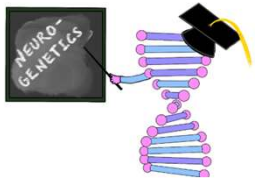
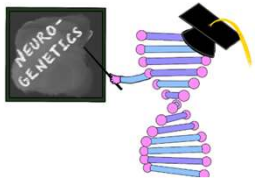
Stroke

MODULE 8



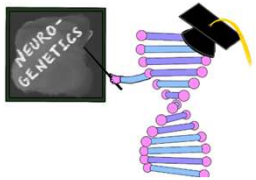
Learning Objectives

- Discuss the differential diagnosis and approach for monogenic causes of stroke
- Describe salient features of autosomal recessive mode of inheritance
- Interpret results of multi-gene panel using OMIM and other resources
- Analyze the role of functional/ biochemical testing for VUS resolution



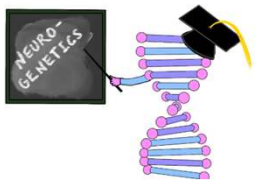
Chief Complaint

- 6-year-old F presented with abnormal eye movements and found to have lacunar infarct in midbrain

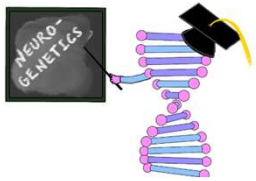


Exercise 1: Differential Diagnosis

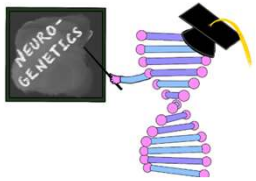
Name of condition	Gene and mode of inheritance	Clinical features and Treatment



Select Monogenic Causes of Pediatric and Adult Stroke				
1	Vascular/ Connective Tissue	CADASIL	*NOTCH3*	Predominantly small vessels, 40-50y
		CARASIL	*HTRA1*	Small vessels, 20-40y, alopecia
		Fabry	*GLA*	Small vessels, 20-50y, cardiac and renal disease
		Aicardi-Goutières	*TREX1*, others	Small vessels, white matter calcifications, chilblains
		Neurofibromatosis	*NF1*	Large vessels, CALMs, Lisch nodules
		COL4A-related disorders	*COL4A1* and *2*	Aneurysm, porencephaly
2	Metabolic/ Mitochondrial	MELAS	*MT-TL1*	No vascular territory, parietal and occipital lobes
		Leigh syndrome	Genetically heterogenous	T2 hyperintensities in basal ganglia and cerebellum
		Organic acidemias (MMA, PA, GA1)		Strokes in deep nuclei during metabolic crisis
		DADA2	*ADA2*	Lacunar infarct in deep nuclei, periodic fever
		Homocystinuria	*CBS*	Marfanoid features, peripheral vein thrombosis, CVA, MI

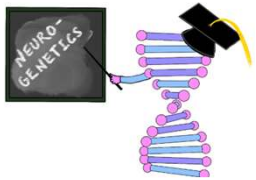


3.	Disorders of Coagulation	Activated Protein C/ Factor V Leiden Resistance	Thrombotic events leading to arterial strokes
		Protein C Deficiency	
		Antithrombin Deficiency	
		Congenital Plasminogen Deficiency	
4.	Miscellaneous	Cerebrotendinous Xanthomatosis	From cholesterol deposits in intracranial vessels
		Alagille syndrome	Cholestasis, dysmorphic features, stroke in 12%
		Sturge Weber syndrome	*GNAQ* mutation in tissue
		Moyamoya	No known gene causation

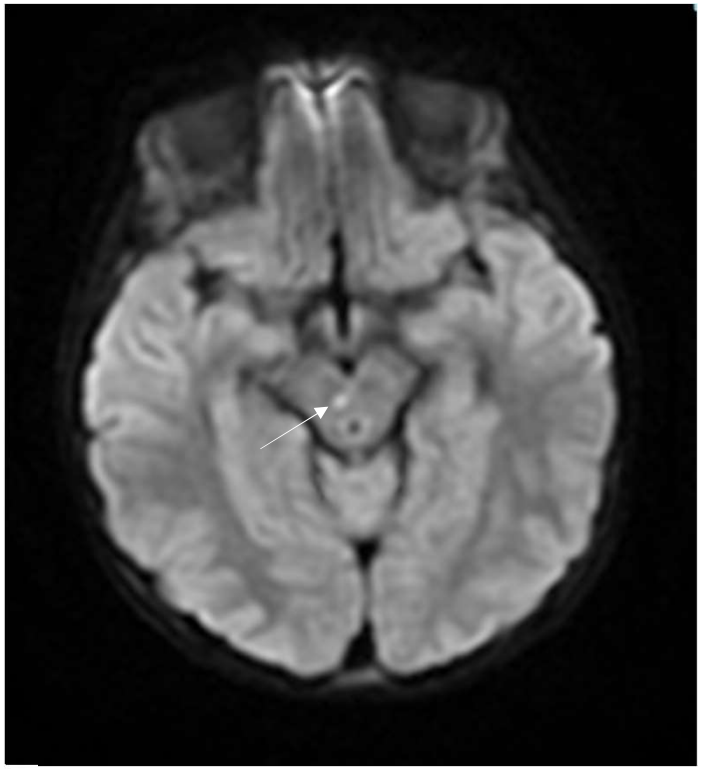


HPI, Exam

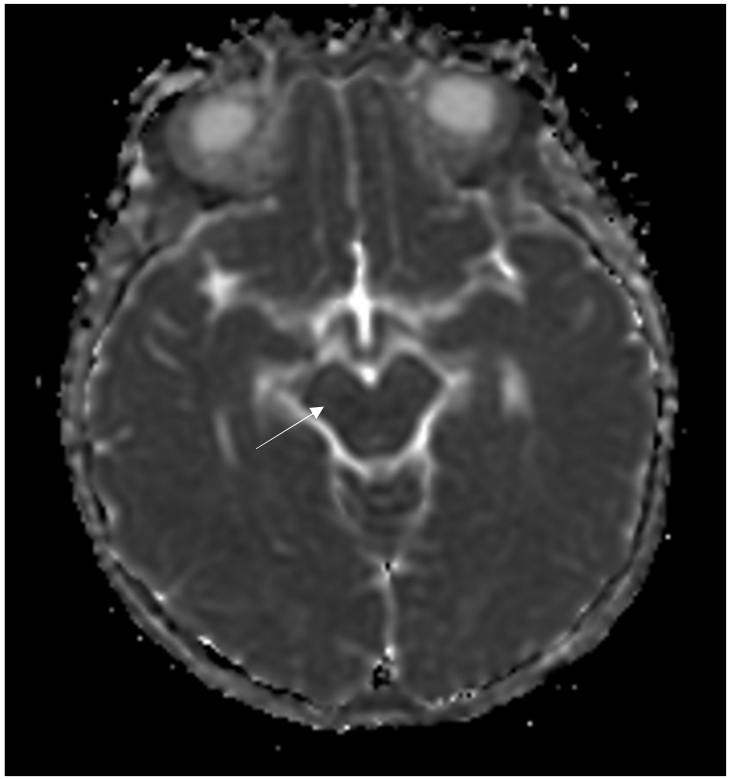
- A 6-year-old previously healthy female presented with one week of intermittent esotropia bilaterally. On initial neurologic examination, her ocular movements were normal, and she had no focal deficits.
- Brain MRI: mildly reduced diffusion in the right midbrain tegmentum consistent with an acute to subacute lacunar infarct. T2 hyperintensity in the right thalamus consistent with prior infarction.
- MRA: mild tortuosity, luminal irregularity, and mild narrowing of the distal right V2 and V3 segments.
- Thrombophilia evaluation, four extremity Doppler ultrasound, rheumatological work up and echocardiogram with bubble study were all unremarkable.



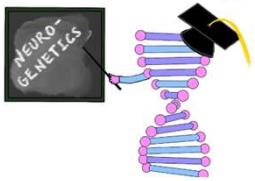
Admission 1: First Infarct



DWI

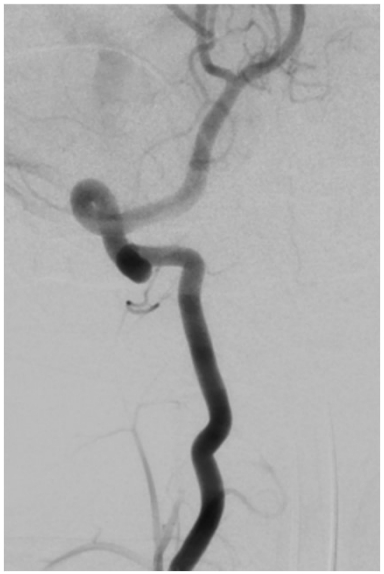


dADC

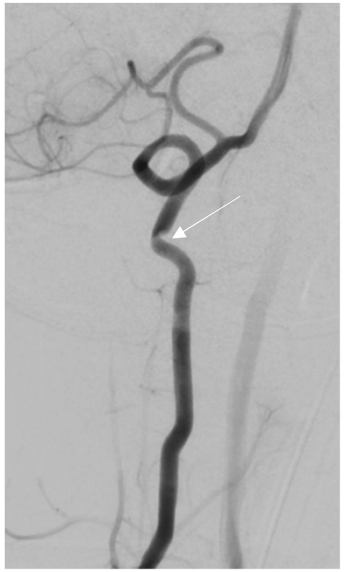


On imaging, had multiple infarcts of different ages

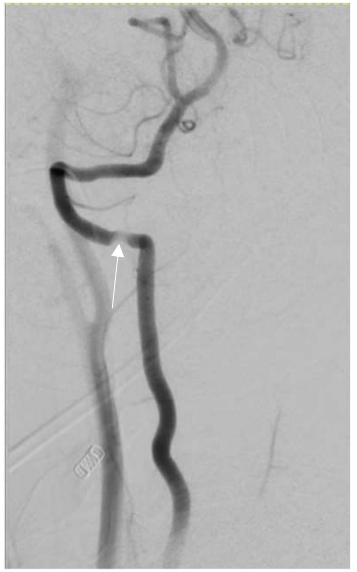
Angiography with Head Rotation Suggested Dynamic Vertebral Artery Compression



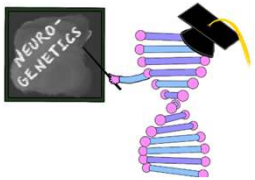
Neutral



Head turn



Head turn

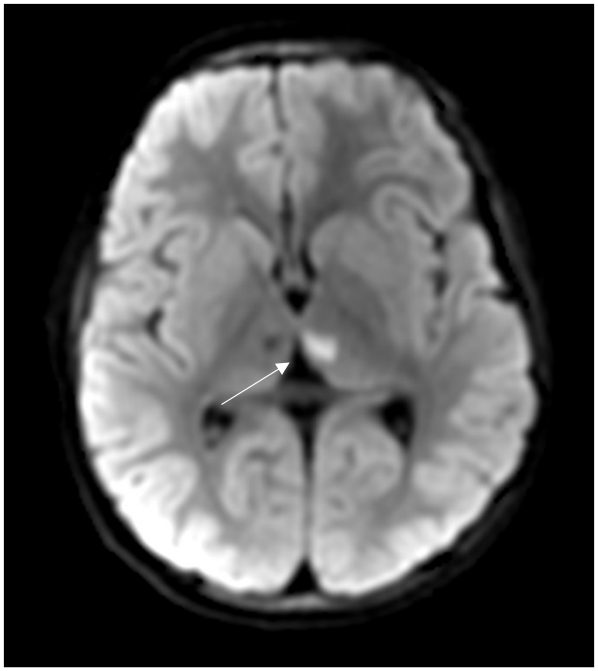


Diagnosed with Bow Hunter syndrome and underwent cervical fusion.
Started on enoxaparin and aspirin.

Admission 2: Repeat Infarct

She returned to the ED two weeks post-op with fevers to 38.5°C and intermittent left eye esotropia x 3 days. Mother mentioned patient has had periodic fever without known infections since infancy.

DWI



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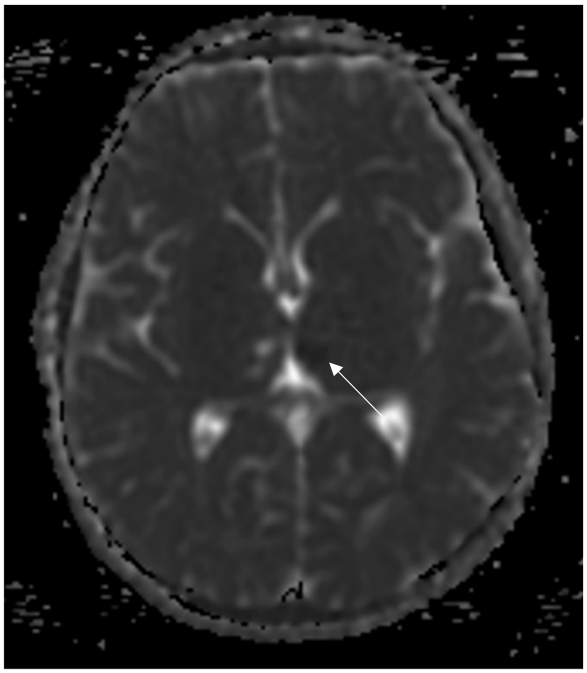
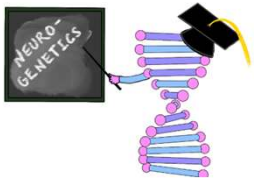
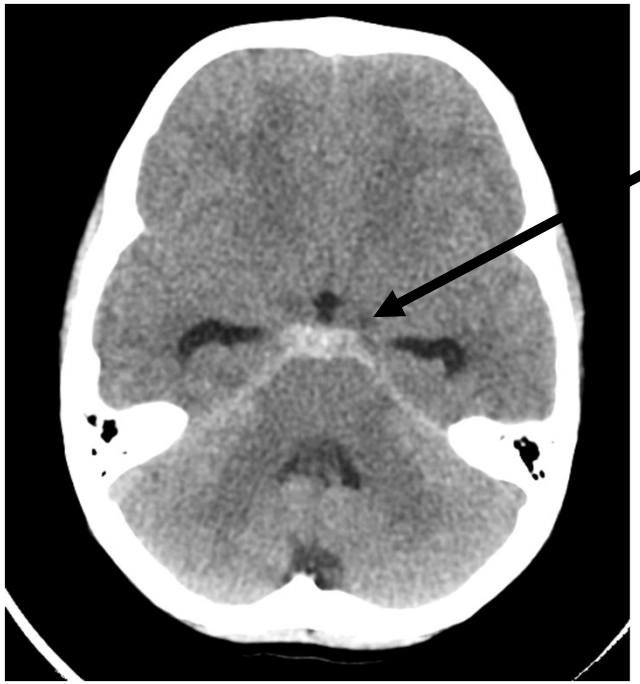


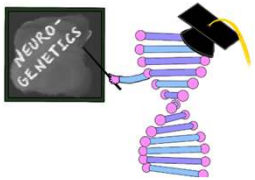


MRI brain demonstrated diffusion restriction in the left medial thalamus consistent with acute ischemic infarct

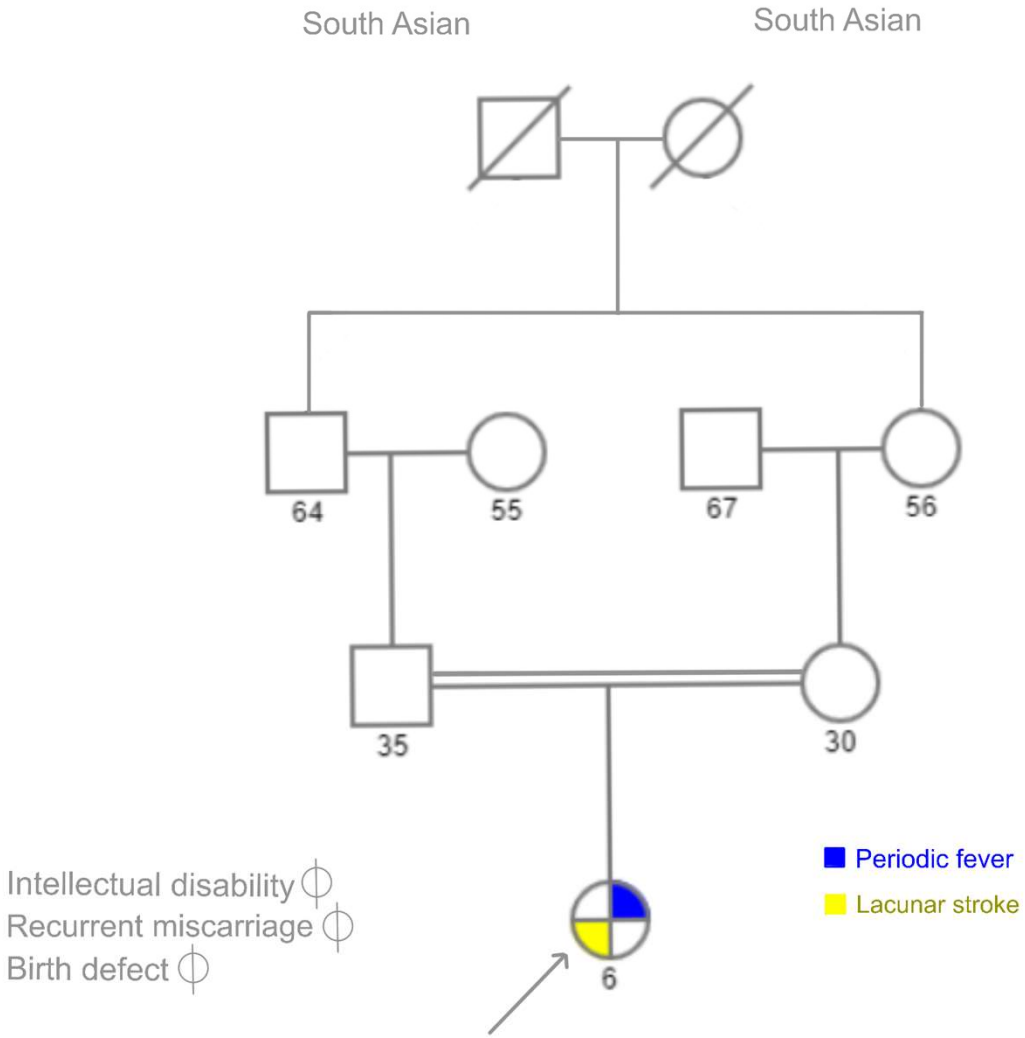
Admission 3: Subarachnoid Hemorrhage, Complicated by Hydrocephalus

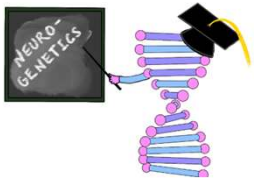


Returned to ED 2 weeks following discharge with headache, emesis, and lethargy



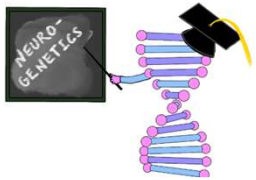
Pre-pontine subarachnoid hemorrhage complicated by hydrocephalus
Anti-coag was stopped
A stroke of genetic etiology was suspected due to consanguinity in family and testing sent


South Asian
South Asian
64
55
67
56
35
30
6
Intellectual disability
Recurrent miscarriage
Birth defect
Periodic fever
Lacunar stroke


NEURO-
GENETICS

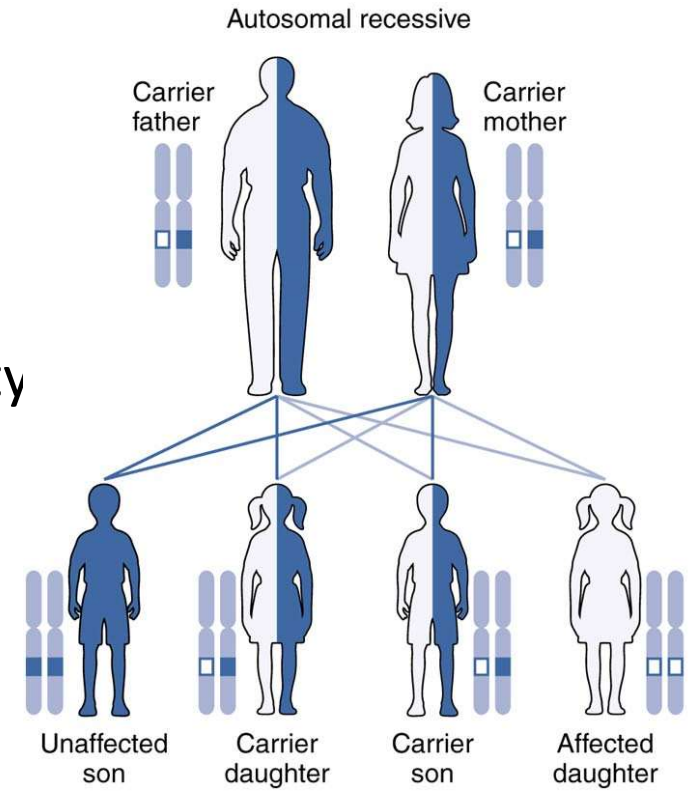
Exercise 2: Mode of Inheritance

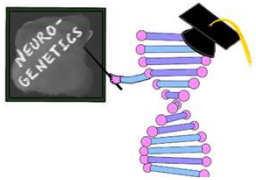
- What could be the possible mode of inheritance?
- If confirmed to have a genetic condition, what would be the estimated recurrence rates in future pregnancies?



Features of Autosomal Recessive Inheritance

- Multiple siblings are affected
- Trait appears in one generation
- Both sexes are affected equally
- There is often a history of consanguinity
- Recurrence risk is 25% if both parents are carriers





Co-efficient of Inbreeding

- The coefficient of inbreeding of an individual is **the probability that two alleles at any locus are identical by descent from the common ancestor(s) of the two parents**.

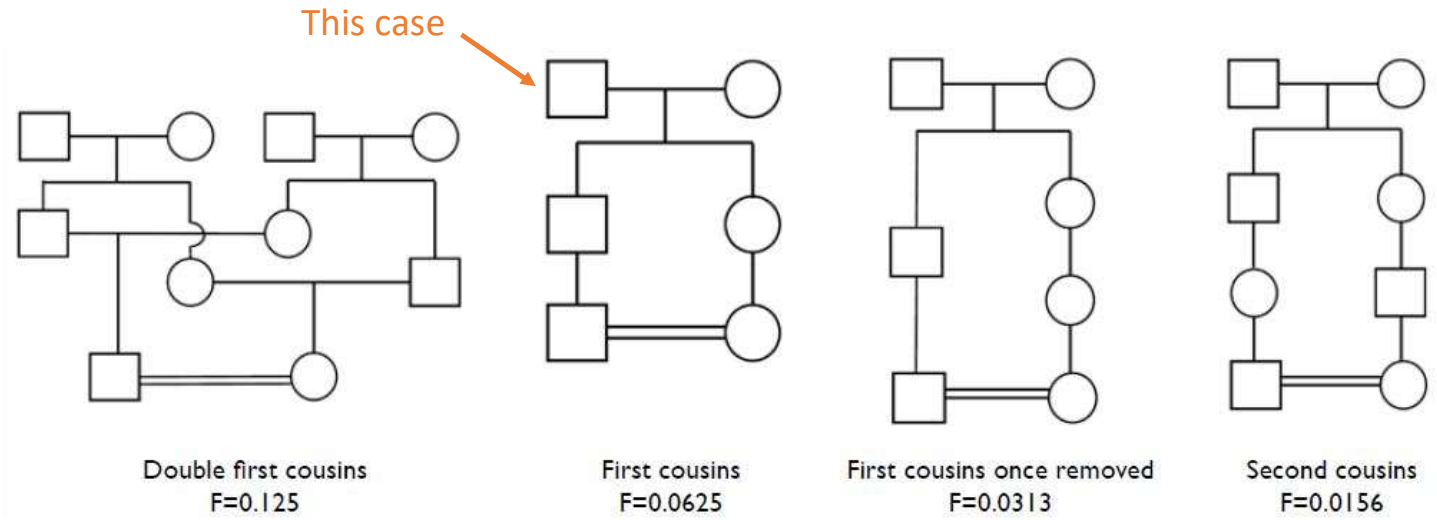
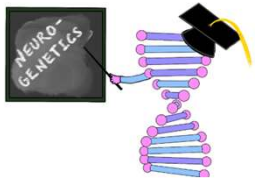
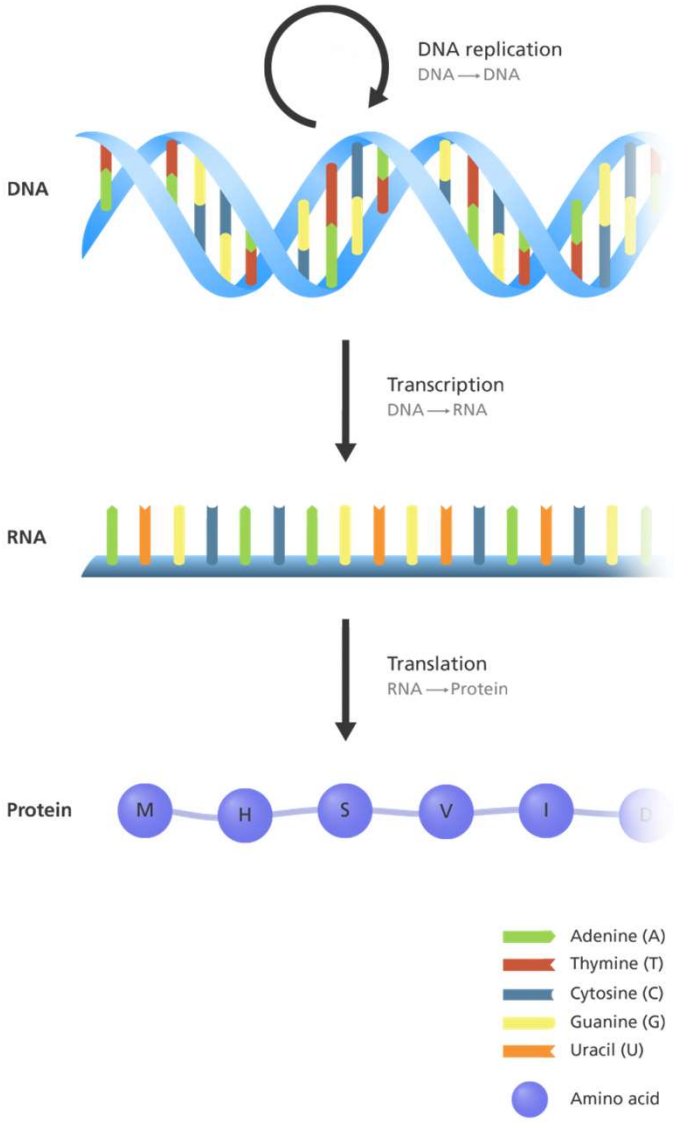


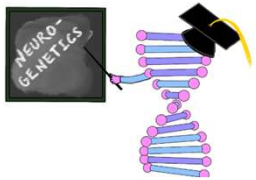
FIG. Family trees of consanguineous marriages with corresponding coefficients of inbreeding (F)



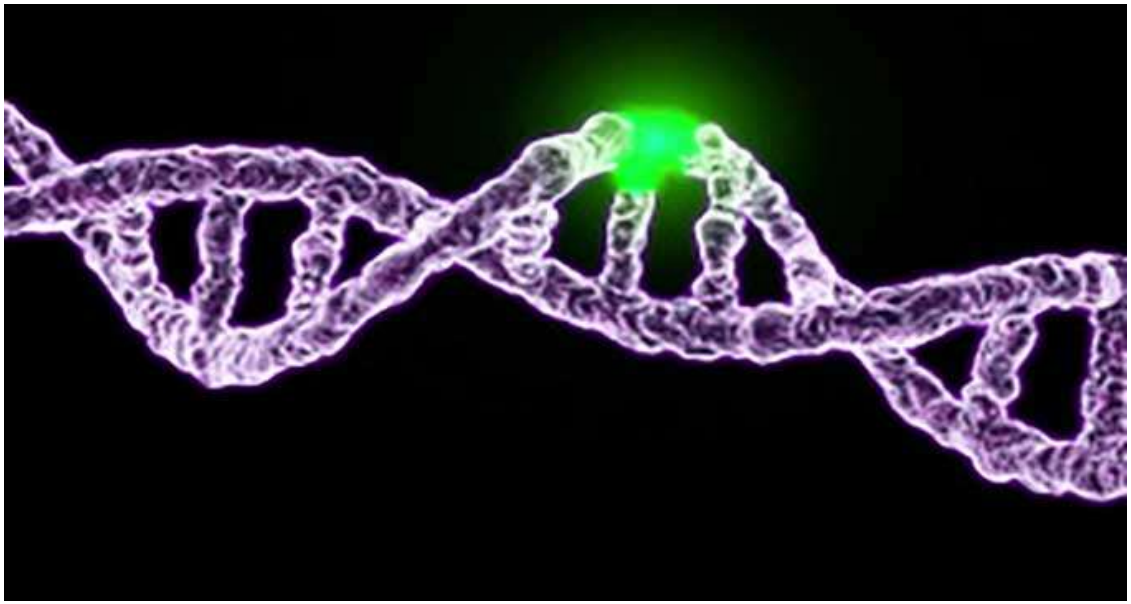
https://www.hkmj.org/abstracts/v25n3/192.htm

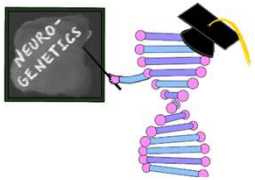
Central Dogma



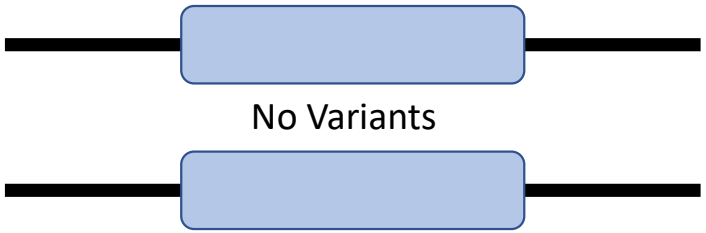


Mutation/Pathogenic Variant



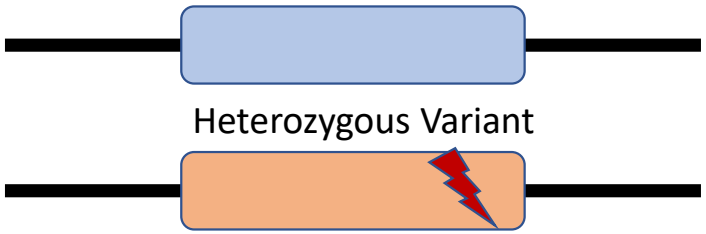


NO DISEASE



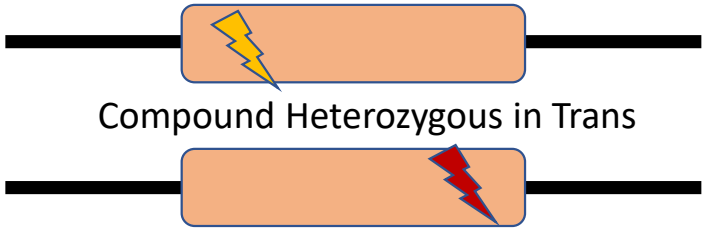
No Variants

Monoallelic/AD



Heterozygous Variant

Biallelic/AR



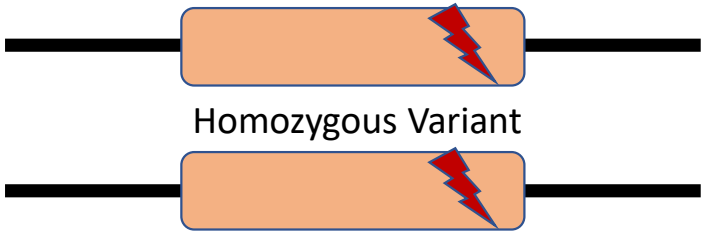
Compound Heterozygous in Trans

Unaffected Allele

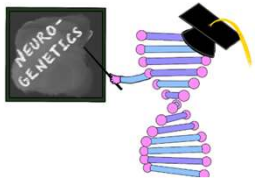
Affected Allele

AD Autosomal Dominant

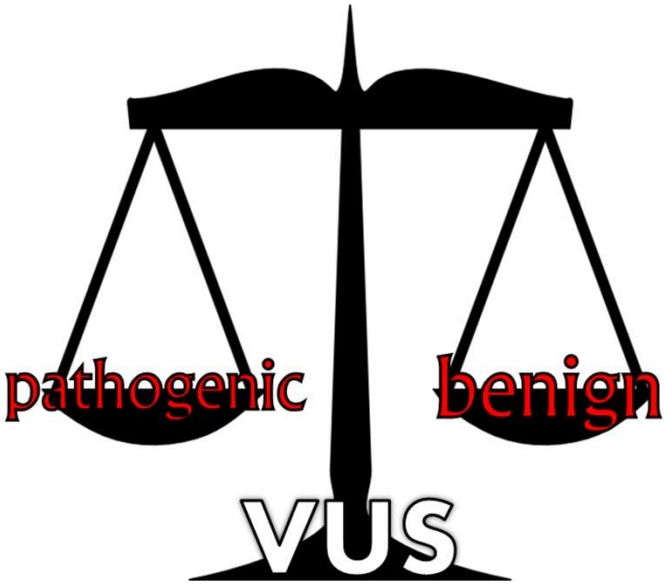
AR Autosomal Recessive



Homozygous Variant



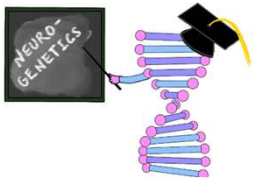
Variant of Uncertain Significance



THEATER

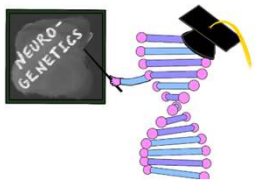
THEATRE

THATERE



Approach for VUS resolution

- **Look at inheritance pattern**
- **Look at phenotypic match**
- Look at variant – present in population? Effect on Protein?
- Familial Segregation Studies
- Functional studies – measure enzyme or transporter activity



Results of Genetic Testing

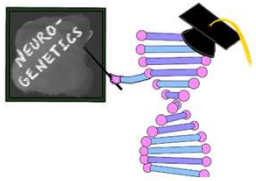
Test performed

Sequence analysis and deletion/duplication testing of the 12 genes listed in the Genes Analyzed section.

Familial Mediterranean Fever Test

- Add-on Additional Periodic Fever Syndromes Genes

Next-generation sequencing multi-gene panel



GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
ADA2	c.746T>G (p.Leu249Arg)	homozygous	Uncertain Significance

Clinical summary

Two Variants of Uncertain Significance, c.746T>G (p.Leu249Arg) (homozygous), were identified in ADA2.

- The ADA2 gene is associated with autosomal recessive deficiency of adenosine deaminase 2 (DADA2) (MedGen UID: 1659861).
- Not all variants present in a gene cause disease. The clinical significance of the variant(s) identified in this gene is uncertain. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
- This variant qualifies for complimentary family studies as part of our VUS Resolution Program. Familial VUS testing is recommended if informative family members are available and are likely to provide additional evidence for future variant reclassification. Details on our VUS Resolution Program can be found at https://www.invitae.com/family.

Variant details

ADA2, Exon 4, c.746T>G (p.Leu249Arg), homozygous, Uncertain Significance

- This sequence change replaces leucine with arginine at codon 249 of the ADA2 protein (p.Leu249Arg). The leucine residue is highly conserved and there is a moderate physicochemical difference between leucine and arginine.
- This variant is present in population databases (rs757520466, ExAC 0.006%).
- This variant has not been reported in the literature in individuals affected with ADA2-related conditions.
- Algorithms developed to predict the effect of missense changes on protein structure and function are either unavailable or do not agree on the potential impact of this missense change (SIFT: "Deleterious"; PolyPhen-2: "Probably Damaging"; Align-GVGD: "Class C0").
- 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.

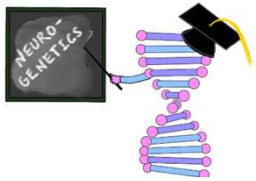
Exercise 2: Genetic Testing

Team A: What is the name of the genetic testing sent? What technology does it use? (Hint: read report)

Team B: What disorder (s) does the gene cause? What is the clinical phenotype? (Hint: report, OMIM)

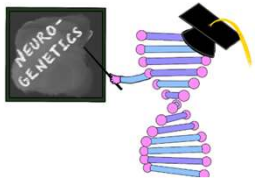
Team C: How is the variant classified? (Hint: report, ClinVar)

Team D: Is there any further biochemical testing that can be done to confirm the pathogenicity of the variant?



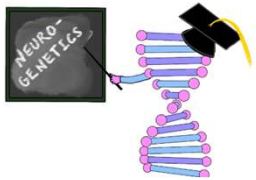
ADA2 Enzyme Activity

Sources of Plasma	Plasma ADA2 Activity, mU per mL mean ± sd (range)
ADA2 deficient patients (n = 55)	0.4 ± 0.5 (0 – 2.5)
ADA2 carriers (n = 46)	5.7 ± 1.9 (2.9 – 11.4)
At risk, not ADA2-deficient (n = 154)	20.9 ± 13.2 (5.3 – 82.8)



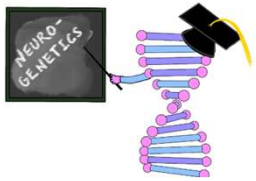
What is a Functional Study?

- Measuring the activity of the protein (enzyme, receptor, channel) in tissue (blood, skin, muscle) encoded by the gene or a biomolecule that is increased or decreased because of the enzymatic block can help in establishing pathogenicity of a genetic variant.
- Traditionally, functional/biochemical testing was ordered first line, but now is often done after NGS (panel or exome) testing
- Functional/biochemical testing can be helpful in cases where a variant is classified as VUS or only a single variant is identified in an AR condition, but there is a strong phenotypic match.



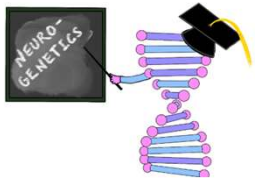
Examples

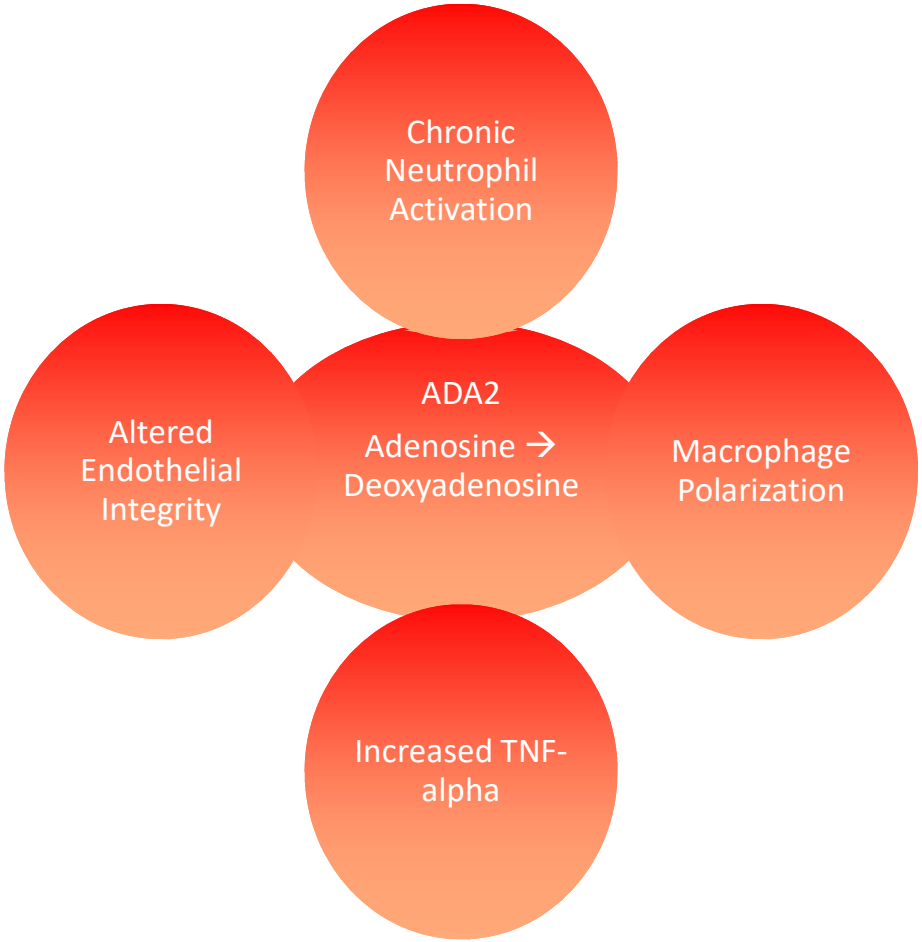
	Gene	Study
1.	*OTC*	Plasma amino acid, urine orotic acid and ornithine transcarbamylase activity in liver
2.	mt DNA variants	Muscle biopsy to measure electron transport chain activity
3.	*PDH1A* variant	Skin biopsy to measure pyruvate dehydrogenase activity
4.	*CFTR* variant	Sweat chloride test
5.	Hemoglobinopathies	Hb electrophoresis

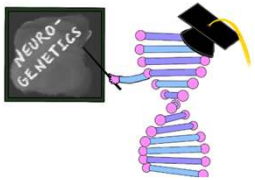


Deficiency of Adenosine Deaminase 2

- Also known as Vasculitis, Autoinflammation, Immunodeficiency and Hematologic Defects Syndrome (VAIHS, OMIM 615688).
- Typical clinical manifestations: fever, elevated inflammatory markers, livedoid rash, and stroke.
- Wide phenotypic variation.
- Additional systemic manifestations include splenomegaly, hepatomegaly, hypertension and abdominal, muscle or joint pain.
- Neuroimaging findings: acute or chronic lacunar infarcts in the deep nuclei and brainstem, usually sparing the subcortical white matter and hemorrhagic and ischemic strokes. Although early cases of **hemorrhagic strokes were in the setting of anti-platelet and/or anti-coagulant agents**, as was the case here, it appears that hemorrhage may also be related to the underlying vasculopathy

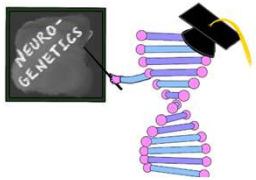



Chronic Neutrophil Activation
Altered Endothelial Integrity
ADA2
Adenosine → Deoxyadenosine
Macrophage Polarization
Increased TNF-alpha


NEURO-
GENETICS

Mechanism and Treatment

- The pathophysiology is related to a defect in nucleotide metabolism that leads to macrophage polarization, neutrophil activation, and endothelial dysfunction.
- **TNF inhibition** is the current treatment paradigm for DADA2 as TNF plays an important role in mediating perivascular inflammation in this disease.
  - TNF inhibitors = effective at preventing further strokes.
- For our patient, it is unclear if the vertebral artery compression played a role in his initial ischemic infarcts.
  - It is conceivable that this finding distracted from the diagnosis of DADA2 but given the location of his strokes, it is impossible to rule it out as a potential etiology of her initial strokes.



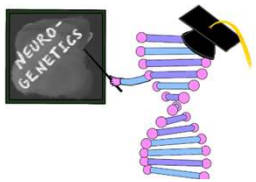
Take Home Points

Monogenic causes of stroke can be broadly classified as vascular, metabolic, and coagulation disorders.

AR inheritance is characterized by members of same generation and both sexes affected. Consanguinity increases risk.

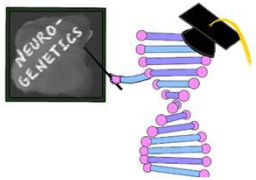
Functional studies can help establish pathogenicity of a genetic variant in select disorders where a tissue biomarker is available.

DADA2 is a disorder of nucleotide metabolism which leads to periodic fevers, rash and lacunar infarcts. Strokes can be prevented with TNFi.



Suggested Reading

- Testai FD, Gorelick PB. Inherited metabolic disorders and stroke part 1: Fabry disease and mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes. Arch Neurol. 2010 Jan;67(1):19-24.
- Barrett KM, Meschia JF. Genetic stroke syndromes. Continuum (Minneap Minn). 2014 Apr;20(2 Cerebrovascular Disease):399-411.
- Tabarki B, Hakami W, Alkhuraish N, Graies-Tlili K, Nashabat M, Alfadhel M. Inherited Metabolic Causes of Stroke in Children: Mechanisms, Types, and Management. Front Neurol. 2021 Mar 4;12:633119.



Acknowledgements

Leads:
- Kuntal Sen (CNMC)
- Louis Dang (UM)

Core members:
- Amitha Ananth (UAB)
- Andrea Gropman (CNMC)
- Education
  - Rachel Gottlieb-Smith (UM)
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