AANP Teaching Rounds: Genotype/Phenotype Correlation in Sudden Unexpected Death in Epilepsy

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Disclosures

- None

NO PHOTOGRAPHY
OR SOCIAL MEDIA SHARING

The authors of this paper are not yet ready to share the results of this study beyond this meeting. No photography or social media sharing is allowed on this paper. Thank you!

Learning Objectives

1. Review epilepsy and SUDEP definitions and epidemiology
2. Describe the gross and microscopic features that should be evaluated in the neuropathologic workup of a seizure disorder
3. Explain the role of genetic testing in cases of epilepsy and how to select cases which may benefit from molecular diagnostics

Definition of Epilepsy / Seizure Disorder

- Diagnosed after 2 unprovoked seizures
- May be focal (partial) or generalized
  - Focal
    - Cognitive, emotional, sensory, and/or motor symptoms
    - Simple (consciousness maintained) or complex
  - Generalized
    - Absence (“petit mal”)
    - Tonic, clonic, or tonic-clonic (“grand mal”) (GTC)
    - Atonic (drop attacks)
    - Myoclonic (muscle jerks)
- Among children
  - 3-5% will have a single febrile seizure in the first five years of life
  - 30 percent will have additional febrile seizures
  - Of these, 3-6% will develop *afebrile* seizures/epilepsy

Epilepsy

- Imparts 2- to 3-fold increase in mortality (vs. age-matched controls)
  - Highest in those under age 50, and first years after dx
  - Often relate to substrate causing sz
    - Tumor, cerebrovascular disease – up to 34% of deaths in epilepsy
- Significant morbidity

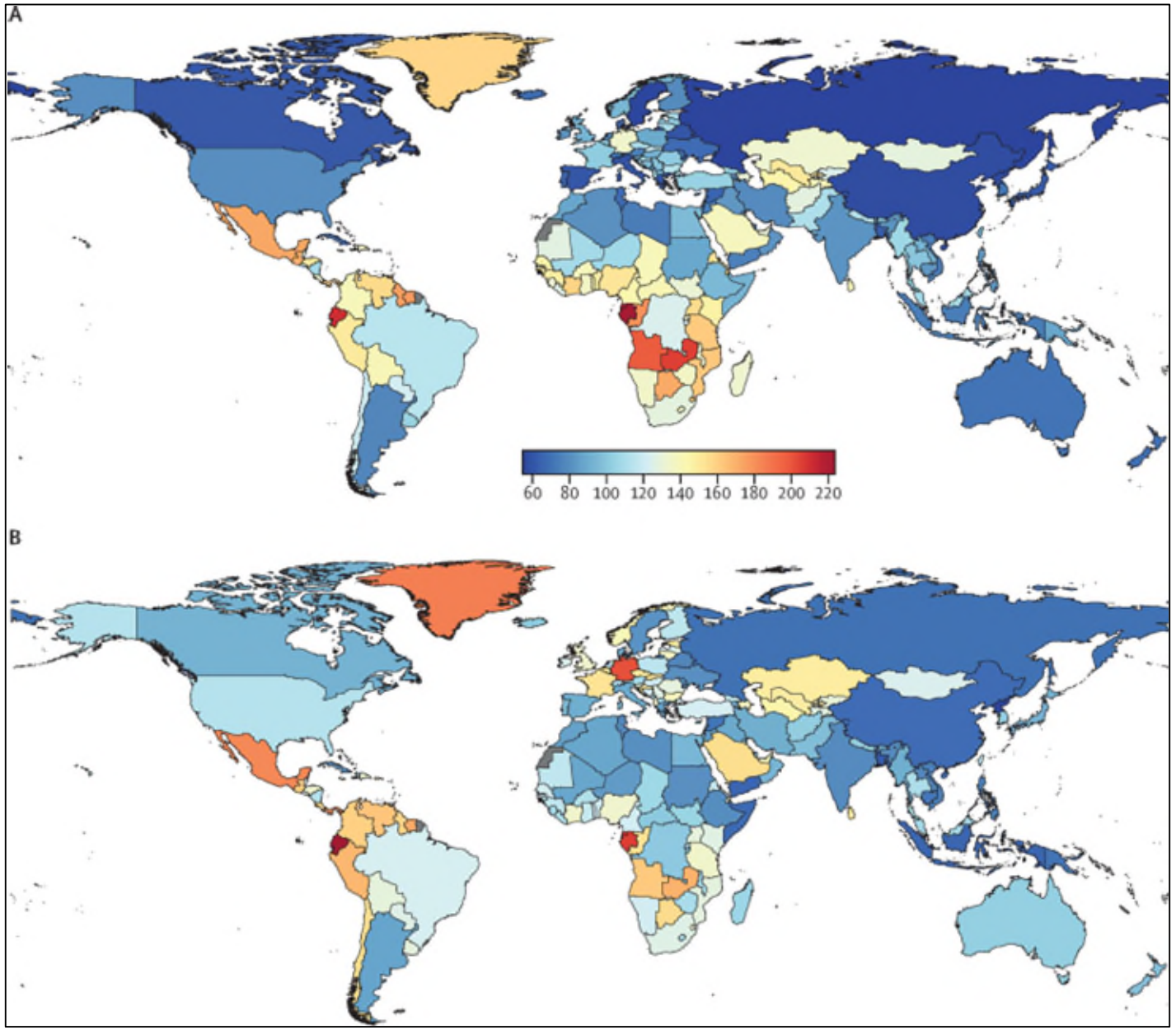
Lancet Public Health. 2025 Feb 24;10(3):e203–e227. doi: 10.1016/S2468-2667(24)00302-5

Global, regional, and national burden of epilepsy, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021

GBD Epilepsy Collaborators†

Author information Article notes Copyright and License information

PMCID: PMC11876103 PMID: 40015291



A. Age-standardized years lived with disability (per100K)
B. Prevalence of idiopathic epilepsy (per100K)

Definition of Sudden Unexpected Death in Epilepsy (SUDEP)

- A fatal complication of epilepsy
- “Sudden and unexpected [death], non-traumatic and non-drowning, without a toxicological or anatomical cause of death after complete autopsy”
- NOT during status epilepticus
- Usage of term
  - Clinical
  - Pathological (i.e., death certification)

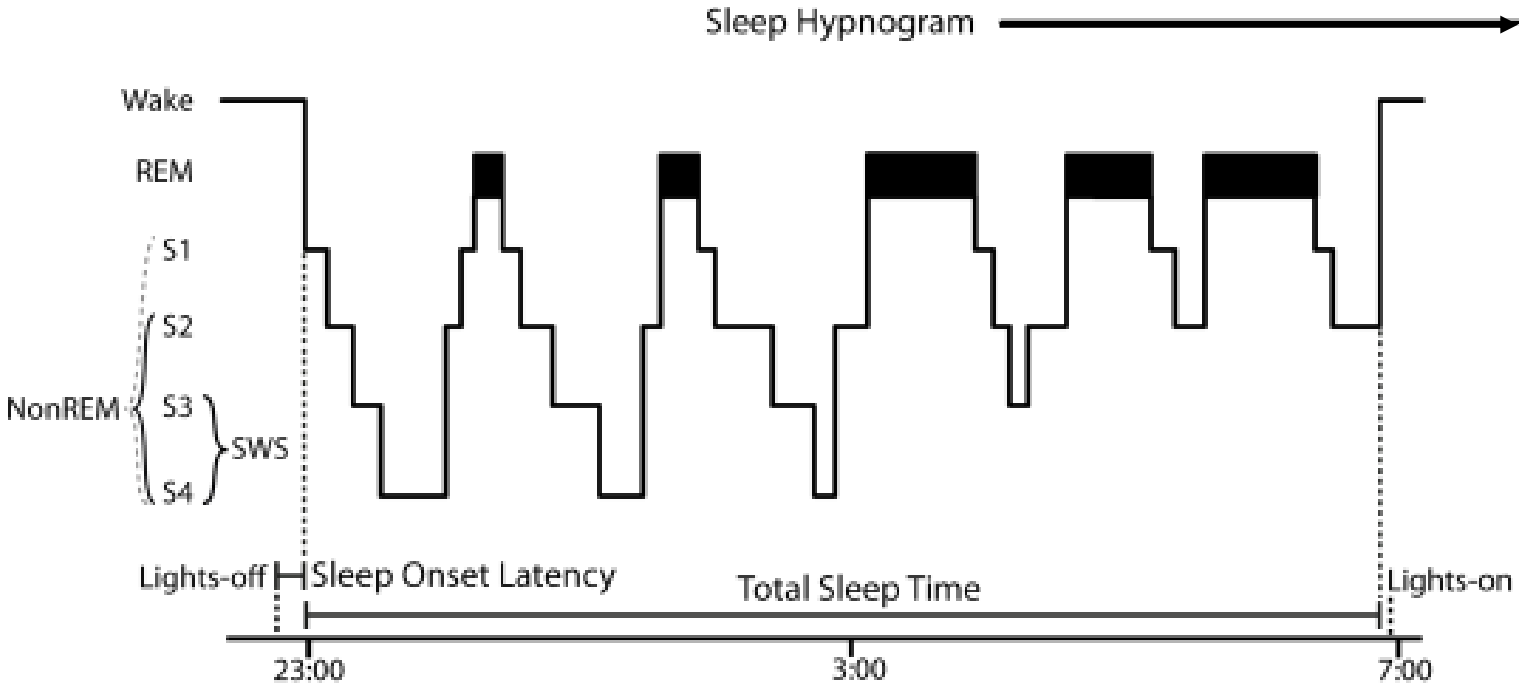
Nashef et al, Epilepsia, 2012

SUDEP

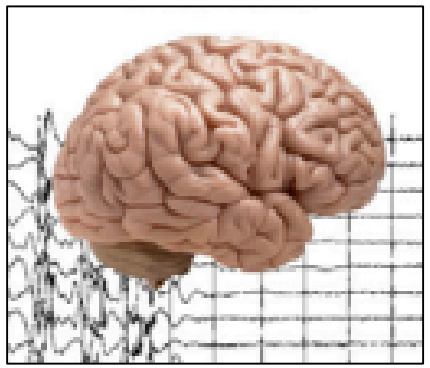
- Accounts for 7.5-17% of all epilepsy-related deaths
  - Up to 50% in medically refractory epilepsy (3 or more anti-seizure meds)
  - Multifactorial causes, including cardiac, respiratory, cerebral
- Incidence 1/1000 adult epileptics, 1/5000 pediatric cases
- Most reported cases of SUDEP are in young adults 18-40 years
- Other risk factors:
  - Male sex
  - GTC → 10-fold higher
  - Presence of three or more GTC seizures per year → 15-fold higher
  - Poor compliance with medication
  - Seizures during sleep (“state change”)

Harden et al, Neurology 2017

SUDEP



Neural Activity	Label	Stage	Type
20 seconds		S1	
	Awake	Awake	Awake
	Sleep Onset	S2 N1	NREM
Sleep Spindles	Light Sleep	S3 N2	NREM
	Slow-Wave	N3	NREM
	Dream	REM	REM



Generalized tonic-clonic seizure

?

Spread to midbrain

Spread to respiratory centers in medulla

Spread to cardiovascular centers in medulla

Ascending arousal system inhibition

Respiratory nuclei inhibition

Serotonin neuron inhibition

Loss of arousal

?

PGES

Postictal Generalized Electro-encephalographic Suppression

?

Hypoventilation / Apnea

Predisposing factors

Lack of Supervision at Night

Prone / face down

Sleep / Sleep Apnea

Intrinsic pulmonary dysfunction

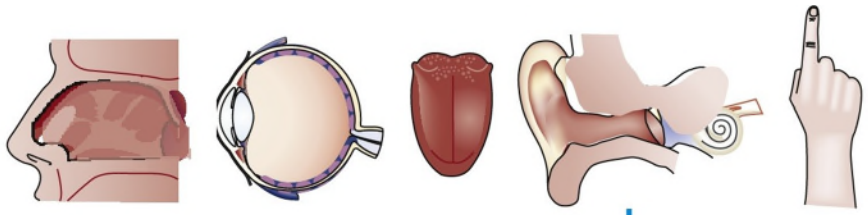
Cardiac autonomic neuron dysfunction

Congenital LQTS

Arrhythmias / Asystole

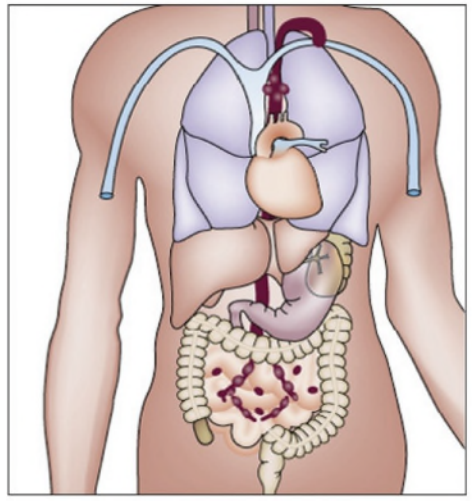
Intrinsic cardiac dysfunction

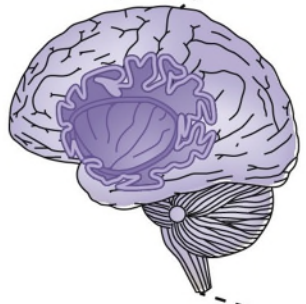
SUDEP / death

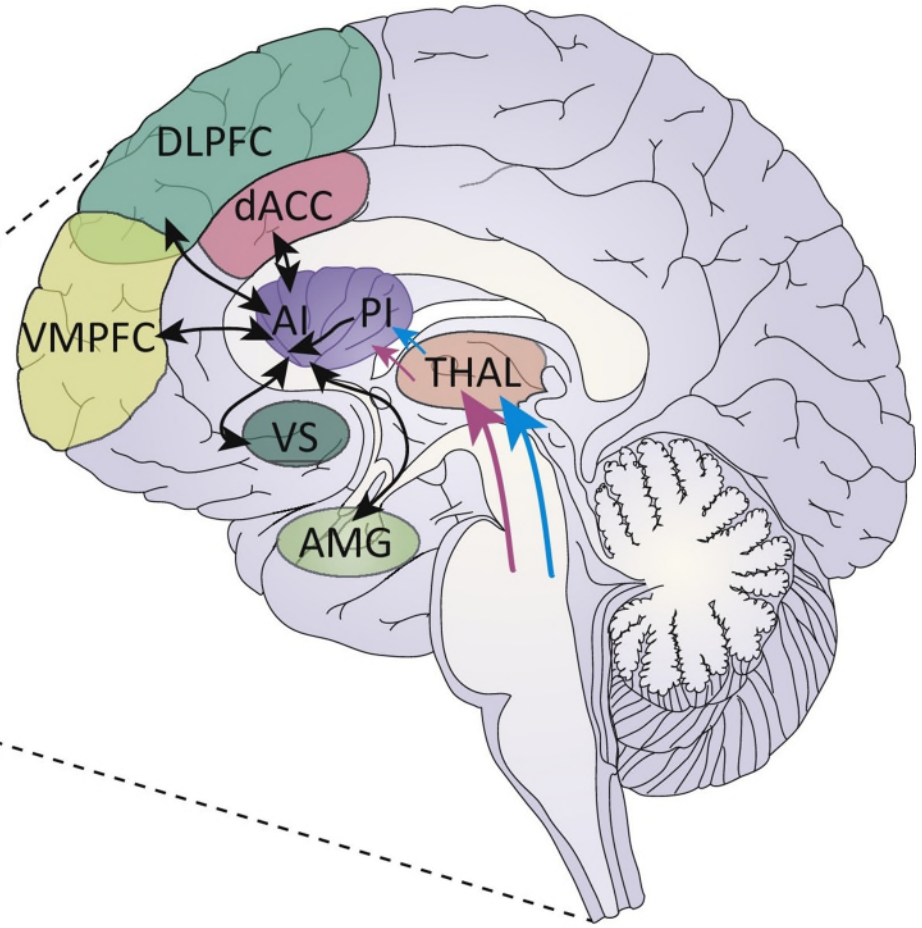


External stimuli

Internal stimuli







Cardunculus

Trends in Neurosciences

Types of SUDEP according to Nashef *et al.*[5]

Type of SUDEP	Definition
Definite SUDEP	Clinical criteria are met, and no other possible cause of death is found on anatomical and toxicological post-mortem examinations. Evidence of a terminal seizure may or may not be present, and status epilepticus must be excluded
Definite SUDEP Plus	Definite SUDEP + comorbidity other than epilepsy identified before or after death may have contributed to the death
Probable SUDEP	Clinical criteria are met, but no autopsy is available or feasible
Probable SUDEP Plus	Probable SUDEP + comorbidity that may have contributed to death
Possible SUDEP	There is evidence for a competing cause of death
Near SUDEP	A person with epilepsy who survives resuscitation for >1 h after a cardiorespiratory arrest that is not due to another identified disorder
Near SUDEP Plus	Near SUDEP + comorbidity that may have contributed to cardiorespiratory arrest
Not SUDEP	The apparent cause of death is not SUDEP
Unknown	Incomplete information

Manner=Natural

Manner=Natural, accident or other

Shlobin et al, Brain Commun, 2024

Overlap between SUDEP and cardiac death

No autopsy

Cardiologist's classification	Neurologist's classification
SCD Criteria (2022 ES C guidelines) • Sudden natural death presumed to be of cardiac cause • 1 h from the onset of symptoms in witnessed cases or within 24 h of last being seen alive if unwitnessed	Probable SUDEP Criteria (Nashef 2012 definition) • Sudden, unexpected, witness or unwitnessed, non-traumatic and non-drowning death occurring in benign circumstances in an individual with epilepsy • With or without terminal seizures • Excluding documented status epilepticus • No other cause of death was identified on anatomical or toxicological post-mortem examination • 1 h from the onset of a known terminal event

Autopsy

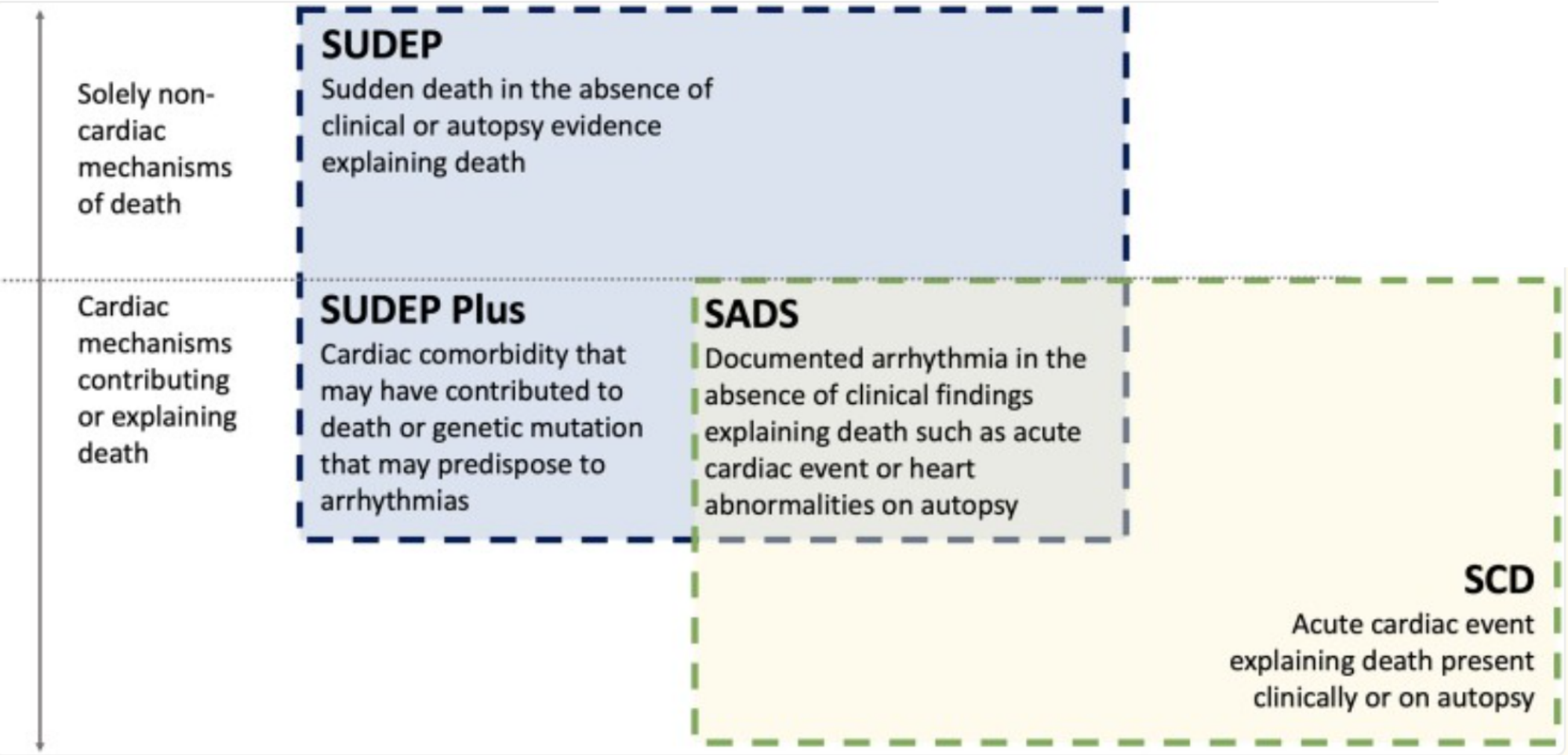
Cardiologist's classification	Neurologist's classification
SADS Criteria (2022 ES C guidelines) • Unexplained sudden death occurring in an individual over 1 year old • No other cause of death was identified on pathological or toxicological post-mortem examination No hx epilepsy	Definite SUDEP Criteria (Nashef 2012 definition) • Sudden, unexpected, witness or unwitnessed, non-traumatic and non-drowning death occurring in benign circumstances in an individual with epilepsy • With or without terminal seizures • Excluding documented status epilepticus • No other cause of death was identified on anatomical or toxicological post-mortem examination • 1 h from the onset of a known terminal event

SCD=Sudden Cardiac Death

SADS=Sudden Arrhythmic Death Syndrome

Shlobin et al, Brain Commun, 2024

Similarities and differences in SUDEP, SADS and SCD.



SADS=Sudden Arrhythmic Death Syndrome SCD=Sudden Cardiac Death

Shlobin et al, Brain Commun, 2024

Neuroanatomical (Structural) Substrates of Epilepsy

- Post-traumatic
- Developmental
  - Disruptive (Acquired)
    - Porencephaly / perinatal infarcts
    - Post-meningitic
  - Malformative
    - Focal cortical dysplasia (FCD) / malformations of cortical development (MCD) / microdysgenesis
    - Neuronal migration disorders / heterotopias

Structural and Other Substrates of SEIZURES (*NOT necessarily = Epilepsy*)

- Neoplastic
- Vascular lesions
  - Vascular malformations
  - Infarcts
- Miscellaneous
  - Alzheimer
  - Multiple sclerosis
- Toxic/metabolic
  - Alcohol withdrawal
  - Agonal metabolic derangements

(Not covering today)

The Hippocampus in Epilepsy

- Hippocampal (mesial temporal) sclerosis
  - Ammon's horn (hippocampal) sclerosis:
    - CA4 (end-folium), +/- CA1, +/- CA3
  - Mesial temporal sclerosis:
    - CA + subiculum + temporal neocortex/parahippocampal gyrus +/- amygdala
  - Cause vs. effect
  - Can accompany any other substrate
- Hippocampal dysgenesis
  - Relationship to personal or family hx of febrile seizures, best recognized in toddlers
  - Evolving understanding over pathognomonic features – no consensus

The Royal College of Pathologists
Pathology: the science behind the cure

Guidelines on autopsy practice:
Deaths in patients with epilepsy including sudden deaths

July 2019

Series authors: Dr Michael Osborn, Imperial College Healthcare NHS Trust

Specialist authors: Professor Maria Thom, Department of Neuropathology, UCL Queen Square Institute of Neurology
Dr Kieren Allinson, Department of Neuropathology, Addenbrookes Hospital

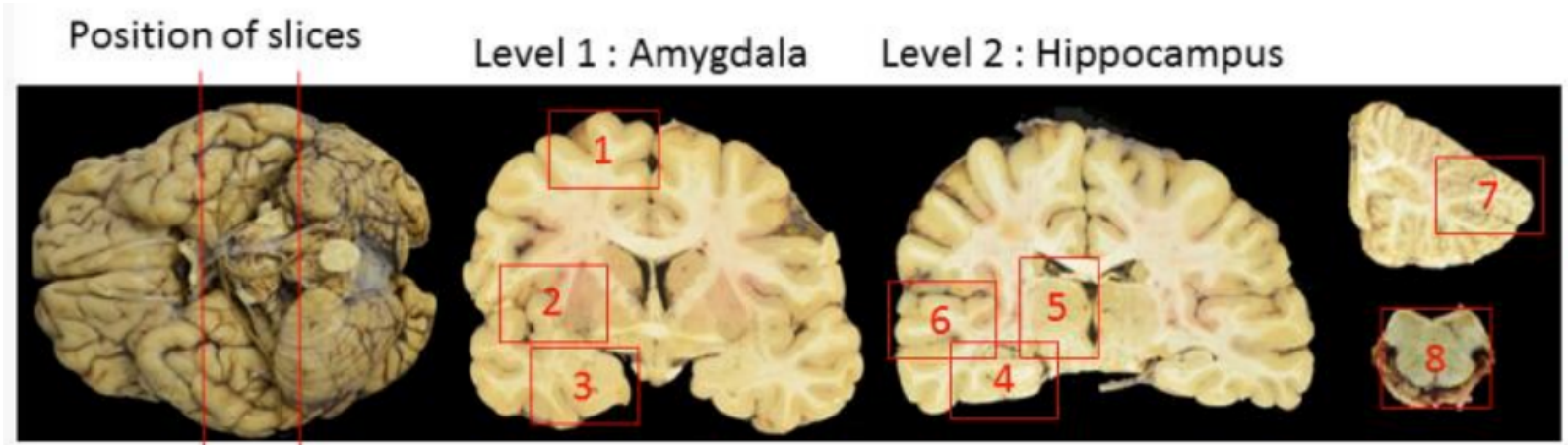
The aims of macroscopic brain examination are to:

- identify the structural cause of epilepsy
  - common lesions identified in SUDEP and epilepsy autopsy series include hippocampal sclerosis, cortical malformations (e.g. cortical dysplasia), vascular malformations/cavernomas, primary brain tumours, old contusions, etc.[17,18]
  - there is no evidence that any single neuropathology is more often associated with SUDEP.[15]
- identify the effects of previous/recent seizures
  - acute neuronal injury/eosinophilic neurones (can be limited to hippocampus or extensive if patient resuscitated for short period)
  - cerebellar atrophy, thalamic atrophy
  - cortical atrophy/scarring from seizures (status epilepticus, mitochondrial disease, epileptic encephalopathies, autoimmune encephalitides)
  - evidence of neurosurgery

Histological examination

Tissue sampling must be taken within the limits of consent from the next of kin or agreement with the coroner. Recommend sampling protocols include any grossly abnormal areas and:

1. vascular watershed region/frontal watershed regions (F1/2): acute hypoxic/ischaemic damage/meningitis/encephalitis/chronic neuronal loss (from previous seizures or episodes of status epilepticus, e.g. laminar atrophy)
2. insular cortex/basal ganglia: acute neuronal injury, hypoxic/ischaemic damage/meningitis/encephalitis
3. amygdala: acute neuronal injury, hypoxic/ischaemic damage/limbic encephalitis/chronic astrocytosis
4. hippocampus: acute neuronal injury (CA1), hypoxic changes/limbic encephalitis/hippocampal gliosis/sclerosis/malformation/neurodegenerative disease
5. thalamus: acute neuronal injury/chronic regional gliosis
6. temporal cortex (T1/2): meningitis/encephalitis/gliosis/global hypoxic changes/chronic atrophy/traumatic brain injury/neurodegenerative pathology
7. cerebellum: acute or chronic atrophy/inflammation
8. medulla: inflammatory disease.



Section guidelines for seizure work-up:

Academic Forensic Pathology

Investigation of Deaths in Seizure Patients

Dr. R. Ross Reichard MD, Rachael Vaubel, MD PhD

First Published September 1, 2014 | Review Article
https://doi.org/10.23907/2014.045

NAME POSITION PAPER

National Association of Medical Examiners Position Paper: Recommendations for the Investigation and Certification of Deaths in People with Epilepsy

Owen L. Middleton, Daniel S. Atherton, Elizabeth A. Bundock, Elizabeth Donner, Daniel Friedman, Dale C. Hesdorffer, Heather S. Jarrell, Aileen M. McCrillis, Othon J. Mena, Mitchel Morey, David J. Thurman, Niu Tian, Torbjörn Tomson, Zian H. Tseng, Steven White, Cyndi Wright, Orrin Devinsky

ABSTRACT

Sudden unexpected death of an individual with epilepsy (SUDEP) can pose a challenge to death investigators, as most deaths are unwitnessed and the individual is commonly found dead in bed. Anatomic findings (e.g., tongue/lip bite) are commonly absent and of varying specificity, limiting the evidence to implicate epilepsy as a cause of or contributor to death. Thus, it is likely that death certificates significantly underrepresent the true number of deaths in which epilepsy was a factor. To address this, members of the National Association of Medical Examiners, North American SUDEP Registry, Epilepsy Foundation SUDEP Institute, American Epilepsy Society, and the Centers for Disease Control and Prevention convened an expert panel to generate evidence-based recommendations for the practice of death investigation and autopsy, toxicological analysis, interpretation of autopsy and toxicology findings, and death certification to improve the precision of death certificate data available for public health surveillance of epilepsy-related deaths. The recommendations provided in this paper are intended to assist medical examiners, coroners, and death investigators when a sudden, unexpected death in a person with epilepsy is encountered. *Acad Forensic Pathol. 2018 8(1): 119-135*

Table 1: Death Investigation of Seizure-Related Deaths

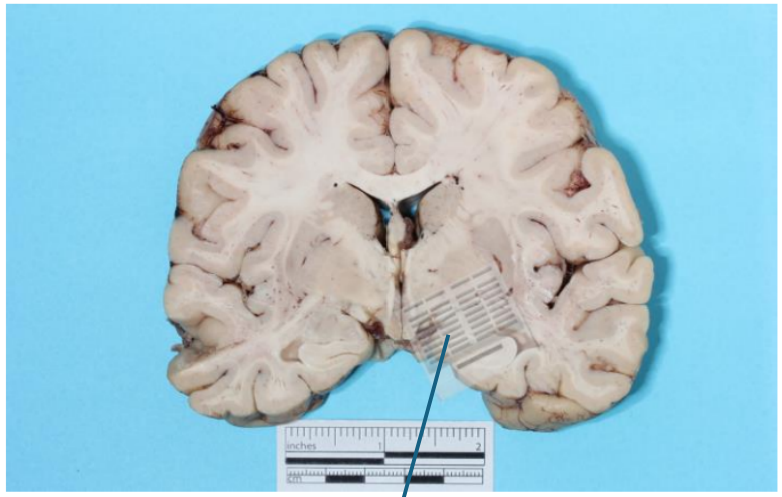
Medical History	
Establish history of epilepsy and rule out seizure mimickers	
Seizure type, frequency and underlying cause (if known)	
Age of onset, prior treatment, and medication compliance	
Comorbid medical conditions including diabetes, cardiac disease, and syncope	
Death Scene Investigation	
Body position	Most sudden unexpected death in epilepsy deaths occur in bed in prone position
Antiepileptic medications at scene	Types of drugs, dosage, date of last refill, number of pills remaining, and prescribing physician
Witnesses/family members	Circumstance of death, medication compliance, seizure frequency, recent changes in seizure activity, substance use history

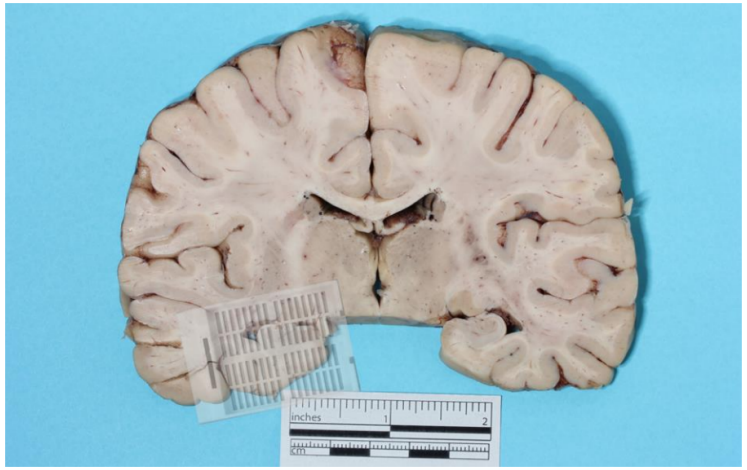
Table 2: Recommended Sections for Microscopic Analysis of the Brain (22)

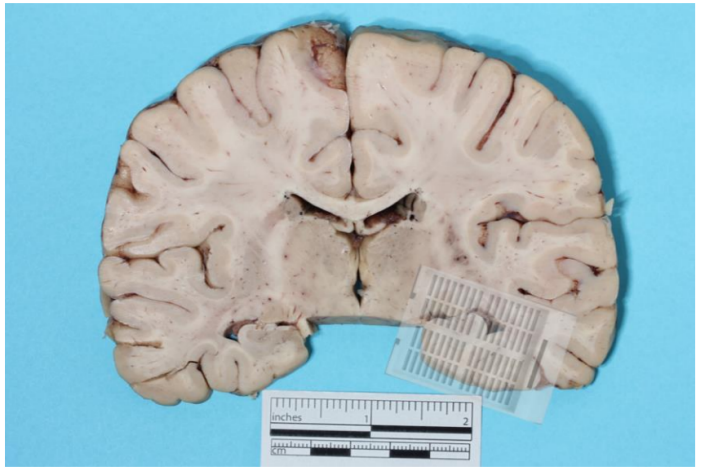
Hippocampus (right and left)
Amygdala (right and left)
Watershed (frontal and parieto-occipital parasagittal regions)
Basal ganglia
Midbrain
Pons
Medulla (at area postrema)
Hypothalamus

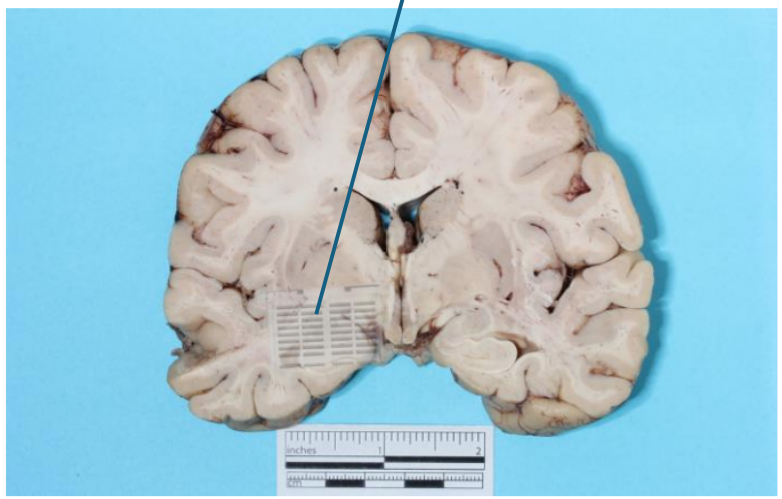
Section guidelines for seizure work-up: NYC OCME

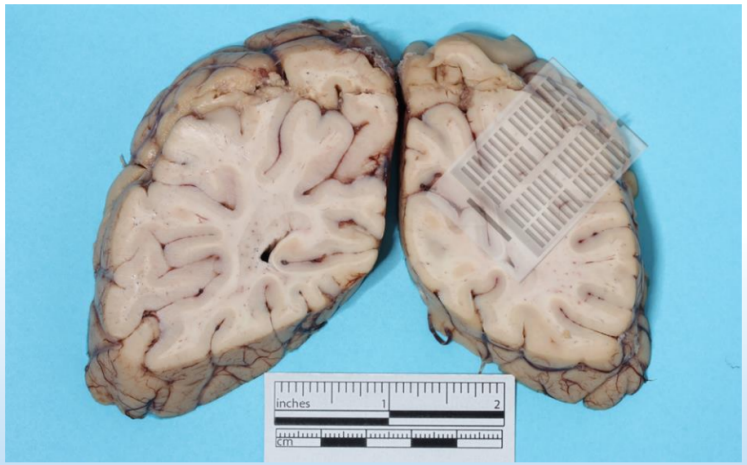
1. Right hippocampus at level of lateral geniculate nucleus and parahippocampal gyrus
2. Left hippocampus at level of lateral geniculate nucleus and parahippocampal gyrus
3. Amygdala, right (notched) and left
4. Parieto-occipital cortex (“triple watershed”)
5. Cerebellar hemisphere including dentate nucleus
6. Any macroscopic abnormality

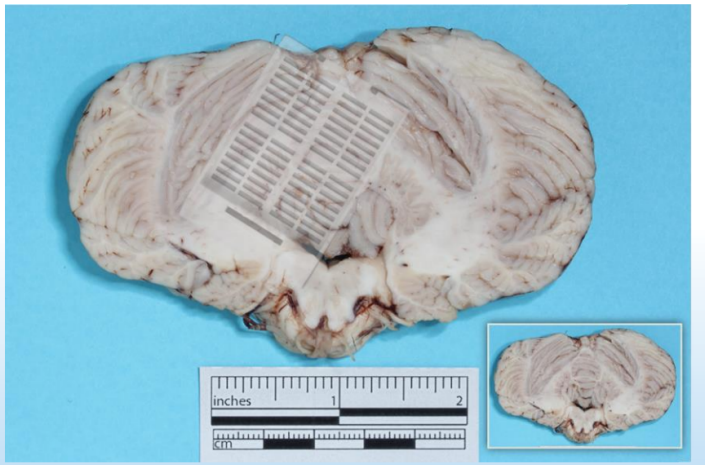












Molecular Testing Studies in Epilepsy

ARTICLE OPEN ACCESS

Multigene Panel Testing in a Large Cohort of Adults With Epilepsy

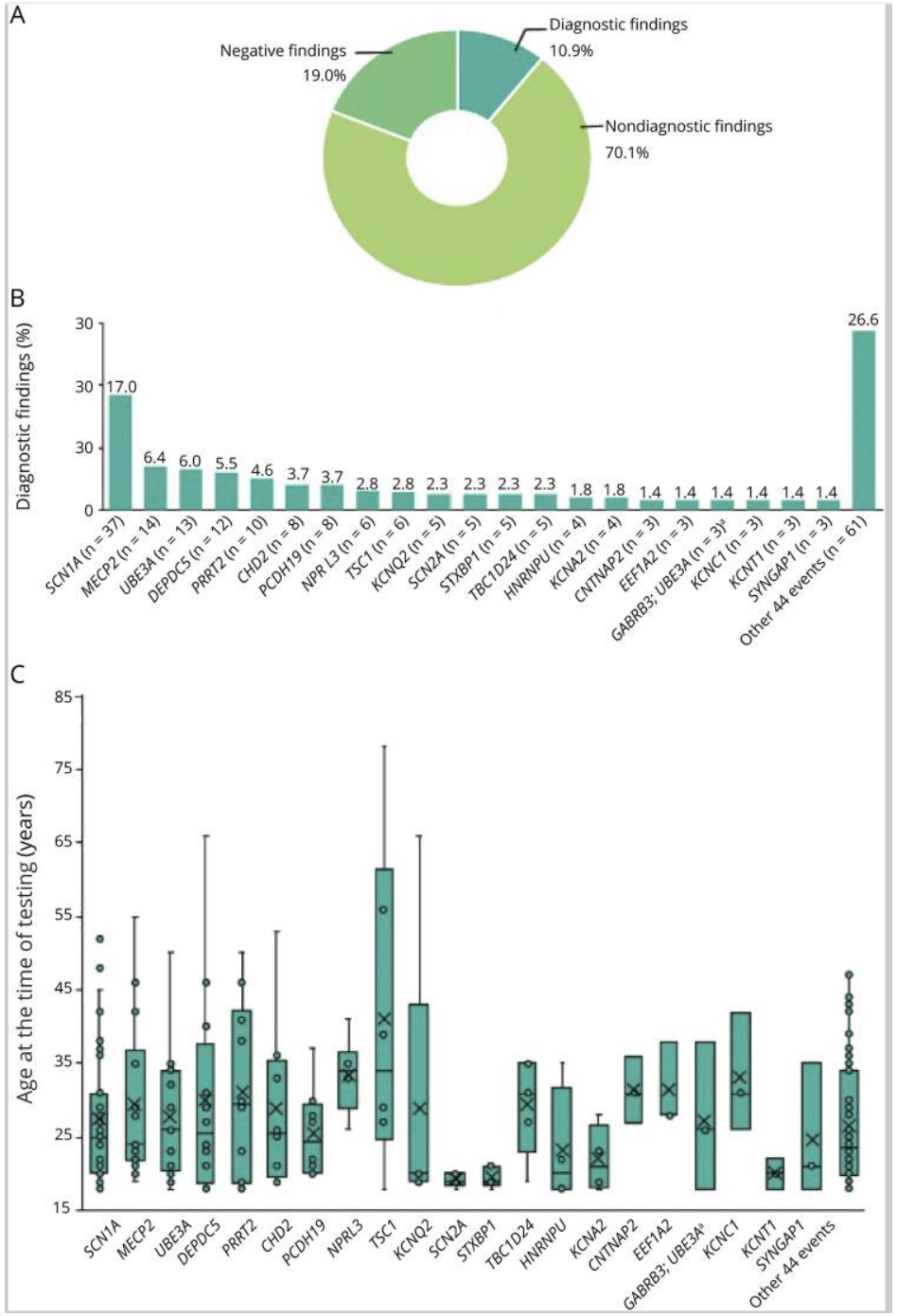
Diagnostic Yield and Clinically Actionable Genetic Findings

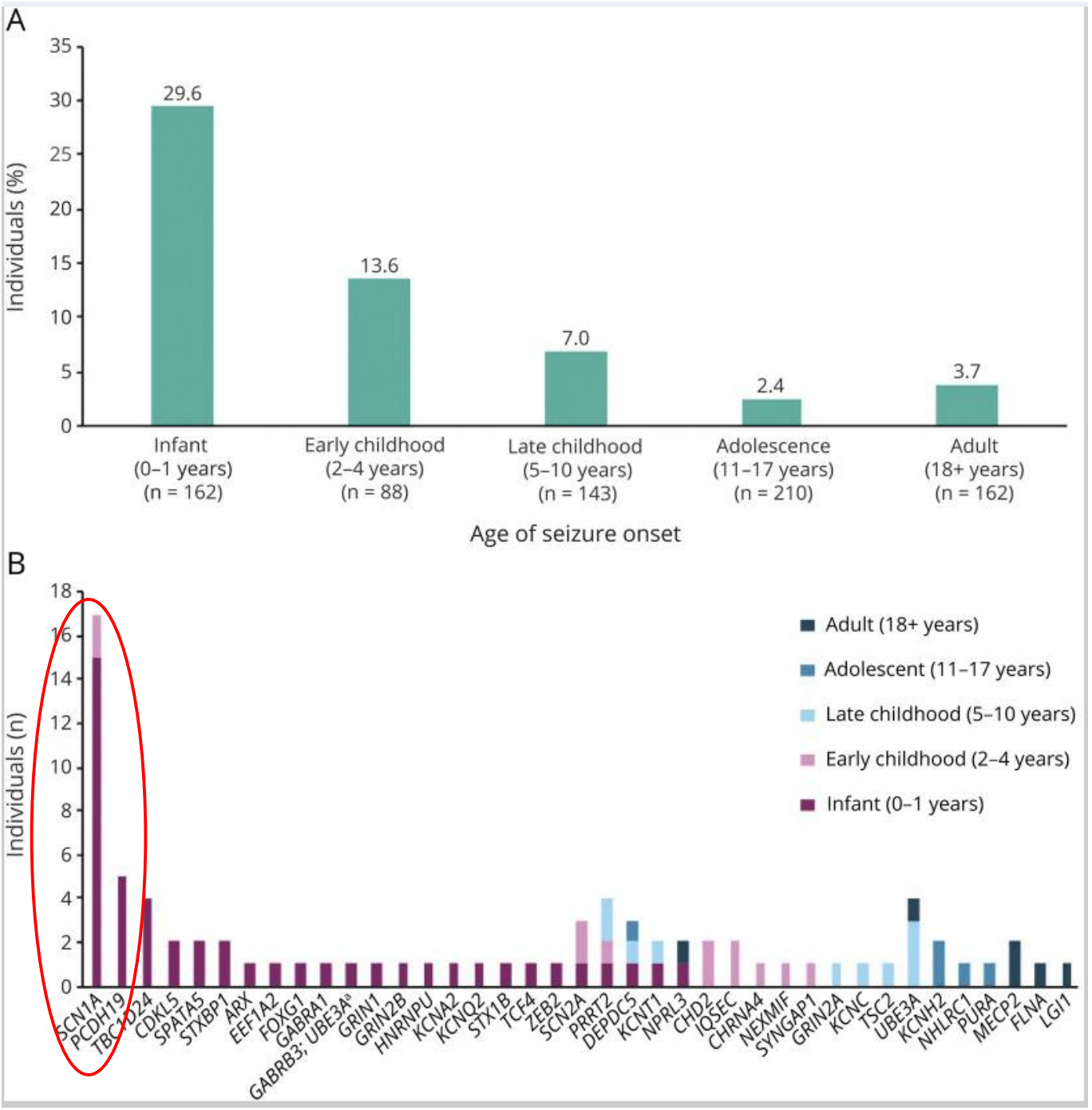
Dianalee McKnight, PhD, Sara L. Bristow, PhD, Rebecca M. Truty, PhD, Ana Morales, MS, Molly Stetler, MS, M. Jody Westbrook, PhD, Kristina Robinson, PhD, Darlene Riethmaier, MS, Felippe Borlot, MD, Marissa Kellogg, MD, Sean T. Hwang, MD, Anne Berg, PhD, and Swaroop Aradhya, PhD

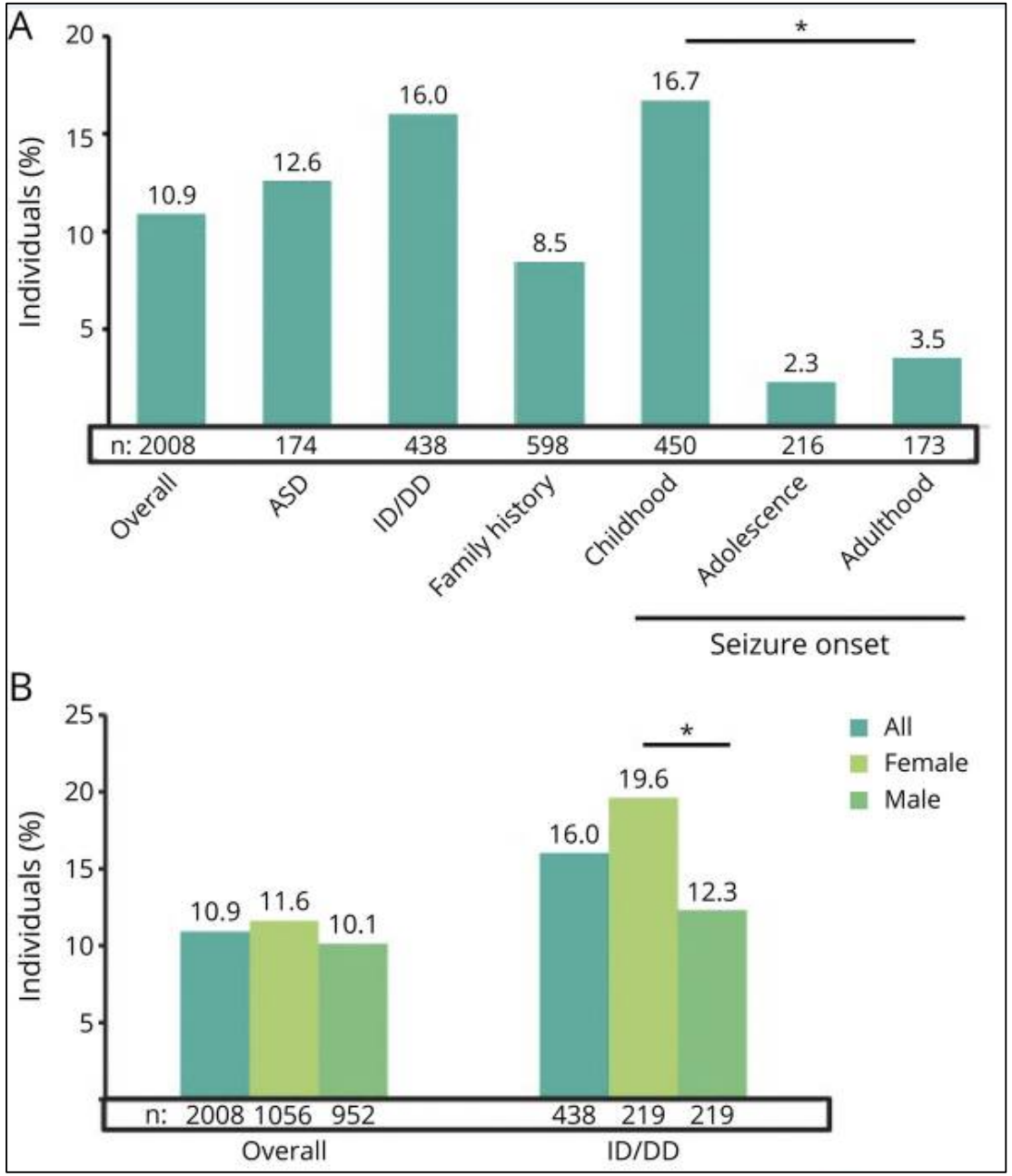
Correspondence
Dr. McKnight
dee.mcknight@invitae.com

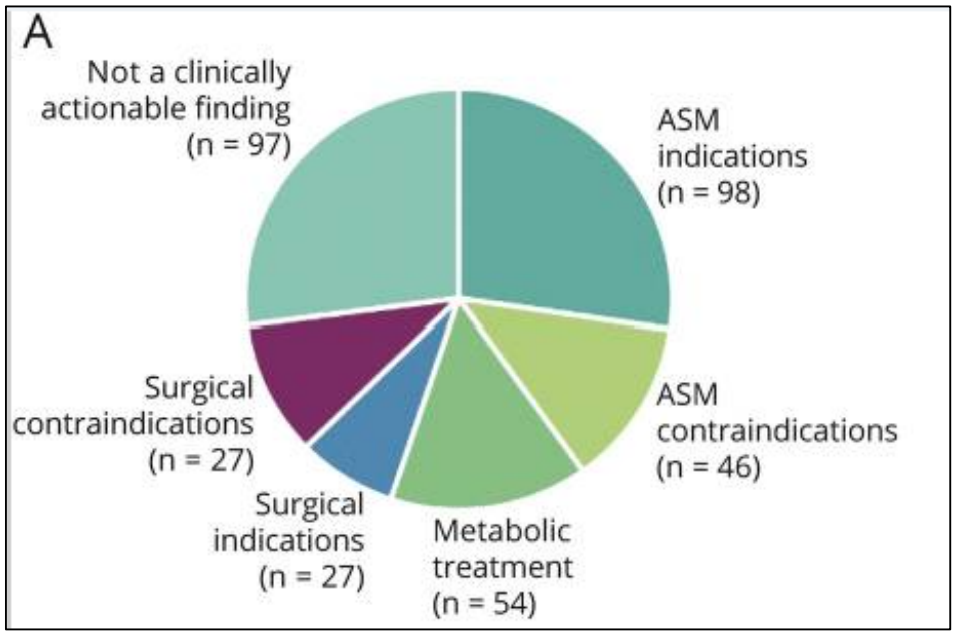
Neurol Genet 2022;8:e650. doi:10.1212/NXG.0000000000000650

- Next-gen sequencing-based, targeted gene panel (89-189 genes), over 5-year period
  - Single-nucleotide variants, small insertions or deletions (indels), structural variants, exon-level copy number variants (CNVs)
  - Classified as benign/likely benign (B/LB), of uncertain significance (VUS), or pathogenic/likely pathogenic (P/LP)
- Unrelated individuals ≥18yo (n=2,008; 52.6%F); mean age at testing 28.7y (range 18-90y)
- Seizure onset grouped as infant (0-1yr), early childhood (2-4y), late childhood (5-10y), adolescence (11-17y) and adult


A
Diagnostic findings
10.9%
Negative findings
19.0%
Nondiagnostic findings
70.1%
B
Diagnostic findings (%)
SCN1A (n = 37)
MECP2 (n = 14)
UBE3A (n = 13)
DEPDC5 (n = 12)
PRRT2 (n = 10)
CHD2 (n = 8)
PCDH19 (n = 8)
NPRL3 (n = 6)
TSC1 (n = 6)
KCNQ2 (n = 5)
SCN2A (n = 5)
STXBP1 (n = 5)
TBC1D24 (n = 5)
HNRNPU (n = 4)
KCNA2 (n = 4)
CNTNAP2 (n = 3)
EEF1A2 (n = 3)
GABRB3; UBE3A (n = 3)[b]
KCNC1 (n = 3)
KCNT1 (n = 3)
SYNGAP1 (n = 3)
Other 44 events (n = 61)
C
Age at the time of testing (years)
SCN1A
MECP2
UBE3A
DEPDC5
PRRT2
CHD2
PCDH19
NPRL3
TSC1
KCNQ2
SCN2A
STXBP1
TBC1D24
HNRNPU
KCNA2
CNTNAP2
EEF1A2
GABRB3; UBE3A[b]
KCNC1
KCNT1
SYNGAP1
Other 44 events


A
Individuals (%)
29.6
13.6
7.0
2.4
3.7
Infant (0–1 years) (n = 162)
Early childhood (2–4 years) (n = 88)
Late childhood (5–10 years) (n = 143)
Adolescence (11–17 years) (n = 210)
Adult (18+ years) (n = 162)
Age of seizure onset
B
Individuals (n)
Adult (18+ years)
Adolescent (11–17 years)
Late childhood (5–10 years)
Early childhood (2–4 years)
Infant (0–1 years)
SCN1A
PCDH19
TBC1D24
CDKL5
SPATA5
STXBP1
ARX
EEF1A2
FOXG1
GABRA1
GABRB3; UBE3A[b]
GRIN1
GRIN2B
HNRNPU
KCNA2
KCNQ2
STX1B
TCF4
ZEB2
SCN2A
PRRT2
DEPDC5
KCNT1
NPRL3
CHD2
IQSEC
CHRNA4
NEXMIF
SYNGAP1
GRIN2A
KCNC
TSC2
UBE3A
KCNH2
NHLRC1
PURA
MECP2
FLNA
LGI1





Clinically actionable genetic findings

Note: NO PATHOLOGY

Ann Neurol. 2016 Apr;79(4):522-34. doi: 10.1002/ana.24596. Epub 2016 Feb 2.

Exome-based analysis of cardiac arrhythmia, respiratory control, and epilepsy genes in sudden unexpected death in epilepsy

Richard D Bagnall [1 2], Douglas E Crompton [3 4], Slavé Petrovski [4 5], Lien Lam [1 2], Carina Cutmore [1 2], Sarah I Garry [4], Lynette G Sadleir [6], Leanne M Dibbens [7], Anita Cairns [8], Sara Kivity [9], Zaid Afawi [10], Brigid M Regan [4], Johan Duflou [2 11], Samuel F Berkovic [4], Ingrid E Scheffer [4 12 13 14], Christopher Semsarian [1 2 15]

Affiliations + expand

PMID: 26704558 DOI: 10.1002/ana.24596

TABLE 1. Characteristics of the SUDEP Cohort

Characteristic	Overall	Male	Female
No. of subjects (%)	61	34 (56)	27 (44)
Age at epilepsy onset(yr) mean ± SD (range)	10.3 ± 8.2 (0–34)	10.9 ± 8.8 (0–34)	9.6 ± 7.5 (0–24)
Age at SUDEP(yr) mean ± SD (range)	28.1 ± 12.0 (1–53)	31.0 ± 11.7 (9–53)	24.4 ± 11.6 (1–40)

SD, standard deviation; SUDEP = sudden unexpected death in epilepsy.

- SUDEP cases (definite, n=54; definite-plus, n=2; and probable SUDEP, n=5)
  - Exome-sequencing and rare variant collapsing analysis with 2,936 control exomes
  - Screened for variants with frequency <0.1% and predicted to be deleterious *in silico*

- De novo mutations in 28 of 61 (46%)
  - 4 (7%) had long QT syndrome variants
  - 9 (15%) had candidate pathogenic variants in dominant cardiac arrhythmia genes
  - 15 (25%) had variants in dominant epilepsy genes
  - DEPCD5 and KCNH2 were among top 30 genes genome-wide

TABLE 3. De Novo Mutation and Pathogenic Variants in Cardiac Arrhythmia Genes

De Novo Mutations and Previously Reported Pathogenic Mutations			
ID/GeneAmino acid (ExAC AC)	**Variant**	**Epileptiform EEG/ Brain MRI**	**Epilepsy Syndrome or Coronial Evidence of Epilepsy**
EP11/*KCNH2*R744* (0)	LQT2 pathogenic	NA/NA	History of epilepsy; infarct of left basal ganglia
EP19/*KCNH2*G924A (0)	LQT2 pathogenic	Yes/hippocampal sclerosis	Mesial temporal lobe epilepsy
EP40/*KCNQ1*Y662* (0)	LQT1 pathogenic	Yes/normal	Dravet syndrome
EP41/*SCN5A*I397V (0)	LQT3 de novo mutation	Yes / normal	Juvenile myoclonic epilepsy
Candidate Pathogenic Variants in Dominant Cardiac Arrhythmia Genes			
EP63/*ANK2*A1027D (0)	LQT4 novel	NA/NA	History of absence seizures and tonic clonic seizure
EP35/*ANK2*S2440N (0)	LQT4 novel	NA/normal	Temporal lobe epilepsy
EP59/*ANK2*I3903N (0)	LQT4 rare	NA/NA	History of seizures
EP39/*AKAP9*I1749T (109)	LQT11 rare	Yes/temporal heterotopia	Structural temporal lobe epilepsy
EP43/*AKAP9*R2607G (0)	LQT11 novel	Yes/NA	Juvenile myoclonic epilepsy
EP21/*HCN4*E1193Q (76)	BrS8 rare	NA/NA	History of nocturnal seizures; focal cortical dysplasia
EP55/*KCNH2*G749A (0)	LQT2 novel	NA/NA	History of epilepsy
EP14/*RYR2*C1489R (20)	CPVT1 rare	NA/NA	History of nocturnal seizures
EP12/*SCN5A*V223G (0)	LQT3 novel	NA/NA	Unspecified diagnosis of epilepsy

ExAC AC = Exome Aggregate Consortium allele count; EEG = electroencephalogram; MRI = magnetic resonance imaging; LQT = long QT syndrome; BrS1 = Brugada syndrome type 1; BrS8 = Brugada syndrome type 8; CPVT1 = catecholaminergic polymorphic ventricular tachycardia type 1; NA = not available.

TABLE 4. De Novo Mutations and Pathogenic Variants in Epilepsy Related Genes

De Novo mutations and Previously Reported Pathogenic Mutations			
ID/GeneAmino acid (ExAC AC)	**Variant Classification**	**Abnormal EEG/Brain MRI**	**Epilepsy Syndrome or Coronial Evidence of Epilepsy**
EP09/*DEPDC5*R843* (0)	FFEVF pathogenic	NA/NA	History of epilepsy; temporal lobe pathology
EP29/*GABRB3*Y182F (0)	EE de novo mutation	Yes/normal	Epileptic encephalopathy
EP38[$]/*PAFAH1B1*G162S (0)	Lissencephaly pathogenic	Yes/NA	Structural focal epilepsy
EP37/*SCN1A*G1480V (0)	GEFS+2 de novo mutation	Yes/normal	Epilepsy with myoclonic-atonic seizures
EP67/*SCN2A*R1882Q (0)	EE11 de novo mutation	Yes/normal	Epileptic encephalopathy
EP73/*SCN2A*N976K (0)	EE11 de novo mutation	Yes/microcephaly	Epileptic encephalopathy
Candidate Pathogenic Variants in Dominant Epilepsy Genes			
EP13/*CHRNA4*F66L (0)	NFLE1 novel	NA/NA	History of epilepsy
EP62/*DEPDC5*S19T (0)	FFEVF novel	NA/NA	History of epilepsy
EP70/*DEPDC5*R286* (0)	FFEVF novel	NA/NA	History of nocturnal epilepsy
EP10/*DEPDC5*R347H (1)	FFVEF rare	NA/NA	History of epilepsy
EP64/*DEPDC5*Q1016* (0)	FFVEF novel	Yes/parietaldysplasia	Structural parietal lobe epilepsy
EP23/*DEPDC5*R1332* (0)	FFVEF novel	Yes/corticaldysplasia	Structural frontotemporal lobe epilepsy
EP66/*KCNQ2*A306V (0)	EE7 novel	Yes/normal	Ohtahara syndrome
EP32/*PCDH19*N509S (0)	EE9 novel	Yes/normal	Juvenile myoclonic epilepsy
EP51/*SCN1B*R96Q (11)	GEFS+1 rare	NA/NA	History of epilepsy
EP38[$]/*SPTAN1*Q425R (6)	EE5 rare	Yes/NA	Structural focal epilepsy

ExAC AC = Exome Aggregate Consortium allele count; EEG = electroencephalogram; MRI = magnetic resonance imaging; "$" = patient has two variants in epilepsy-related genes; NFLE1 = nocturnal frontal lobe epilepsy; FFEVF = familial focal epilepsy with variable foci; GEFS+1 = genetic epilepsy with febrile seizures plus type 1; GEFS+2 = genetic epilepsy with febrile seizures plus type 2; EE = early infantile epileptic encephalopathy; NA = not available.

Molecular Testing in Sudden Death Associated with Epilepsy in a Forensic Office:

Preliminary Genotype-Phenotype Correlations

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YingYing Tang, MD, PhD
Jansen Seheult, MD, MsC
Rebecca Folkerth, MD

NYC
Office of Chief
Medical Examiner

NYU
School of Medicine
Department of Forensic Medicine

Methods

- Cases received (retro- and prospectively) over a 3-year period
- Inclusion criteria:
  - "Epilepsy" or "Seizure" on the death certificate, *OR*
  - Cases in which the Medical Examiner (ME) requested molecular genetics for a seizure disorder or syndrome (e.g., Lennox-Gastaut)
- Exclusion criteria:
  - Seizures due to trauma, alcohol/drug withdrawal, or other terminal metabolic event
- Standard NP examination for epilepsy, including examination of the formalin-fixed brain and histologic sections (epilepsy protocol)
  - Additional histologic sections on pediatric cases (standard pediatric protocol), and as dictated by the findings
- Definition *a priori* of NP features of interest (described later)
- Sequence analysis of 139 genes:
  - *ADAR, ADGRG1, ADGRV1, ALDH7A1, ALG13, AP3B2, ARFGEF2, ARHGEF9, ATPIA2, CACNA1A, CACNA1C, CACNA1E, CACNA1H, CACNA2D1, CACNA2D2, CASK, CC2D2A, CDKL5, CHD2, CHRNA2, CHRNA4, CHRNB2, CLCN2, CNPY3, CNTNAP2, CPA6, CRLF1, CSF1R, CSTB, DEPDC5, DNM1, DOLK, DAD, DYRK1A, EEF1A2, EFHC1, EPM2A, FGFR3 (Chr4:1803571), FGF12, FKTN, FLNA, FOLR1, FOXG1, GABRA1, GABRB1, GABRB3, GABRD, GABRG2, GAMT, GCH1, GFAP, GLI2, GLRA1, GNAO1, GNB5, GRIN1, GRIN2A, GRW2B, HCN1, HCN2, HCN4, HNRNPU, IER3IP1, KCNA1, KCNA2, KCNAB2, KCNB1, KCNC1, KCND2, KCNH2, KCNJ2, KCNJ10, KCNMA1, KCNQ1, KCNQ2, KCNQ3, KCNT1, KCTD7, KMT2D, LGI1, MECP2, NHLRC1, NOTCH3, NPRL2, NSD1, PAFAH1B1, PCDH19, PLCB1, PMM2, PNKP, PNPO, POLG, POLR3.4, POLR3B, PPP3C4, PRICKLE1, PRRT2, RELN, RPGRIP1L, RYR2, SCARB2, SCN1A, SCN1B, SCN2A, SCN41, SCN5A, SCN8A, SCN9A, SEPSECS, SIK1, SLC12A5, SLC13A5, SLC25A22, SLC2A1, SLC35A2, SLC6A1, SLC6A8, SMC1A, SPTAN1, STX1B, STXBP1, SUOX, SURF1, SYN1, SYNGAP1, SZT2, TBC1D24, TBCD, TCF4, TMYM67, TPP1, TSC1, TSC2, TUBA1A, TUBB2A, TUBB2B, TUBB3, TUBB4A, VPS13A*

Molecular Genetics Panel

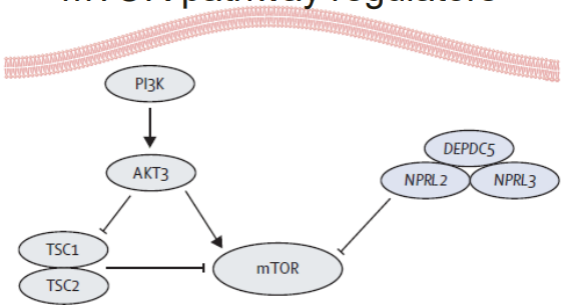
Included

- Genes known associated with dominant epilepsy syndromes
- Genes known associated with early childhood onset epileptic encephalopathy
  - Includes some neuronal migration genes
- Voltage-gated channel genes
- Folate transport deficiency
- GABA-Receptor genes and GABAergic pathway genes
- TSC1 and TSC2 genes (Tuberous sclerosis proteins 1 and 2)

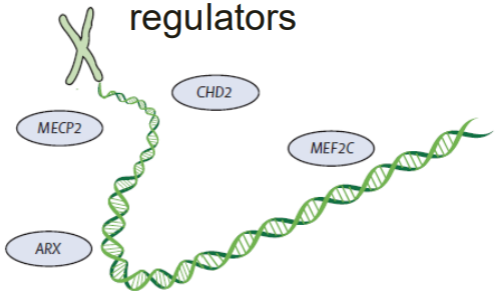
Not included

- Diseases on New Born Screening panel (Autosomal Recessive)
- Mitochondrial Disorders
- Epigenetic diseases/Imprinting diseases (Angelman syndrome)
- Epilepsy due to large-scale DNA rearrangements
- New disease-genes reported after 2017

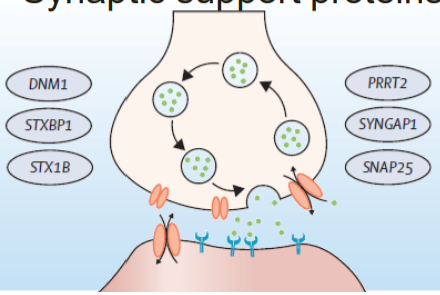
mTOR pathway regulators



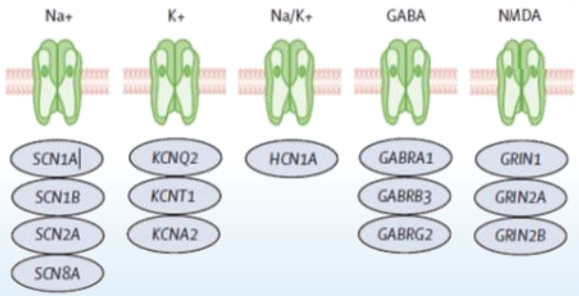
Chromatin remodeling/transcription regulators



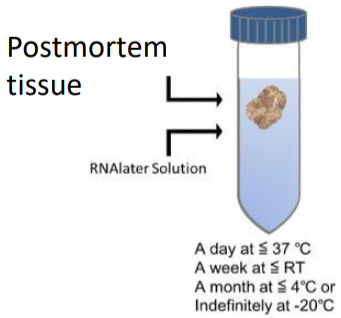
Synaptic support proteins



Ion channels (including receptors)



Molecular Methodology



RNA Stabilization Tissue Preservative

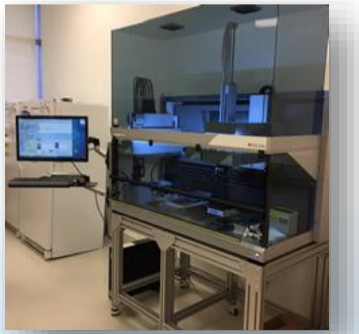
Blood Spot Card

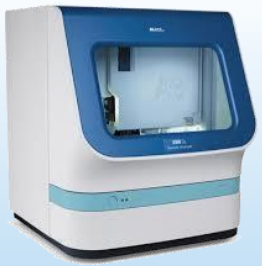
DNA extraction and quantification

DNA fragmentation and library preparation

Next Generation Sequencing

Sequencing (Low coverage region and variant confirmation)





Variant Interpretation and Reporting

Automated Sequencing Data Analysis Process

Primary NGS Data Analysis
- Quality filtering and reads alignment

Secondary NGS Data Analysis
- Variant Annotation

Tertiary NGS Data Analysis
- Variant database + Variant Interpretation

Sequencing Data Analysis
- Alignment and Annotation

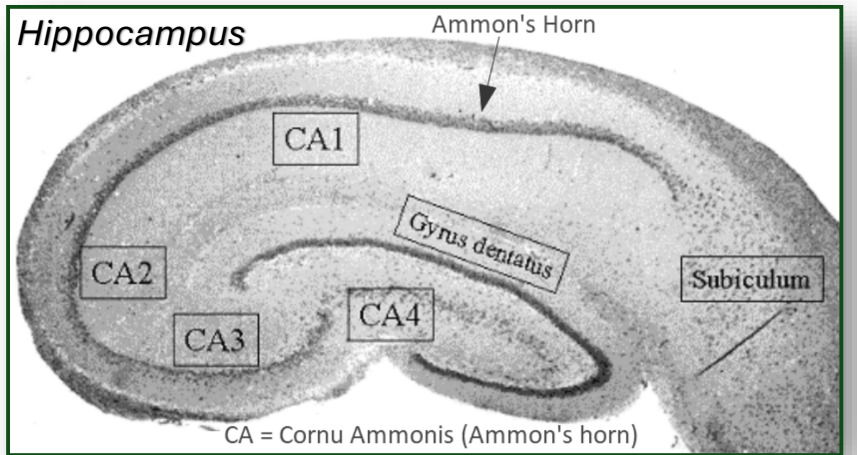
Clinical databases: ClinGen, OMIM, ClinVar, HGMD, ARVD, etc
Publications: pubmed search
MAF in 1000 genome, ESP6500, ExAC, gnomAD
In silico predictions: PP2, SIFT/proven, MutationTaster
Medical history/Neuropathological findings

Pathogenic/Likely Pathogenic | **Benign/Likely Benign**

Variant of Uncertain Significance (VUS)

Neuropathologic Features of Interest

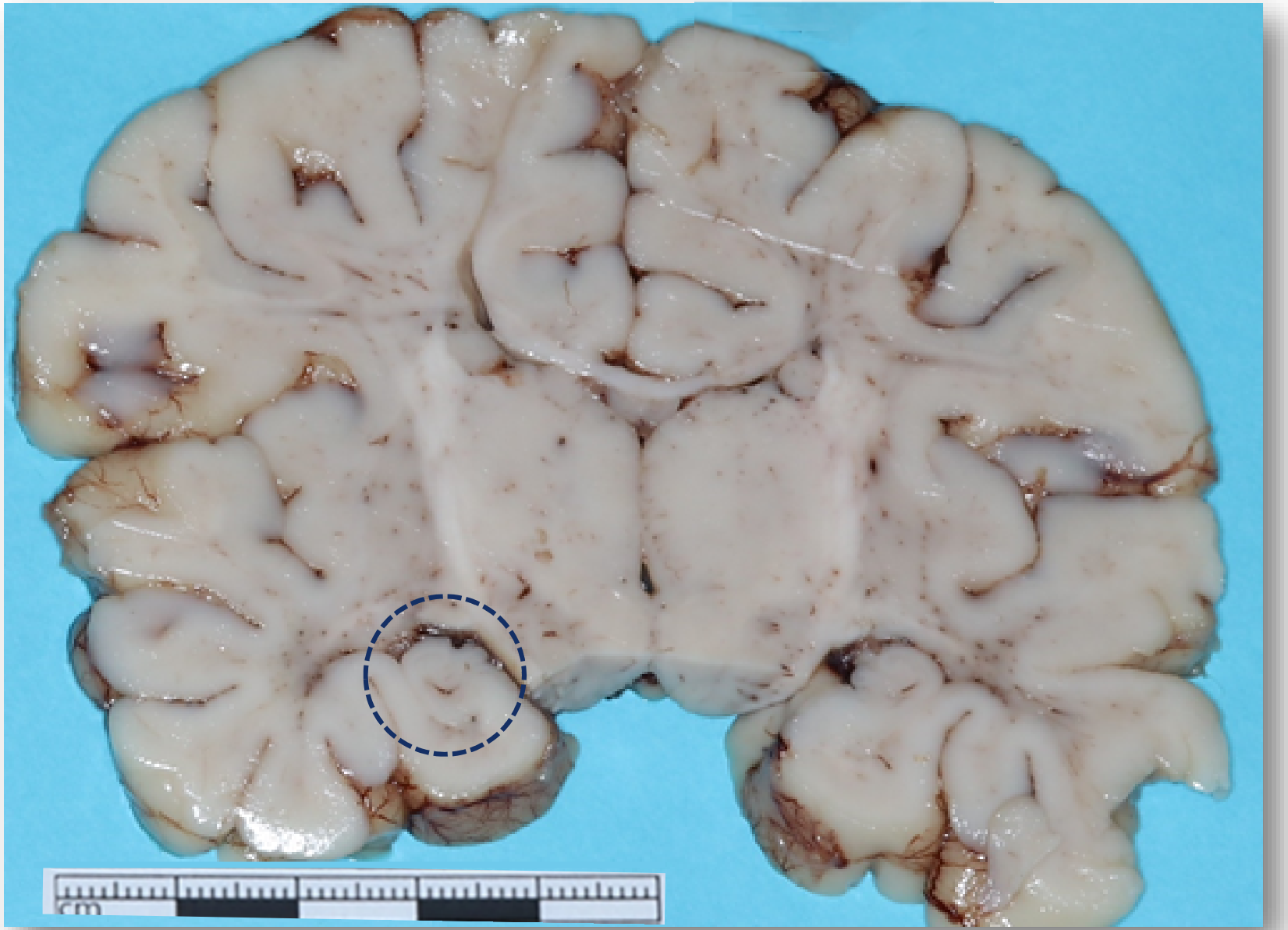
- Macroscopic Evidence of Dysgenesis
  - Hippocampal asymmetry, e.g., incomplete hippocampal infolding
  - Other findings, e.g., gyral abnormalities, nodular heterotopia
- Microscopic Lesions
  - Hippocampal dysgenesis: dentate gyrus (DG) irregularities/bilamination*
  - Hippocampal sclerosis:
    - End-folium sclerosis (EFS; neuronal loss and gliosis, CA4)
    - Mesial temporal sclerosis (MTS; neuronal loss and gliosis, CA4 and CA1/CA3 ± amygdala)
  - Dysplasia/Neuronal migration disorders, e.g., polymicrogyria (PMG), pachygyria, focal cortical dysplasia (FCD), subarachnoid glioneuronal heterotopia (SAGNH), rhombic lip heterotopia
  - Other epilepsy-associated findings, e.g., cerebellar atrophy



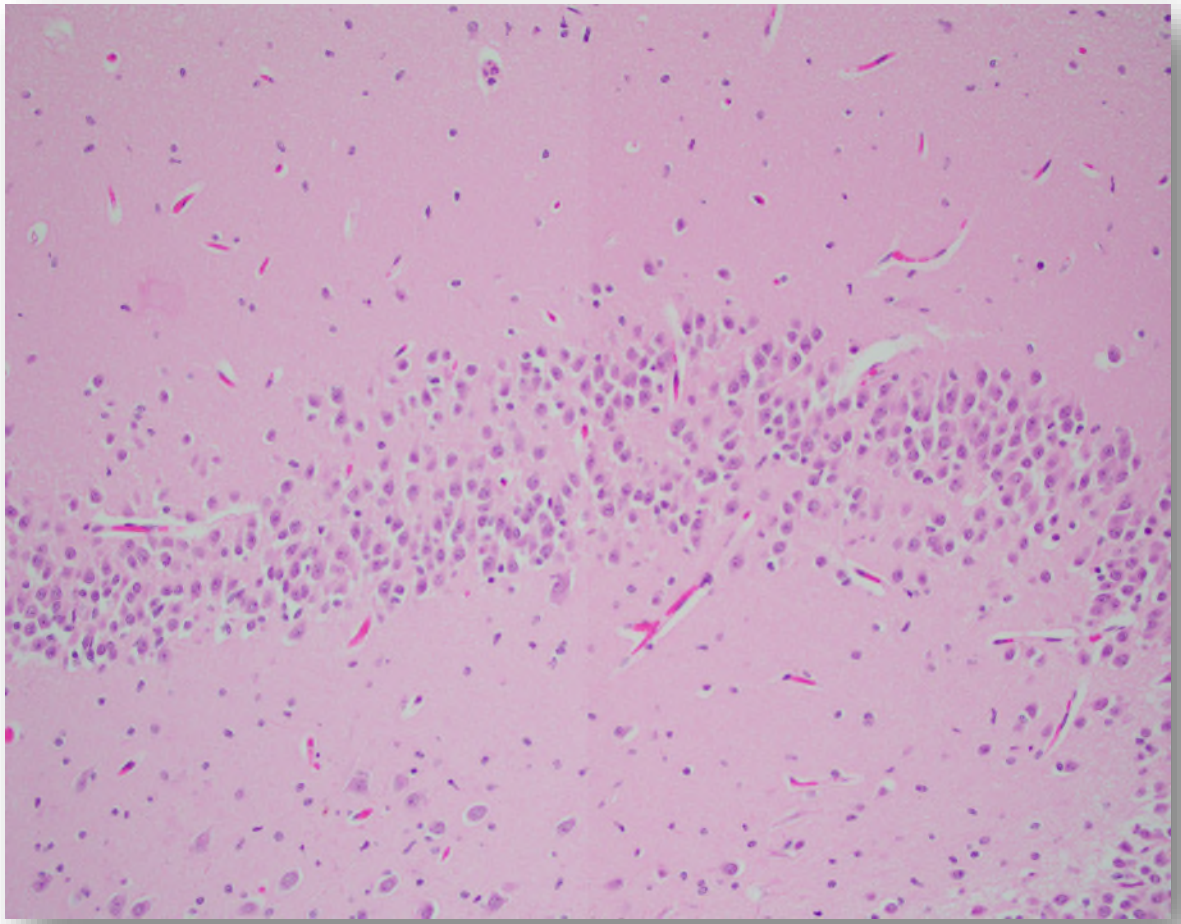
per Kinney et al. 2016

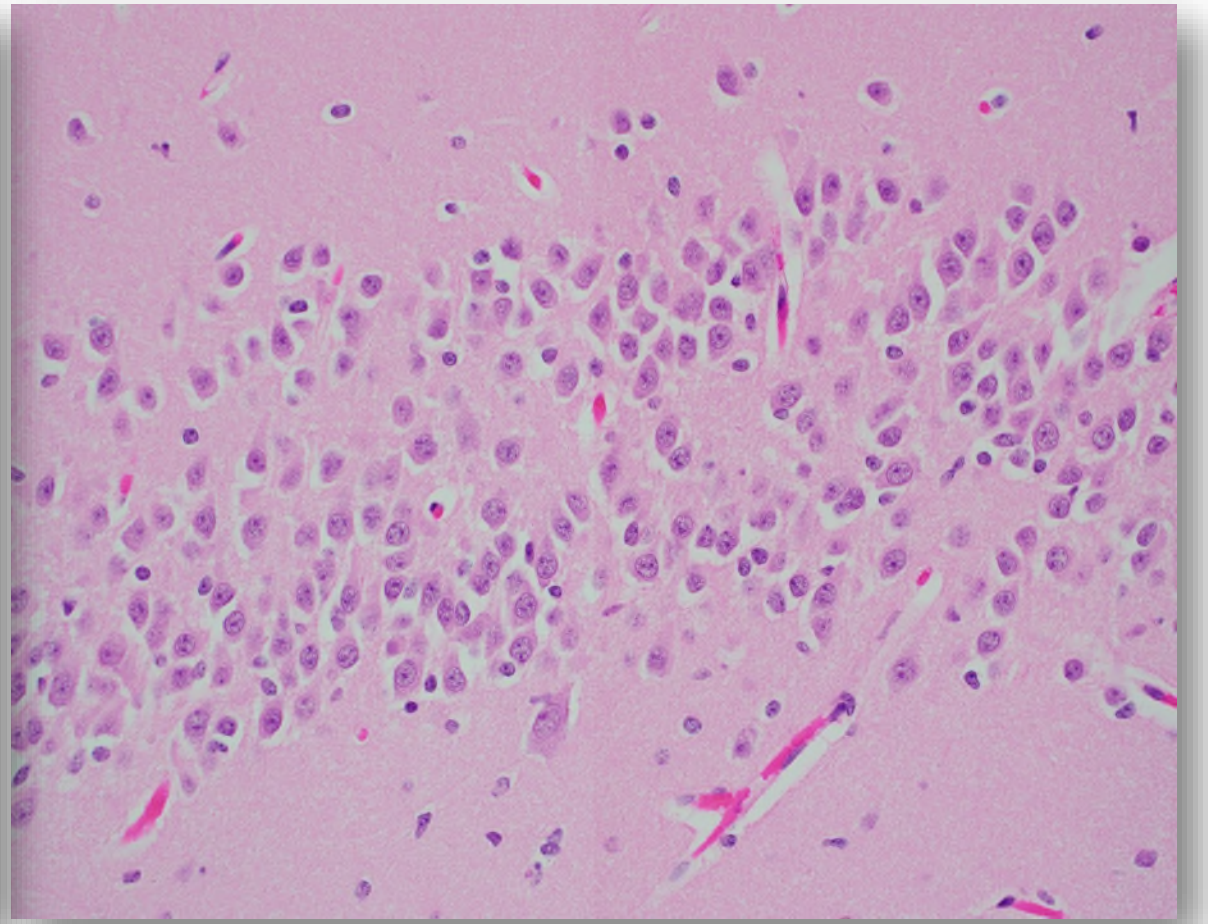
(Hippocampal Formation Maldevelopment and Sudden Unexpected Death across the Pediatric Age Spectrum)

Example: Asymmetric Hippocampi (Dysgenesis)

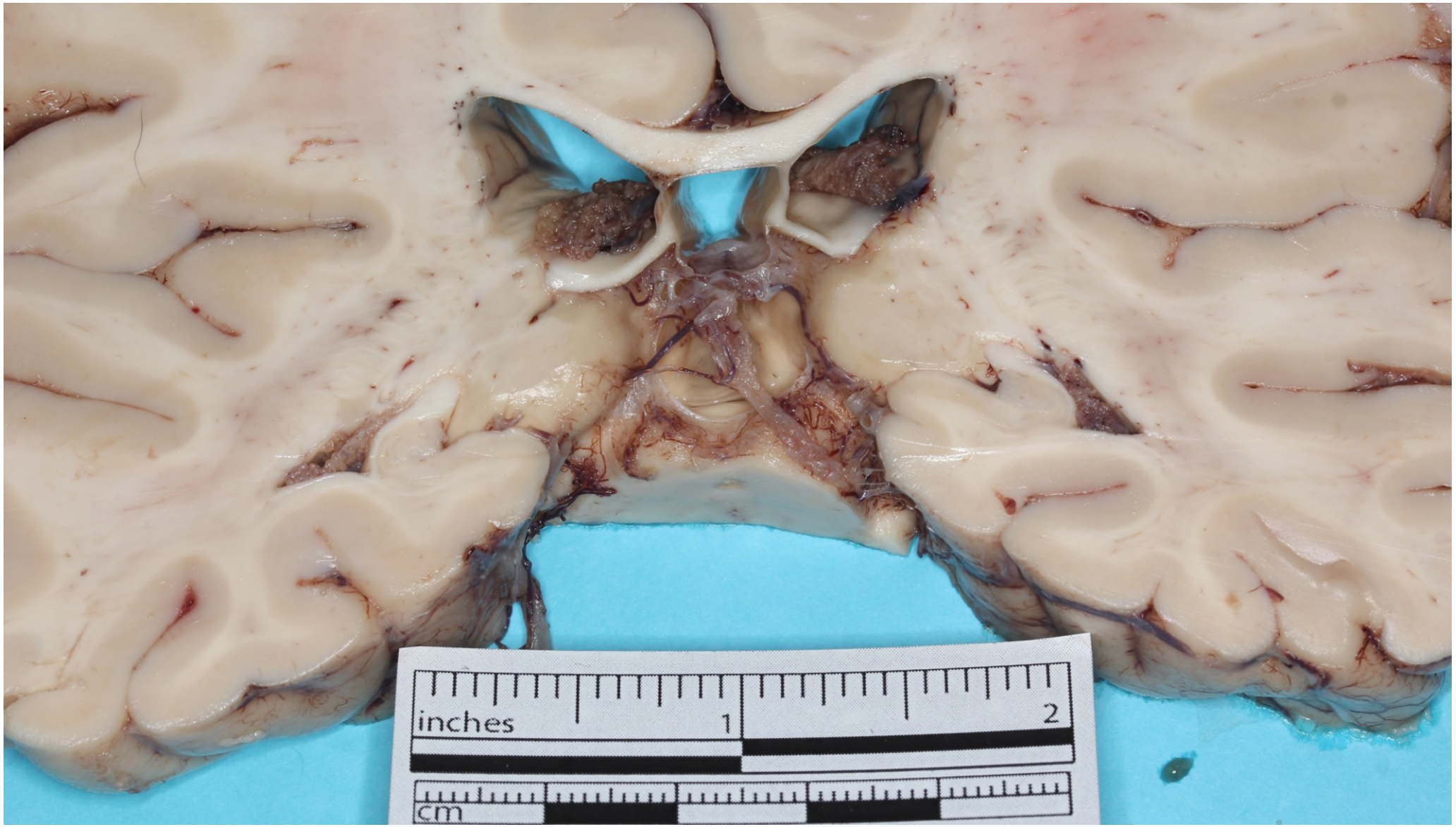


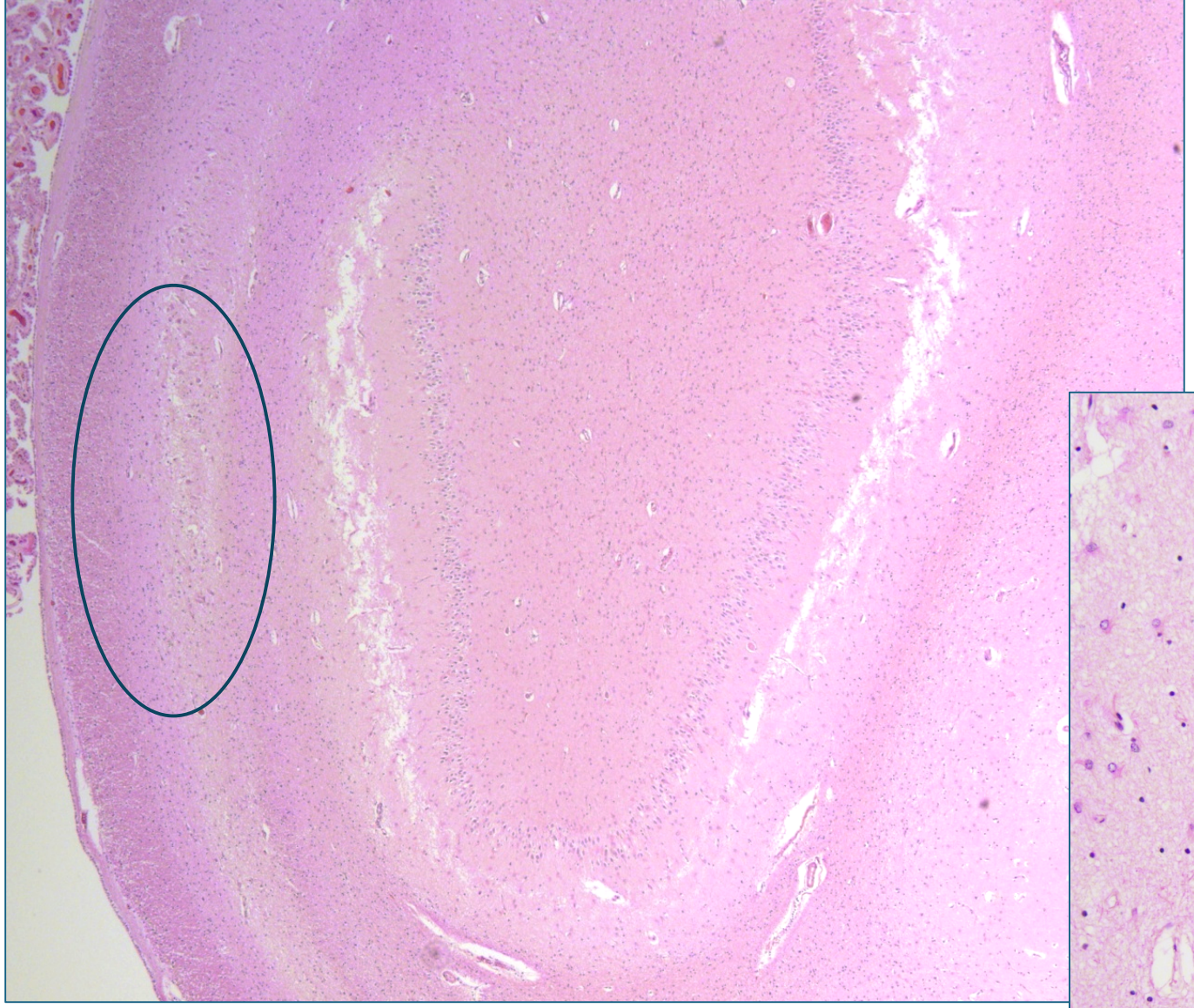
Example: Dentate Gyrus (DG) Bilamination

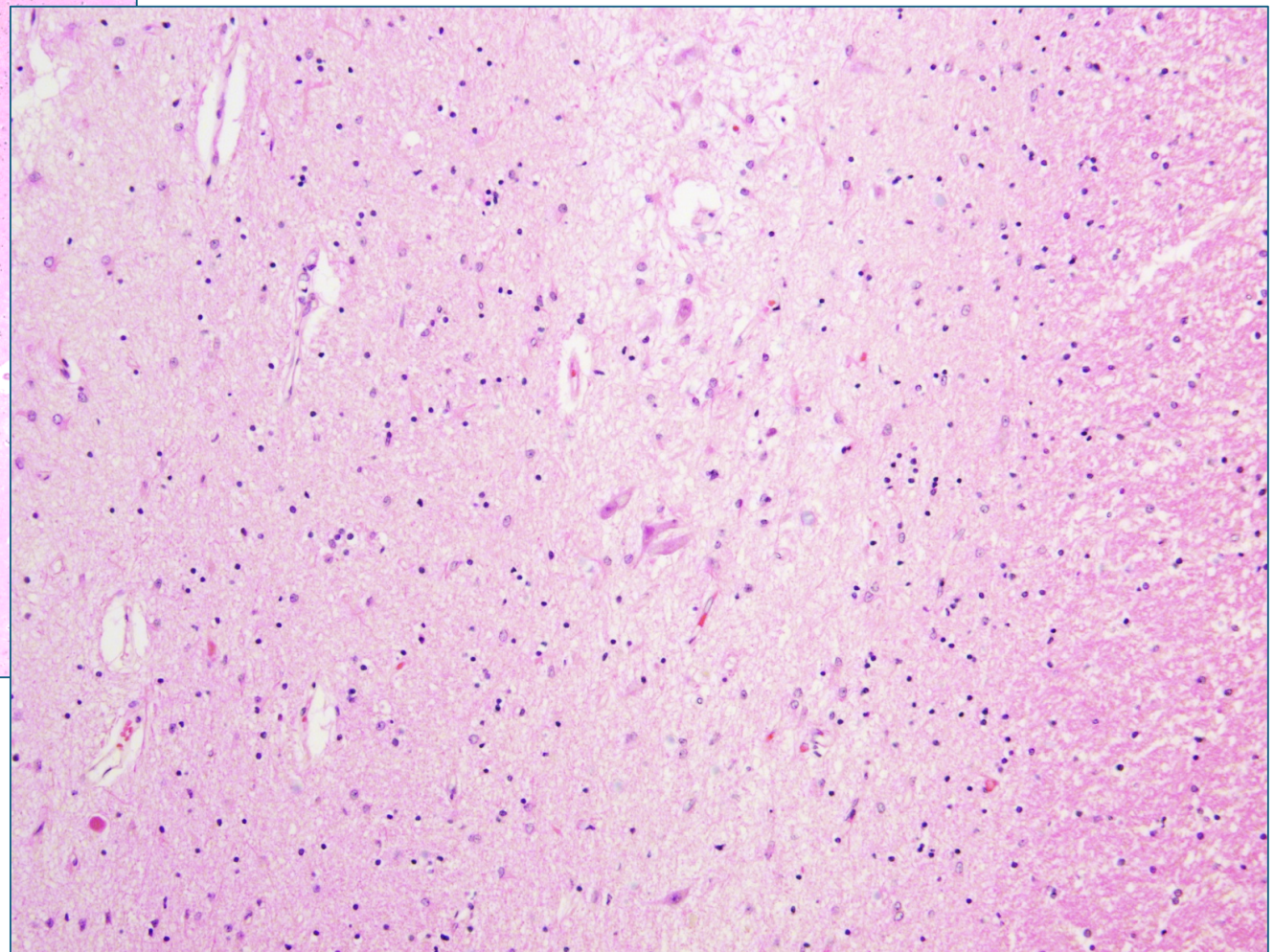




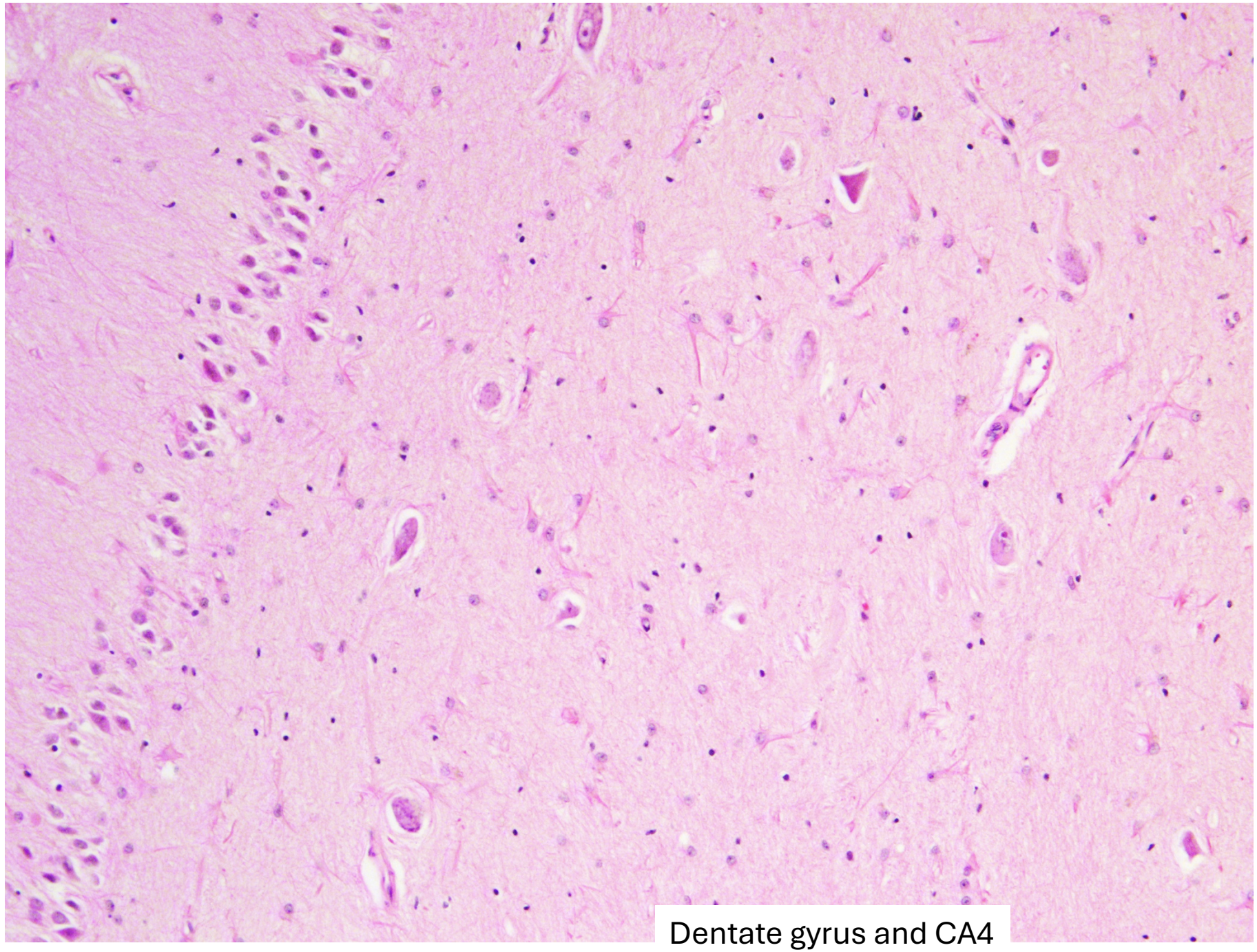
Example: MTS







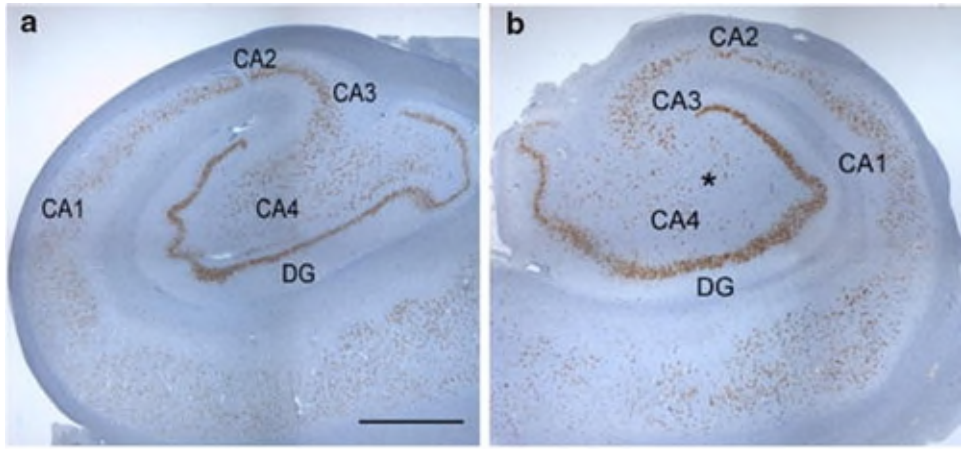
Neuronal loss and gliosis CA1



Dentate gyrus and CA4

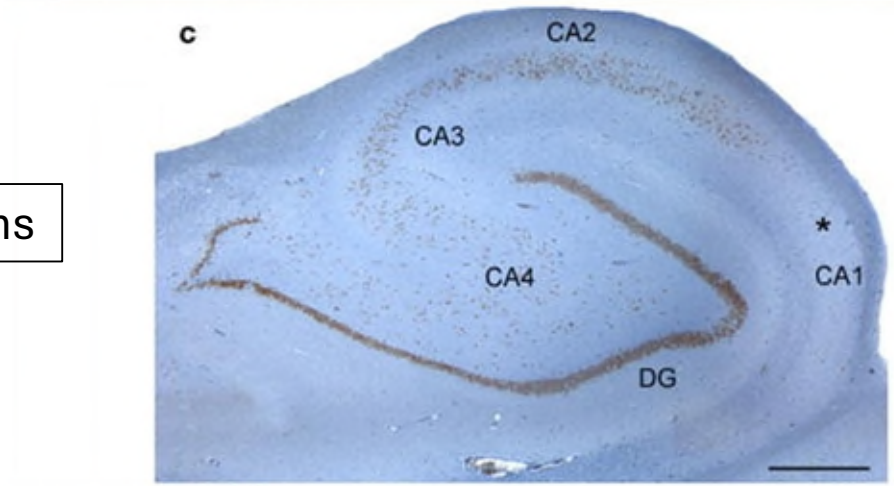
Normal

End-folium sclerosis

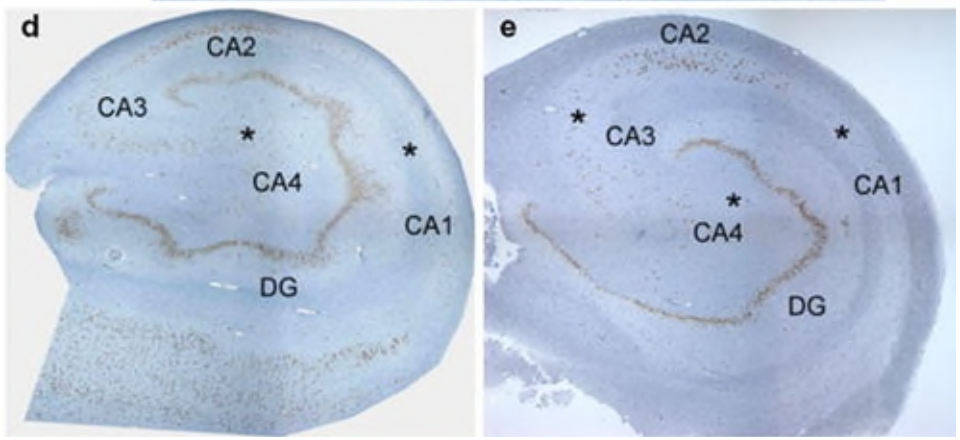


NeuN immunostains

CA1 only

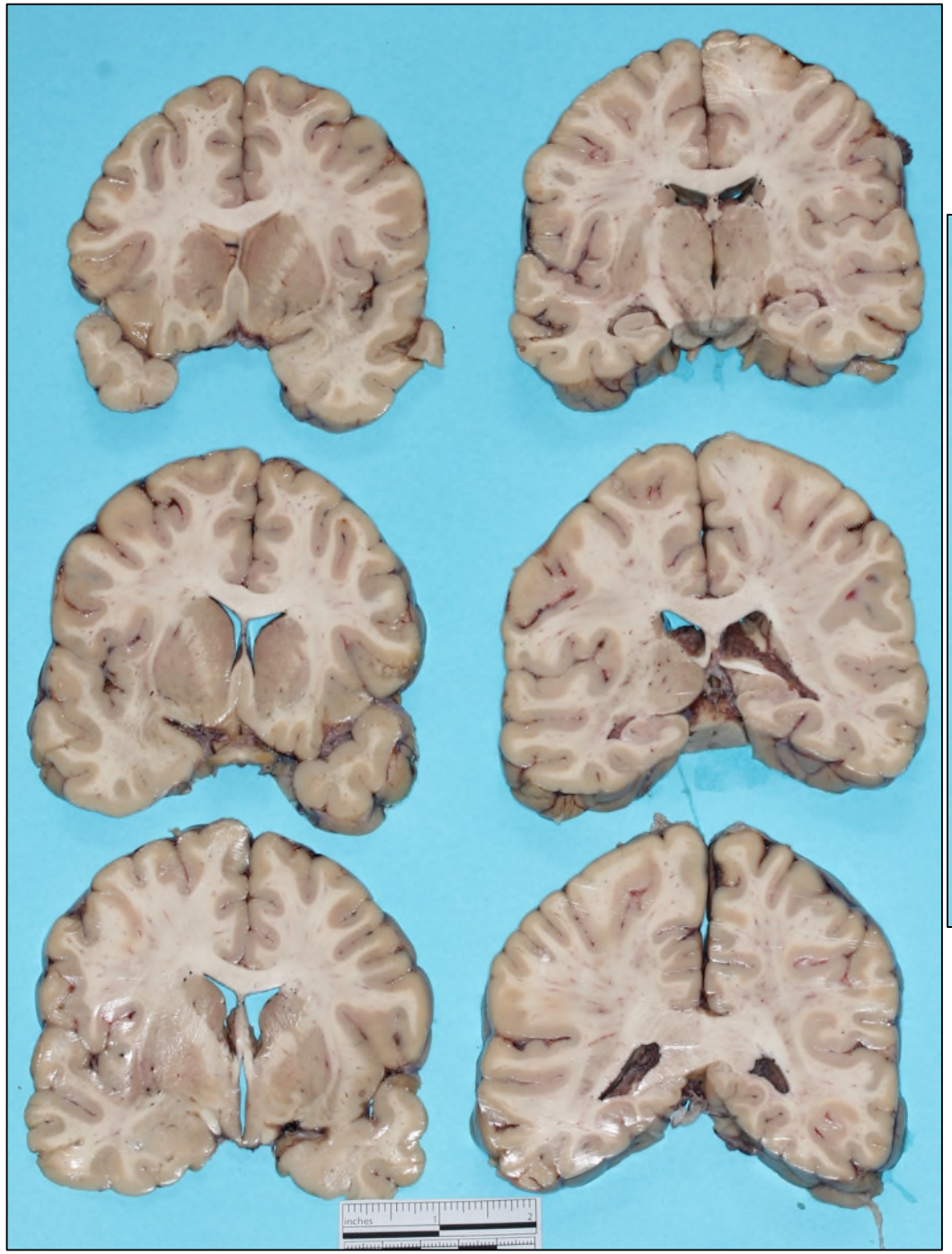


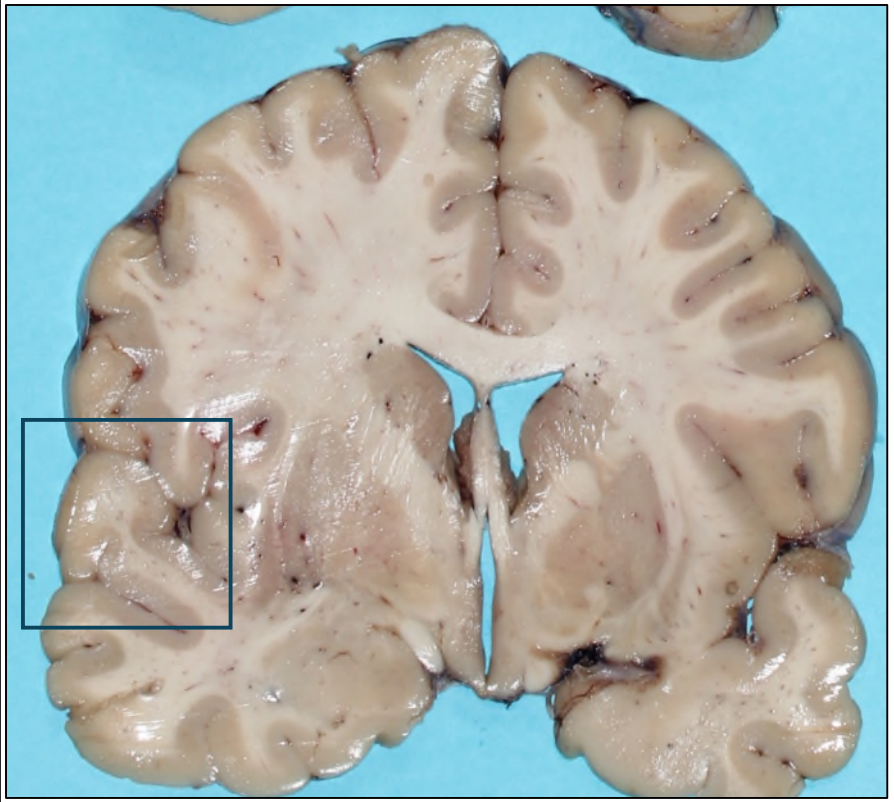
CA1 + CA3 + CA4



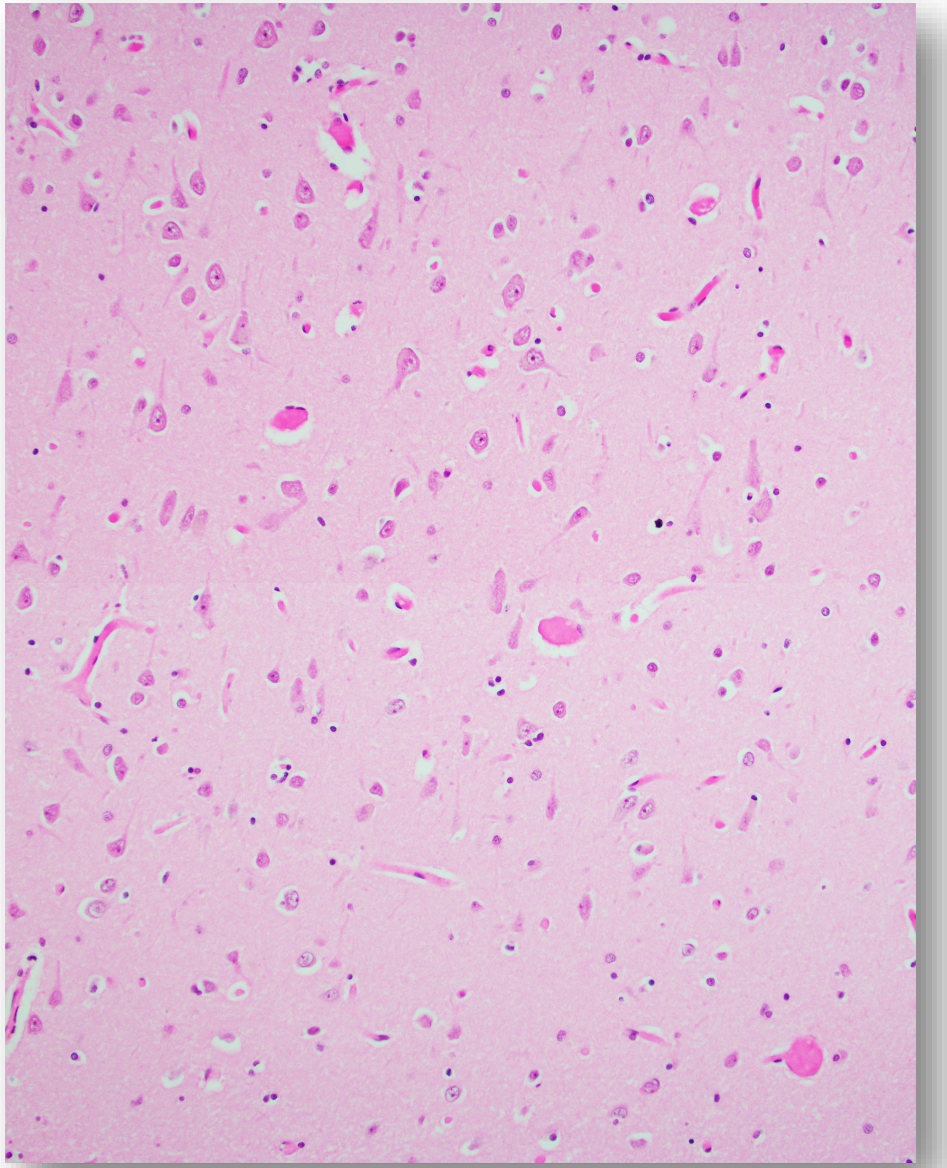
Blumcke et al, 2007

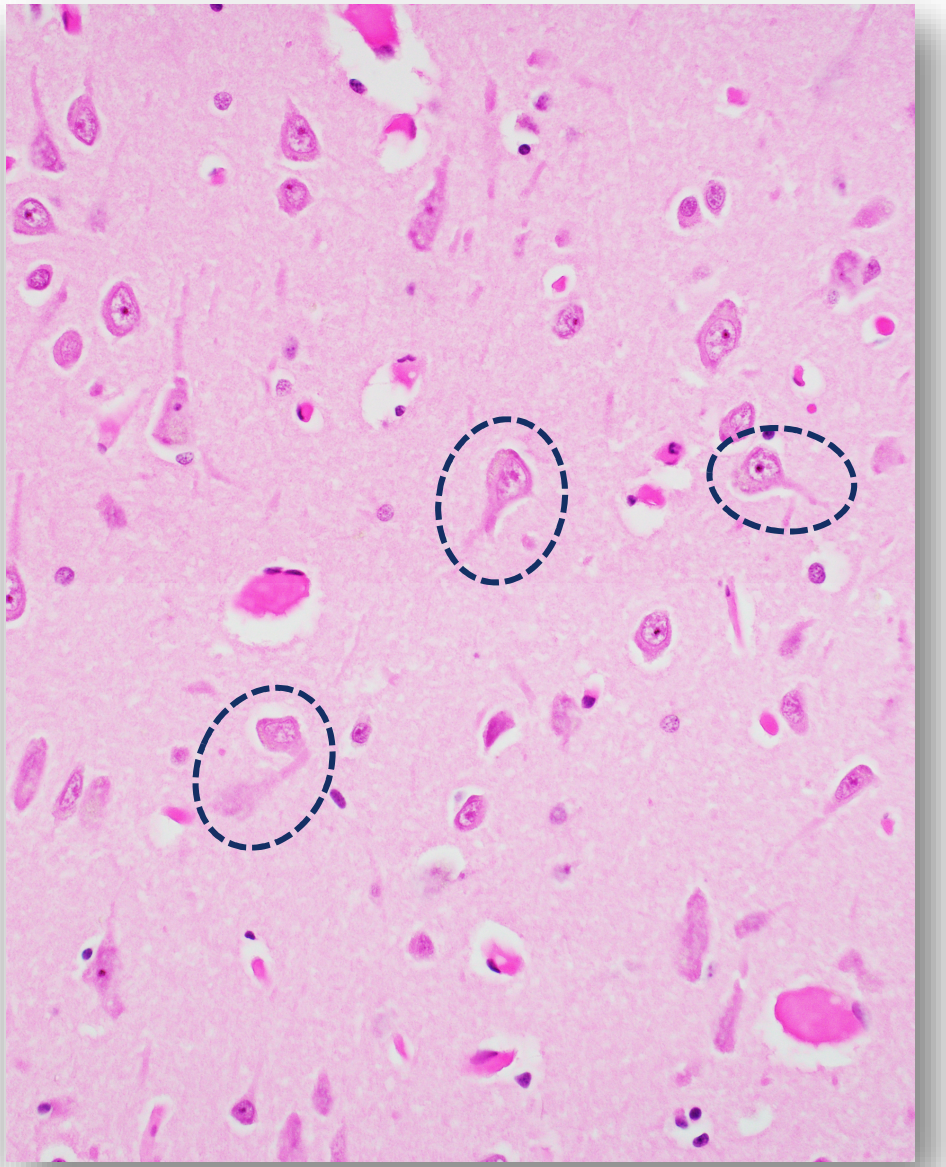
Example: Focal Cortical Dysplasia

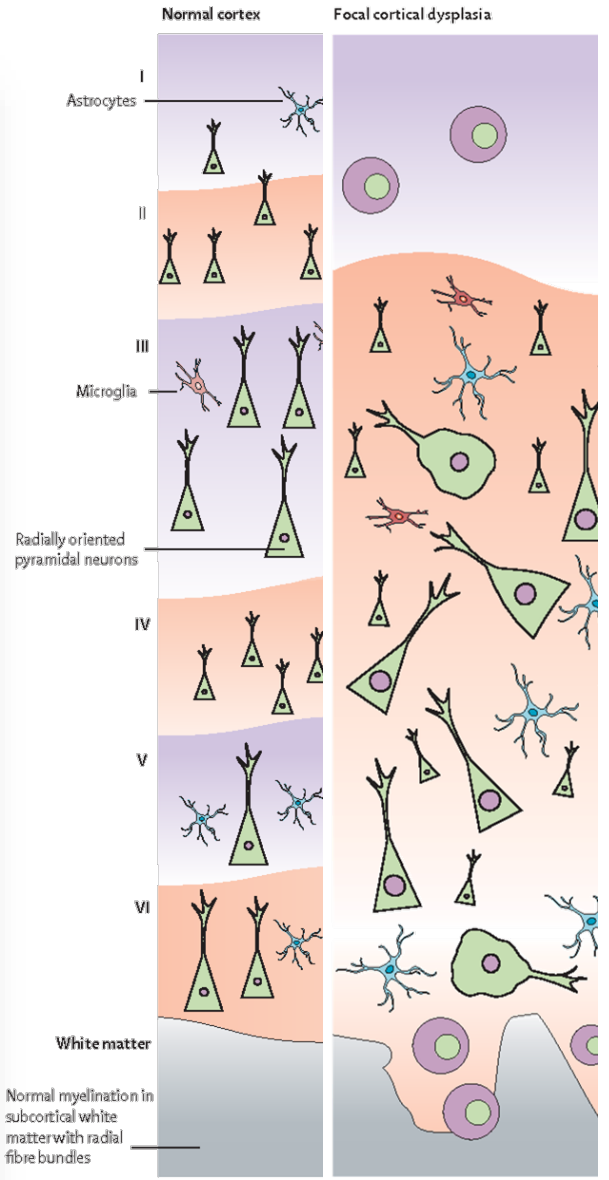




Example: Focal Cortical Dysplasia

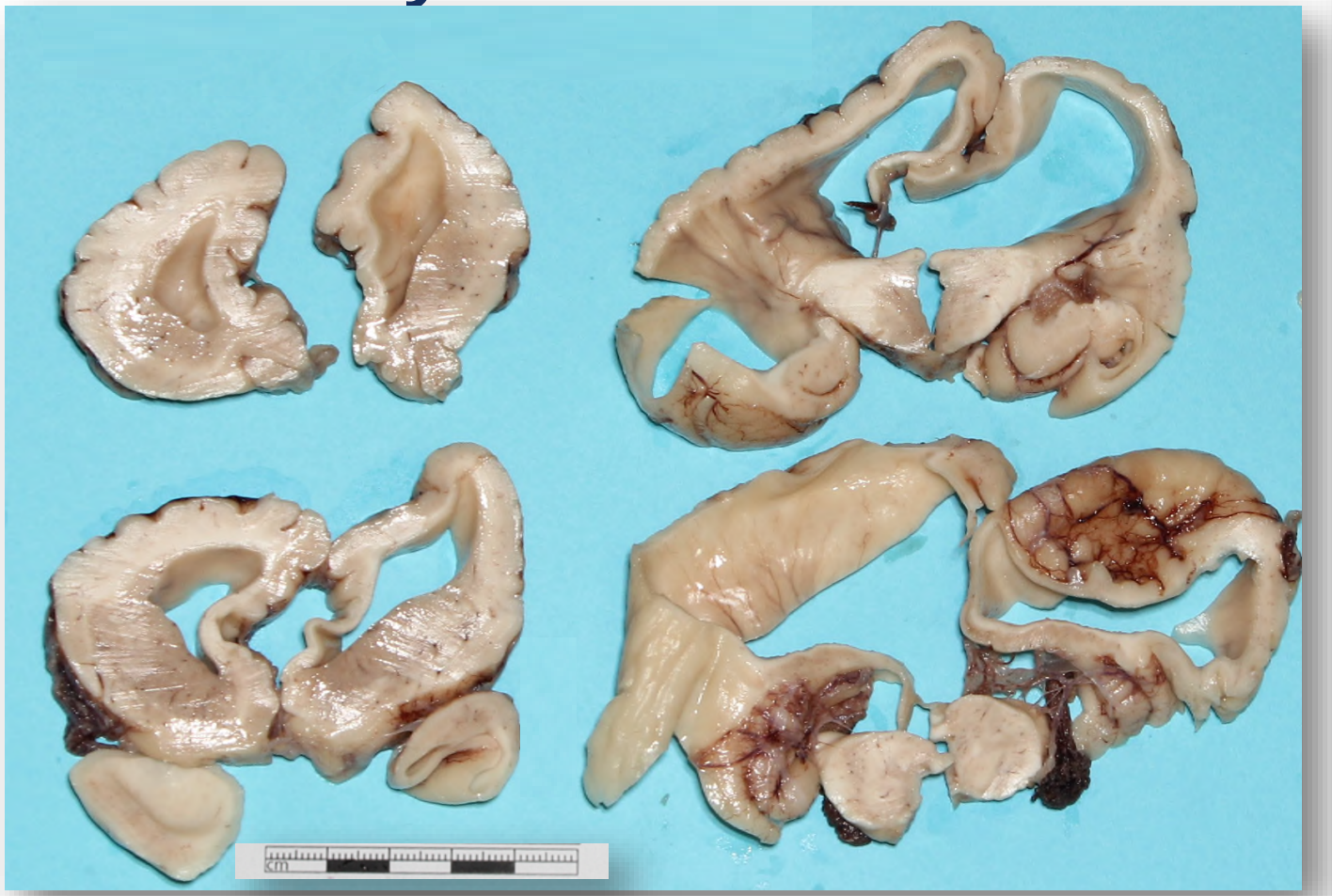


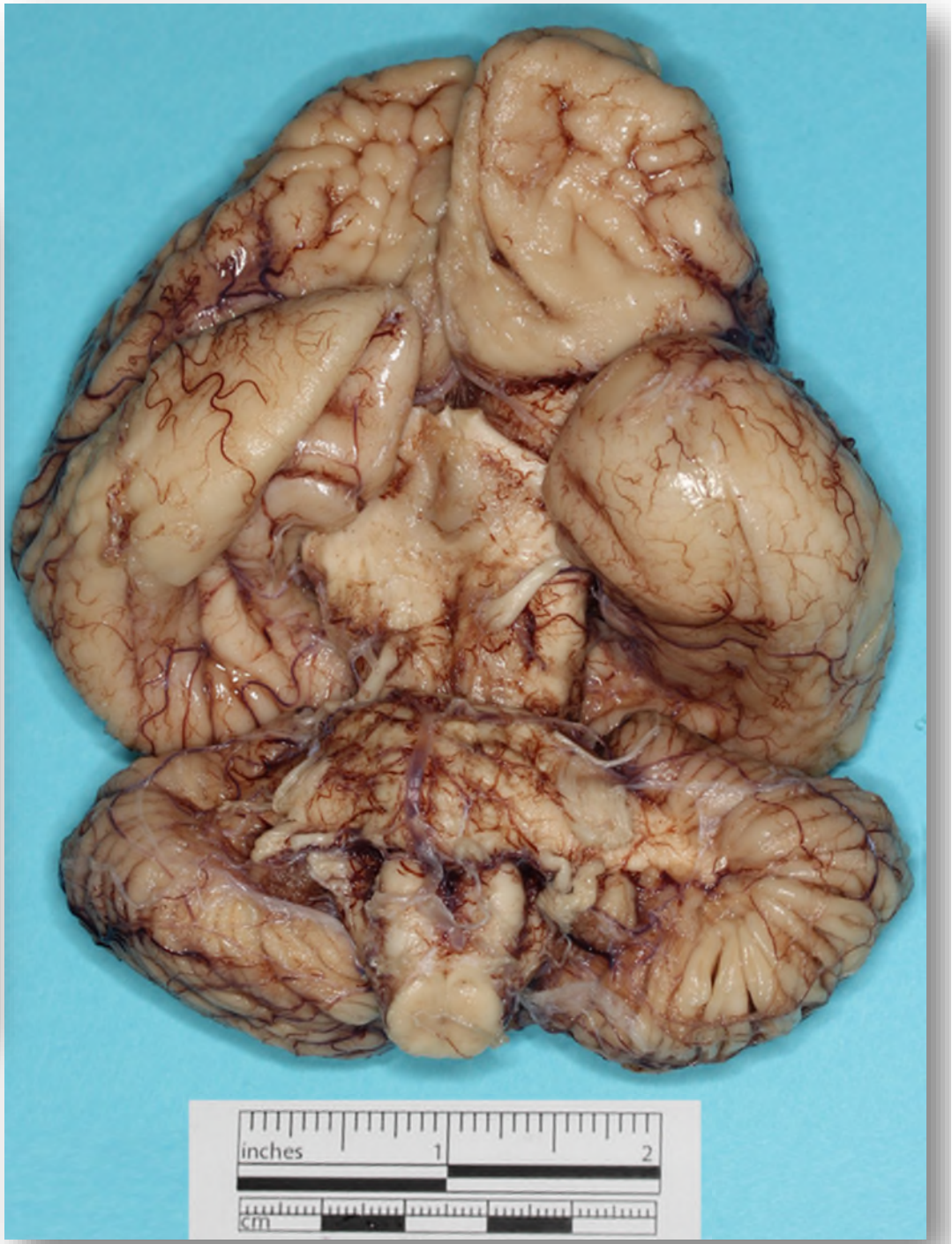


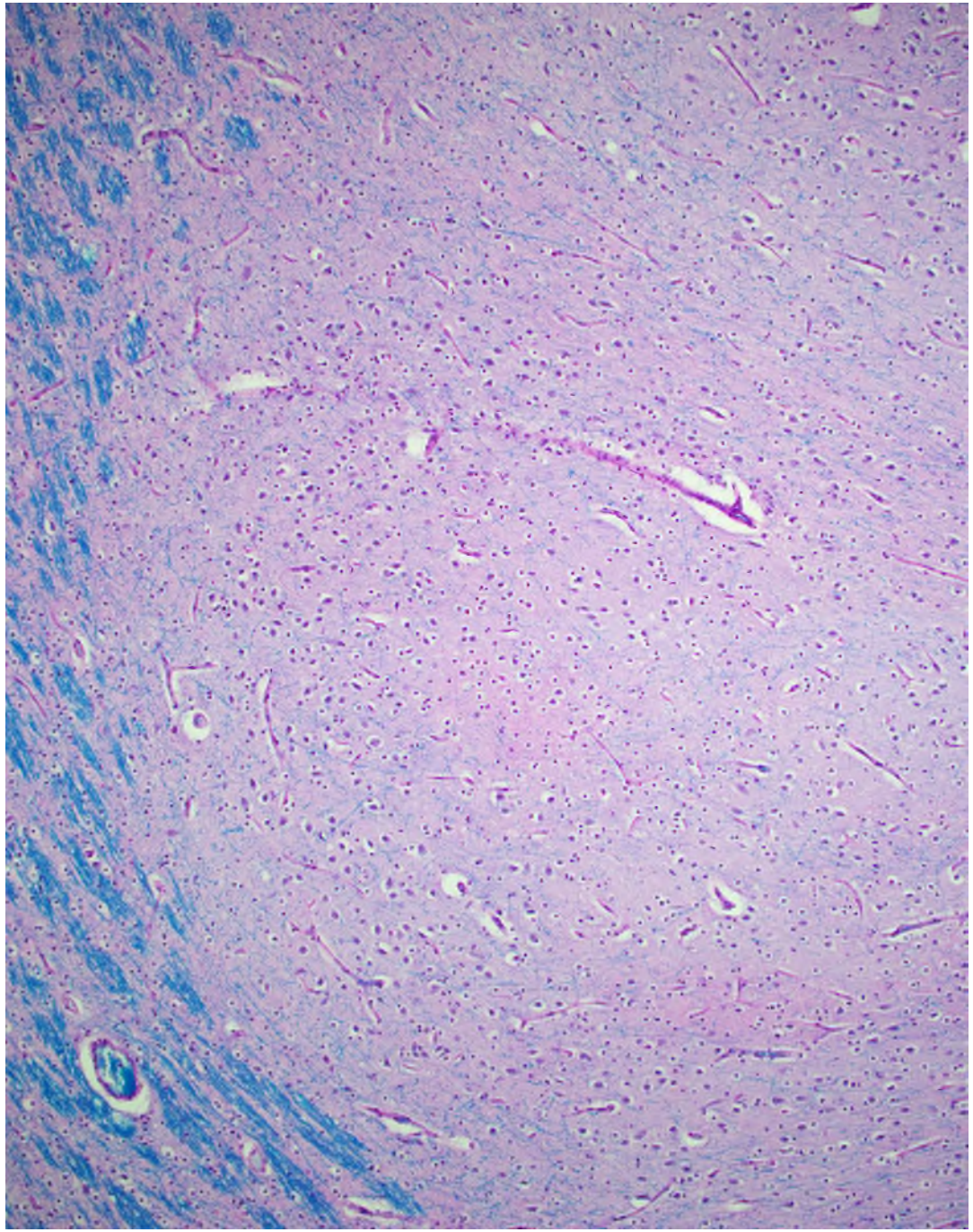


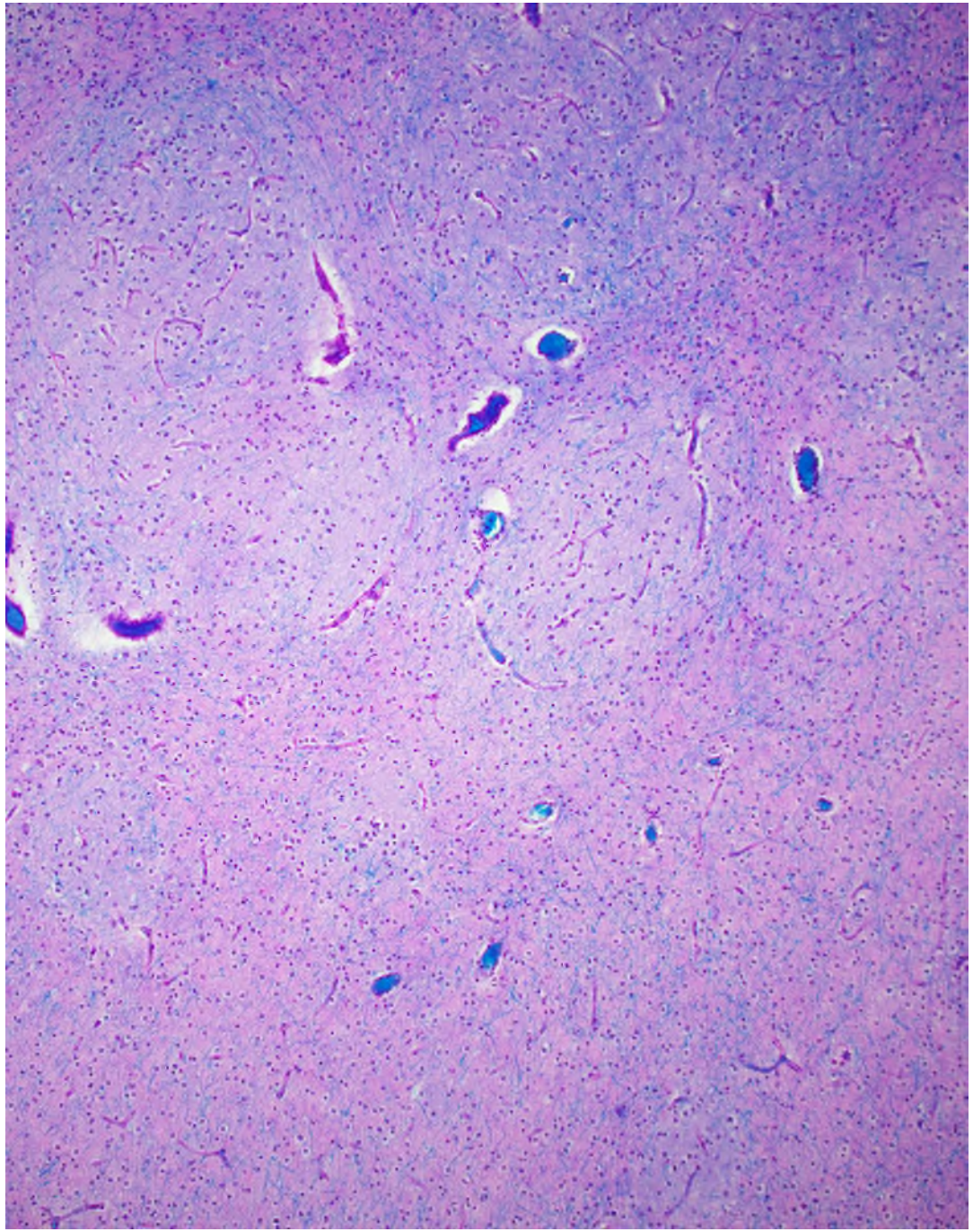
Sisodiya SM. Lancet Neurol. 2009;8(9):830-43.

Example:
Cortical Gyral Abnormalities

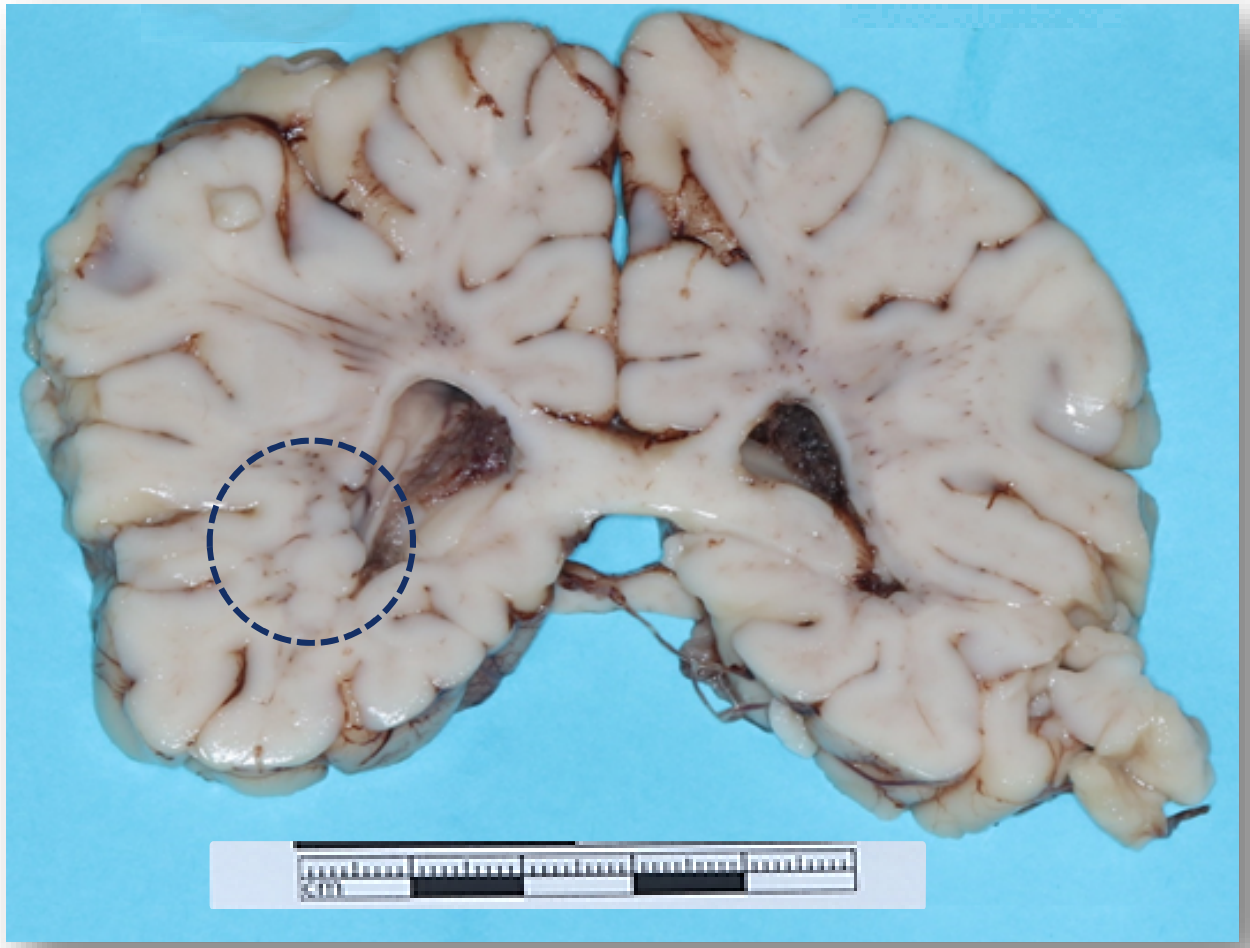


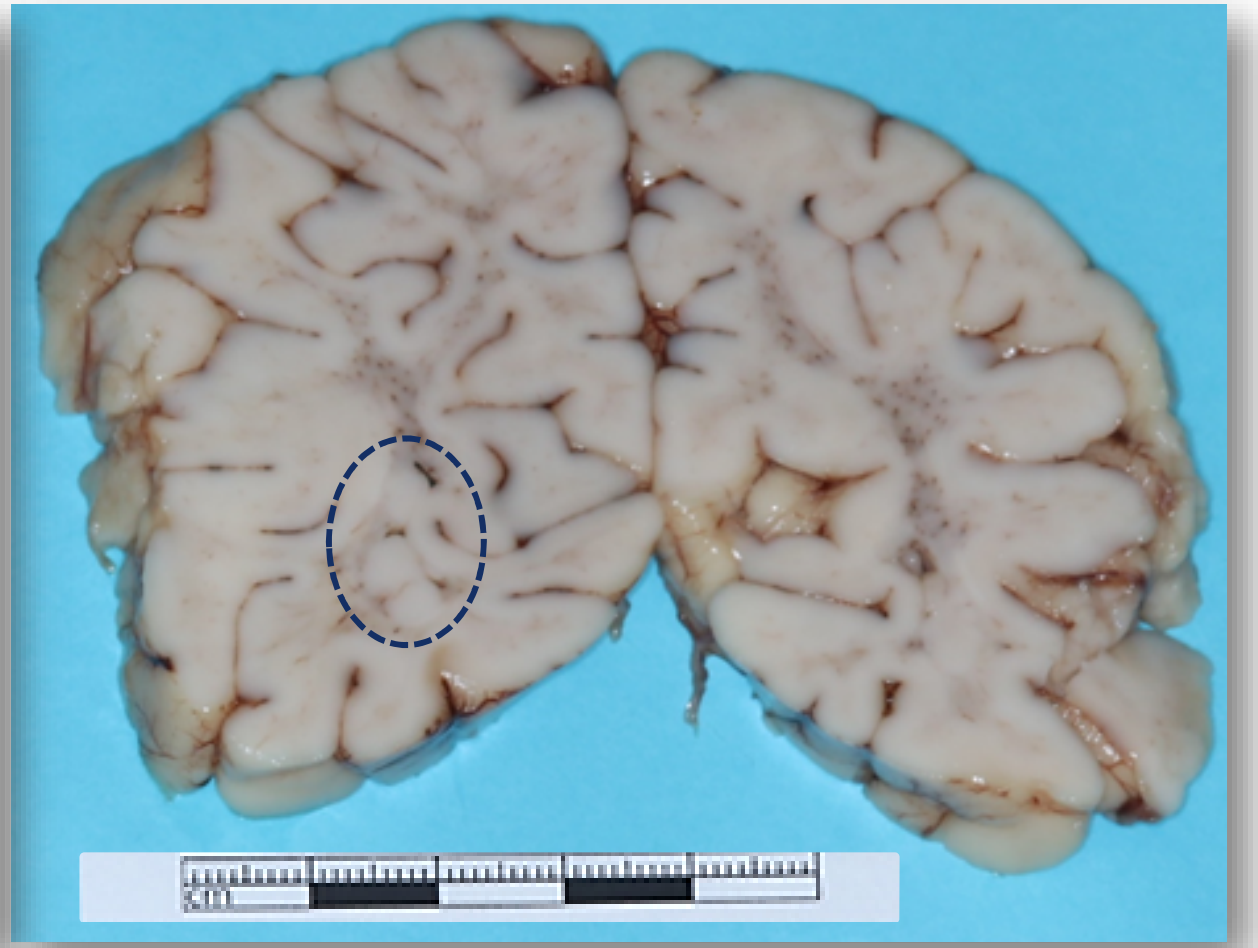




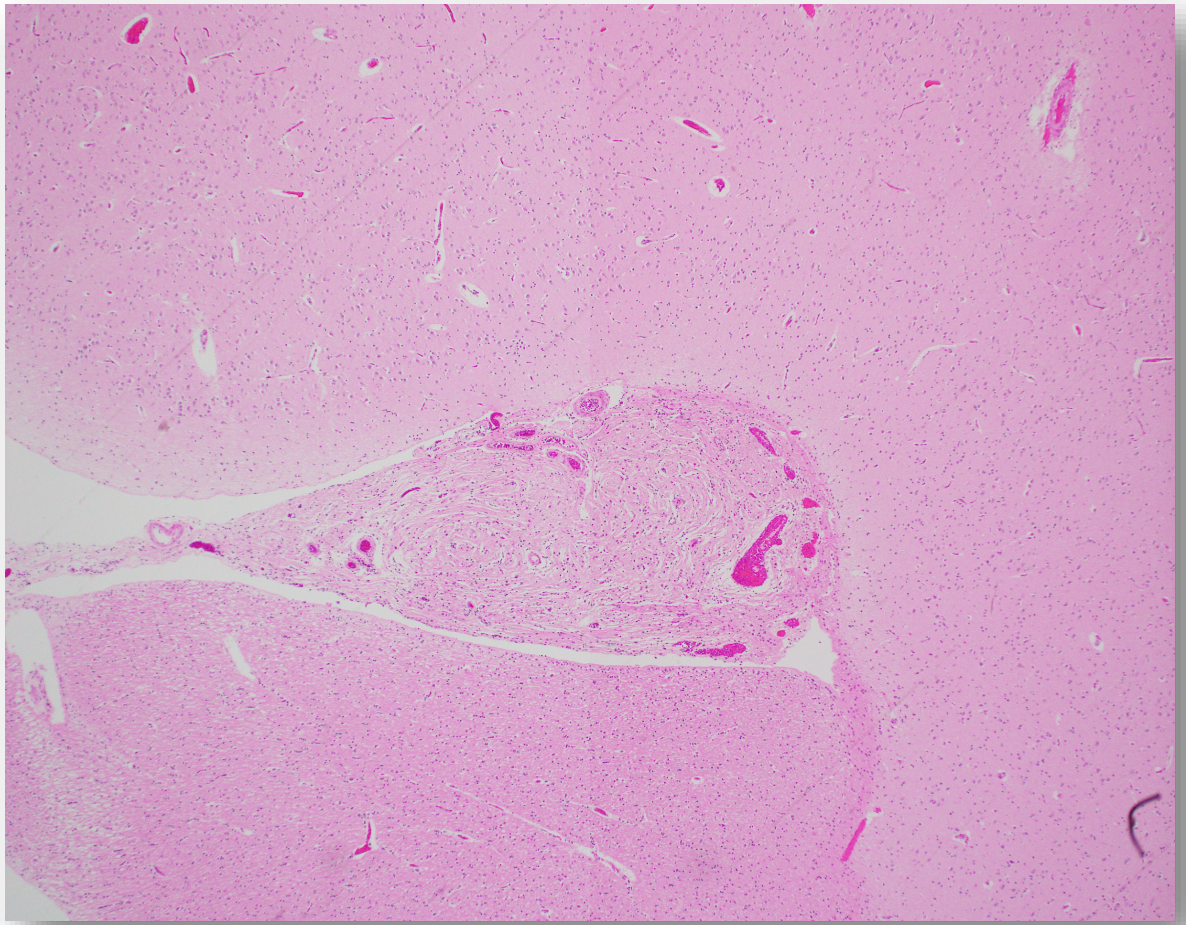


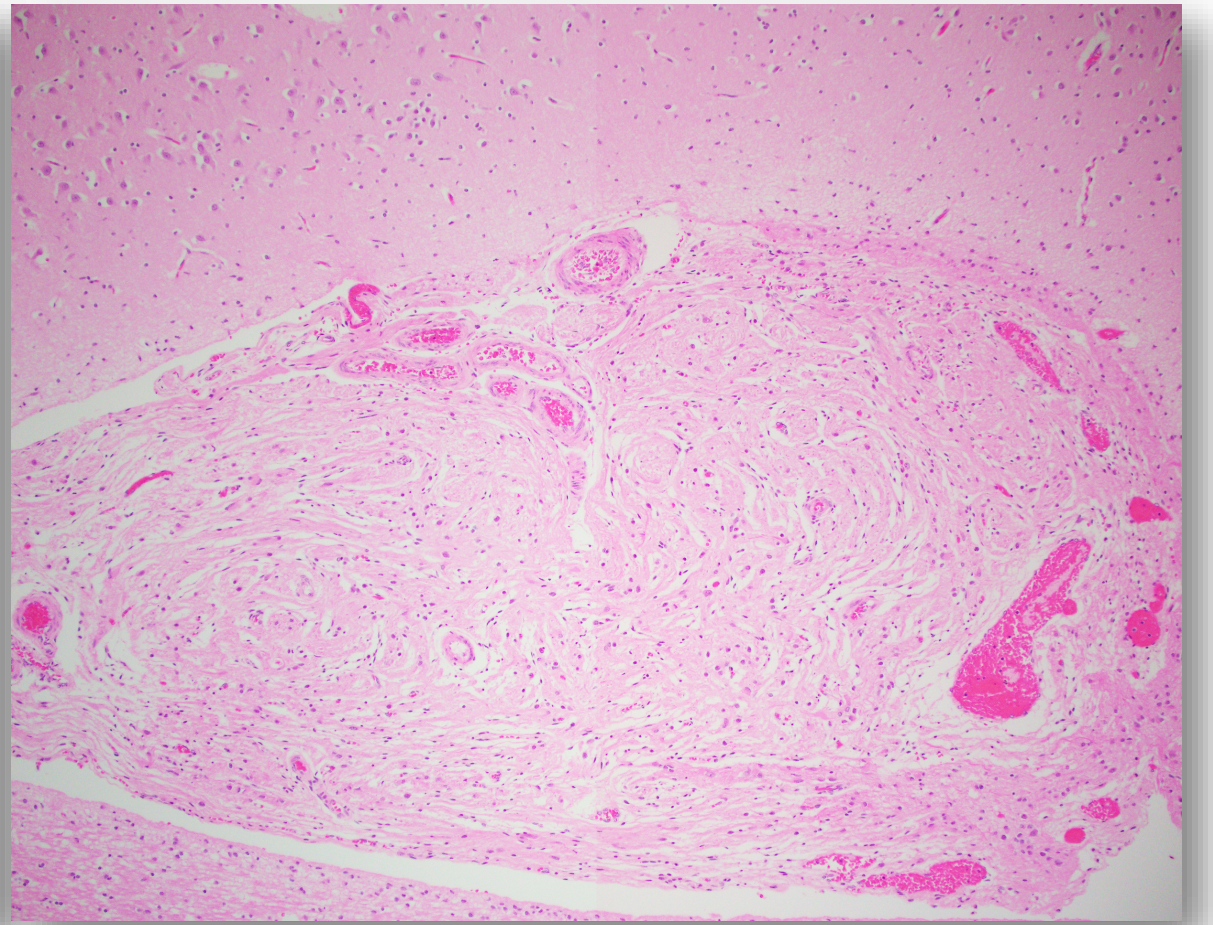
Example:
Periventricular Nodular Heterotopia (PVNH)





Example:
Subarachnoid Glioneuronal Heterotopia (SAGNH)





Results

- Molecular testing (with full NP workup): n = 65
- Age: 2wks-47yr (median, 22.6yr)
- Sex: 31M, 24F, and 1TGF
- Race: 34 Black, 10 White, 19 Hispanic (14 WH, 5 BH), 2 Asian Pacific

- **Pathogenic or likely pathogenic variants: n = 3 (4.6%)**

  - With NP findings:
    - **CACNA1H** (frameshift) with MTS
    - **SCN1A** (Dravet Syndrome) (nonsense) with EFS and SAGNH
    - **SCN2A** (splice-site missense) with EFS

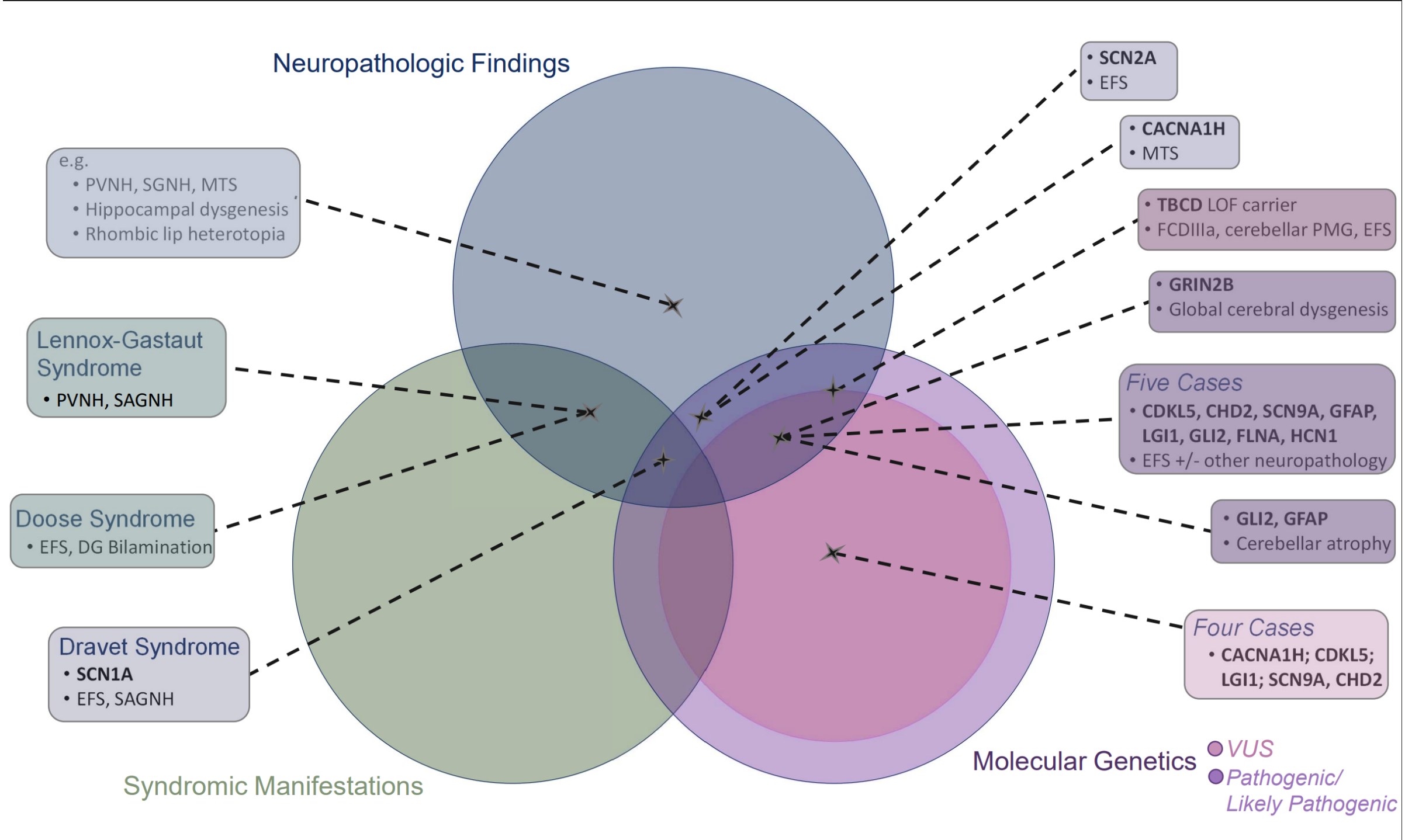
Results

- **Variants of uncertain significance (VUS): n = 37 (56.9%)**
  - With NP findings: n = 33 cases (50.8%)
    - **GRIN2B** with global cerebral dysgenesis (*in silico* prediction is consistently deleterious)
    - **SPTAN1** with cerebral dysgenesis, including hippocampal dysgenesis
    - **CRYAB** AR variant carrier with features of cerebral dysgenesis
    - **GLI2, GLRA1, KMt2D** with Down Syndrome, cerebellar hypotrophy and features of hippocampal dysgenesis
    - **CDKL5** with megalencephaly (1800gm) and bilateral mesial temporal sclerosis
    - Six others had EFS, with or without other NP
      - **FLNA**, **HCN1**; **GLI2**, **NSD 1**; **SCN9A**; **DSC2**; **NSD1**; **CHD2**
    - Six had microscopic findings of uncertain significance (e.g., ≥1 microscopic DG irregularities) without EFS
      - **CACNA1E**; **LGI1**; **SCN9A**, **CHD2** splice-site; **GLI2**, **GFAP**; **HCN1**; **CACNA1C**
    - VUS for autosomal recessive disorder: **TBCD** (loss-of-function) carrier with EFS, FCD IIIA, and cerebellar polymicrogyria

  - Without NP Findings: n = 4 cases (6.2%)
    - **CDKL5** novel splice-site; **CACNA1H**; **CACNA1E**, **NOTCH3**; **RYR2**, **MYPN**

Results

- **Likely benign/benign variants: n = 39 (60%)**
  - With NP findings: n = 35 cases (53.8%)
    - Known syndrome:
      Doose syndrome and DG bilamination
      Lennox-Gastaut syndrome and cerebral dysgenesis
    - No diagnosed syndrome:
      One with cerebral polymicrogyria and focal disorganization of cerebellar cortex
      Two with cerebral dysgenesis and hippocampal dysgenesis
      Two with macroscopic **and** microscopic evidence of hippocampal dysgenesis
      Twenty-eight with microscopic findings of dentate gyral irregularities with or without other findings
  - Without NP findings: n = 4 cases (6.2%)


Neuropathologic Findings
e.g.
• PVNH, SGNH, MTS
• Hippocampal dysgenesis
• Rhombic lip heterotopia
Lennox-Gastaut Syndrome
• PVNH, SAGNH
Doose Syndrome
• EFS, DG Bilamination
Dravet Syndrome
• SCN1A
• EFS, SAGNH
Syndromic Manifestations
• SCN2A
• EFS
• CACNA1H
• MTS
• TBCD LOF carrier
• FCDIIIa, cerebellar PMG, EFS
• GRIN2B
• Global cerebral dysgenesis
Five Cases
• CDKL5, CHD2, SCN9A, GFAP, LGI1, GLI2, FLNA, HCN1
• EFS +/- other neuropathology
• GLI2, GFAP
• Cerebellar atrophy
Four Cases
• CACNA1H; CDKL5; LGI1; SCN9A, CHD2
Molecular Genetics
VUS
Pathogenic/ Likely Pathogenic

Summary and Conclusions

- Epilepsy results from a number of structural brain abnormalities
  - A subset results from “channelopathies” overlapping with cardiac arrhythmogenic syndromes
- Autopsies (and ideally molecular testing) are required for accurate diagnosis and certification in cases of sudden unexpected death
  - Forensic settings are front-line
  - Identification of genetic variants may impact the care of surviving family members
- Genotype-phenotype correlation in epilepsy is in its infancy – stay tuned!!

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