case of Charcot Marie Tooth disease

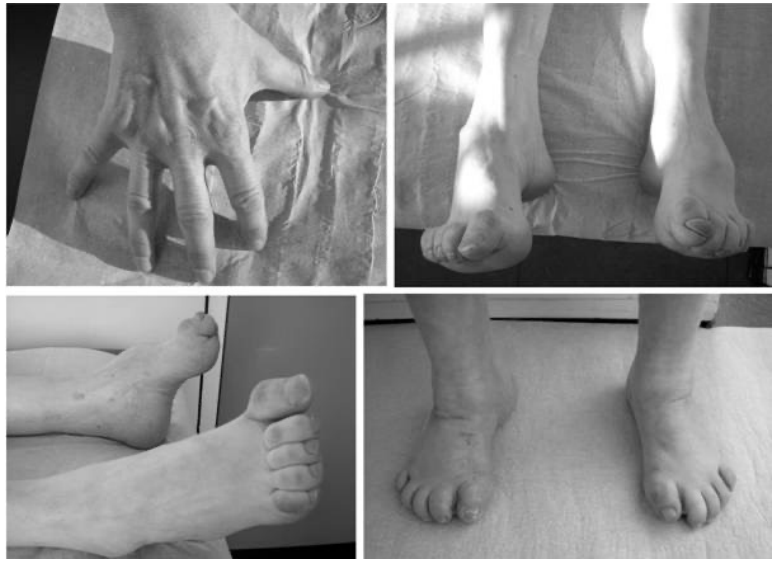
A different class of mutations in HisRS leads to a different disease: a peripheral neuropathy

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT



CMT causes degeneration of the peripheral nerves, leading to muscle weakness in the body's extremities.

- Charcot-Marie-Tooth disease (CMT) is a neurological disorder named after the three physicians who first described it in 1886 — Jean-Martin Charcot and Pierre Marie of France, and Howard Henry Tooth of the United Kingdom.
- CMT is the most commonly inherited peripheral nerve disorder affecting about 1 in 2,500 people. CMT causes damage to the peripheral nerves, which carry signals from the brain and spinal cord to the muscles, and relay sensations, such as pain and touch, to the brain and spinal cord from the rest of the body.



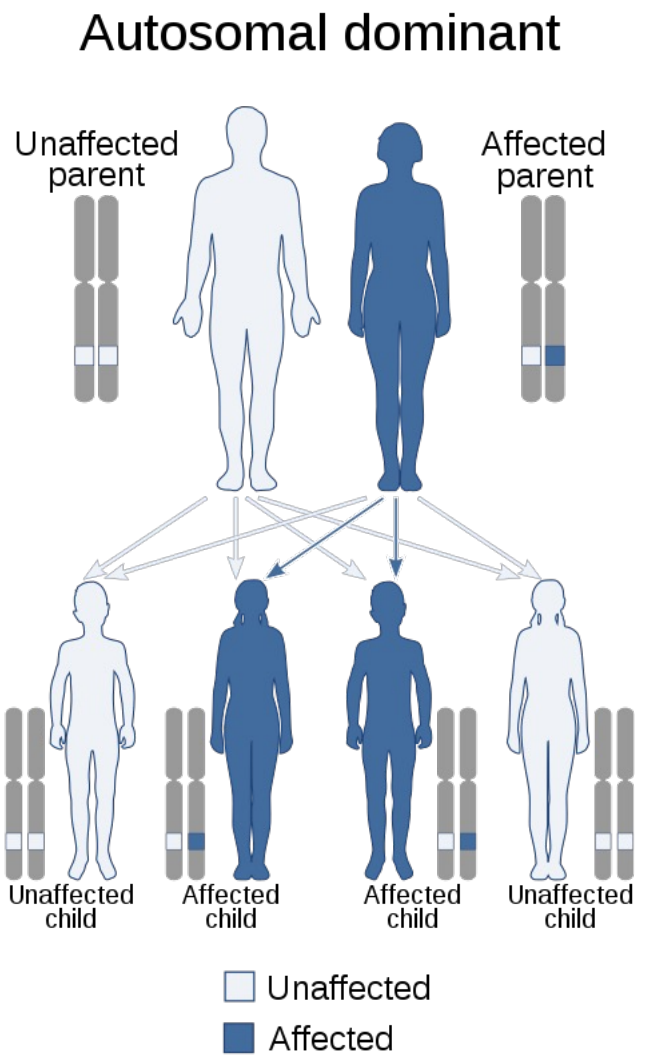
- CMT causes muscle weakness and atrophy, loss of sensation in the feet, the lower legs, the hands and the forearms. Often causes contractures (stiffened joints due to abnormal tightening of muscles and associated tissues), curvature of the spine (scoliosis). Severe end of the CMT spectrum, disease can affect nerves other than those that go to and from the extremities. If the nerves that enervate diaphragm or intercostal (between the ribs) muscles are affected, respiratory impairment can result.

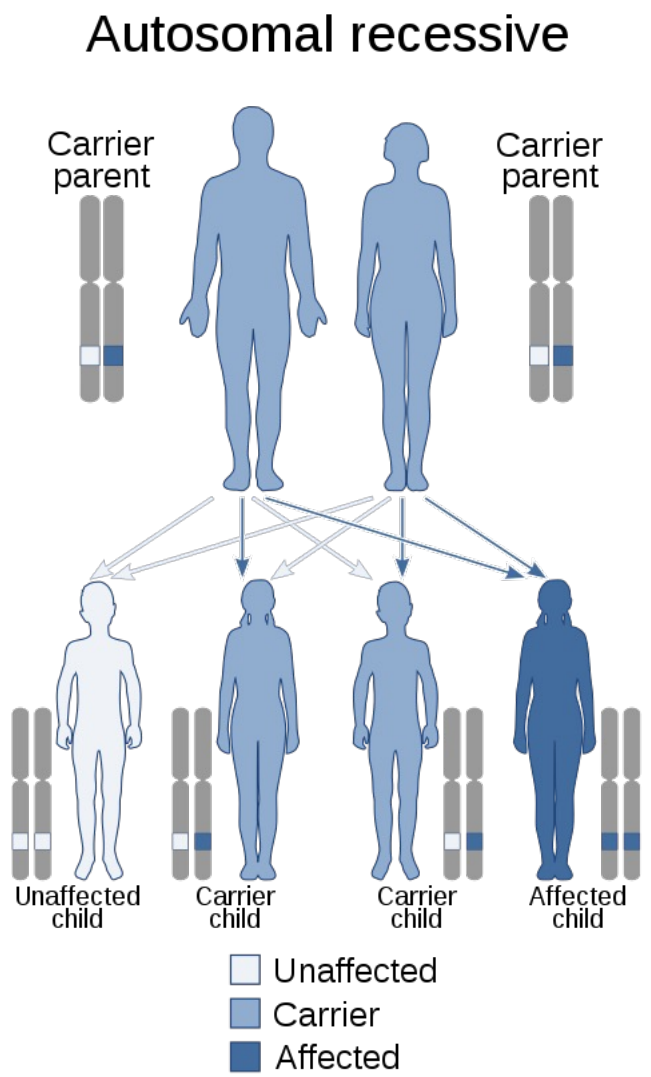
Peripheral neuropathies can be inherited in a dominant fashion

The Usher type III disease I just discussed is an example of an **autosomal recessive disease**. In order for the children to express the disease, they must an inherited **two defective alleles**, one from each parent.

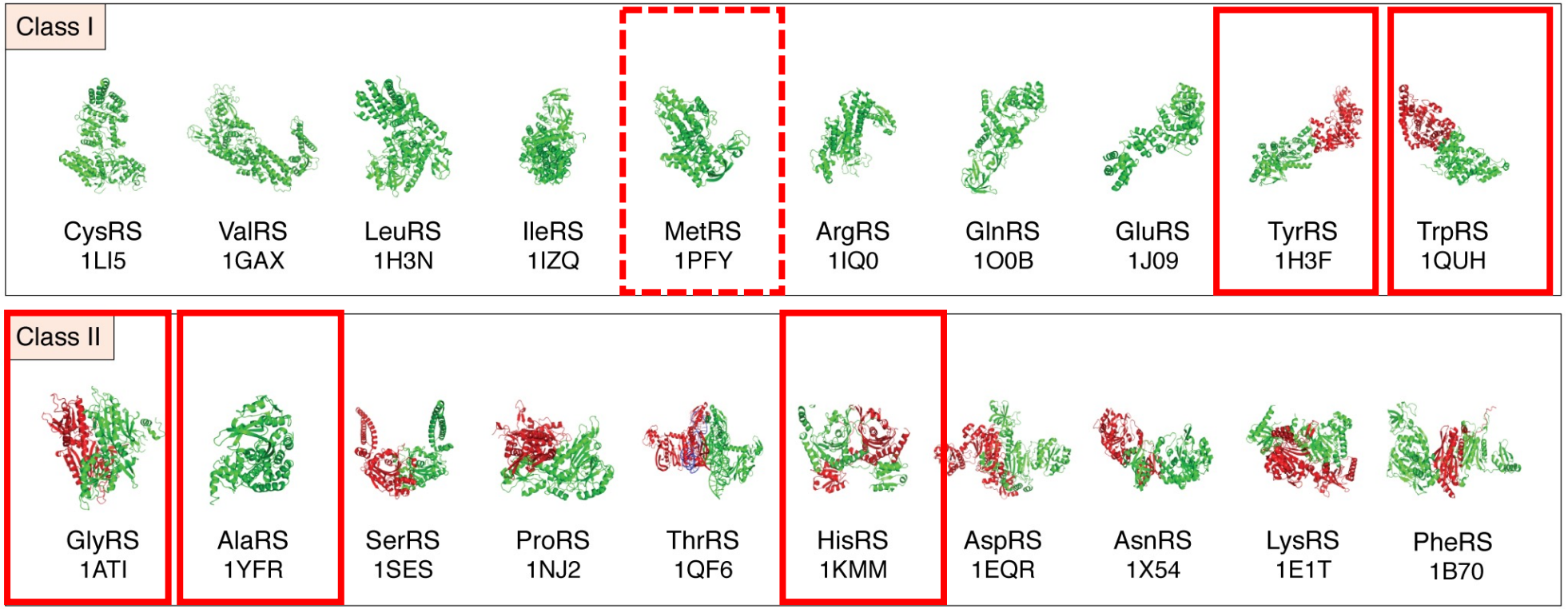
By contrast, in a disease that is inherited in an autosomal dominant fashion, a child need only inherit a single defective allele from a single parent.

A Third case is epitomized by X- and Y-linked diseases, which show a sex-linked inheritance pattern. (Women are carriers and boys often express the disease.)





Mutations in numerous aminoacyl-tRNA synthetase genes give rise to Charcot Marie Tooth Disease

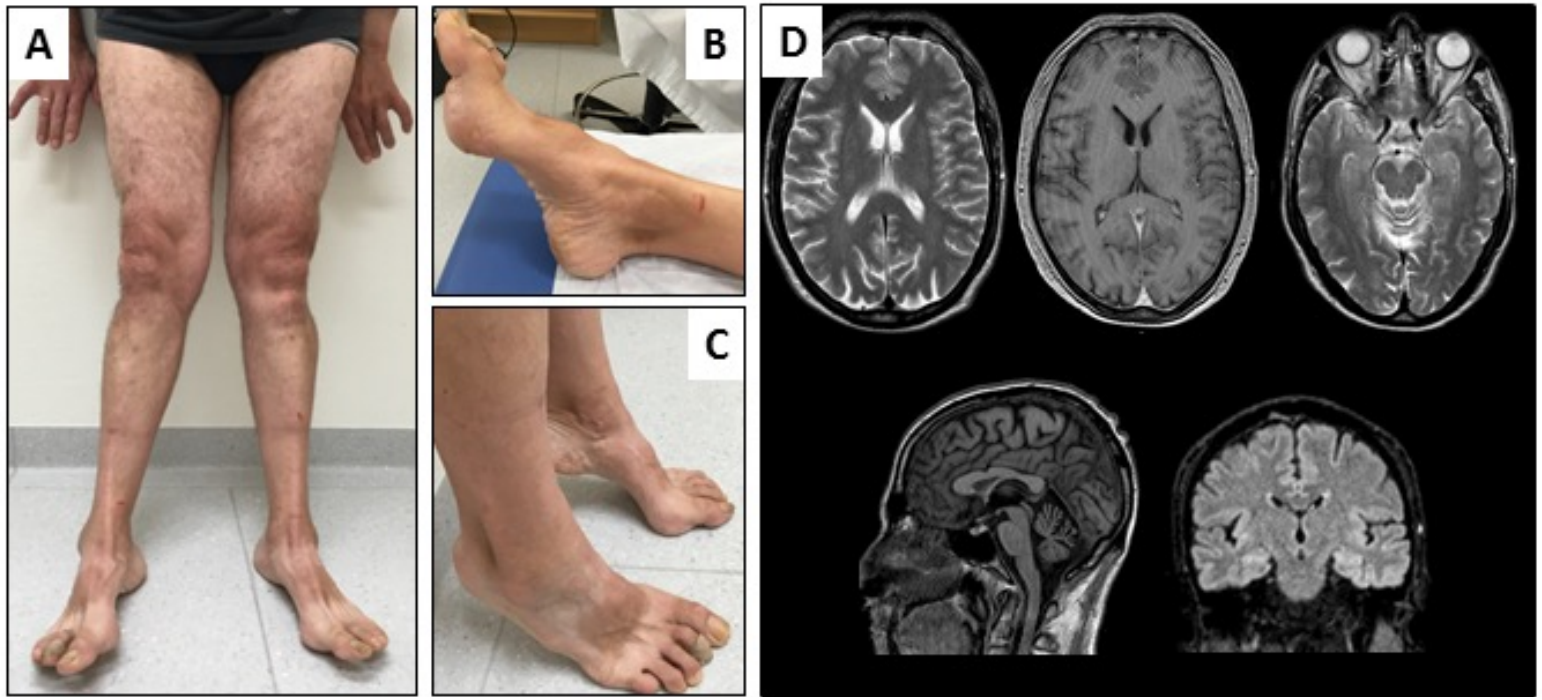


- In humans, 37 different genes.
- 17 cytoplasmic
- 18 mitochondrial
- two dual function. synthetases
- Mutations reported in 5 (or six different ARS loci.

- All CMTs linked mutations in ARS genes are Type 2: associated with axonal degeneration. First example of ARS linked CMT was a mutation in GARS. Mutations reported in both Class I and Class II ARSs, but (with exception of MetRS) restricted to those ARS that form dimers.

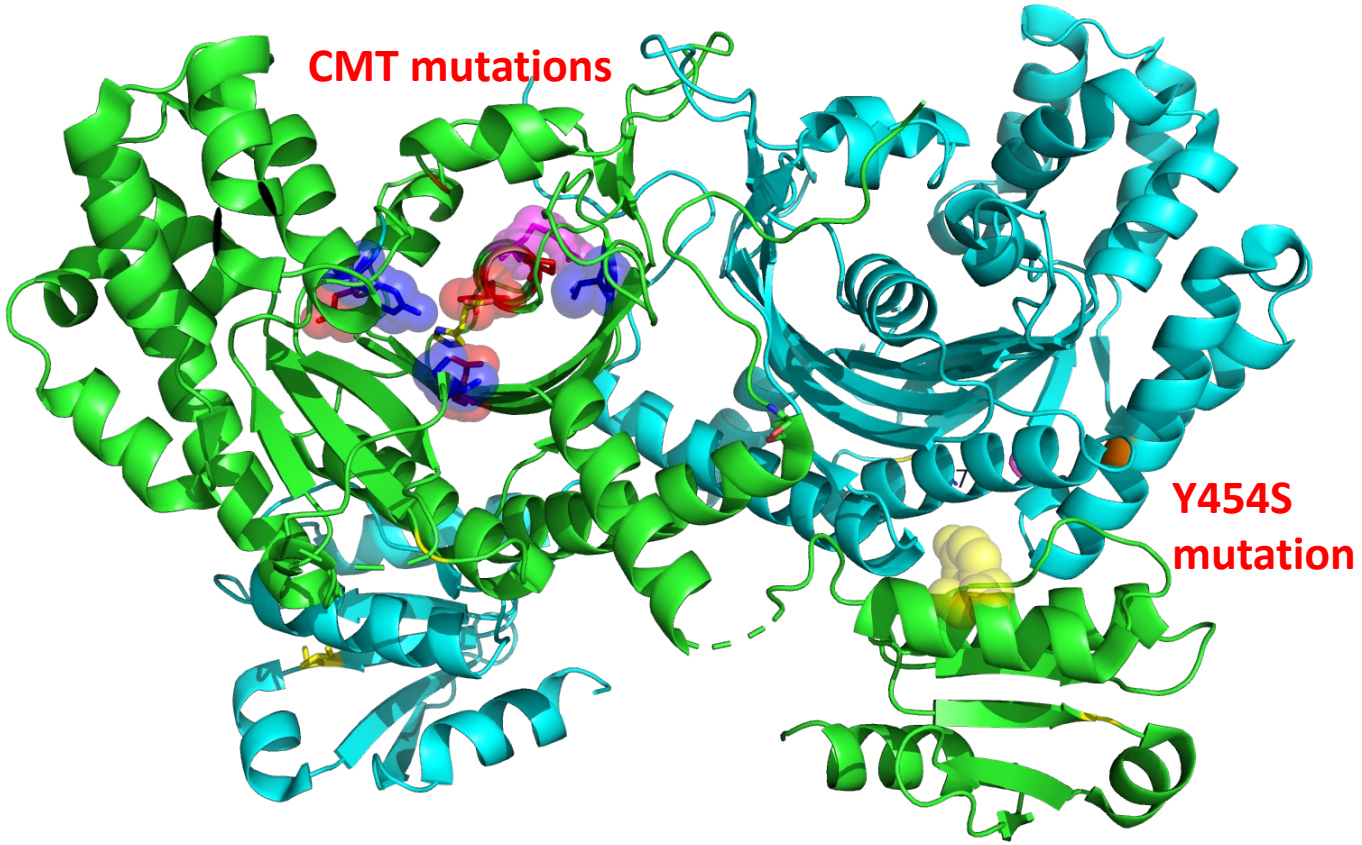
A different class of mutations in HisRS leads to a different disease: a peripheral neuropathy

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

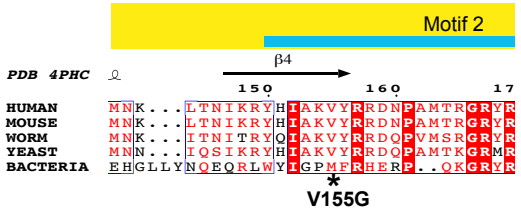


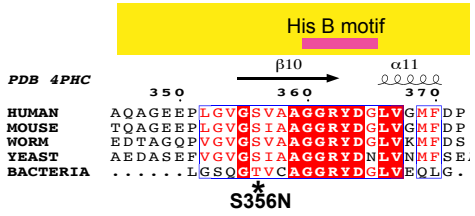
- Patient is 49 yr old late onset case of peripheral neuropathy. Also exhibits cerebellar atrophy, cognitive deficit And the classic muscle wasting and distorted foot arch that is seen with CMT cases.

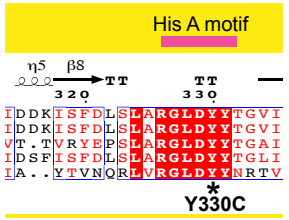
Mutations in HARS give rise to Charcot Marie Tooth Disease



- Relative to the HARS mutation associated with type 3B Usher syndrome, mutations associated with CMT **cluster in the active site**, proximal to the site of amino acid, ATP, and tRNA binding. Suggest a more pronounced effect on catalytic function.
- Mutations affect conserved residues in eukaryotic enzymes.



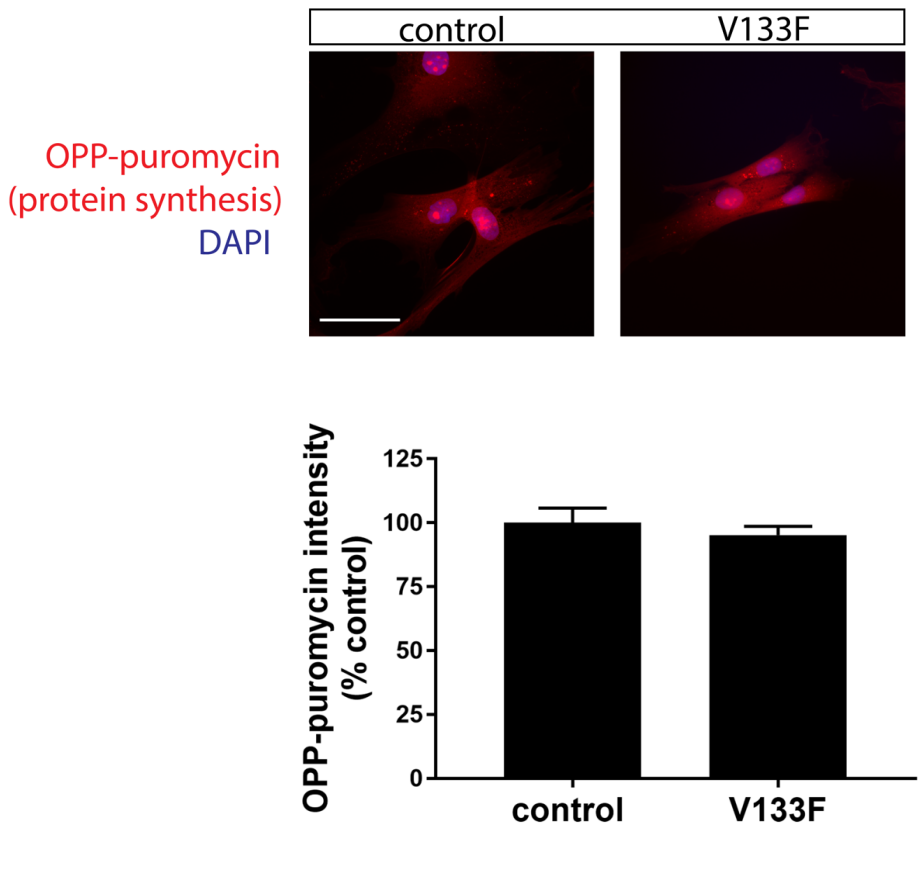


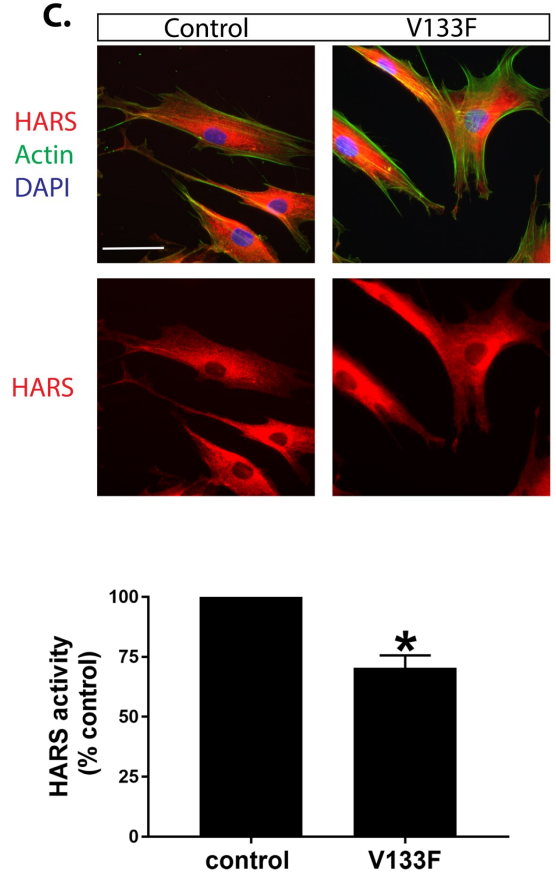


Using patient cells to test the effects of peripheral neuropathy mutations

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

- When proteins are assessed for catalytic function, see a sharp reduction in activity.
- However, when activity- especially overall protein synthesis is assessed in patient skin cells , the effects are very slight.
- Thus, using the cells that are most readily available from patients may not give a conclusive result.





The zebrafish is a model of neuronal development

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

Embryo

- Rapid Development
- Optical Clarity
- Genetic malleability

Larva

- Rapid Development
- Optical Clarity
- Genetic malleability

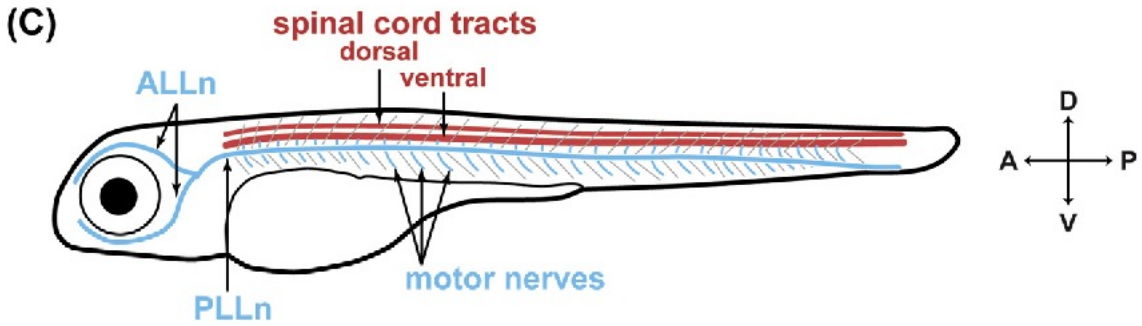
Adult

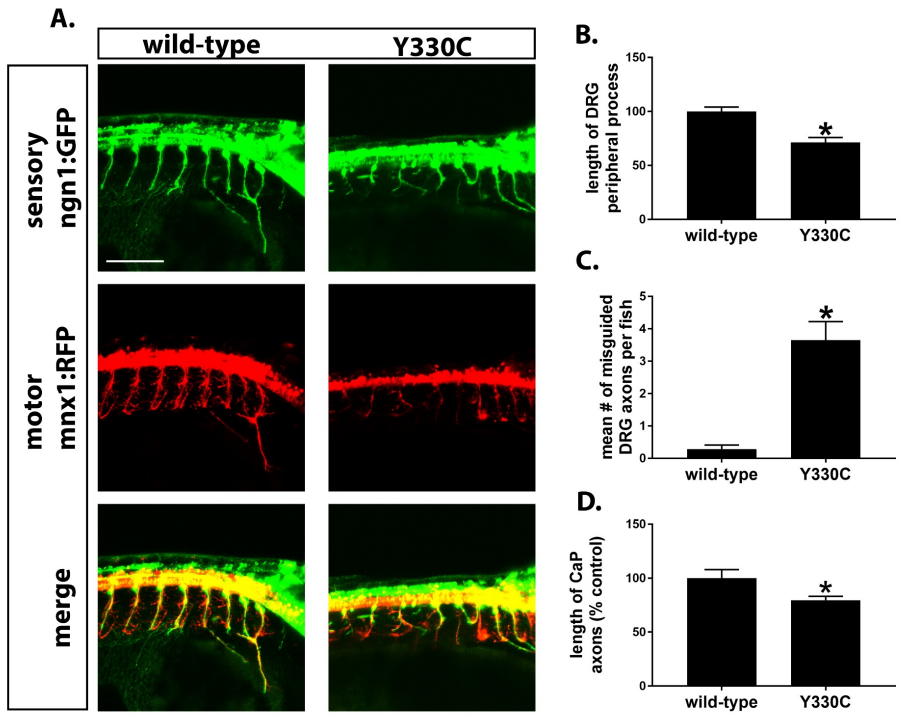
- Rapid Development
- Optical Clarity
- Genetic malleability

Using genetically marked zebrafish to follow neuronal development

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

- In these experiments, We inject a piece of DNA into the zebrafish that encodes for the mutant version of our gene of interest.
- The zebrafish lines we used are “marked” such that motor neurons are fluorescent red, and the sensory neurons are marked fluorescent green. Experimentally we then measure the length of longest axon (dorsal root ganglion); control measurements include fish body length.
- We found that expression of the mutant proteins led a decrease in the length of the DRG, with only minimal effect on the overall body length. Further, the effect of the mutant protein could be mimicked by low concentrations of a drug (cycloheximide) a drug that inhibits protein synthesis.





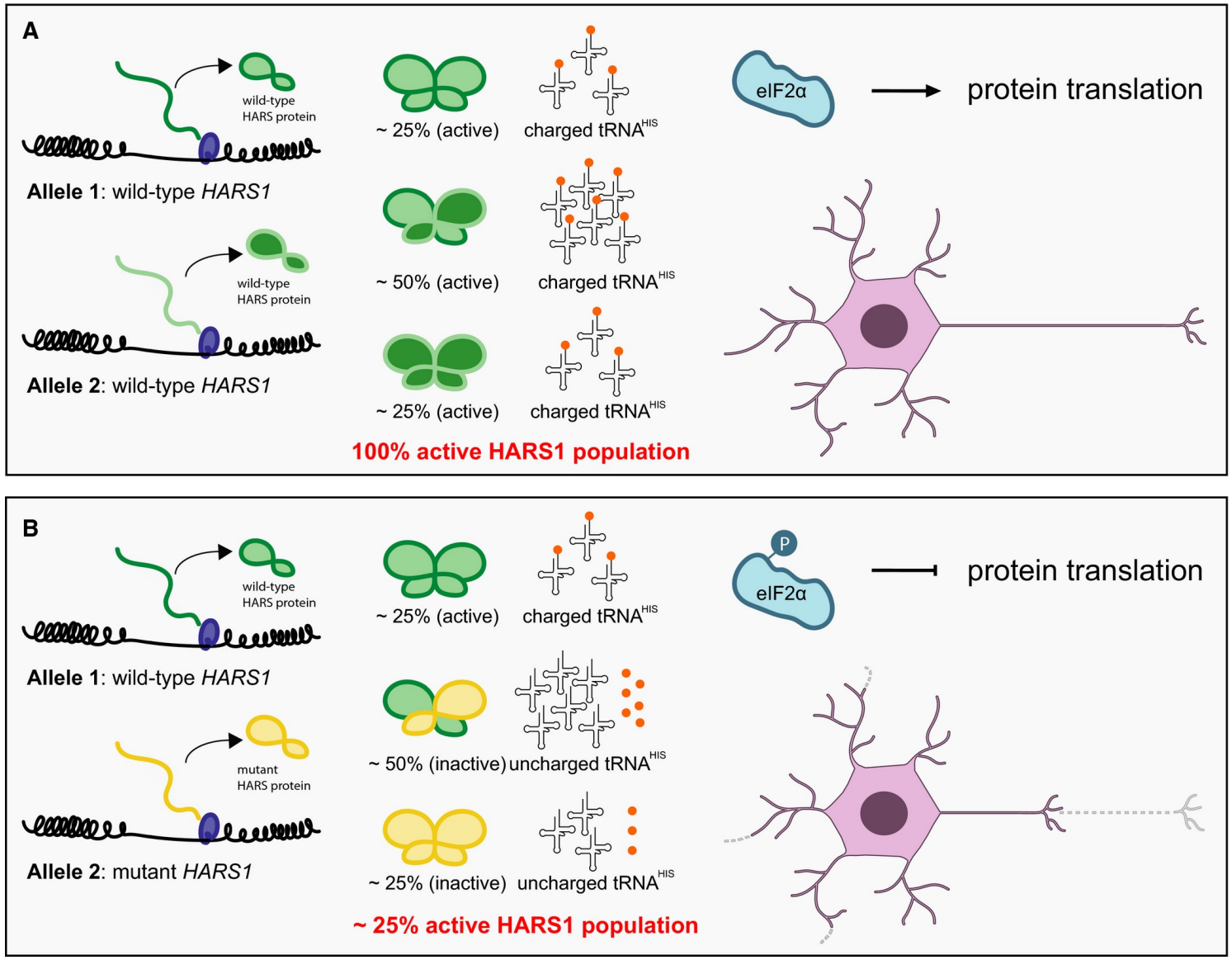
Comparing Usher Syndrome to CMT

	Usher Syndrome	Charcot Marie Tooth
System Affected	**Sensory System: hearing and sight**	**Peripheral nervous system**
Genetics	Autosomal recessive	Autosomal dominant
Protein stability	Mutant protein is temperature sensitive	Protein stable
Protein catalytic activity	Protein loses catalytic activity but only at elevated temperatures	Protein catalytic activity is sharply reduced
Impact on protein synthesis	Cells shows reduced histidine incorporation at elevated temperature	Protein synthesis minimally affected in fibroblasts, but substantially reduced in a neurite producing cell lines
Model organism impacts	Morpholino knockdown leads to smaller eye, and fewer neuromasts	over-expression of mutant proteins leads to shortening of the dorsal axon

Why does only a single mutation cause disease?

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

- A dominant negative mutation can bring about disease by interfering with the function of the wild type protein.
- This can be understood best when the active is a dimer of two subunits.
- When the wild type and mutant subunits mix, they may create a version of the enzyme that cannot function, owing to inability to release product.
- In this a dominant negative allele can be seen to be more deleterious than a complete loss of function that might not make any protein at all.



Meyer-Schuman & Antonellis, 2020

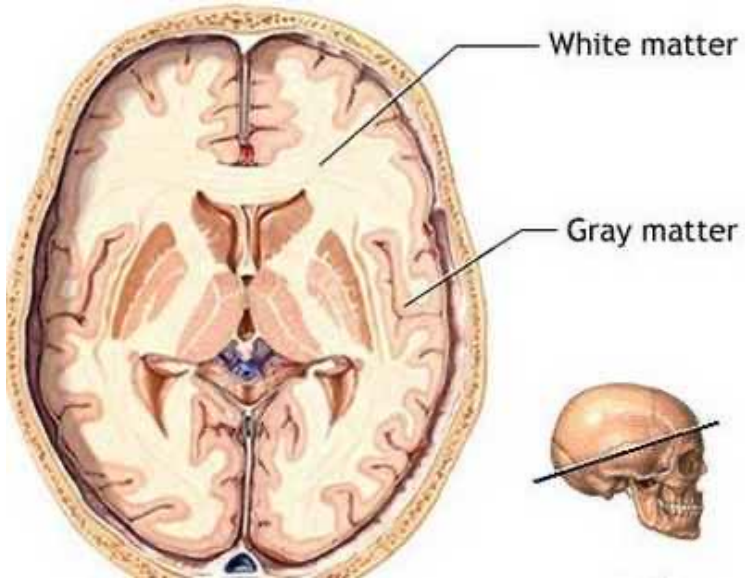
Is there an approach to "cure" a genetically inherited disease?

- The typical strategy that a pharmaceutical company uses a develop therapies is to identify a key protein/enzyme target, and then identify small molecules that can inhibit its activity. (Example: protein kinases and cancer.)
- Inherited diseases are challenging target, because most of the time, the problem is **too little activity of the target protein**, and not too much. Hence, interest in "rescuing activity" by use of gene therapy.
- Dominant negative diseases are particularly challenging, because you only want to inhibit the *activity of the bad subunit*. Very difficult to identify drugs that are specific for the defective subunit.
- For ARS linked CMT, one strategy may involve raising tRNA levels to promote release of aminoacylated product from WT-mutant heterodimer.

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

Part Four
Recesssive Diseases: Impacts on Brain Development

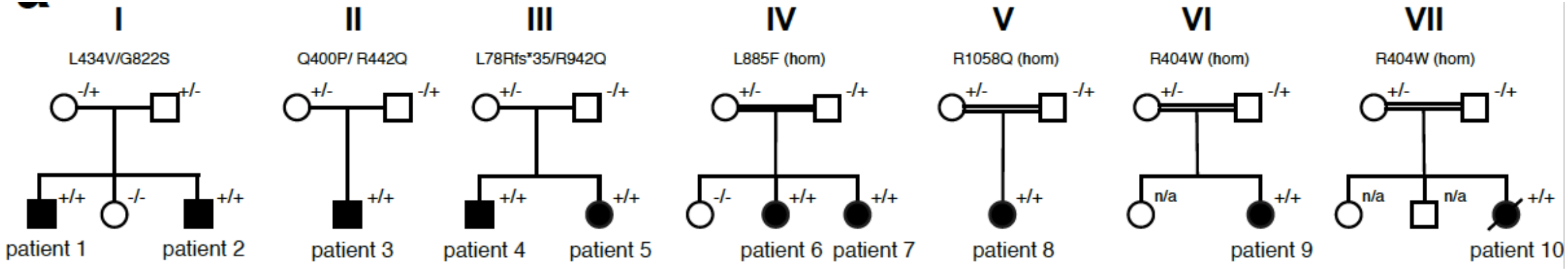
Biallelic mutations are associated with white matter diseases: developmental leukoencephalopathies



- White matter: The white matter of your brain and spinal cord is composed of bundles of axons. These axons are coated with myelin, a mixture of proteins and lipids, that helps conduct nerve signals and protect the axons. White matter's job is to conduct, process, and send nerve signals up and down the spinal cord.

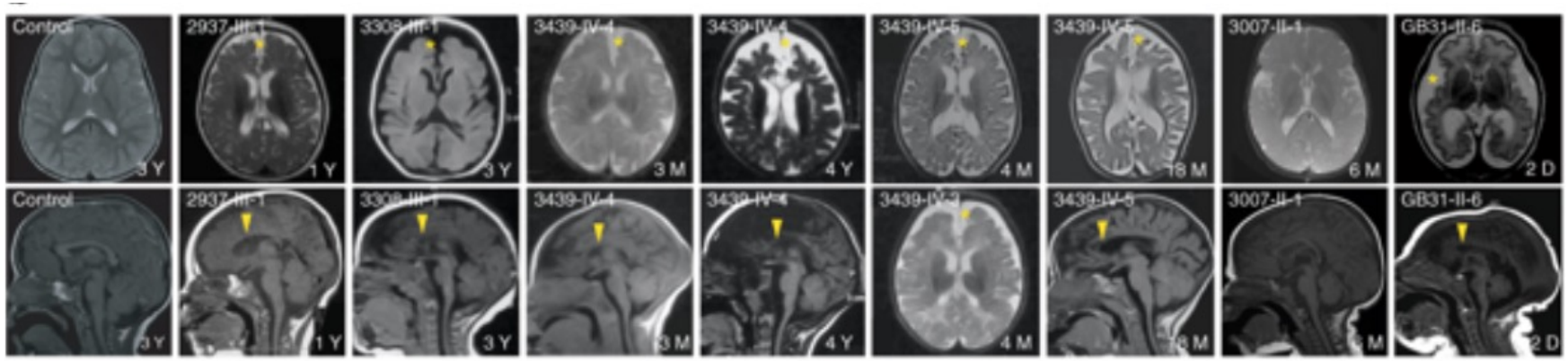
- Hypomyelination with brainstem and spinal cord involvement: DARS
- Leukodystrophy (loss of myelination and white matter): RARS
- Congenital visual impairment and progressive microcephaly: KARS
- Development delay with progressive microcephaly and intractable seizures: QARS
- Early onset epileptic encephalopathy with myelination defect: AARS

VARS linked leukoencephalopathy

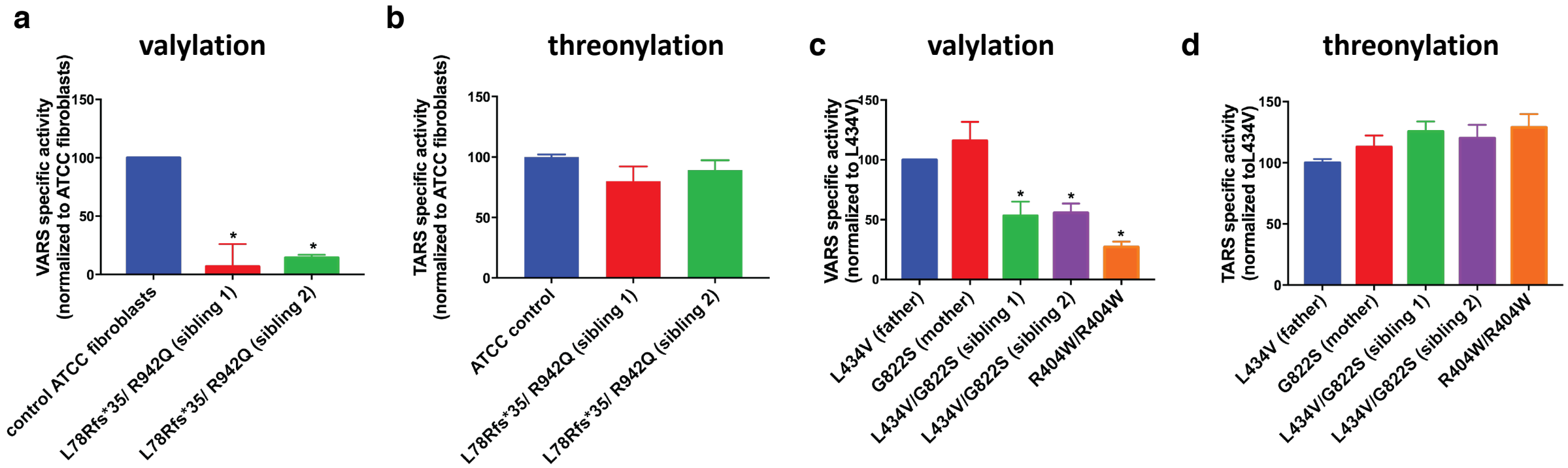


- ✓ All patients had global development delay
- ✓ Severe progressive microcephaly
- ✓ Epileptic seizures
- ✓ Profound intellectual disability

- ✓ axial or midline sagittal brain MRIs demonstrated diffuse cortical atrophy (yellow star) and thinned corpus callosum (yellow arrow) compared to control.



Affected patients all show a loss of ValRS specific activity, but not ThrRS activity.

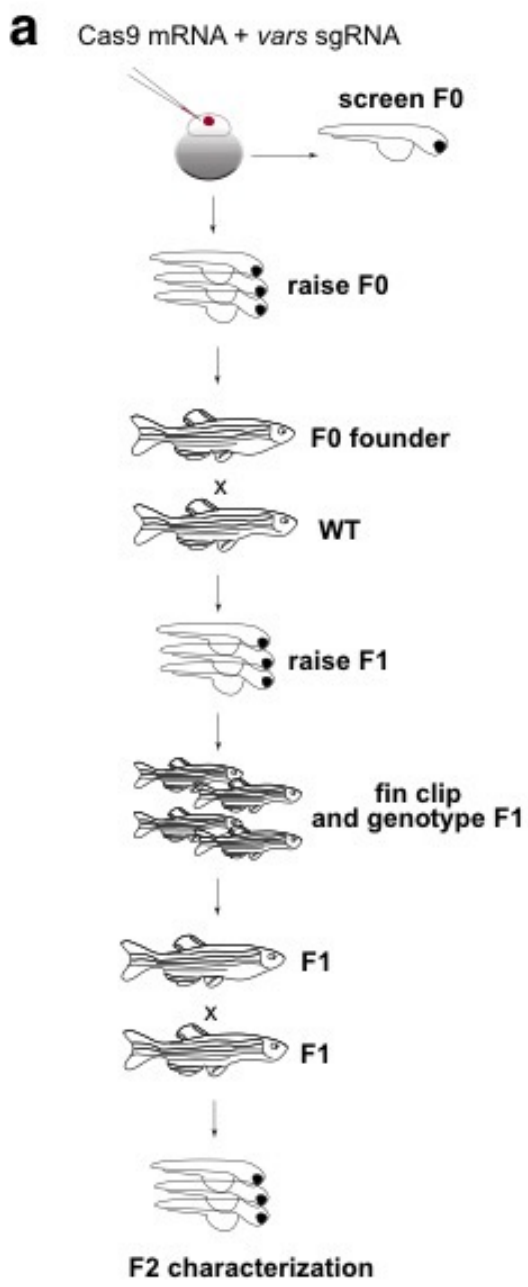


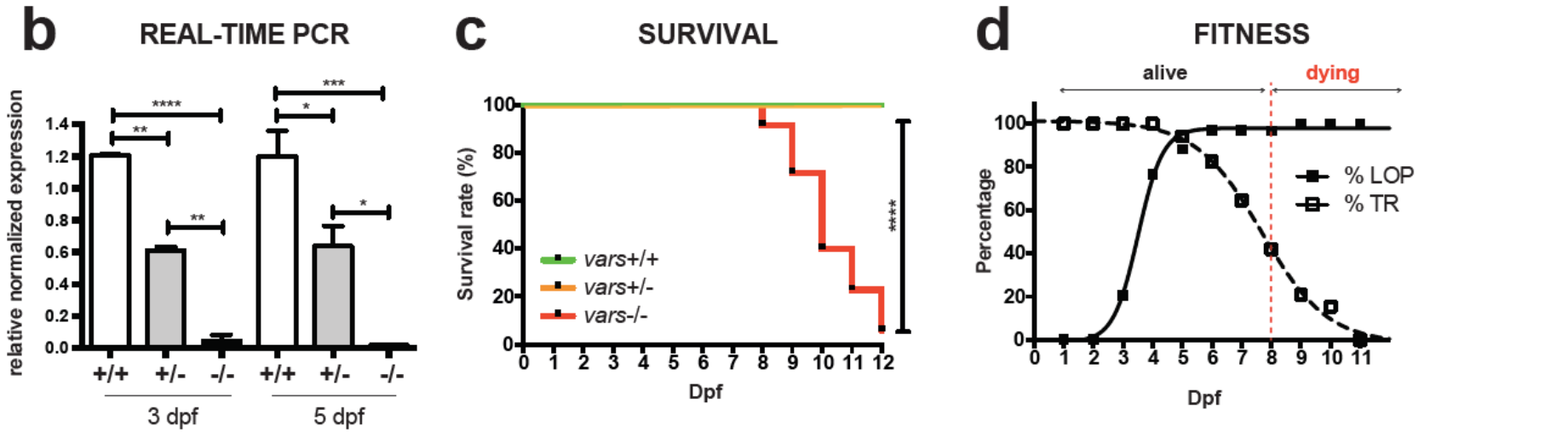
- All mutants tested are associated with a decrease in aminoacylation typically on the order of 50%, relative to either control fibroblasts or relative to parents.
- Western blots suggest that VARS protein levels in the compound heterozygotes are significantly reduced

Sierkierska et al. *Nature Communications 2019*

Generation and characterization of VARS knockoutfish created by CRISPR

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

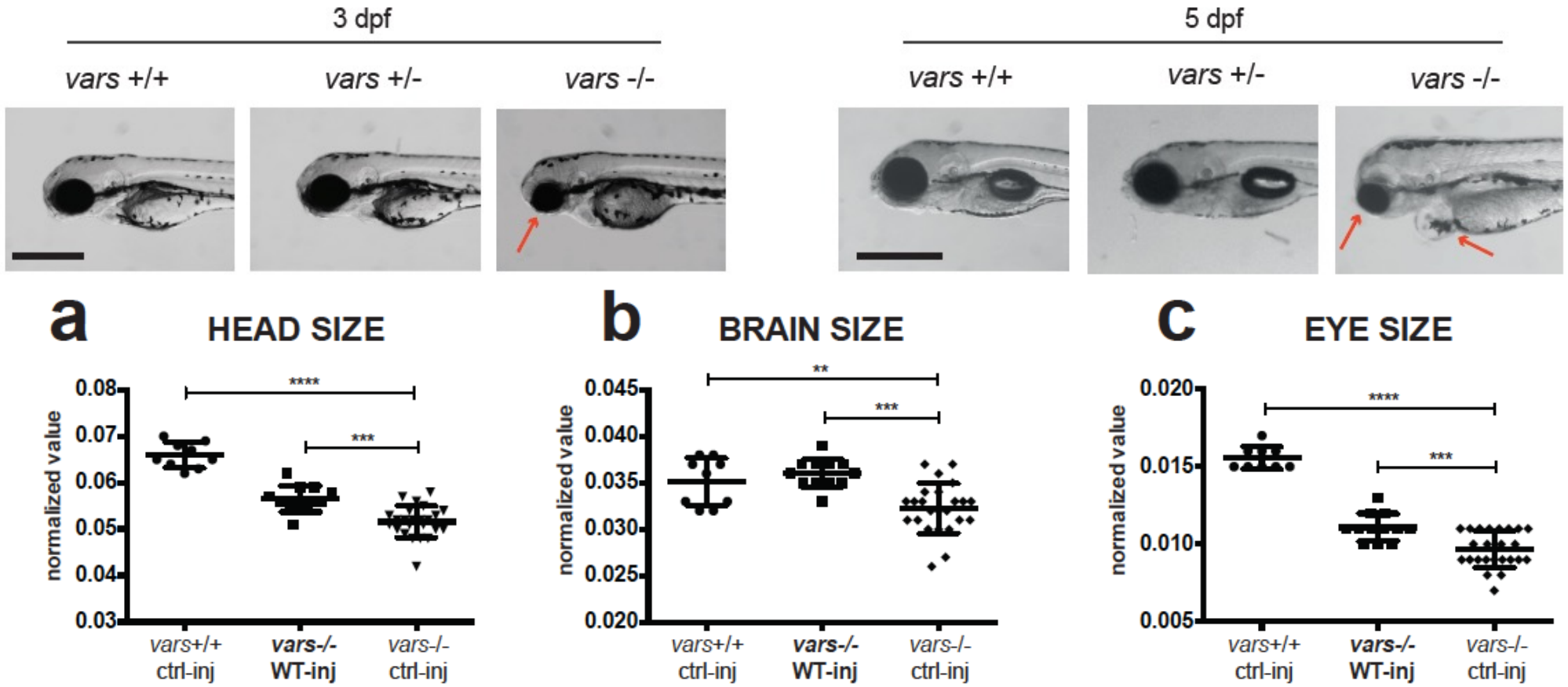




- The success of the CRISPR is indicated by the decrease in the mRNA levels at 3 and 5 days.
- Premature death occurs in the homozygous recessives at 8-12 dpf
- At day 7, you can see a loss of touch response (TR) gain of loss of posture

Sierkierska et al. *Nature Communications 2019*

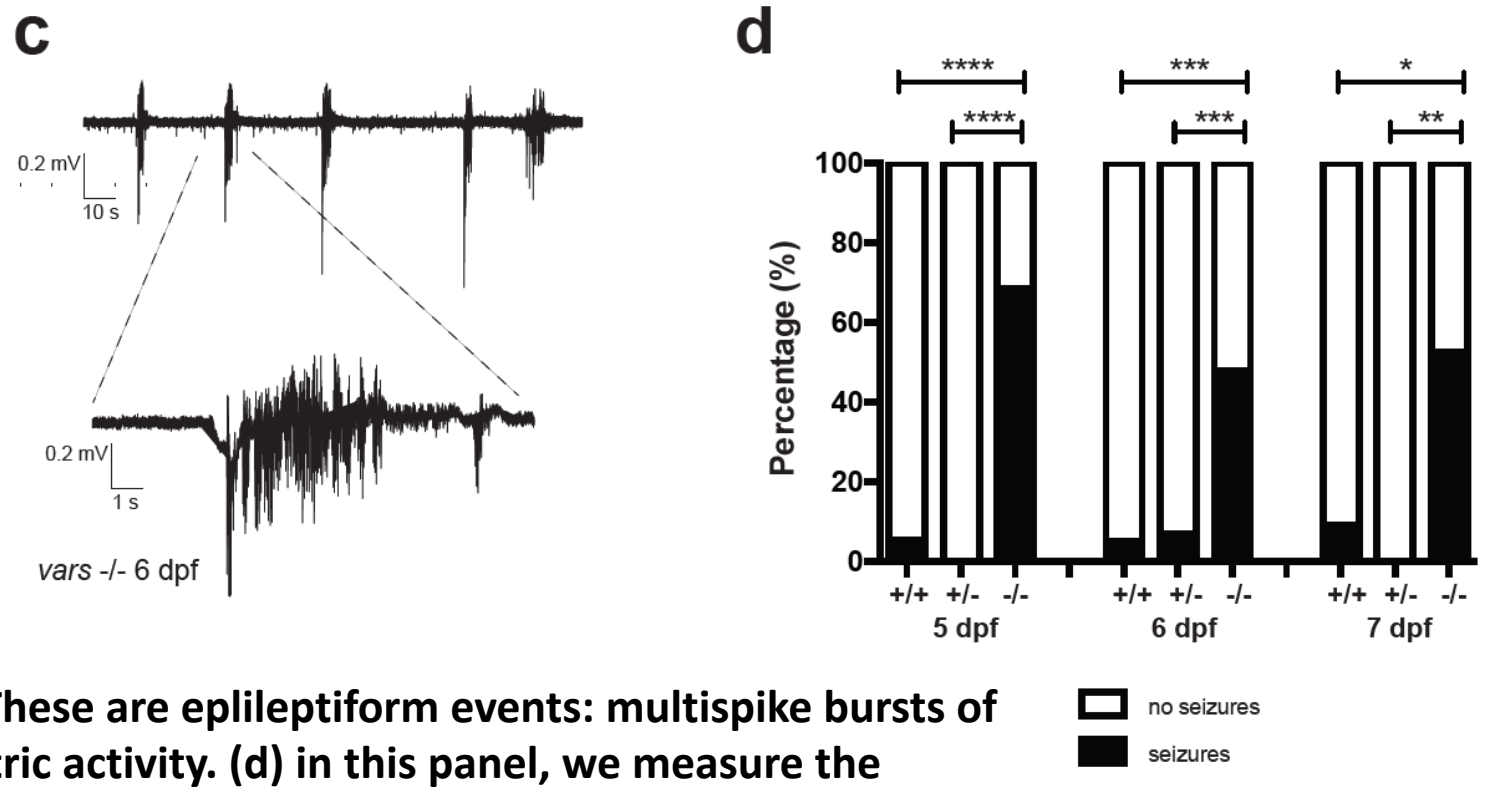
Small eye and head phenotypes associated with the VARS knockout in Zebrafish



- Black and white imaging of VARS mRNA in the wt, heterozygote, and homozygous recessive:
- At three days, you can see the smaller eye and swollen heart
- At five days, these same trends are more pronounced.

Sierkierska et al. *Nature Communications 2019*

Epileptiform events observed in VARS knockout zebrafish



(c) These are eplileptiform events: multispike bursts of electric activity. (d) in this panel, we measure the percentage of seizures. Statistically different from wt at each time point

The data at left indicate that zebrafish in which VARS has been disrupted by CRISPR, electrophysiology recordings of the animal's brains indicate changes in electrical activity that resemble the epilepsy seen with human patients.

Sierkierska et al. *Nature Communications 2019*

Mutations in cytoplasmic AARS Genes are associated withcat least three different classes of neurological diseases

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

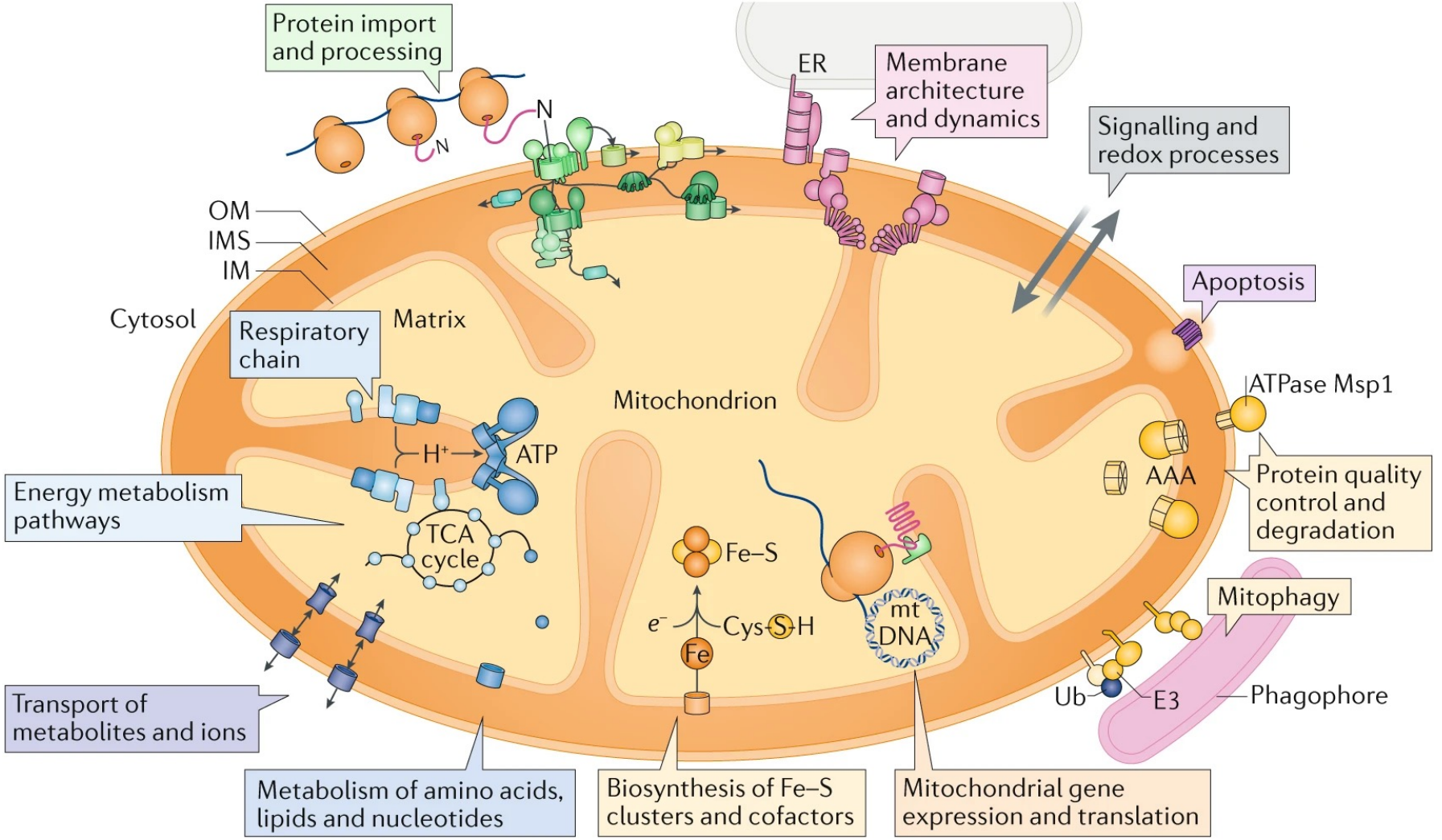
Group I: Peripheral Neuropathy	Group II: Sensorineural pathology	Group III: Severe Neurological Developmental Disorders
Inheritance: Autosomal Dominant	**Inheritance:** Autosomal Recessive	**Inheritance:** Autosomal Recessive; Compound heterozygous
Protein Effect: Strong loss of catalytic function; protein is stable	**Protein Effect:** mild effect on catalytic function, Temp. Sens.	**Protein Effect:** decreased protein, levels,loss of catalytic function,
Principal Phenotype: Inherited peripheral neuropathy, CMT	**Principal Phenotype:** Usher Syndrome, or non-syndromic hearing loss	**Principal Phenotypes:** microcephaly, leukoencephalopathy, epilepsy
ARS Involved: AlaRS, GlyRS, HisRS, TyrRS, TrpRS, LysRS, MetRS	**ARS Involved:** HisRS, LysRS,	**ARS Involved:** AlaRS, HisRS, SerRS, GluProRS, ValRS, AspRS, ArgRS, AIM1, and AIMP2
Alleles studied here: HARS V133F HARS Y330C HARS S356N	**Alleles studied here:** HARS Y454S HARS R362N	**Alleles studied here:** VARS Y454S HARS D206Y NARS G123C

- There are additional mutations known in cytoplasmic AARS genes that are associated with multifunction disorders that impact on multiple organ systems not including the nervous system.

A closer look into Mitochondrial Protein synthesis

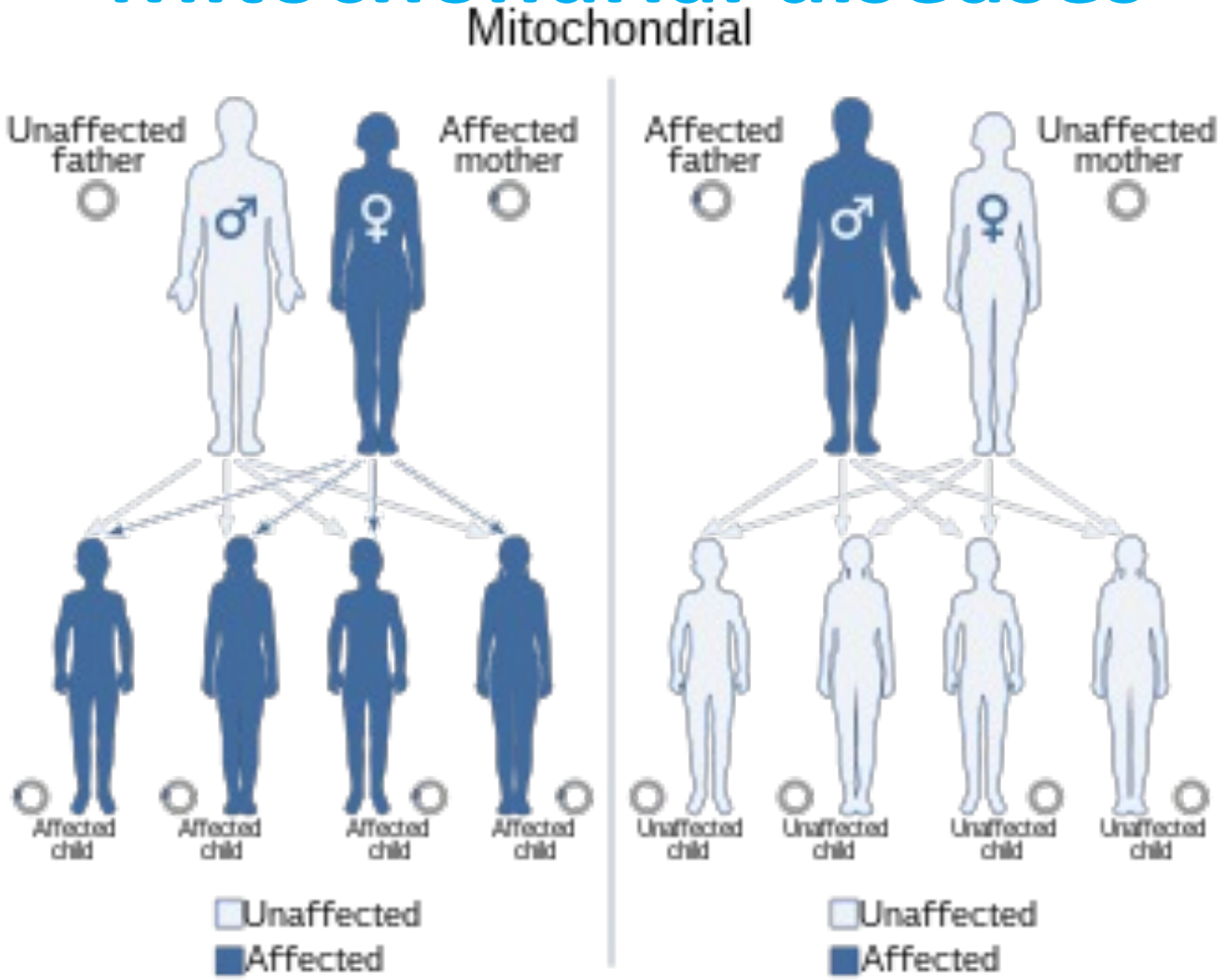
The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

- This figure shows the four main compartments that make up mitochondria: the outer membrane, inner membrane space, inner membrane, and matrix.
- Note the multiple functions: energy production, metabolism of amino acid and lipids, biosynthesis of iron sulfur clusters.
- Protein import is a particularly critical aspect of mitochondrial physiology.



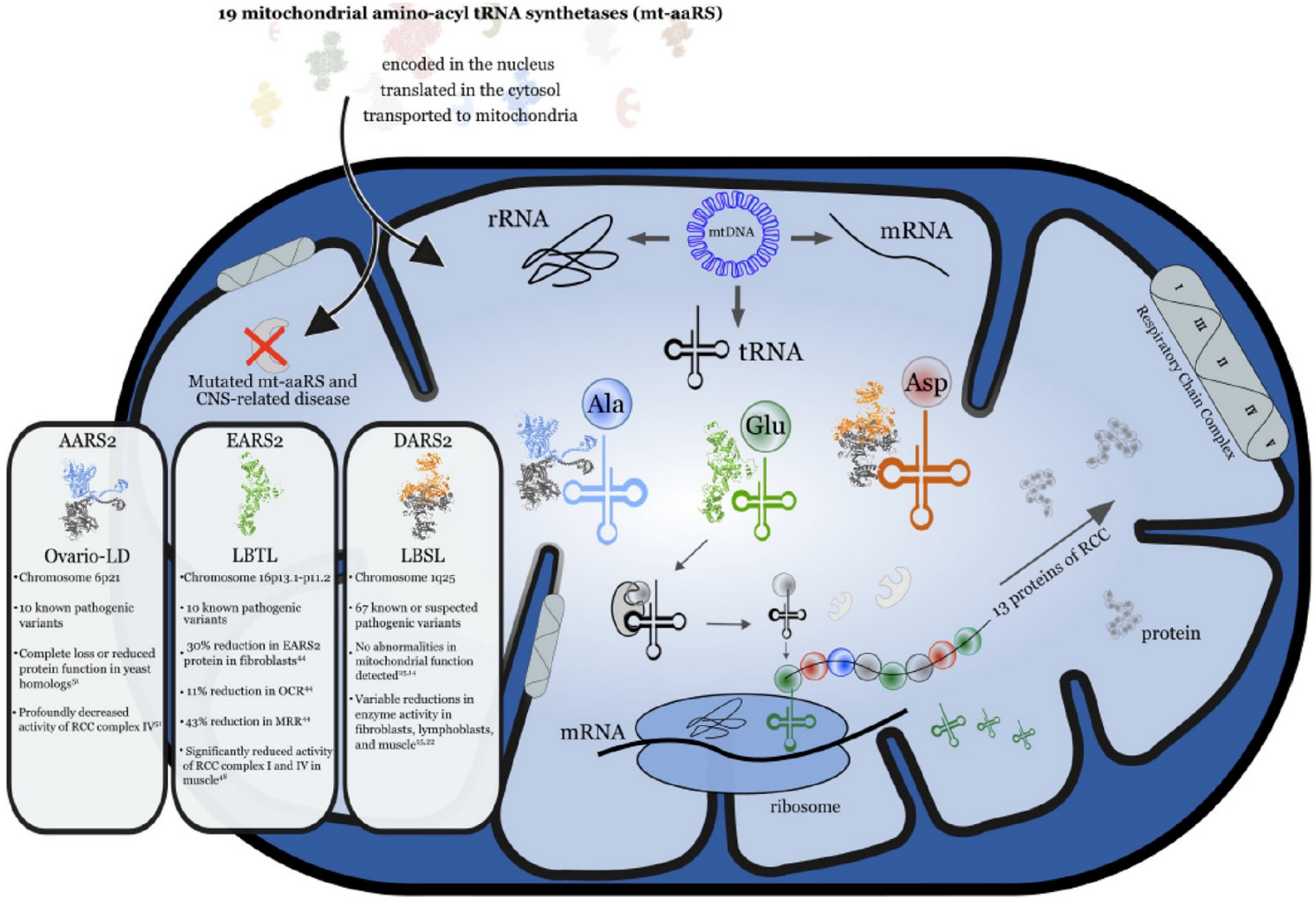
The complex genetics of Mitochondrial diseases

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT



- Mitochondrial physiology is complicated because the the inheritance patterns of the **imported proteins** and **the mitochondrial tRNAs** are different.
- The imported proteins are subject to classical *Mendelian genetics*, while the tRNAs show *mitochondrial inheritance.*
- *Complexity results from variability in number of mitochondria per cell, and percent of mutant genes.*

Making the connection to the work of colleagues at Hopkins…



- I would like to conclude by showing a slide from a review by my colleagues at Johns Hopkins whose work focuses on diseases associated with mutations in the mitochondrial tRNA synthetase genes.

CREDITS

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

People

Jamie Abbott
Susan Robey Bond
Terry Wellman
Patrick Mullen
Pryce Patterson
Shannon Lozito
Karen Lounsbury
Jon Ramsey

Collaborators

Alicia Ebert
Ashley Waldron
Anthony Antonellis
Hannah Stamberger
Victoria Siu
Christel Tran
Shenela Lakhani
Katherine Ross
Ola Siekierska
Reza Maroofian

Support

NIGMS 5P20GM103449-21
NIGMS GM54899
Hearing Health Foundation
Larner College of Medicine