# NEUROGENETICS IN CLINICAL CARE

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# Learning Objectives

- Define key terms used in genetics
- Describe implications of genetic test results
- Describe different methods of genetic testing and when to use them

# Human Genome

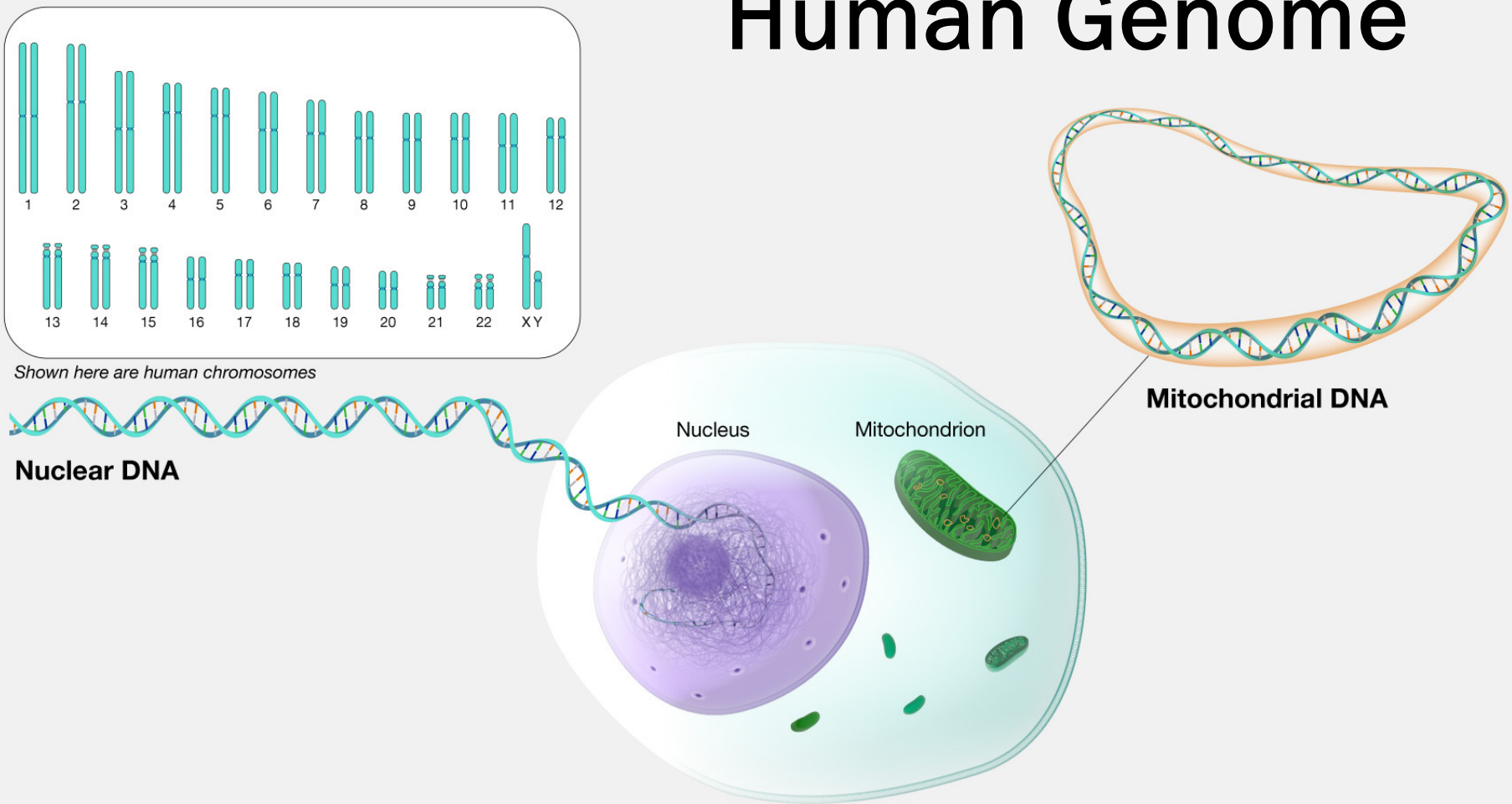
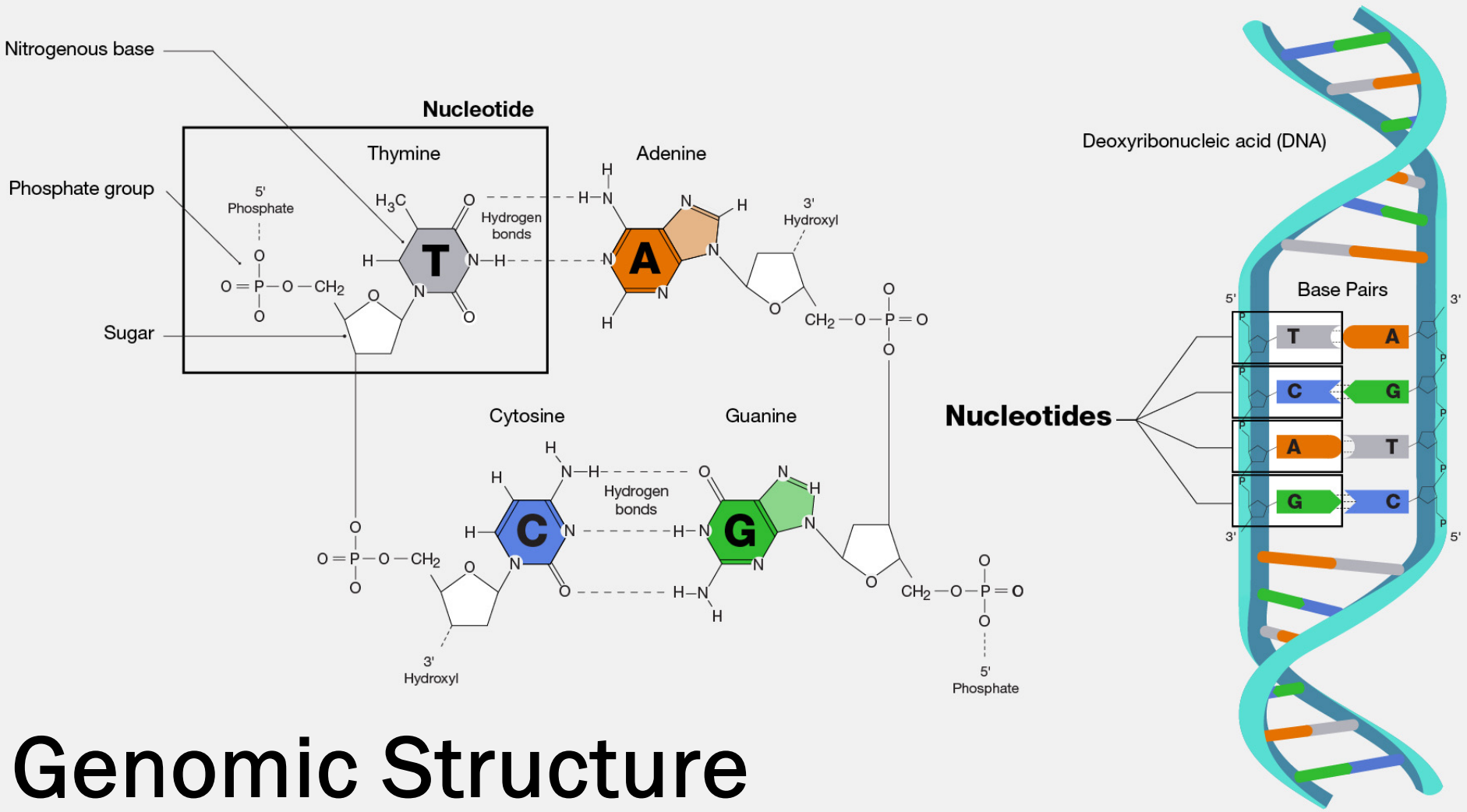



Image: NHGRI Glossary




# Genomic Structure

Image: NHGRI Glossary

# Translation

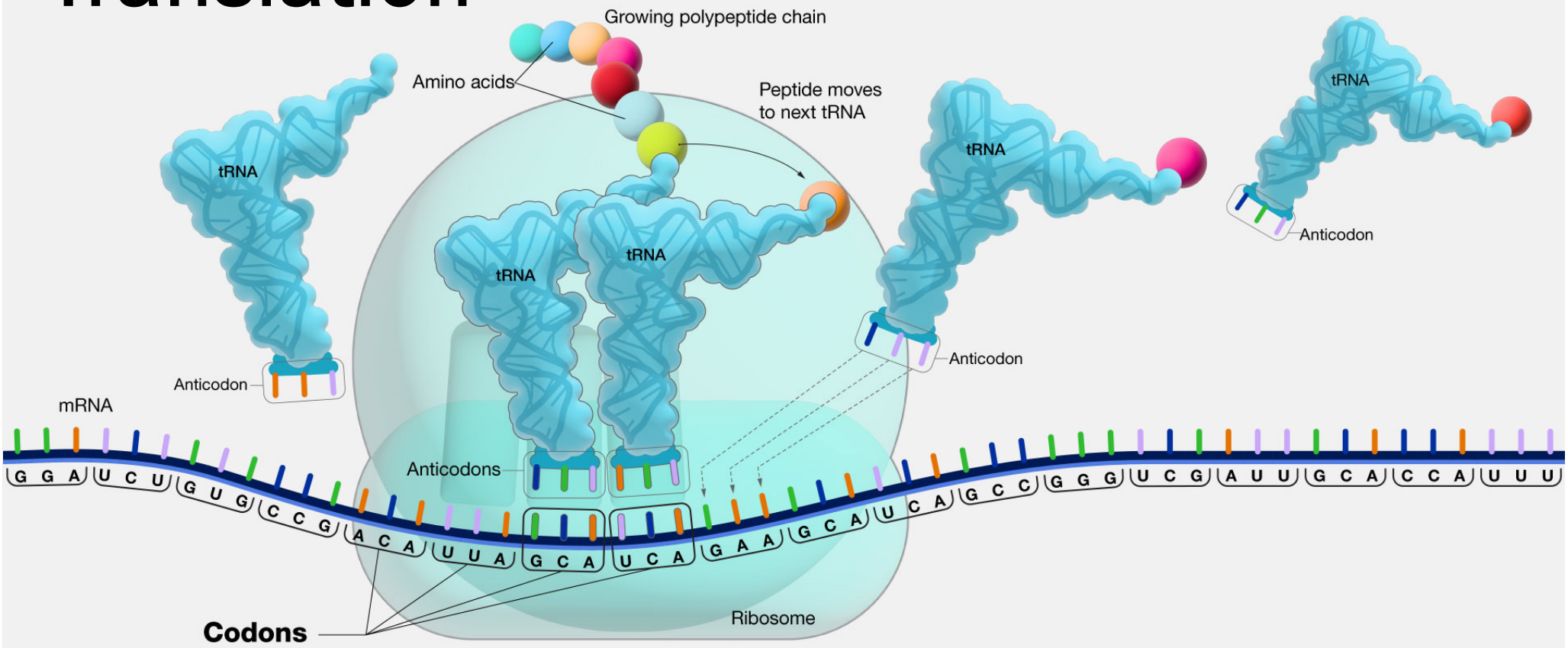
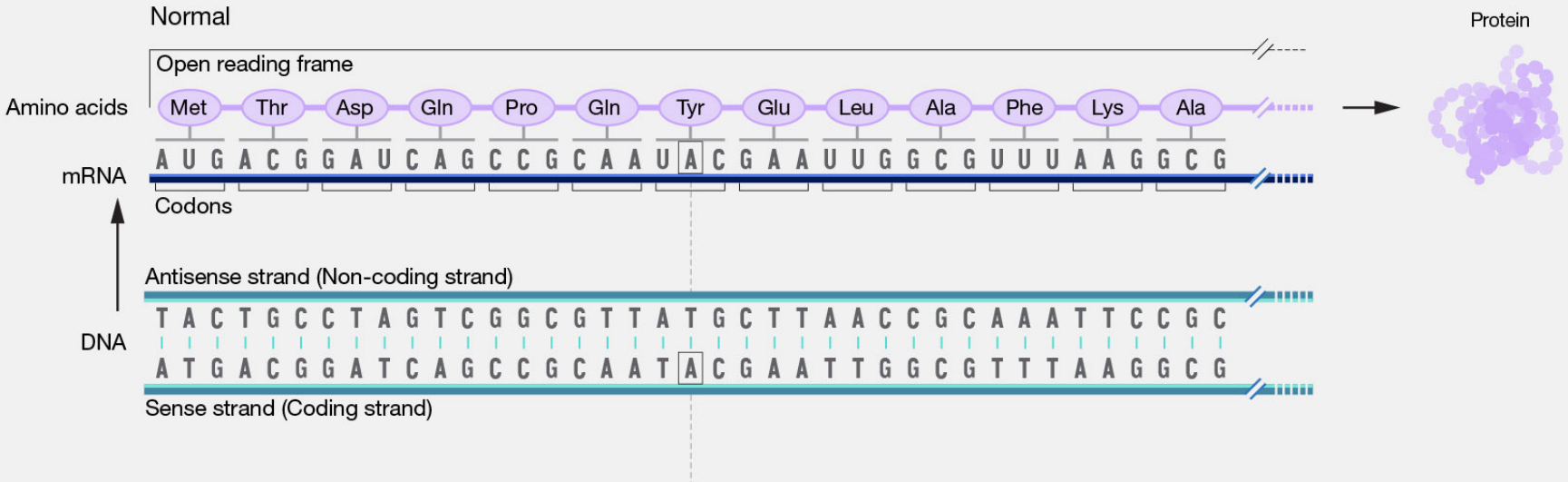



Image: NHGRI Glossary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Codons
Antisense strand (Non-coding strand)
DNA
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A T G A C G G A T C A G C C G C A A T A C G A A T T G G C G T T T A A G G C G
Sense strand (Coding strand)


Starting sequence
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Deletion
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Substitution
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Insertion
. . . G T C G A G T C T A T G C G C T A T C G C T . . .

# Diagnosing Genetic Disease

- Select appropriate test
- Select a lab
- Receive and disclose result
- Incorporate into clinical management plan

# What do we all want from genetic testing?

Diagnosis
Treatment
Understanding of Disease

What do I have
How will this testing help me
Will I lose my job, my insurance
What about my kids, my family

Many individuals at risk for an autosomal dominant neurodegenerative disease choose NOT to do predictive testing

# Implications of Genetic Test Results

## For the Patient

- Diagnosis
- Treatment
- Prognosis
- Clinical trial eligibility

## For the Family

- Paternity/non-paternity
- Consanguinity
- Psychosocial issues
- Family planning

# Genetic Counseling

Genetic Counselors earn Masters degree in Genetic Counseling and Licensure

- Important even in late adult-onset disease because of consequences to family
- Genetic counselors explore perceived risks/benefits to testing
- Educates patient and family to inheritance, penetrance
- Counsels at results visit
- Necessary for predictive testing.

There is a current dearth of genetic counselors, but there are innovative genetic counseling services becoming more available if referral to a genetics clinic is not feasible

# Additional crucial patient implications we address in counseling

- Is now the right time to test?
- For diseases like HD, FTD or other life limiting diseases
  - *Do you have counseling, any history of suicidality, who are your support people*
- We may find variants we just don't understand yet, so we may give you uncertainty rather than answers
- We may find something unexpected, yet medically important.

# Questions for patients to ponder

- How might testing influence my relationship with spouse/significant other? Relationships with friends and family?
- What are my coping strategies?
- Who is in my support network? Would it be helpful to meet with a professional counselor and/or spiritual support person?
- How might testing influence my short and long-term plans? Family planning decisions? Career decisions?
- What financial planning should be done prior to testing (such as life, disability, or long-term care insurance)?

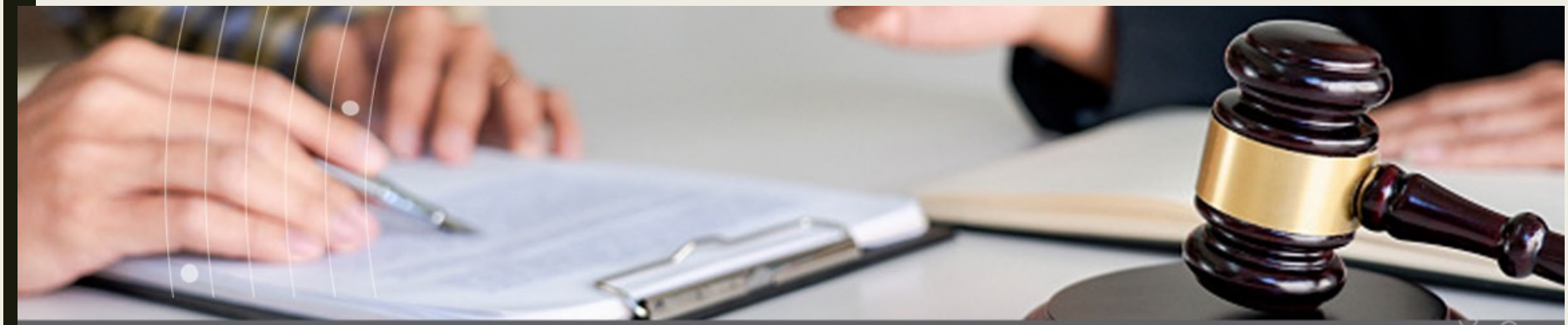
# Insurance issues to know before patients test

- Health insurance and employment protected by GINA
- Long term disability, life insurance NOT protected

# GINA

- Genetic Information Nondiscrimination Act (GINA)
- Passed in 2008 during the Bush administration
- Prohibits discrimination based on genetic information
  - *Limited to employers and health insurance companies*


Image: NHGRI – Genetic Discrimination



National Human Genome Research Institute

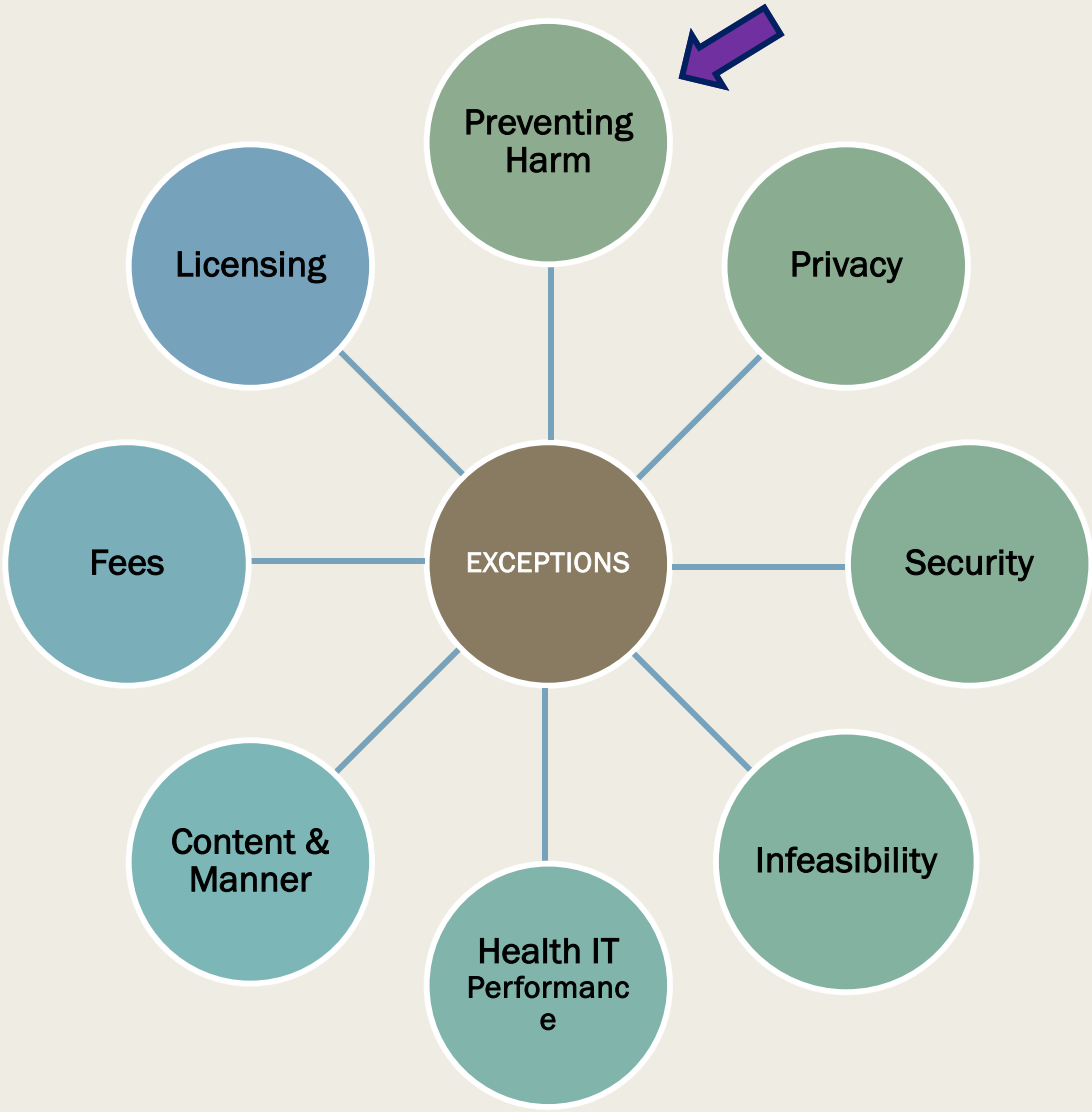
# Genetic Information Nondiscrimination Act Patient Resource

NHGRI's 1-page PDF available for download

# CURES Act

- The 21st Century Cures Act was signed into law in 2016
- Goal was to “accelerate the discovery, development, and delivery of 21st century cures, and for other purposes.”
- Changed with the 21st Century Cures Act Final Rule
  - *The Information Blocking Provision of the Cures Act Final Rule mandated that patients have unencumbered, free access to their electronic health information (effective April 5, 2021)*
  - *The Final Rule expanded to include to all EHI on October 6, 2022.*

# Exceptions to Information Blocking



# Genetic Counseling Resources

## Commercial Labs

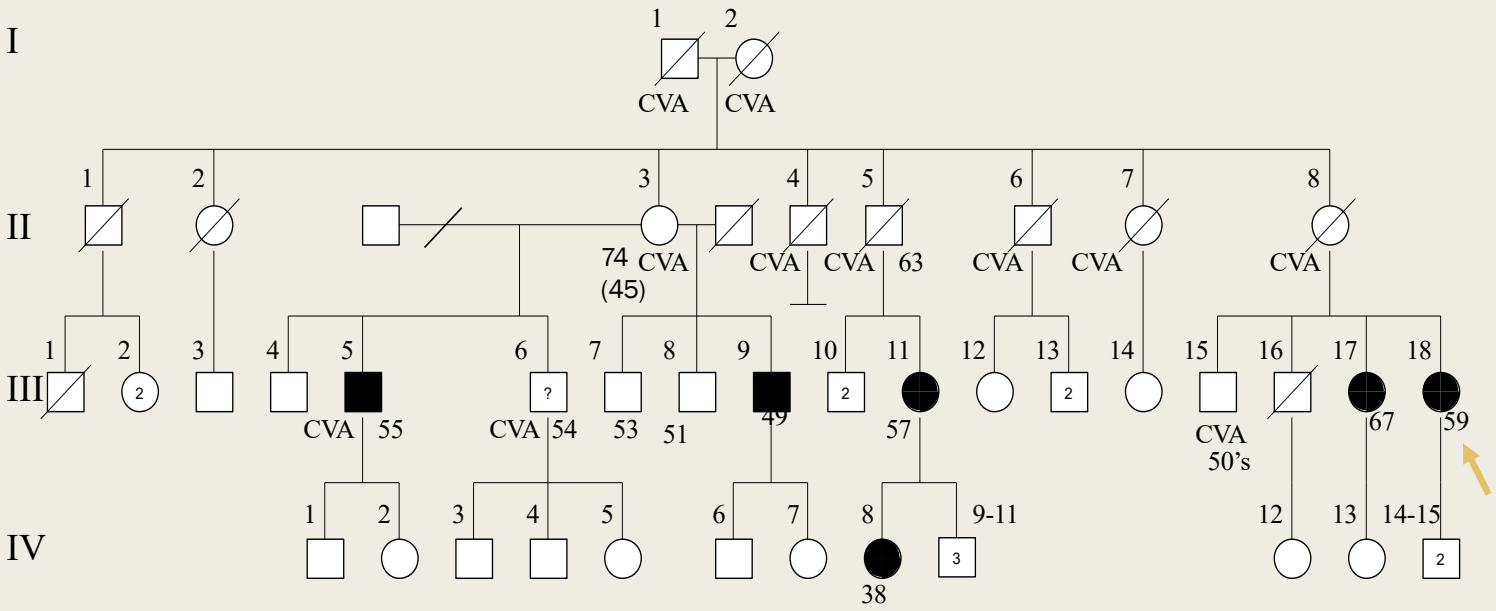
- Many commercial labs that specialize in genetic testing offer support to ordering providers
- Some offer genetic counseling to patients (pre- and post-test counseling)

## Contract Genetic Counseling Services

- Genome Medical
- Genetic Support Foundation

# Case

- 59 year old female awoke with hemibody tingling: no weakness, confusion, headache, visual disturbance
- Admit for stroke workup and local hospital
  - *Carotid studies negative*
  - *Echocardiogram unremarkable*
- MRI with bilateral patchy confluent subcortical lesions
- No history of smoking, diabetes, BP 90/60 on average
- Started on 325 mg ASA per day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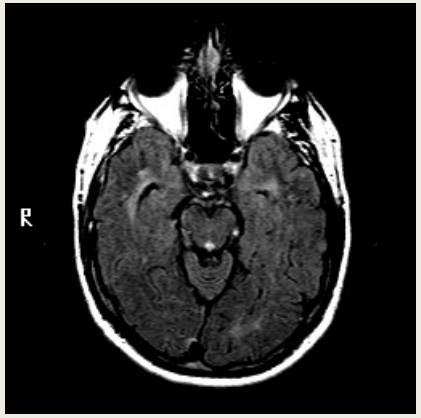
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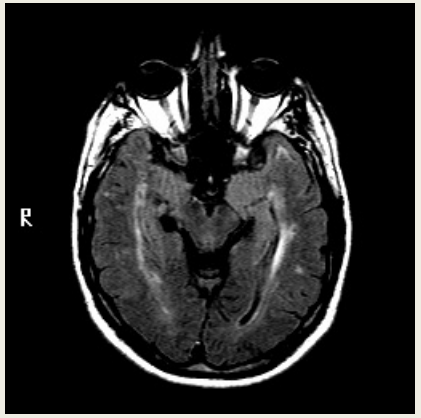
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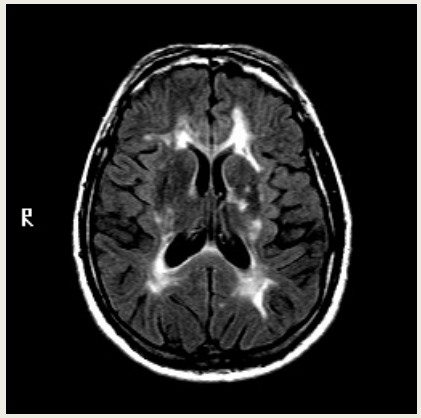
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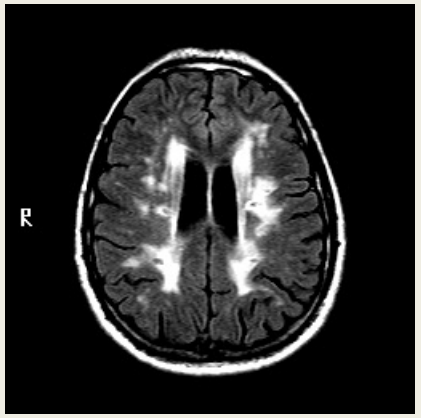
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# *Diagnoses often considered*

- Multiple Sclerosis
- Primary CNS angiitis

## *Other disorders causing similar stroke Symptoms:*

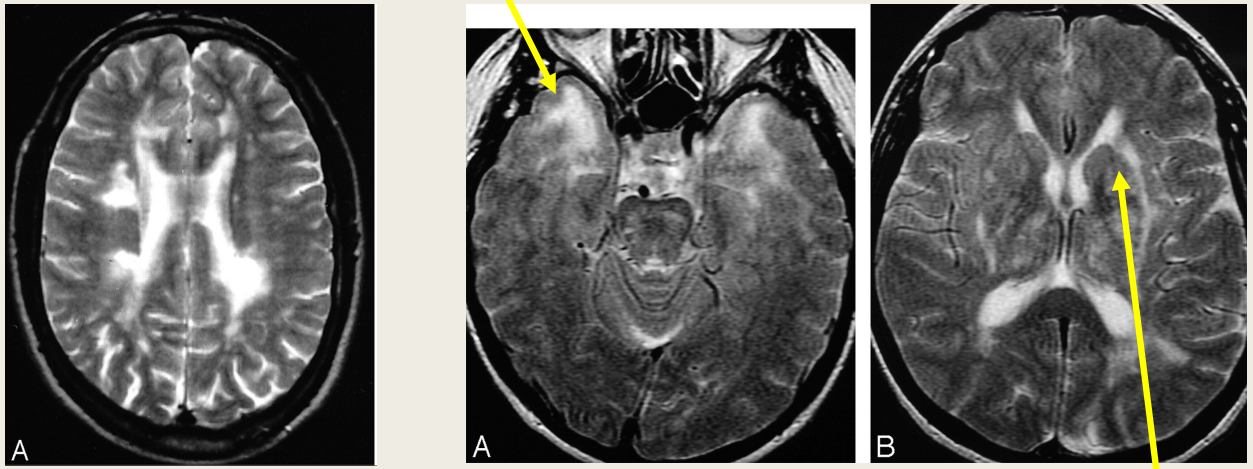
- Connective tissue Diseases (COL4A1/2)
- CARASIL
- Hereditary endotheliopathy with retinopathy, nephropathy and stroke (TREX1)
- Familial dyslipidemia, homocystinuria
- Fabry's Disease

# CADASIL

- Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy
  - *Genetic small vessel disease*
- Early onset stroke, migraines, TIA, later onset dementia, seizure psychiatric symptoms/signs

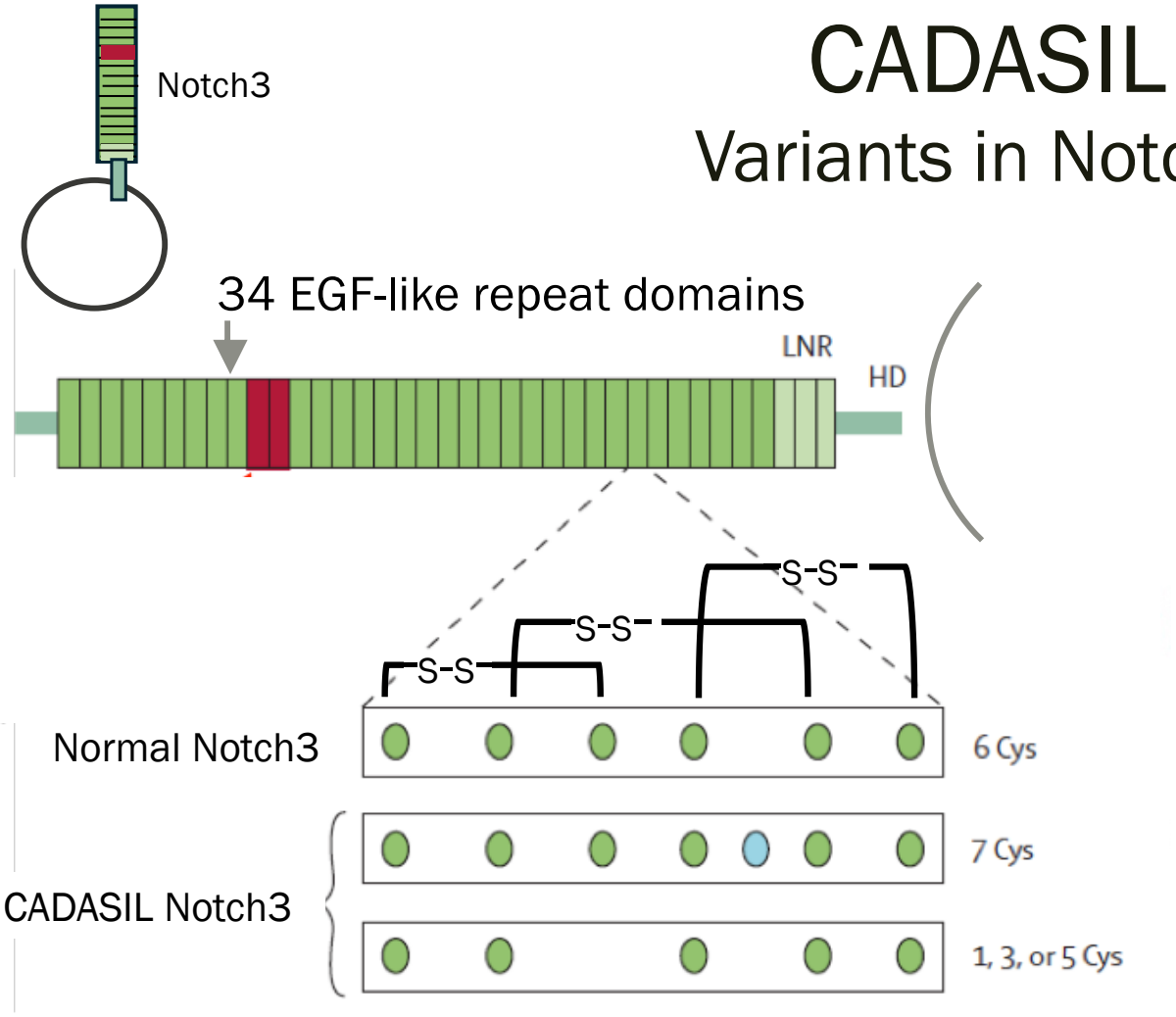
# MRI

Anterior temporal lobe T2 intensities




External Capsule

# CADASIL
## Variants in Notch3




-S-S- "Di-sulfide Bond"

Cys Cysteine Amino Acid

Chabriat et al Lancet Neuro 2009
Muinoz et al. IJMS 2017

| Gene/Transcript | Variant Position | Variant | Zygosity/Inheritance | Classification | Disease Association |
|---|---|---|---|---|---|
| *NOTCH3* NM_000435.2 | chr19 g.15302947C>T | c.503G>A, p.Cys168Tyr | Heterozygous, Unknown | Likely Pathogenic | Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), autosomal dominant |

***NOTCH3* c.503G>A p.Cys168Tyr, Likely Pathogenic**

The p.Cys168Tyr variant in the *NOTCH3* gene has not been previously reported in association with disease and was absent from large population databases, including the Genome Aggregation Database (http://gnomad.broadinstitute.org/). This variant disrupts a cysteine predicted to form disulfide bond and is located in a calcium-binding EGF domain of the NOTCH3 protein. Cysteine-altering variants in this domain are a common type of pathogenic variant associated with CADASIL (Rutten 2016, Rutten 2019). *In silico* tools predict that the p.Cys168Tyr variant is deleterious; however, these predictions have not been tested directly. Using ACMG guidelines, this variant was classified as likely pathogenic for autosomal dominant cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (ACMG evidence codes used: PM1_strong, PM2_supporting, PP3, PP4).

## RESULT: UNCERTAIN

### Variant(s) of Uncertain Significance identified.

| GENE | VARIANT | ZYGOSITY | VARIANT CLASSIFICATION |
|---|---|---|---|
| | | heterozygous | Uncertain Significance |
| KIAA1161 | | heterozygous | Uncertain Significance |
| NOTCH3 | c.1144G>T (p.Gly382Cys) | heterozygous | Uncertain Significance |
| ACADS | | heterozygous | Benign (reportable variant) |

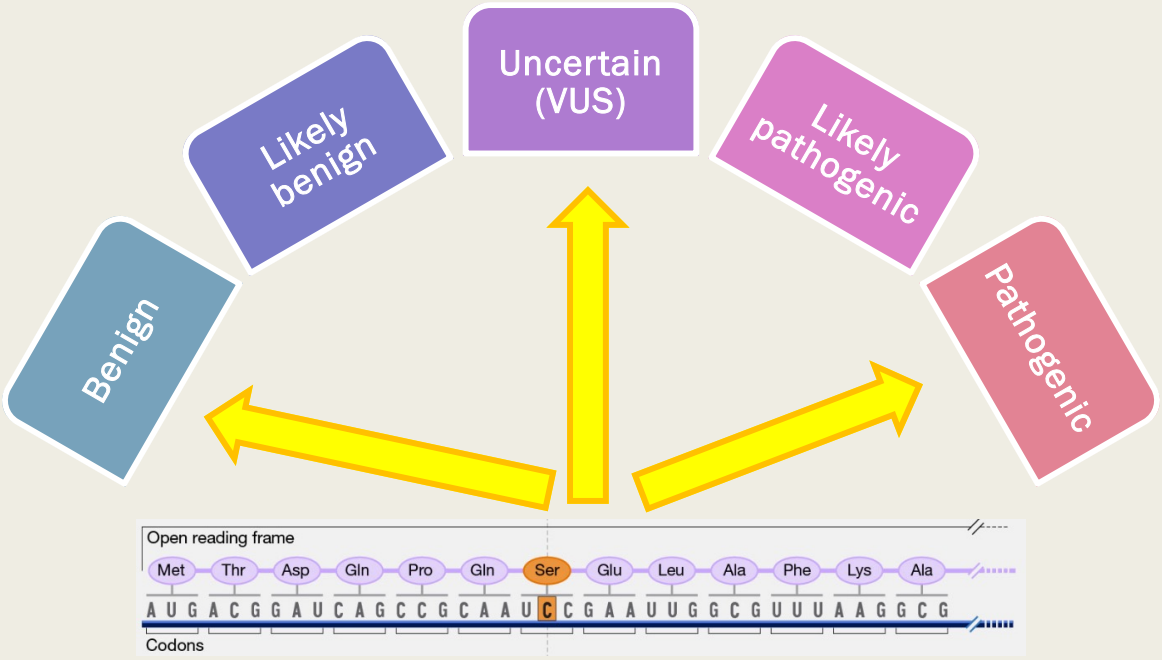
**About this test**

This diagnostic test evaluates 446 gene(s) for variants (genetic changes) that are associated with genetic disorders. Diagnostic genetic testing, when combined with family history and other medical results, may provide information to clarify individual risk, support a clinical diagnosis, and assist with the development of a personalized treatment and management strategy.

NOTCH3, Exon 7, c.1144G>T (p.Gly382Cys), heterozygous, Uncertain Significance

- This sequence change replaces glycine with cysteine at codon 382 of the NOTCH3 protein (p.Gly382Cys). The glycine residue is highly conserved and there is a large physicochemical difference between glycine and cysteine.
- This variant is not present in population databases (ExAC no frequency).
- This missense change has been observed in individual(s) with clinical features of CADASIL (PMID: 14710716, 32277177; Invitae).
- Advanced modeling of protein sequence and biophysical properties (such as structural, functional, and spatial information, amino acid conservation, physicochemical variation, residue mobility, and thermodynamic stability) performed at Invitae indicates that this missense variant is expected to disrupt NOTCH3 protein function.
- In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance.

# Variant Classification




Richards et al. (2015), PMID: 25741868

| | Benign | | Pathogenic | | | |
|---|---|---|---|---|---|---|
| | **Strong** | **Supporting** | **Supporting** | **Moderate** | **Strong** | **Very strong** |
| **Population data** | MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2 | | | Absent in population databases PM2 | Prevalence in affecteds statistically increased over controls PS4 | |
| **Computational and predictive data** | | Multiple lines of computational evidence suggest no impact on gene /gene product BP4<br><br>Missense in gene where only truncating cause disease BP1<br><br>Silent variant with non predicted splice impact BP7<br><br>In-frame indels in repeat w/out known function BP3 | Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3 | Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5<br><br>Protein length changing variant PM4 | Same amino acid change as an established pathogenic variant PS1 | Predicted null variant in a gene where LOF is a known mechanism of disease PVS1 |
| **Functional data** | Well-established functional studies show no deleterious effect BS3 | | Missense in gene with low rate of benign missense variants and path. missenses common PP2 | Mutational hot spot or well-studied functional domain without benign variation PM1 | Well-established functional studies show a deleterious effect PS3 | |
| **Segregation data** | Nonsegregation with disease BS4 | | Cosegregation with disease in multiple affected family members PP1 | Increased segregation data → | | |
| **De novo data** | | | | De novo (without paternity & maternity confirmed) PM6 | De novo (paternity and maternity confirmed) PS2 | |
| **Allelic data** | | Observed in *trans* with a dominant variant BP2<br><br>Observed in *cis* with a pathogenic variant BP2 | | For recessive disorders, detected in trans with a pathogenic variant PM3 | | |
| **Other database** | | Reputable source w/out shared data = benign BP6 | Reputable source = pathogenic PP5 | | | |
| **Other data** | | Found in case with an alternate cause BP5 | Patient's phenotype or FH highly specific for gene PP4 | | | |

Richards et al. (2015), PMID: 25741868

# Penetrance

The chance that a genomic variant will cause a phenotype

## COMPLETE PENETRANCE

- A variant with full penetrance will **ALWAYS** result in a phenotype
- Sometimes called full penetrance

## INCOMPLETE PENETRANCE

- A variant with incomplete penetrance **MAY** result in a phenotype
- Sometimes called reduced penetrance

# HUNTINGTON DISEASE

| Huntington's status | | CAG repeat length |
|---|---|---|
| **Unaffected** | Normal | 10-26 |
| | Intermediate allele | 27-35 |
| (maybe) **Affected** | Reduced penetrance | 36-39 |
| **Affected** | Full penetrance | 40+ |

Image: Huntington Disease Association

# Different degrees of disease

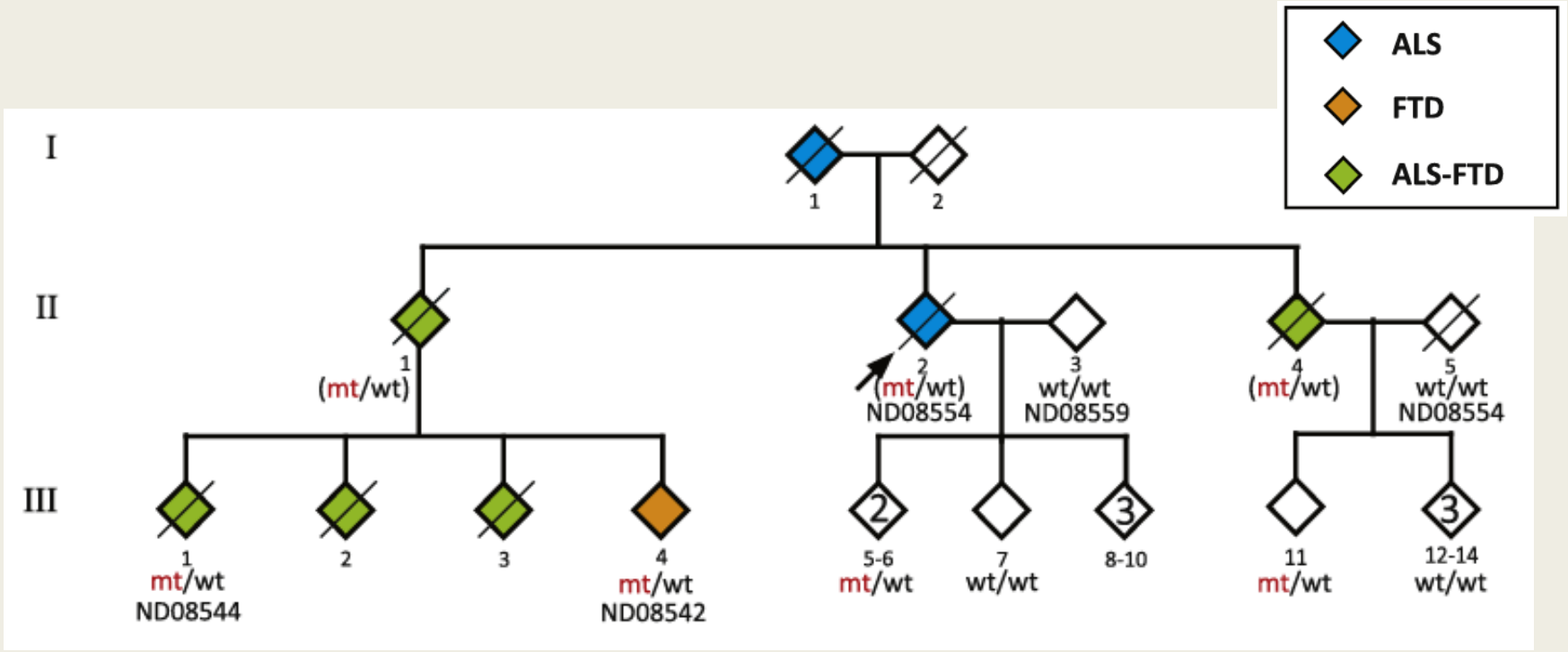
## PLEIOTROPY

- Pathogenic variants that cause disease in multiple tissues:
  - Familial Hemiplegic Migraine, Spinocerebellar Ataxia 6, Episodic Ataxia (CACANA1)

## VARIABLE EXPRESSIVITY

- Occurs when a genetic variant results in varying degrees of a phenotype
  - FTD/ALS, Cerebral Cavernous Malformation

# Variable Expressivity in C9orf72




Renton et al. (2011), PMID: 21944779

# Case

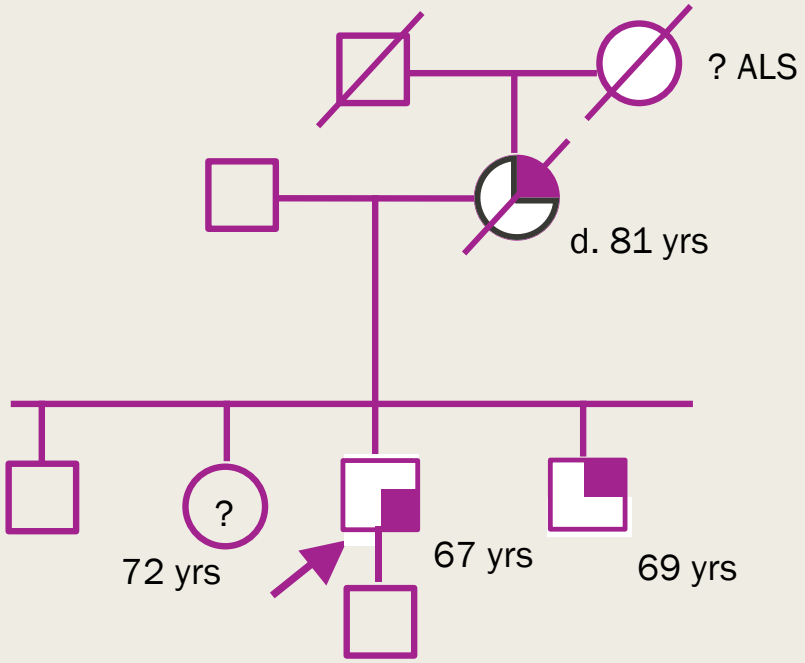
## HPI

- 67 yr old presents with 1 year history of gait instability after fall
- No concerns for weakness, cognition, vision, sensory changes
- No significant past medical history
- Recently retired from sales.
- Referred because family history of ataxia

## Exam

- Pleasant, appropriate
- Increased tone on right side (arm)
- Intact strength. Possibly upgoing toe on right.
- Normal finger nose finger, rapid alternating movements, finger tapping
- MRI no structural abnormalities

# Family History




Spinocerebellar Ataxia (SCA)

Spasticity

- Brother with dx of SCA had 6 year history of progressive ataxia with documented cerebellar signs and brain MRI showing cerebellar atrophy
- Mother had been followed in clinic with diagnosis of SCA
- Unclear history of maternal grandmother. Suspected sister with some cognitive changes though no evaluation

# What to test?

- Brother: Testing for C9orf72, a 300 gene movement disorder panel, SCA1 and SCA3 in 1 year earlier:

  **Negative**

- After meeting with patient, we proceeded with repeat expansion panel SCAs, DRPLA, FRDA

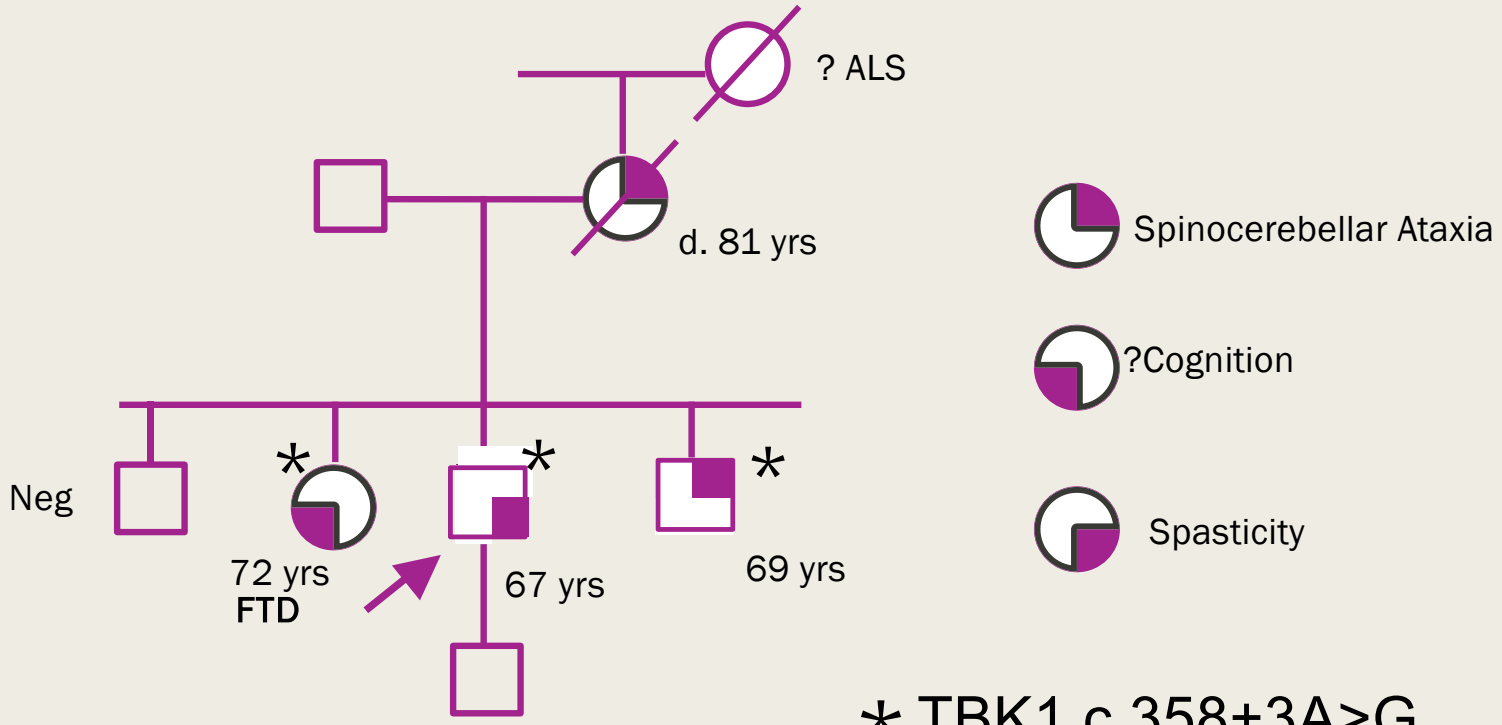
  **Negative**

# Returns 2 years later

- With now bilateral lower extremity weakness, right greater than left
- Diffuse hyperreflexia bilateral upgoing toes
- EMG with trace positive sharp waves in thoracic paraspinal and gastrocnemius muscles, chronic denervation in right C6 –7, L5 and S1 innervated muscles

# Family History

ALS panel done showed **TBK1 c.358+3A>G** variant.
Predicted to alter splicing and thus expression of protein

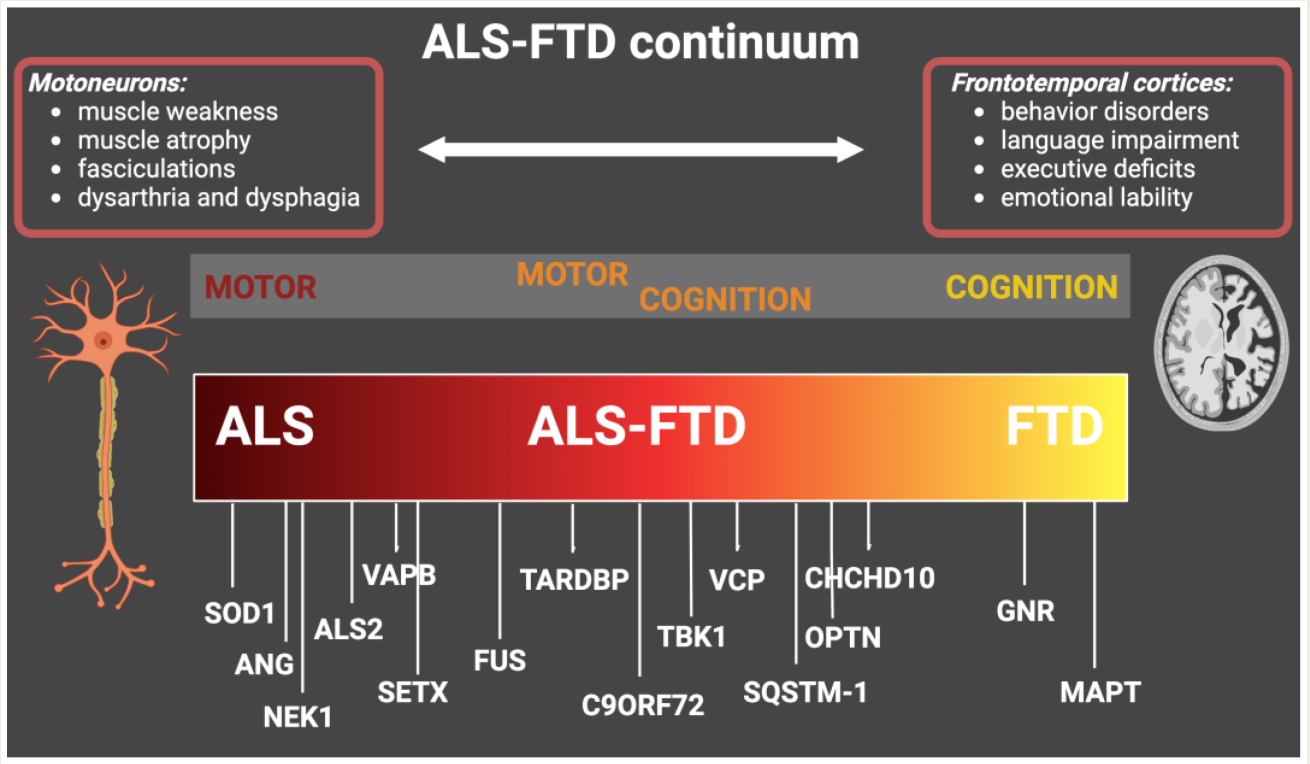



* TBK1 c.358+3A>G

# *TBK1* Frontotemporal Dementia/Motor Neuron Disease

- Broad clinical spectrum including:
  - *amnestic dementia,*
  - *corticobasal syndrome,*
  - *primary progressive aphasia,*
  - *parkinsonism*
  - *primary lateral sclerosis, spinal muscular atrophy, ALS*
- Adult onset
  - *50% TBK1 variant carriers symptomatic by age 70*
- Pathologically it is associated with TDP-43 pathology in brain

# Frontotemporal Dementia/Motor Neuron Disease




De Marchi et al. Genes 2023

# Testing

- 10% of sporadic behavioral variant FTD will be found to have pathogenic variant
- 10-17% sporadic ALS will be found to have pathogenic or likely pathogenic variant
- ~6% of sporadic ALS or FTD caused by C9orf72 expansion
- 25-40% familial FTD or ALS is caused by C9orf72 expansion

- In the case of negative testing but clinically suspected genetic disease we reconsider genetic testing every 1-2 years
- Families can bank DNA (for cost)

Testing is done with genetic counseling

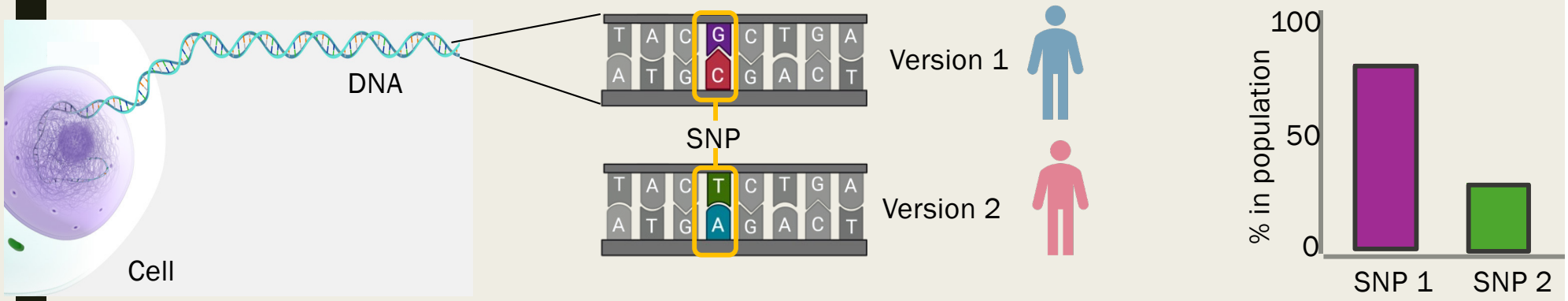
Confirming a variant segregates with the phenotype gives confidence of pathogenicity

Consider the testing landscape regularly

# Human Variation

# Leverage our genetic differences to find biology of disease

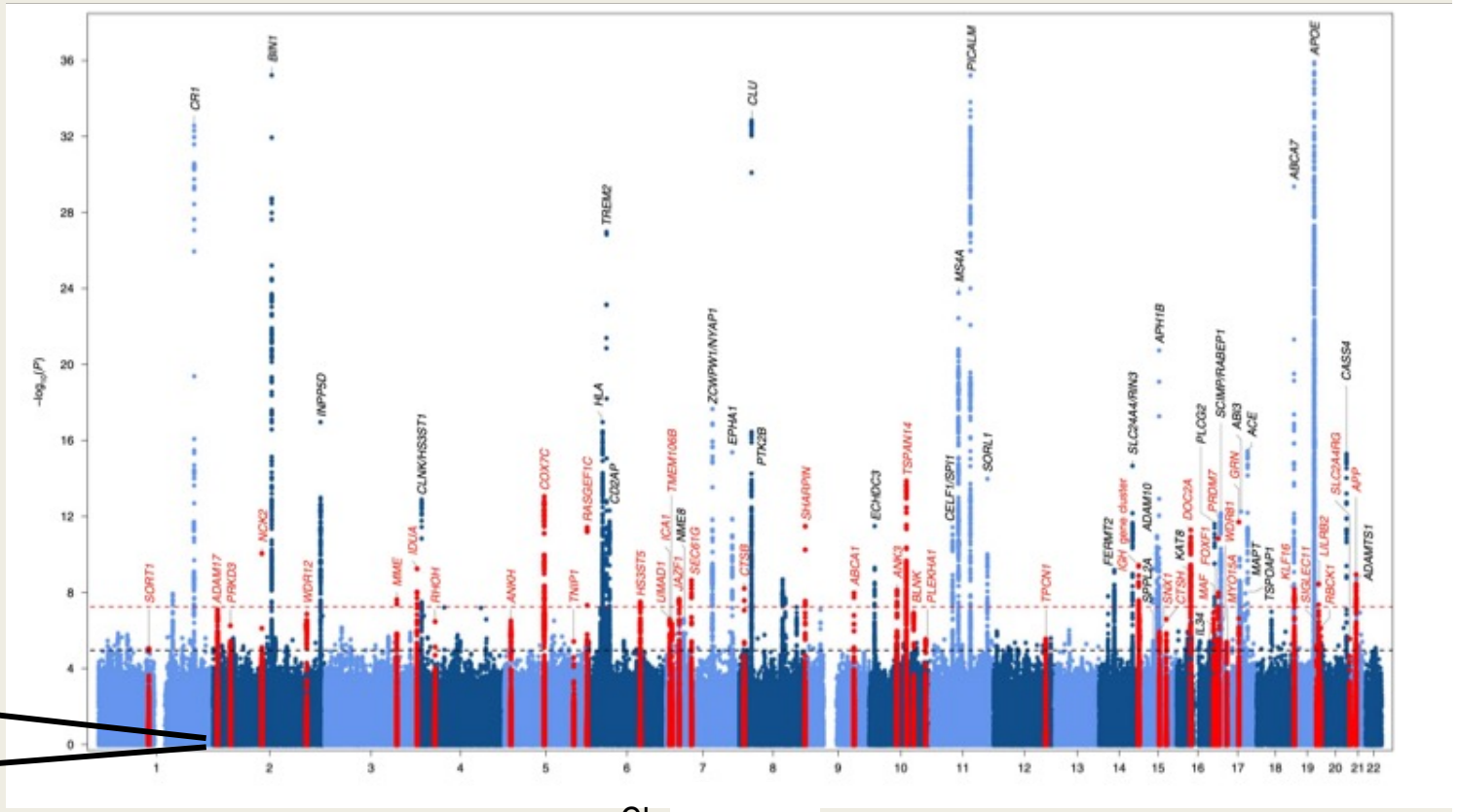
SNP: Single Nucleotide Polymorphisms



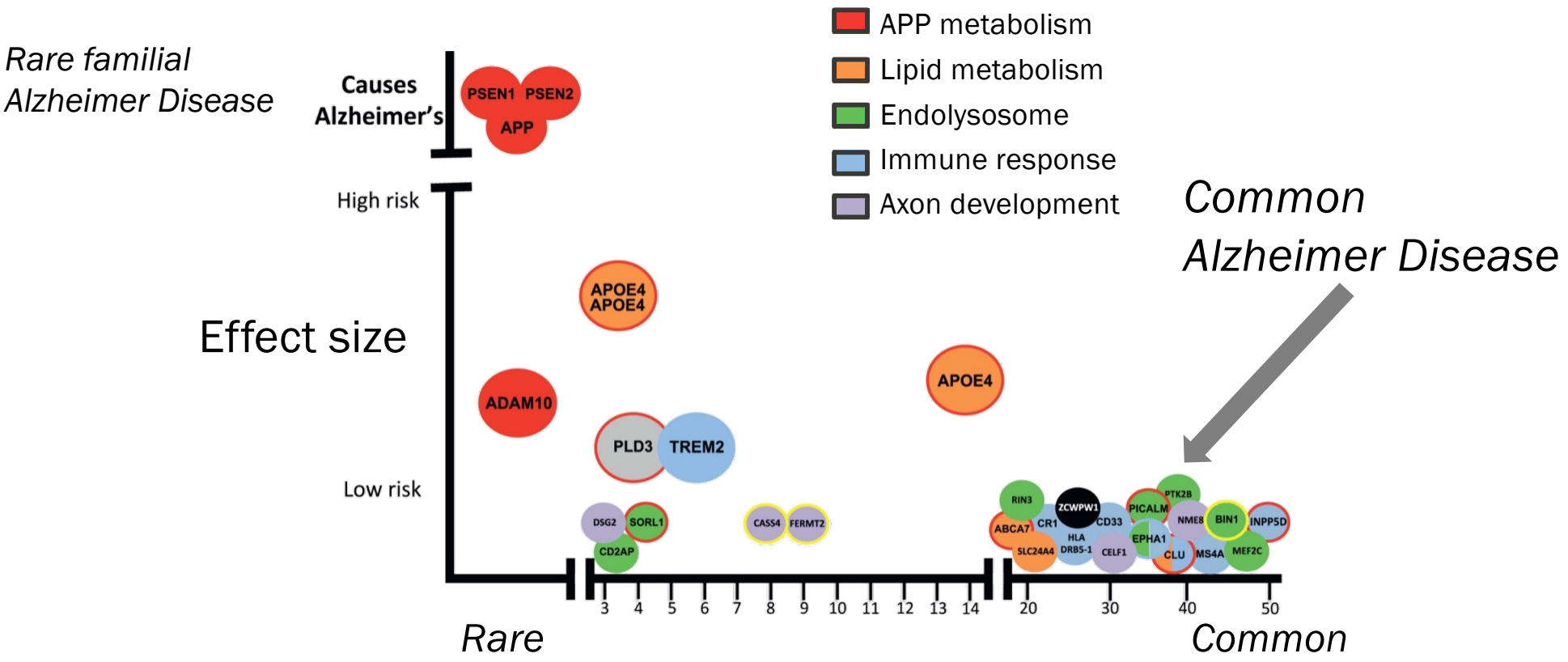

Use this variation in SNPs to correlate DNA regions with disease

# Genome Wide Association Studies
## Measure the association of SNP with AD

We identify regions of DNA that may contain genes contributing to AD risk

# Different SNPs, common genetic variants, are associated with AD risk




Karch and Goate, Biol Psych 2014