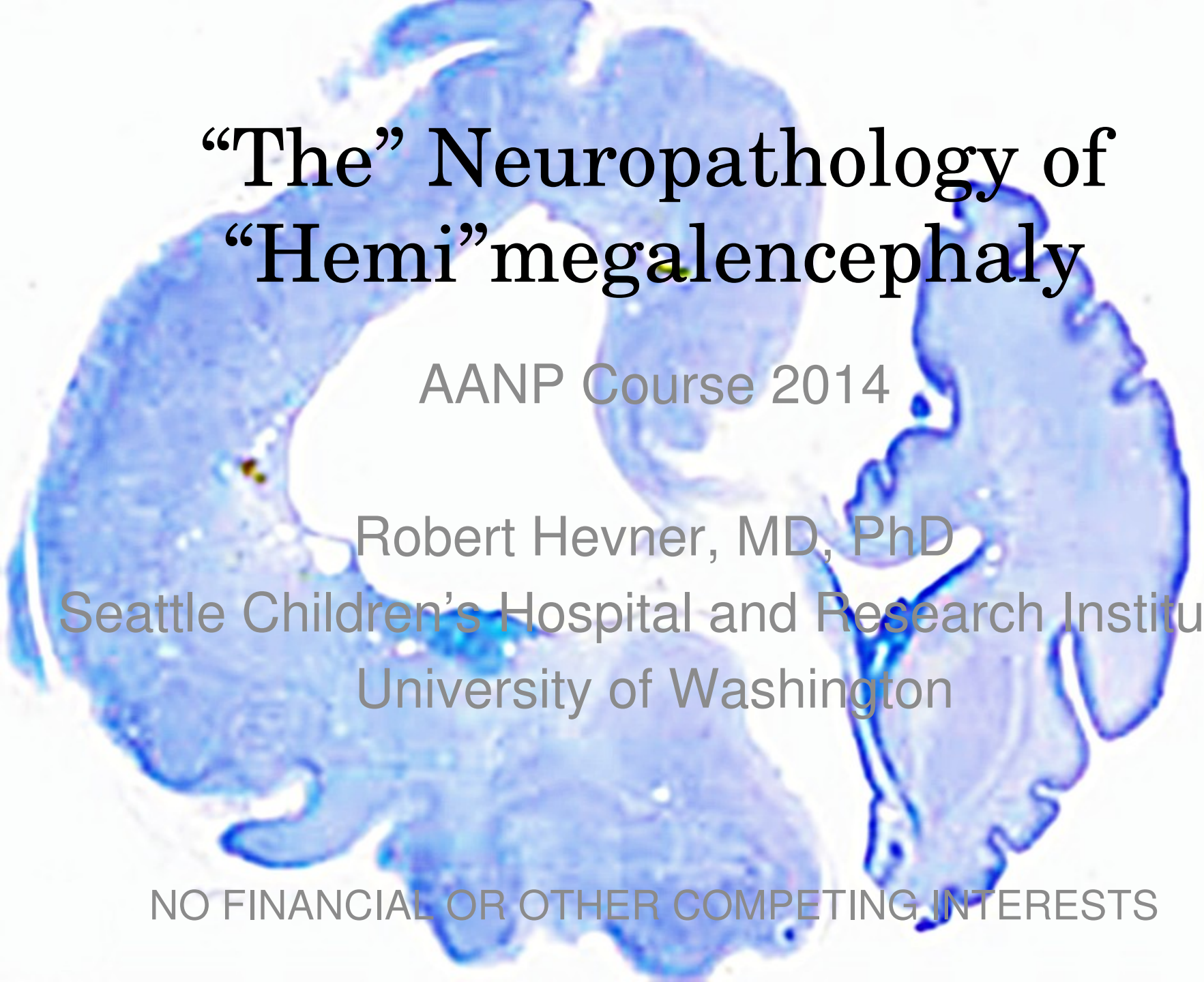

"The" Neuropathology of "Hemi"megalencephaly

AANP Course 2014

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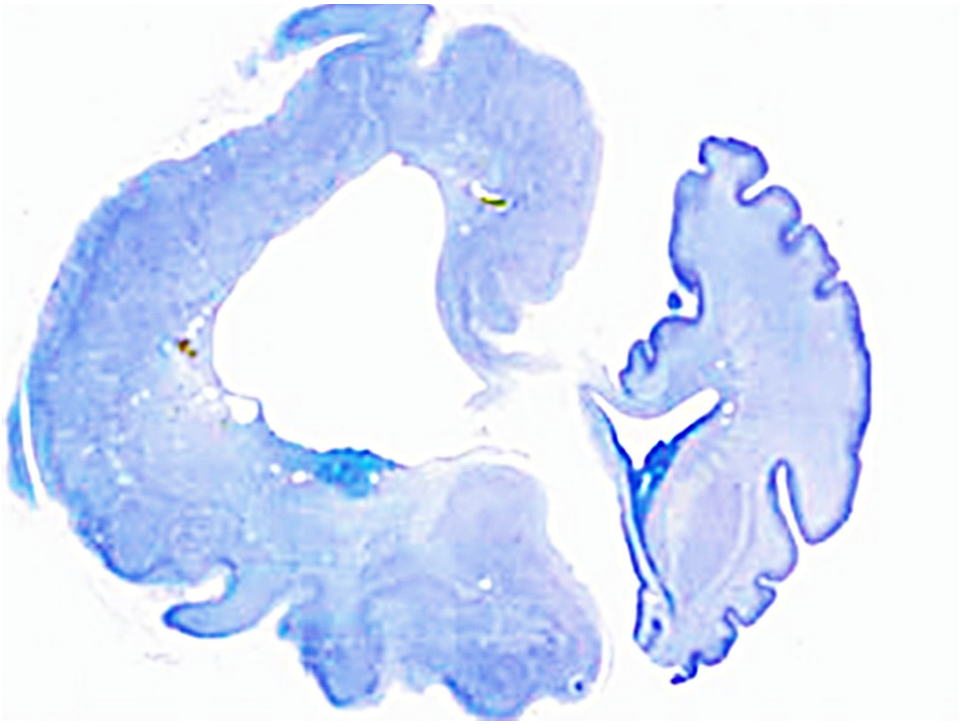
Seattle Children's Hospital and Research Institute
University of Washington

NO FINANCIAL OR OTHER COMPETING INTERESTS

Overview

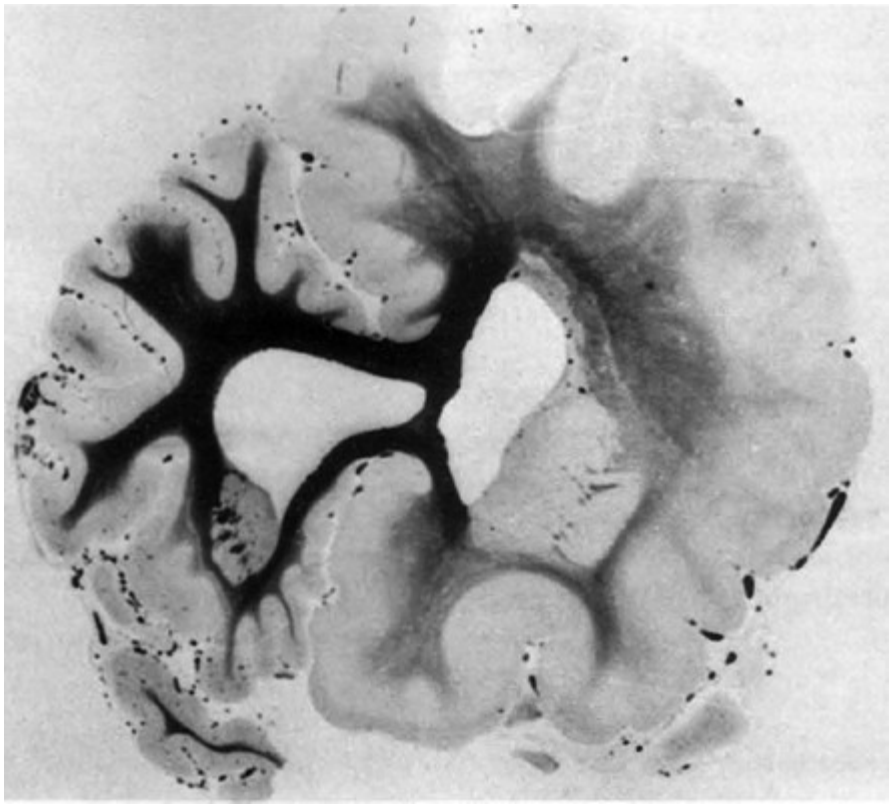
- Review previous pathological studies of HME
- Present new cases from our series (Seattle Children's Hospital and Research Institute)
- Present unpublished findings from an animal model of megalencephaly with *Akt3* mutation

Examples of HME



Left HME (associated with LNS)
Fetus: 32w+2d gestation
Brain: 510 gm (ex. 217)

Boer et al. (2007) *Neuropathol. Appl. Neurobiol.*



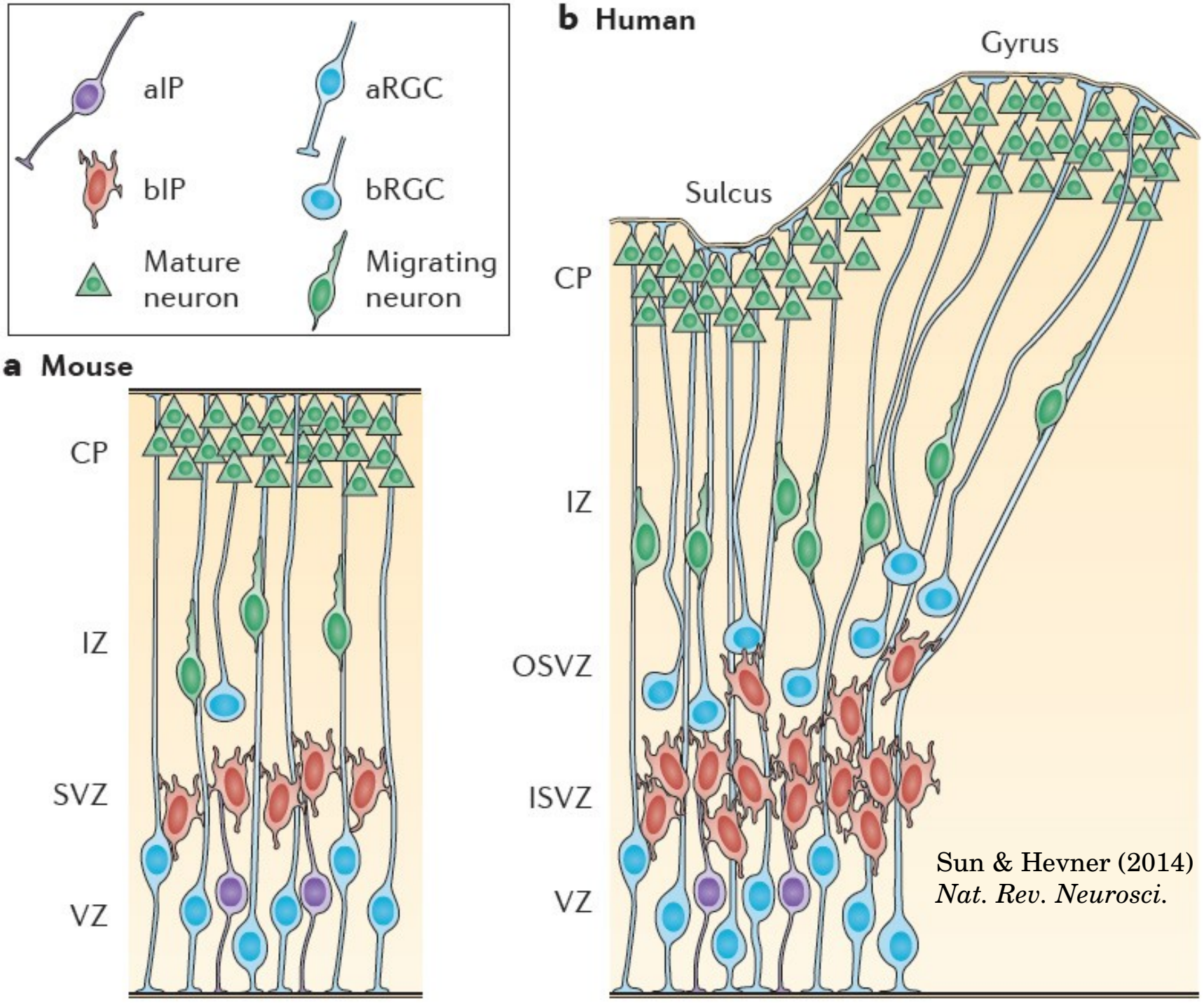
Right HME (sporadic)
Child: 4 years

Robtain et al. (1988) *Neuropathol. Appl. Neurobiol.*

Definition of HME

- “a rare malformation of cortical development characterized by enlargement and cytoarchitectural abnormalities of one cerebral hemisphere” (Aronica et al., 2012)

- “cases where most (at least three lobes) or all of one cerebral hemisphere was larger compared with the opposite hemisphere on MRI.” (Salamon et al., 2006)



Sun & Hevner (2014)
Nat. Rev. Neurosci.

Clinical Manifestations of HME

- **Seizure disorder**, usually with onset during infancy or early childhood, often intractable and requiring hemispherectomy
- Developmental delay
- Cerebral palsy / motor disorders

History and Epidemiology of HME

- First described by Sims (1835)
- M = F
- R = L
- Monozygotic twins can be discrepant (Salamon et al., 2006)
- 1–3 cases per 1,000 epileptic children
- Detectable as early as 22 GW (Manoranjan & Provias, 2010)

Syndromes Associated with HME (~50% of cases)

- Proteus syndrome
- Linear nevus sebaceus (LNS)
- CLOVES
- Hypomelanosis of Ito
- Klippel-Trenaunay-Weber
- Neurofibromatosis
- Tuberous sclerosis
- MCAP
- MPPH

Macroscopic Pathology of HME

- Degree of cerebral asymmetry varies:
  - Contralateral micrencephaly (Salamon et al., 2006)
  - Contralateral “normal”
  - Contralateral overgrowth (MEG or bilateral HME)
- Lobar distribution in hemispheres: variable
- Brain structures other than cortex may be involved: thalamus, brainstem, cerebellum
- White matter may be more overgrown (Kato et al., 1996)

Histopathology of HME

- Diverse and heterogeneous

Table 2 Frequency of histopathological features on the affected side of HME cases with intractable epilepsy ($n = 23$)

Pathologic feature	None (%)	Mild (%)	Severe (%)
PMG	39	39	22
Cortical dyslamination	0	43	57
Balloon cells	65	22	13
Cytomegalic neurons	4	39	57
Immature-appearing cells	30	70	0
Excessive white matter neurons	0	48	52
Calcifications	17	74	9
Glial/neuronal heterotopias	39	61	0

Salamon et al. (2006) *Brain*

Morphometry of CNs in HME

- Neuron packing densities usually abnormal in HME, but this varies by layer
- Increased neuron size in HME (+27%) is overall not statistically significant in large series (Salamon et al., 2006)
- Increased dendritic spines on HME neurons

Increased Dendritic Spines in HME

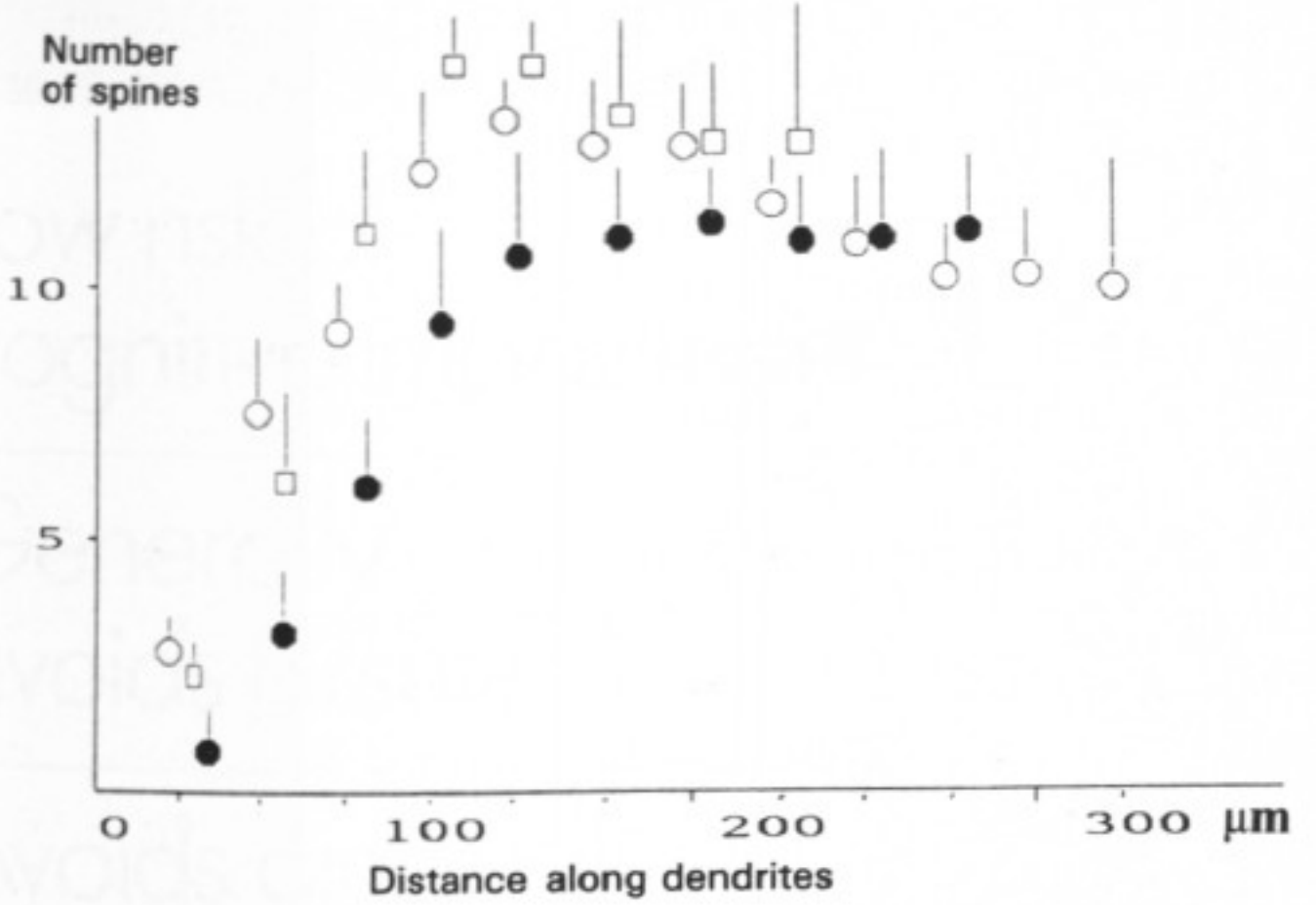
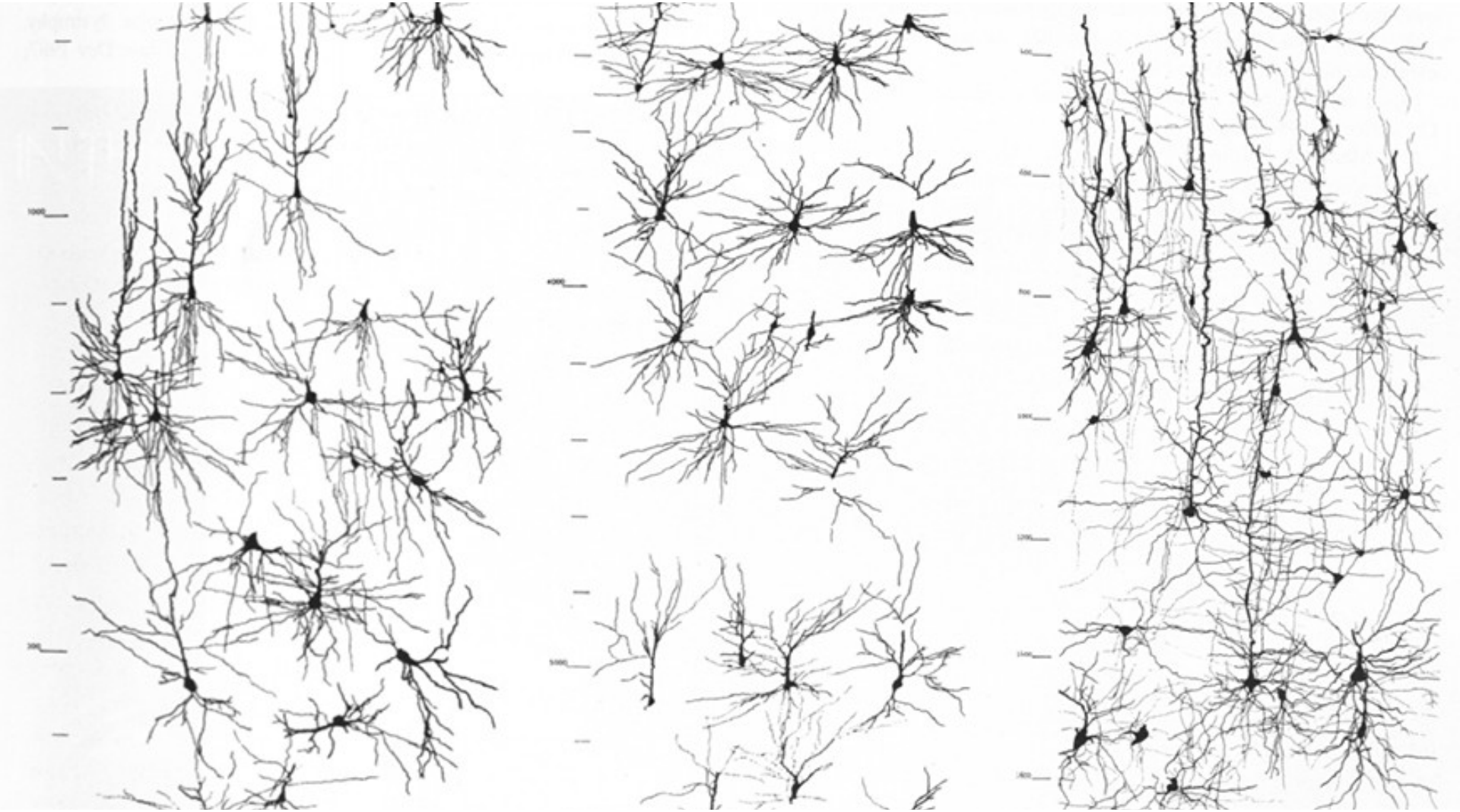


Figure 5. Distribution of spines on basal dendrites of large and moderate sizes of neurons in the visual cortex (Patient 7). ○ = large neurons, □ = moderate neurons, and ● = controls.

Takashima et al. (1991) *Ped. Neurol.*

Neuron Morphology in HME (Golgi)



Takashima et al. (1991) *Ped. Neurol.*

AKT-mTOR Signaling in HME

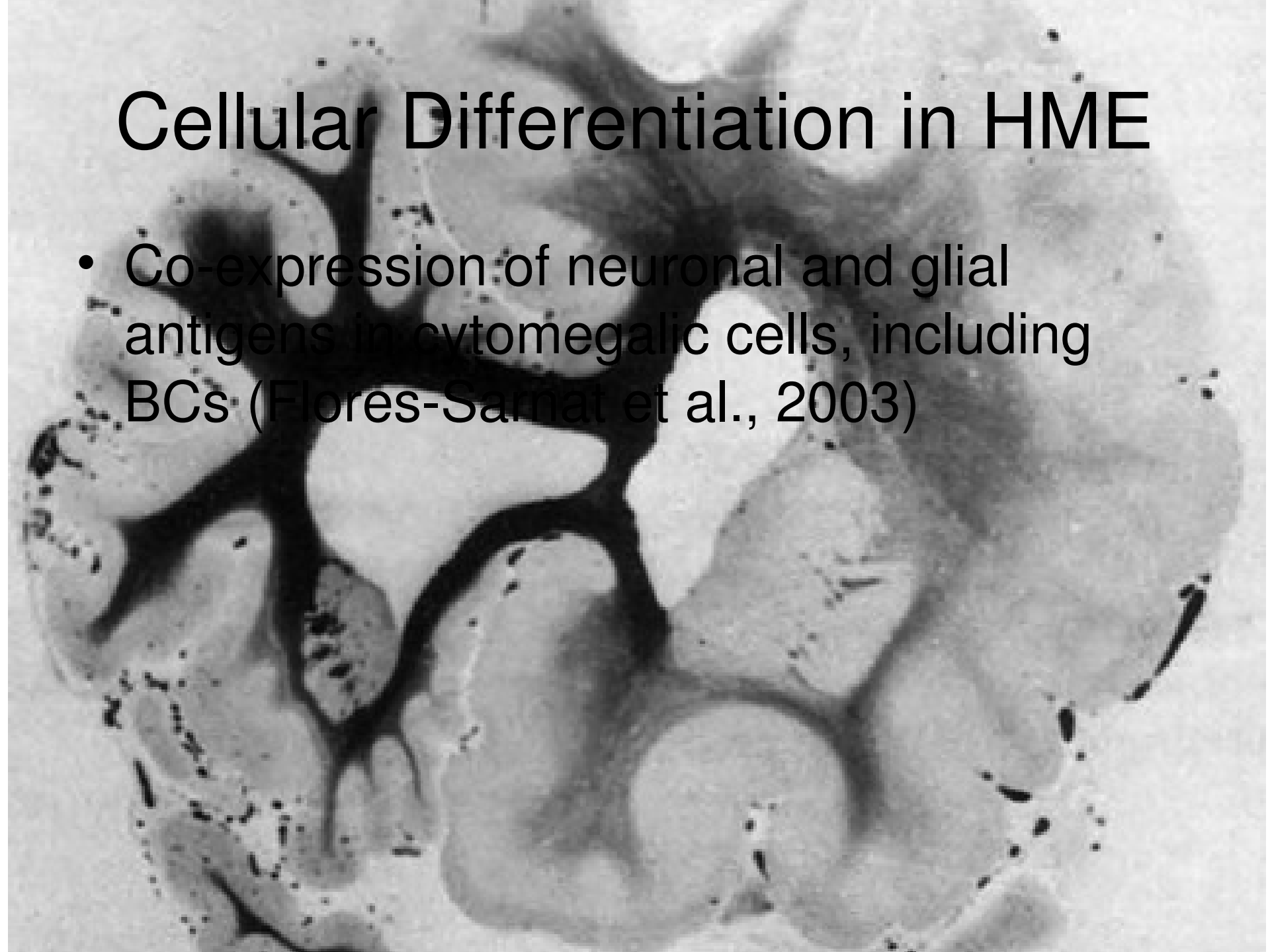
- **Phospho-S6+ (P-S6+)** cytomegalic neurons (CNs; >80%) and balloon cells (BCs); cytoplasmic marker of **mTOR** activation (Crino, 2005; Aronica et al., 2007; Boer et al., 2007)
- P-p70S6K expressed (Aronica et al., 2007) or not expressed (Crino, 2005) in cytomegalic cells

Wnt/β-catenin signaling in HME

- **CyclinD1+ (CCND1+)** neuronal lineage CNs and RCs (87%) (Aronica et al., 2007); nuclear marker of **Wnt/β-catenin** signaling
- *MYC*, *WISP1*, *CCND1* mRNAs (reporters of Wnt/β-catenin activity) upregulated (Crino, 2005)
- Total β-catenin protein upregulated; P-β-catenin (inactivated form) downregulated (Crino, 2005)

Cellular Differentiation in HME

- Co-expression of neuronal and glial antigens in cytomegalic cells, including BCs (Flores-Sarnat et al., 2003)

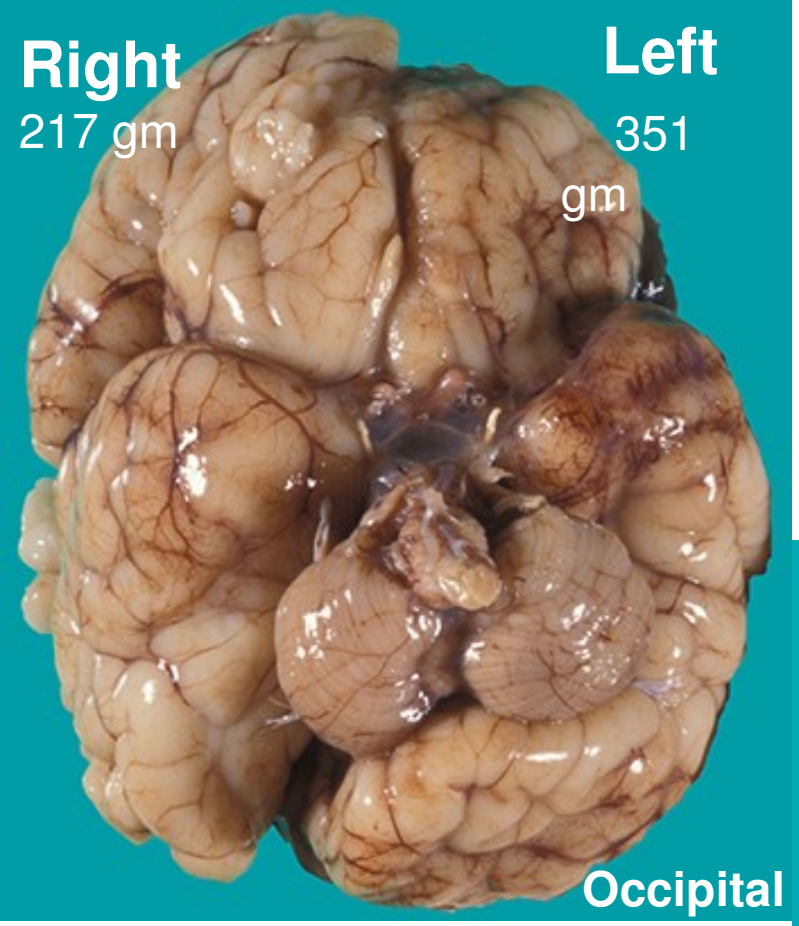


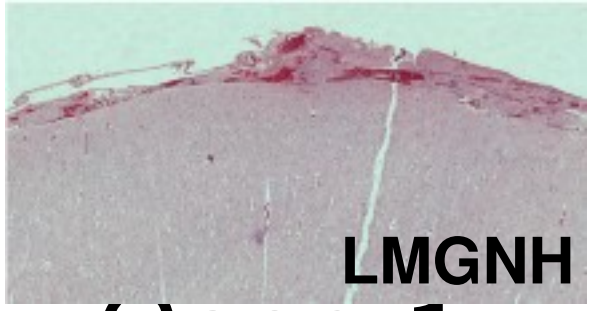
New HME Cases with Genetics

- Too early for confident genotype-phenotype correlations but this is a goal
- “Most of the macroscopic and histologic findings described above were reconfirmed in our three cases…” (Flores-Sarnat et al., 2003)
- Possible new observation:
  - Dentate gyrus hypoplasia (patterning defect?)

Case 1: HME-CLOVES

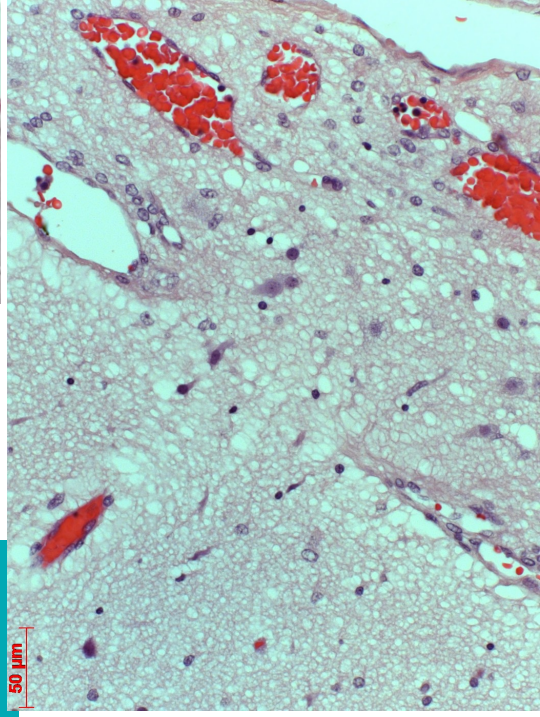
- Premature baby born at 33 GW
- Asymmetric somatic overgrowth noted at birth; Proteus syndrome was considered
- Cranial U/S and CT showed **Left HME**
- Died at 11 days old, due to sepsis (Clostridium) and multi-organ failure
- Brain weight 590 gm (expected 278 gm)
- CLOVES highly linked to ***PIK3CA*** mutations (although this case not sequenced yet)

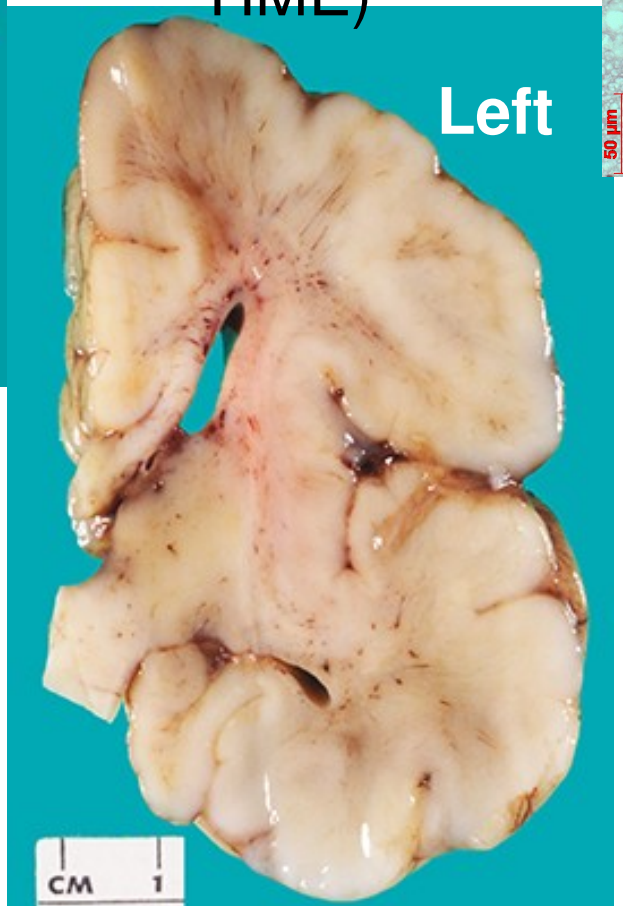


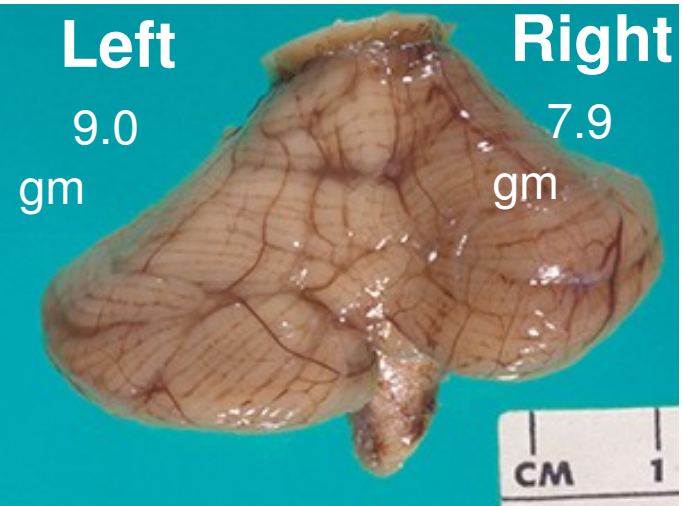


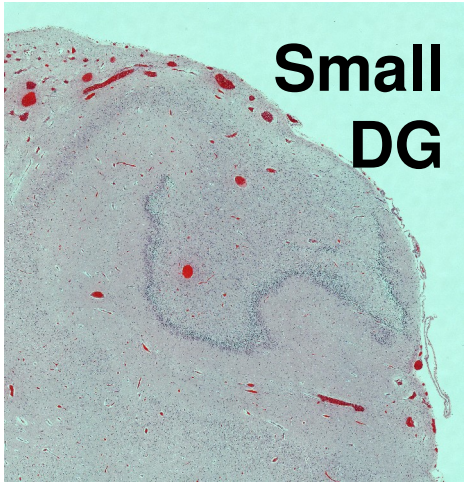
Case 1

(CLOVES-HME)





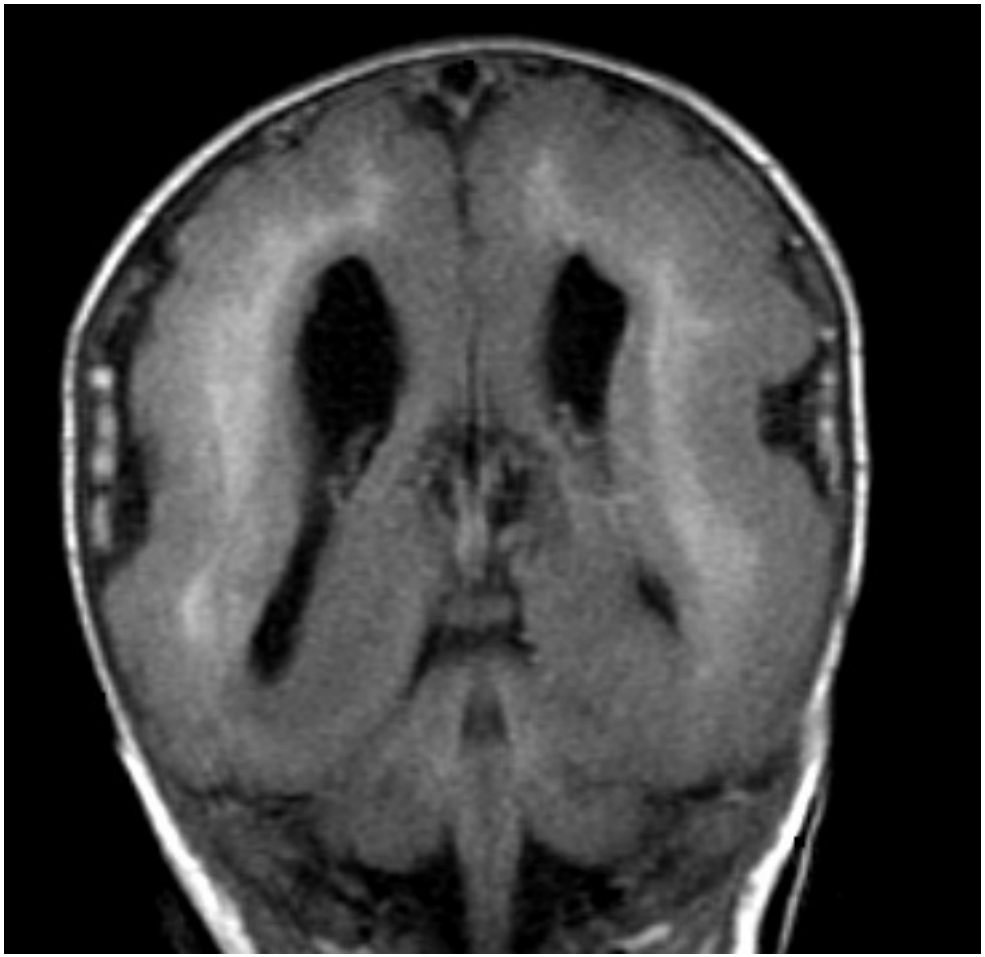




Case 2: Bilateral HME

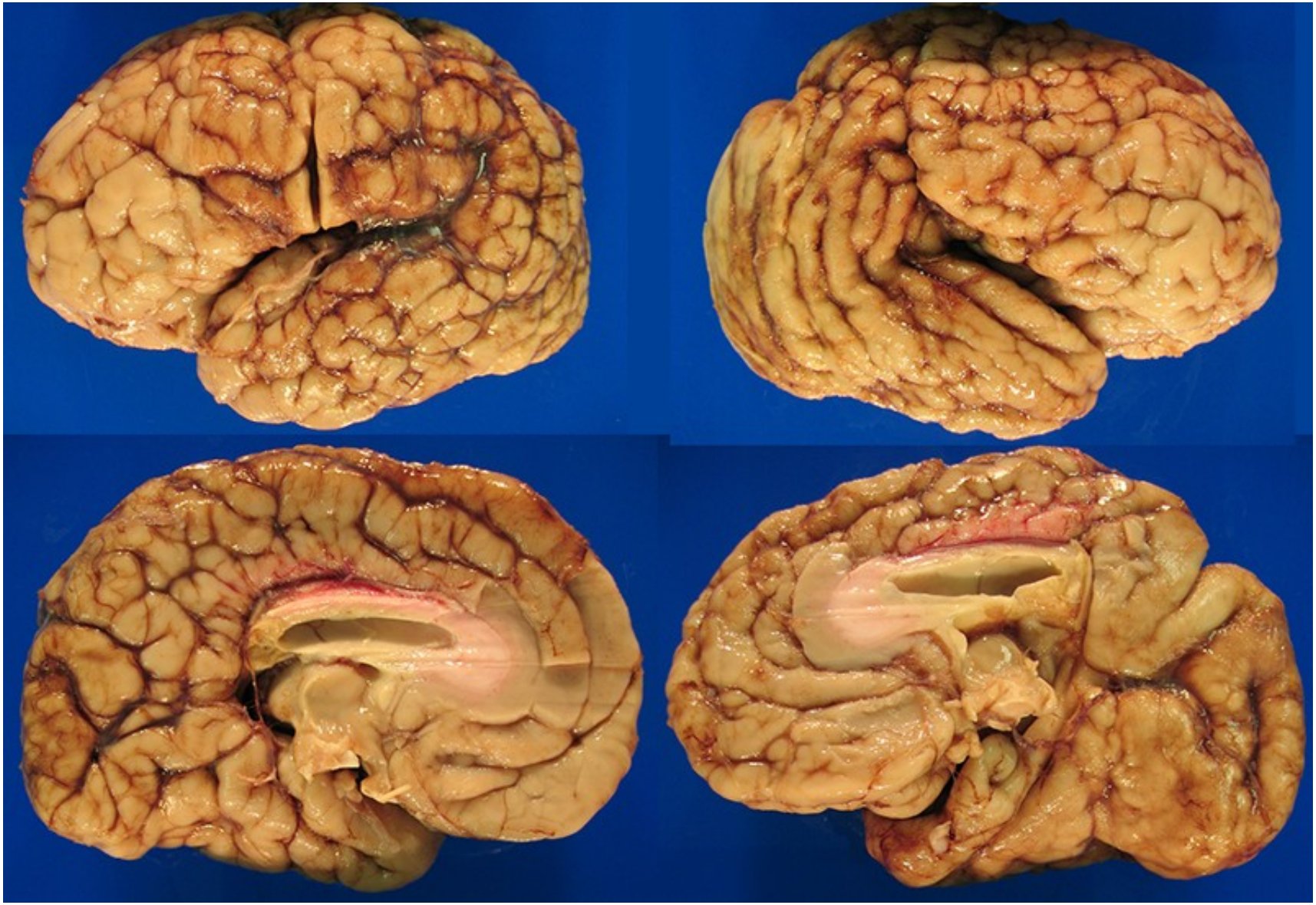
- Term infant with macrocephaly, and seizures beginning within a few days of birth
- MR showed bilateral cortical dysplasia
- Died from complications at 11 weeks postnatal
- Brain weight = 905 gm (expected 506 gm)
- Gene not identified (yet).

Case 2: “Bilateral HME”

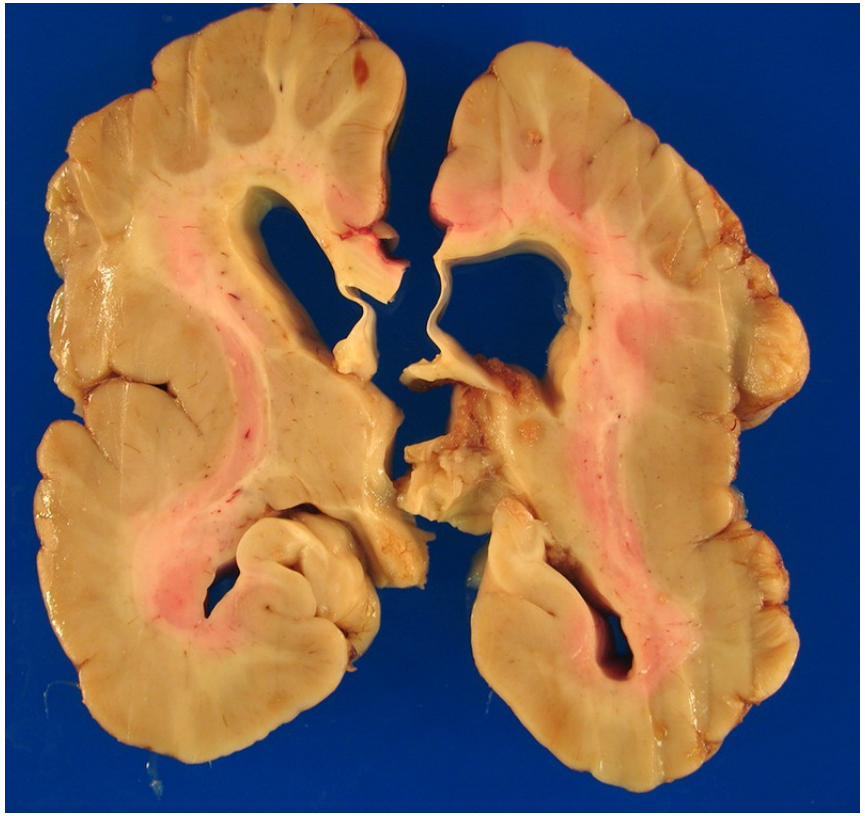


Pachygyria (R parietal)

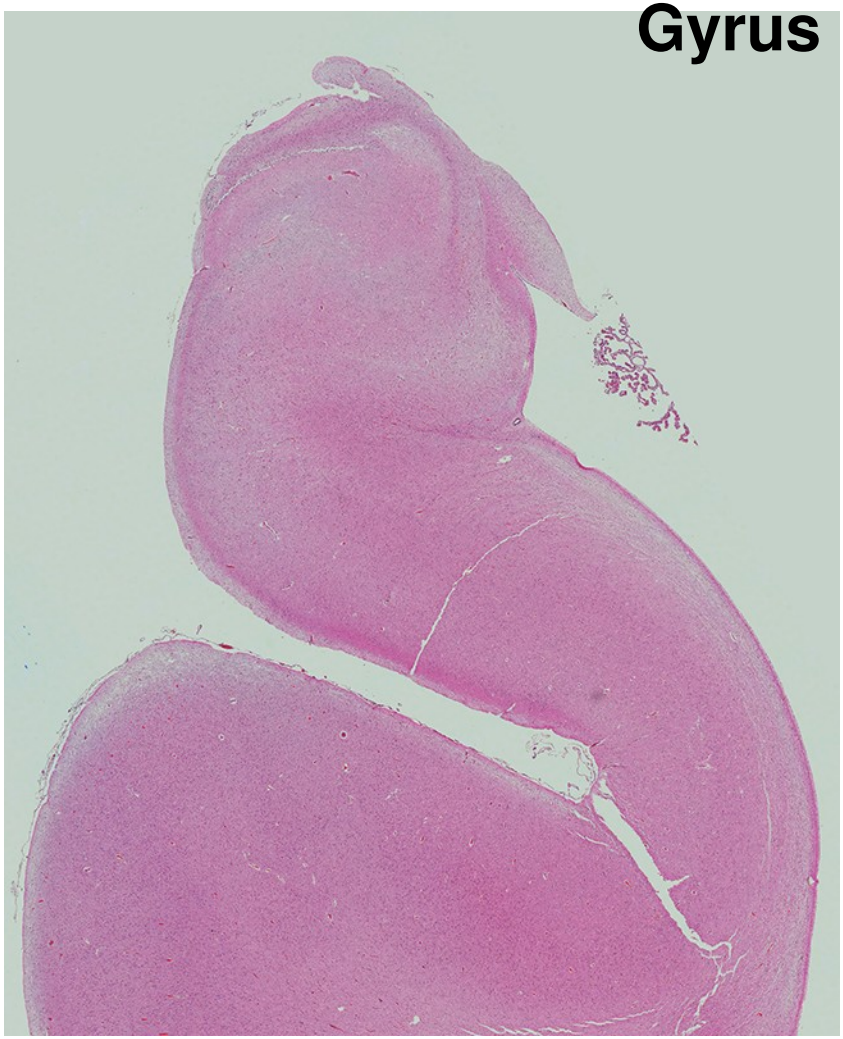
Case 2: “Bilateral HME”



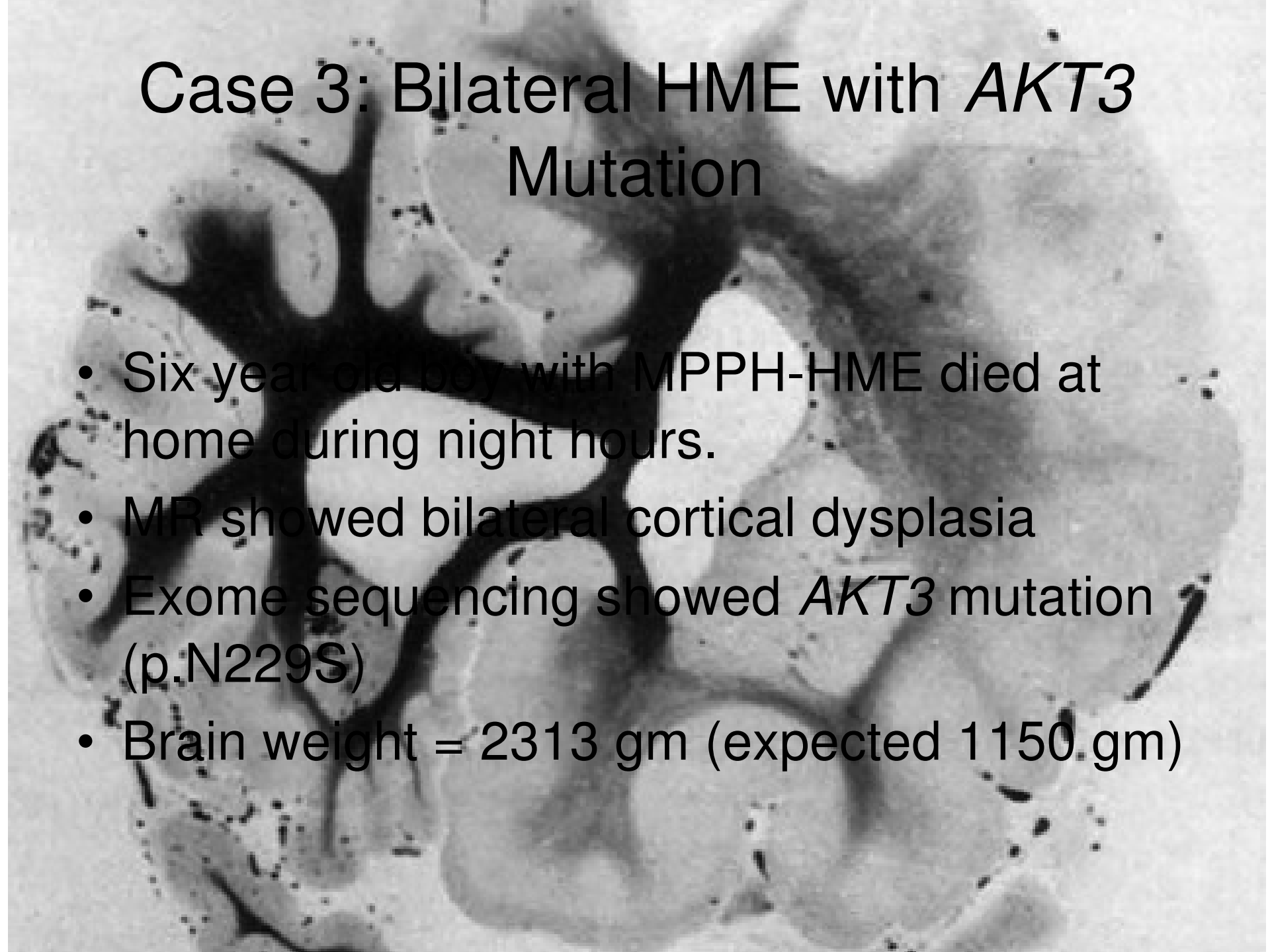
Case 2: “Bilateral HME”



Small Dentate Gyrus

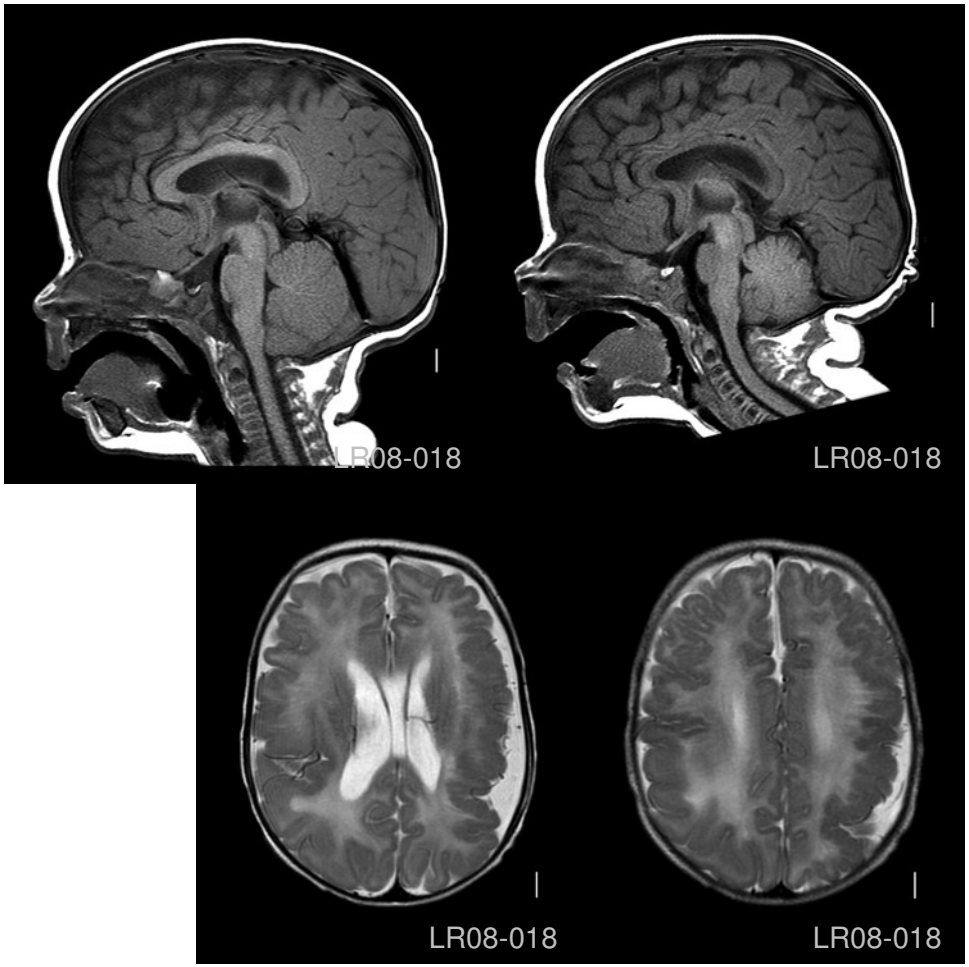


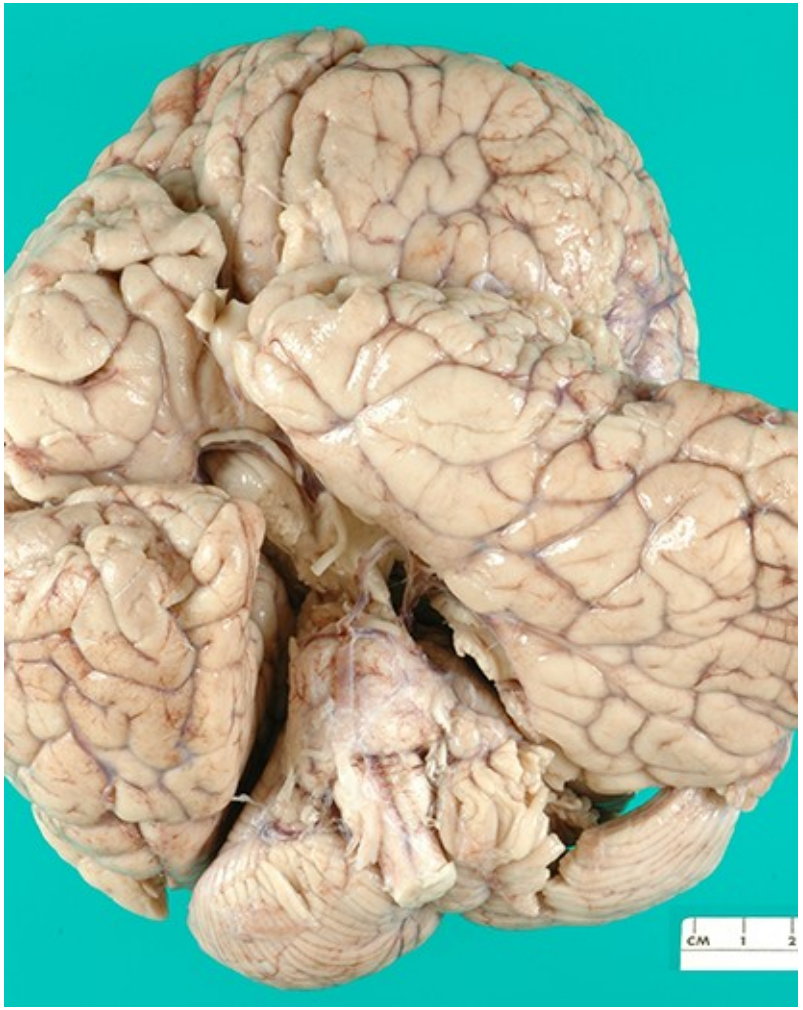
Case 3: Bilateral HME with *AKT3* Mutation



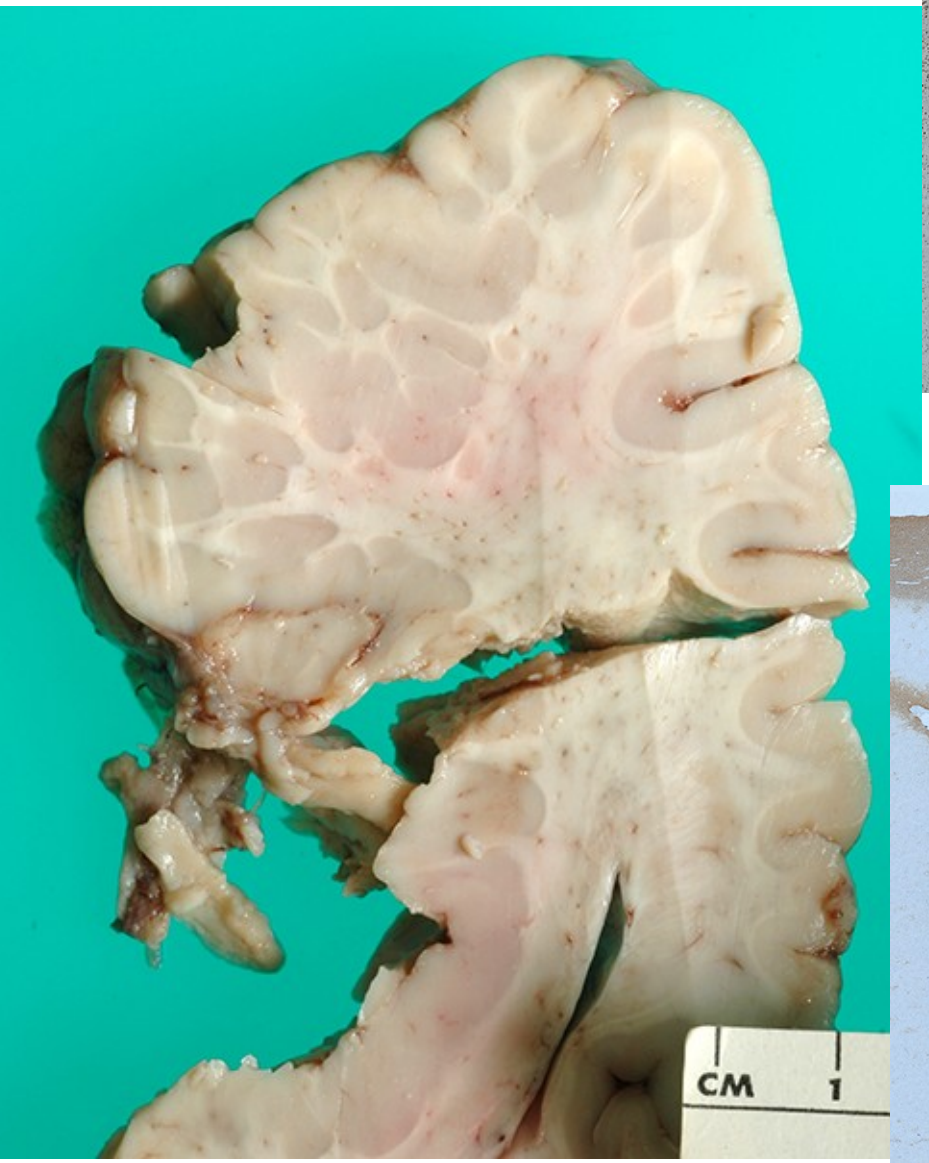
- Six year old boy with MPPH-HME died at home during night hours.
- MR showed bilateral cortical dysplasia
- Exome sequencing showed *AKT3* mutation (p.N229S)
- Brain weight = 2313 gm (expected 1150 gm)

Case 3: “Bilateral HME – *AKT3*”

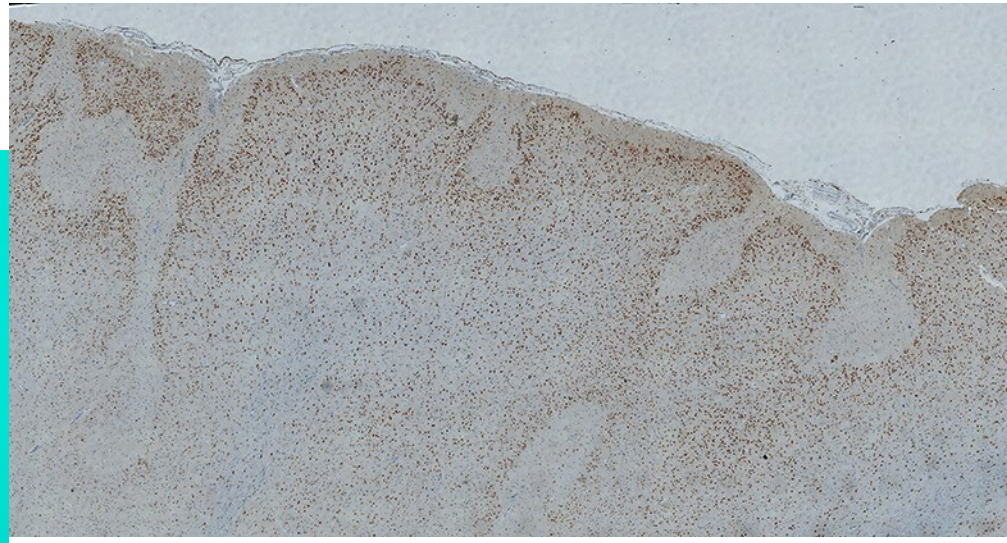




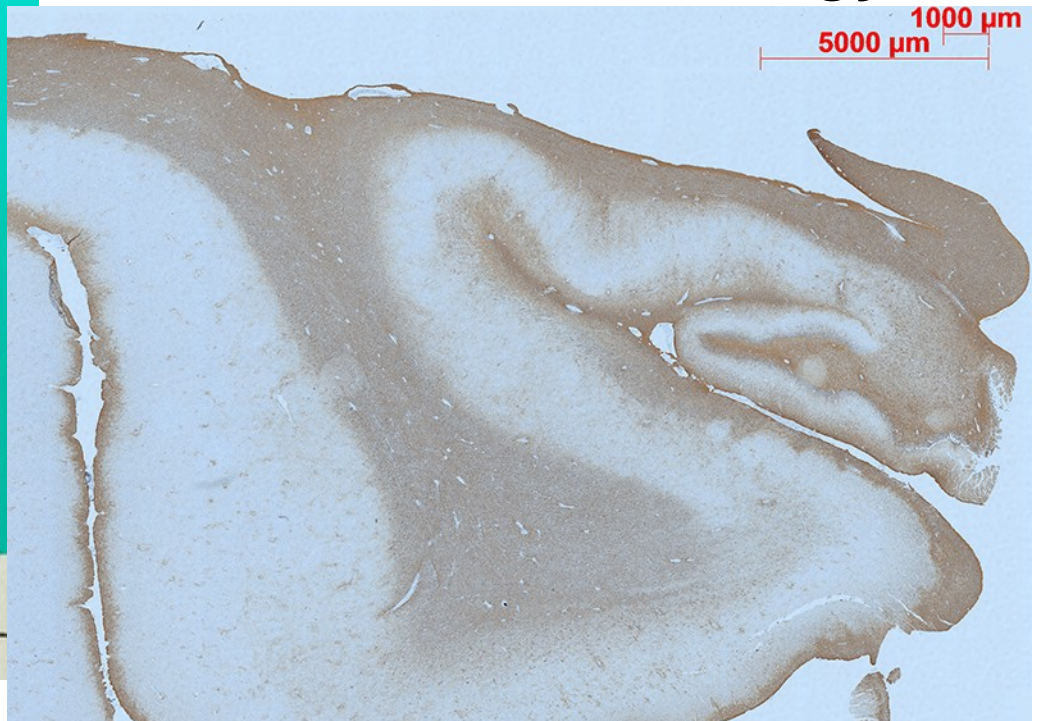
Case 3:
"Bilat. HME – *AKT3*"



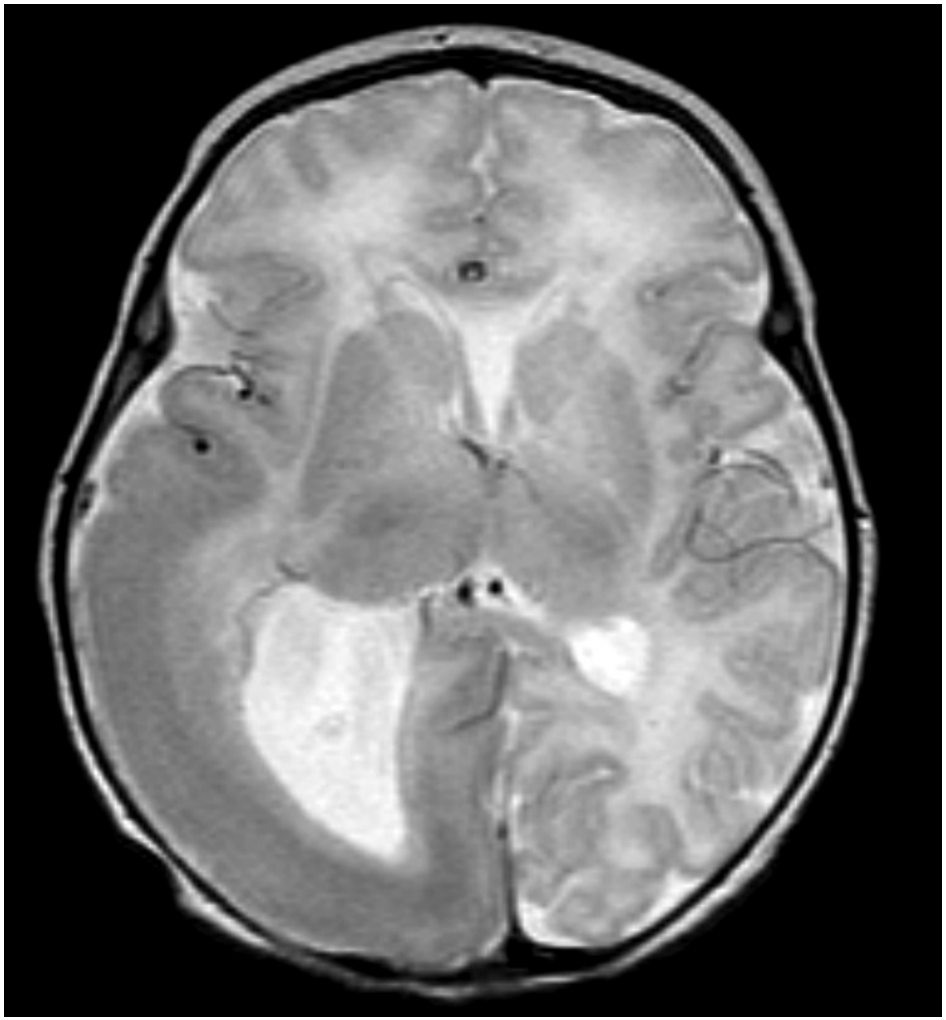
PMG (Left inferior temporal)



Small dentate gyrus



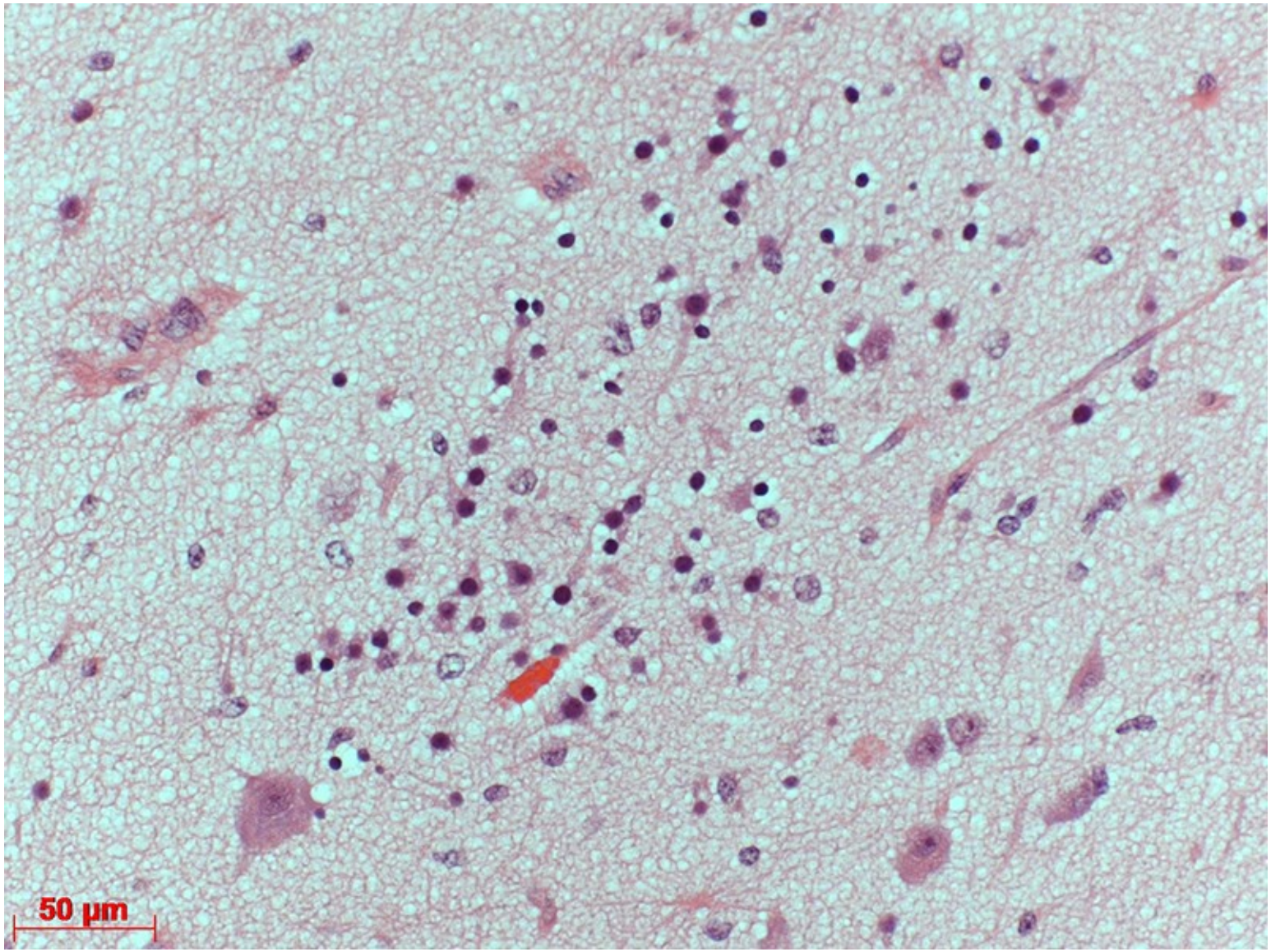
Case 4: “R HME”



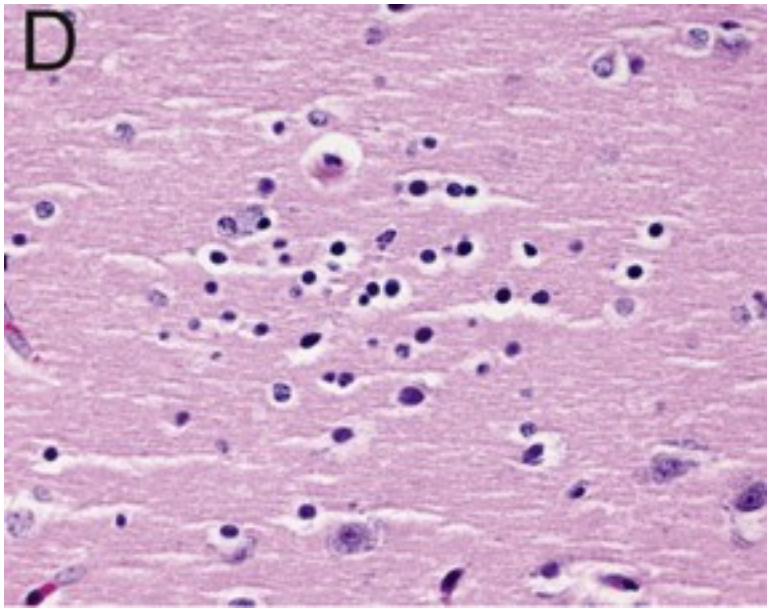
- 4 week old girl with neonatal seizures.
- MR showed R HME
- Gene not identified (yet)

Case 4:
“R HME”

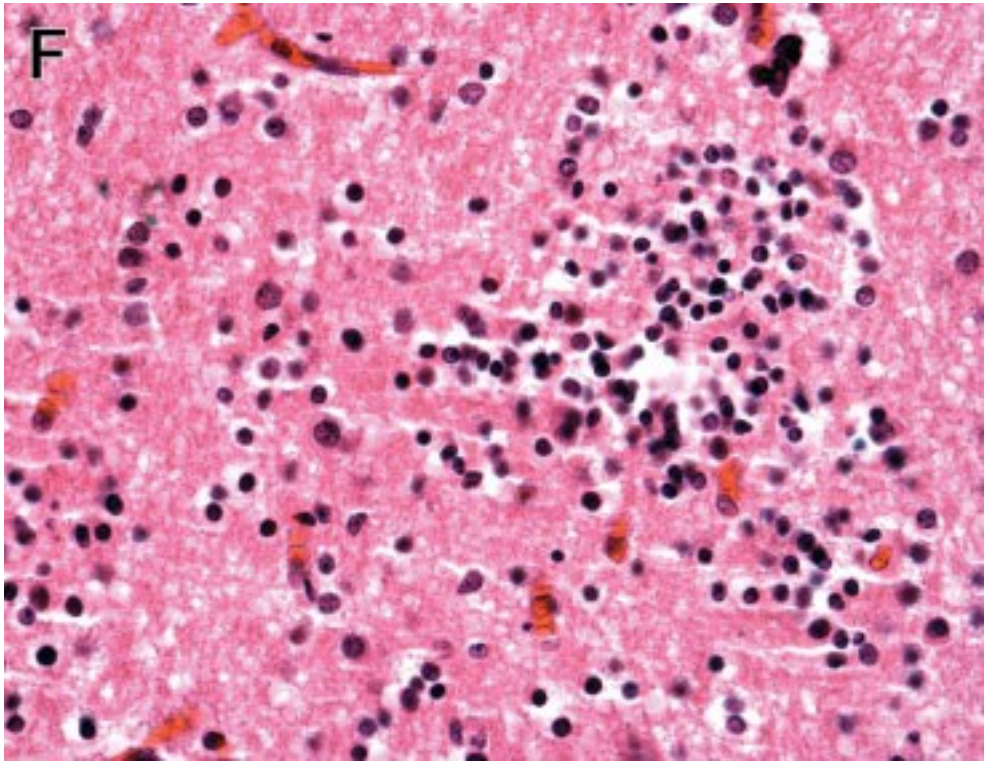
Clusters of small immature cells in cortex



Clusters of Immature Cells in HME

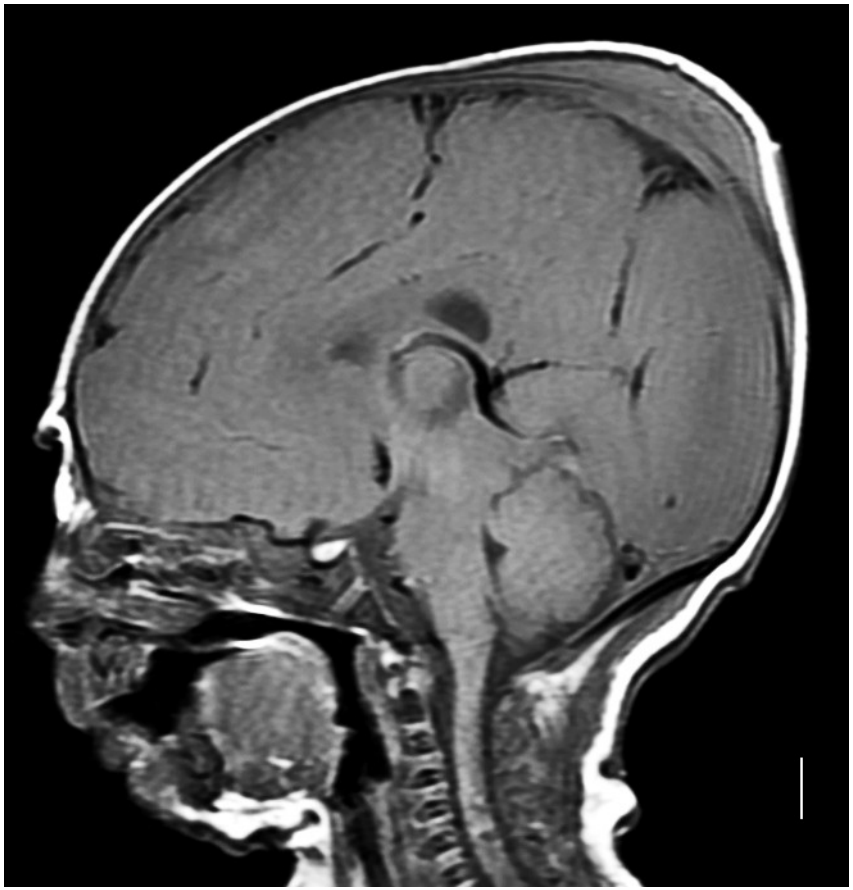


Poduri et al. (2012) *Neuron*



Salamon et al. (2006) *Brain*

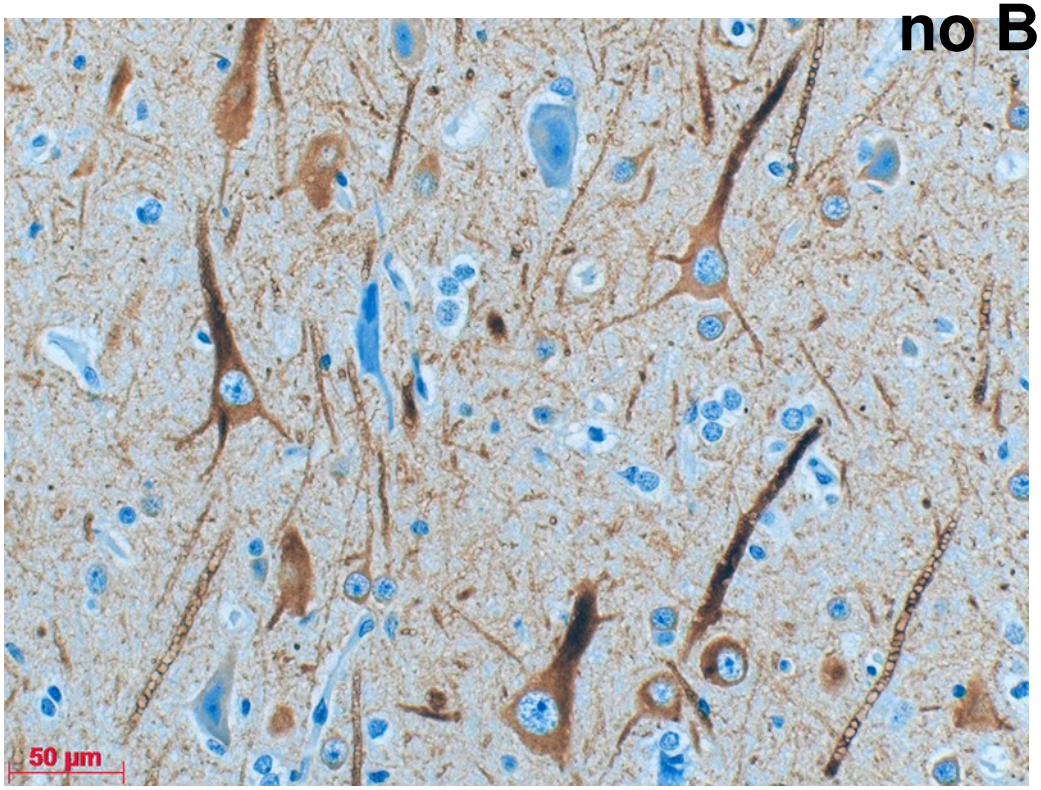
Case 5: “L HME – *AKT3* – Mosaic”



- 11 month old girl with seizures.
- MR showed L HME, L eye enlarged, L cerebellum dysplastic
- Exome sequencing showed *AKT3* mutation (p.E17K) at ~30% mosaic

Case 5: “L HME – *AKT3* – Mosaic”

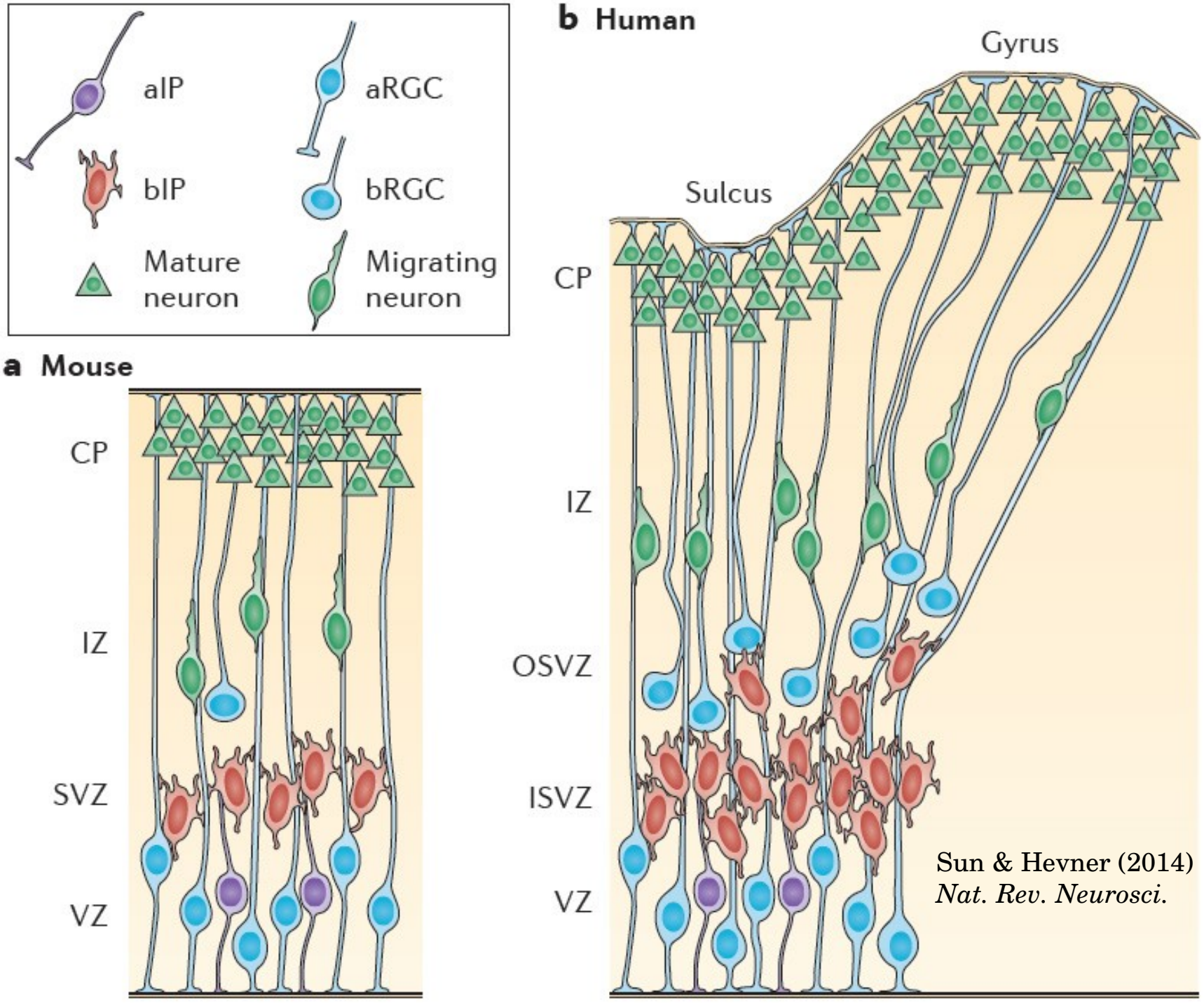
Main finding: CNs scattered in cortex; no BCs



MAP2

Summary of HME Neuropathology

- Distribution of lesions is variable, not necessarily restricted to, or fully involving one hemisphere
- White matter often enlarged, hypomyelinated
- Histopathology is highly variable but often includes pachygyria, PMG, heterotopic WM neurons, heterotopia; in contrast, BCs << half of cases; neuronal cytomegaly is often mild
- Autopsy cases seem to show frequent Dentate Gyrus hypoplasia
  - Sarnat et al. (2012) reported irregular thickness of DG



Sun & Hevner (2014)
Nat. Rev. Neurosci.

An Animal Model of MEG

Human Molecular Genetics, 2011, Vol. 20, No. 5 **988–999**
doi:10.1093/hmg/ddq544
Advance Access published on December 15, 2010

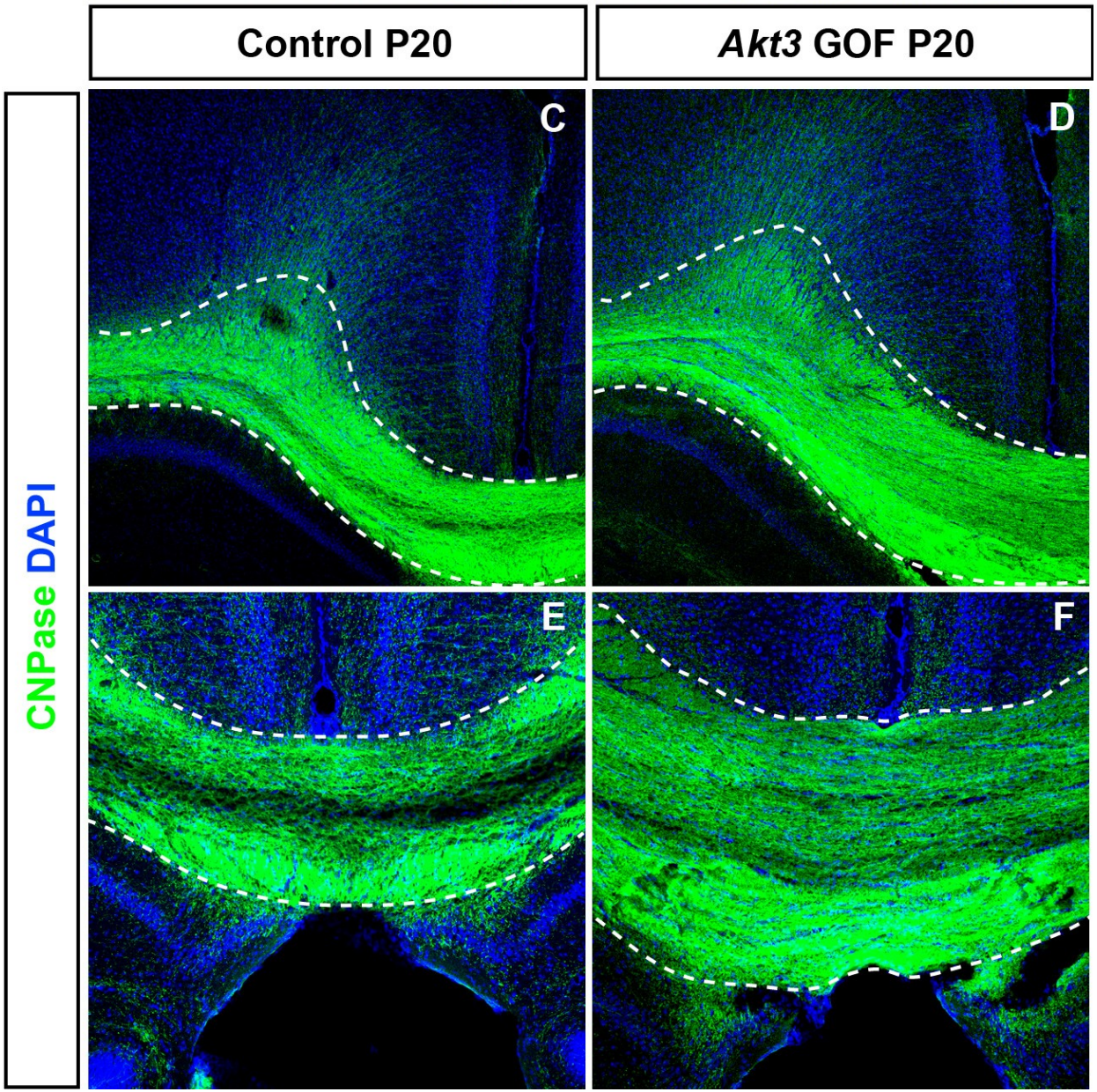
A novel *Akt3* mutation associated with enhanced kinase activity and seizure susceptibility in mice

Satoko Tokuda[1], Connie L. Mahaffey[1], Bobby Monks[2], Christian R. Faulkner[3], Morris J. Birnbaum[2], Steve C. Danzer[3] and Wayne N. Frankel[1,*]

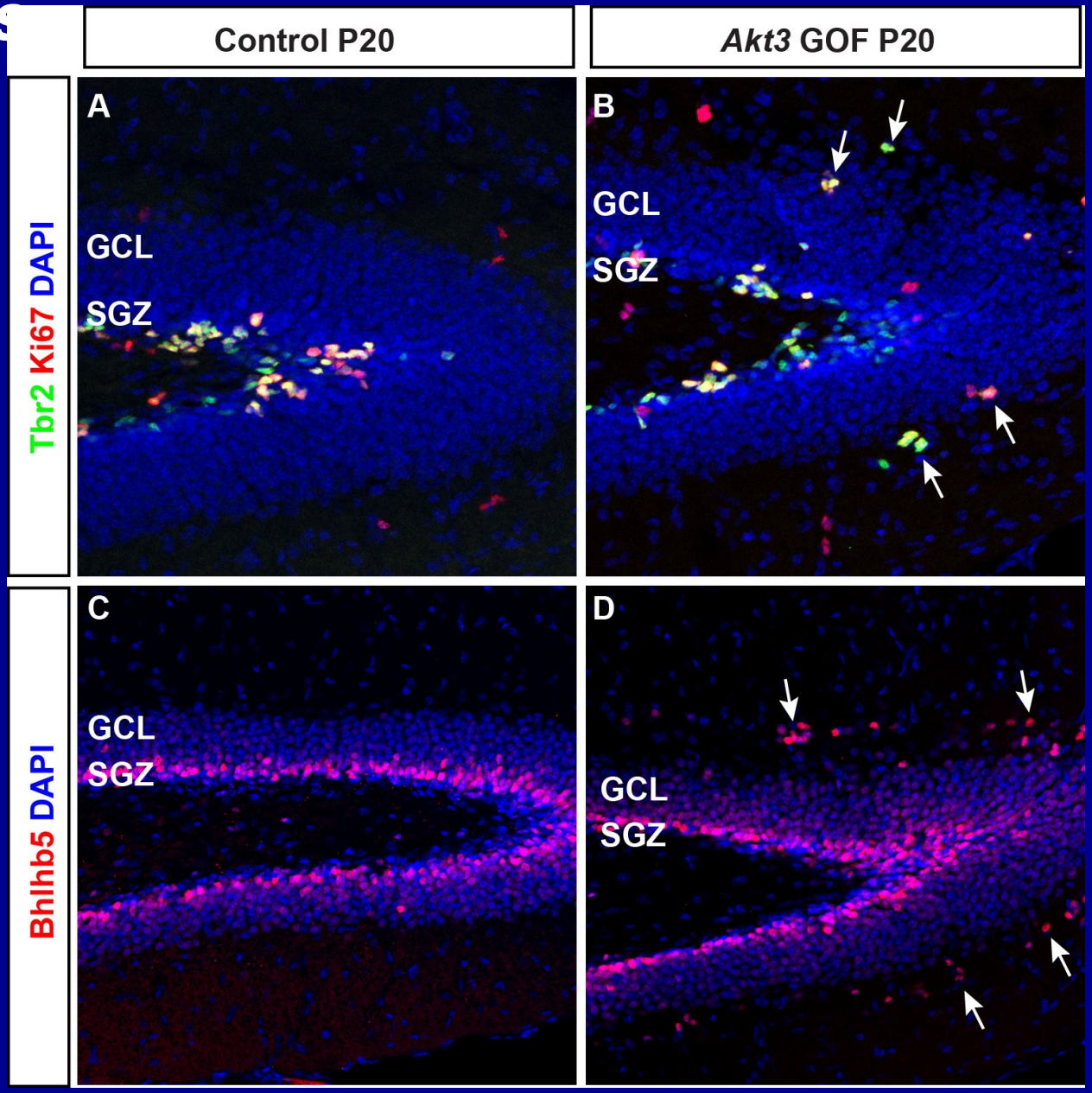
[1]The Jackson Laboratory, Bar Harbor, ME 04609, USA, [2]Institute for Diabetes, Obesity and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA and [3]Department of Anesthesia, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

- ENU mutagenesis screen for seizure prone mice
- *Akt3* mutation identified (p.D219V)
- Spontaneous seizures from 6 months age (hets)
- Enlarged brains (15-20% increased weight)

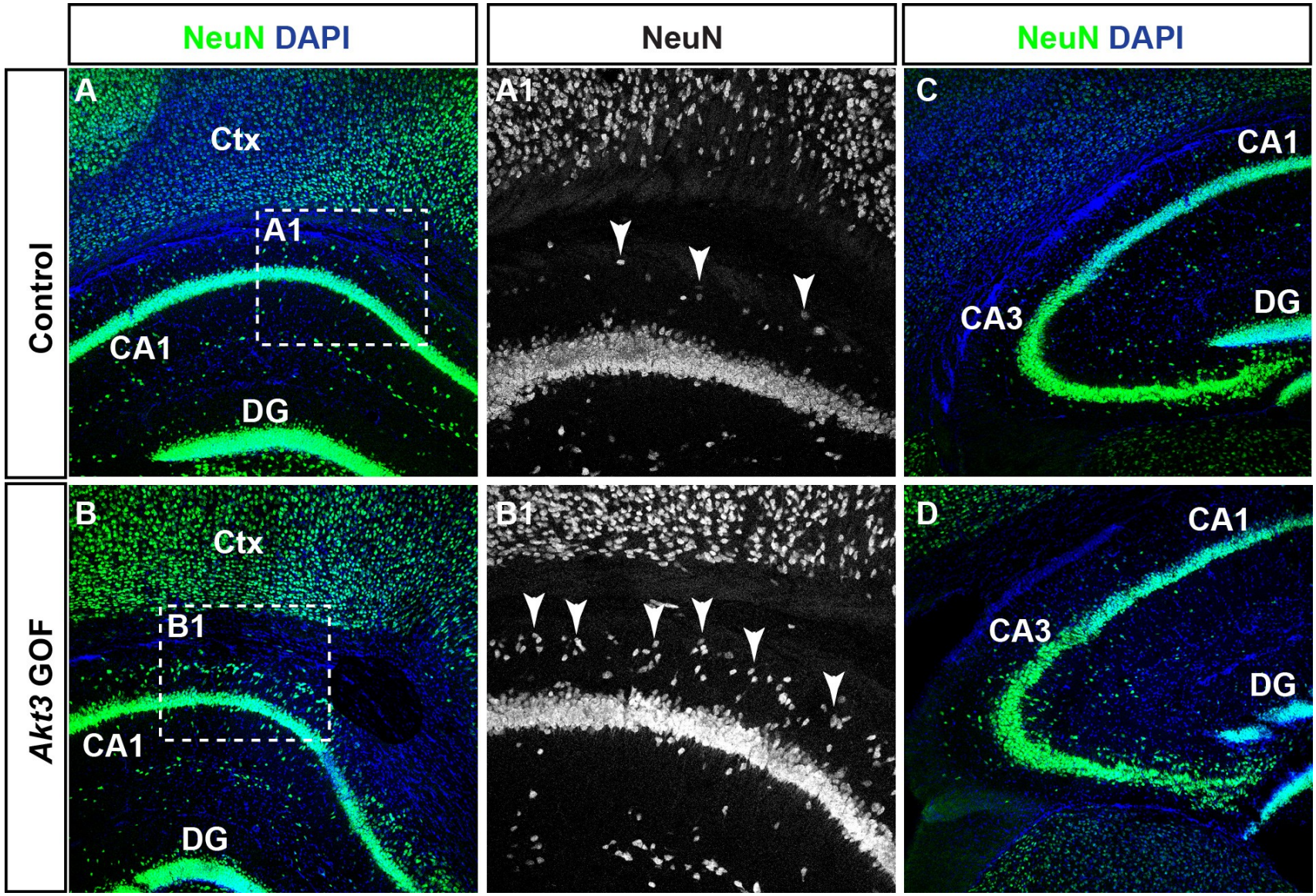
Increased white matter volume in Akt3^{D219V} *mice*



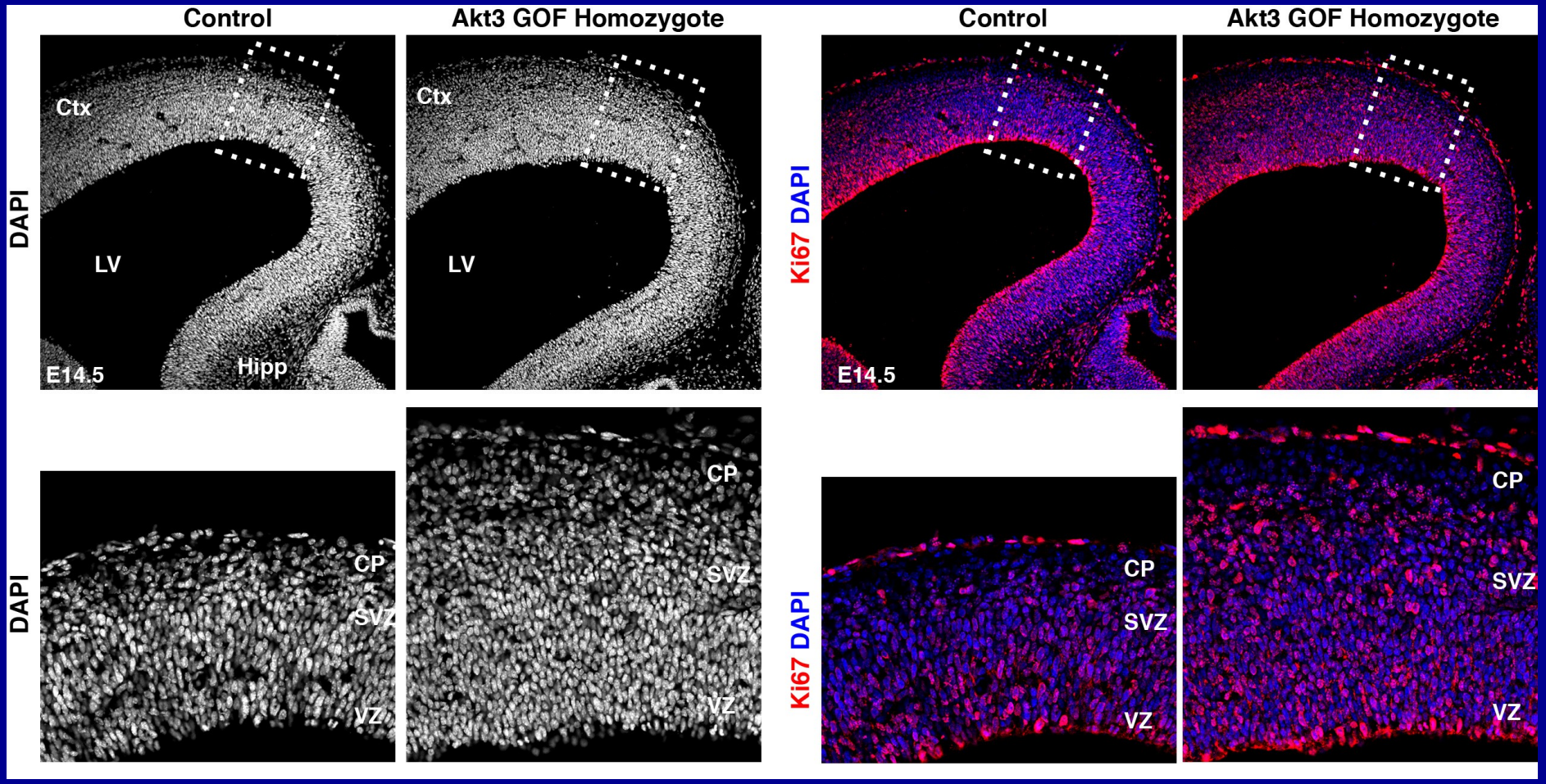
Ectopic progenitor cells in postnatal dentate gyrus



Akt3 GOF – Hippocampal Abnormalities



Early hyperplasia of cerebral cortex, E14.5



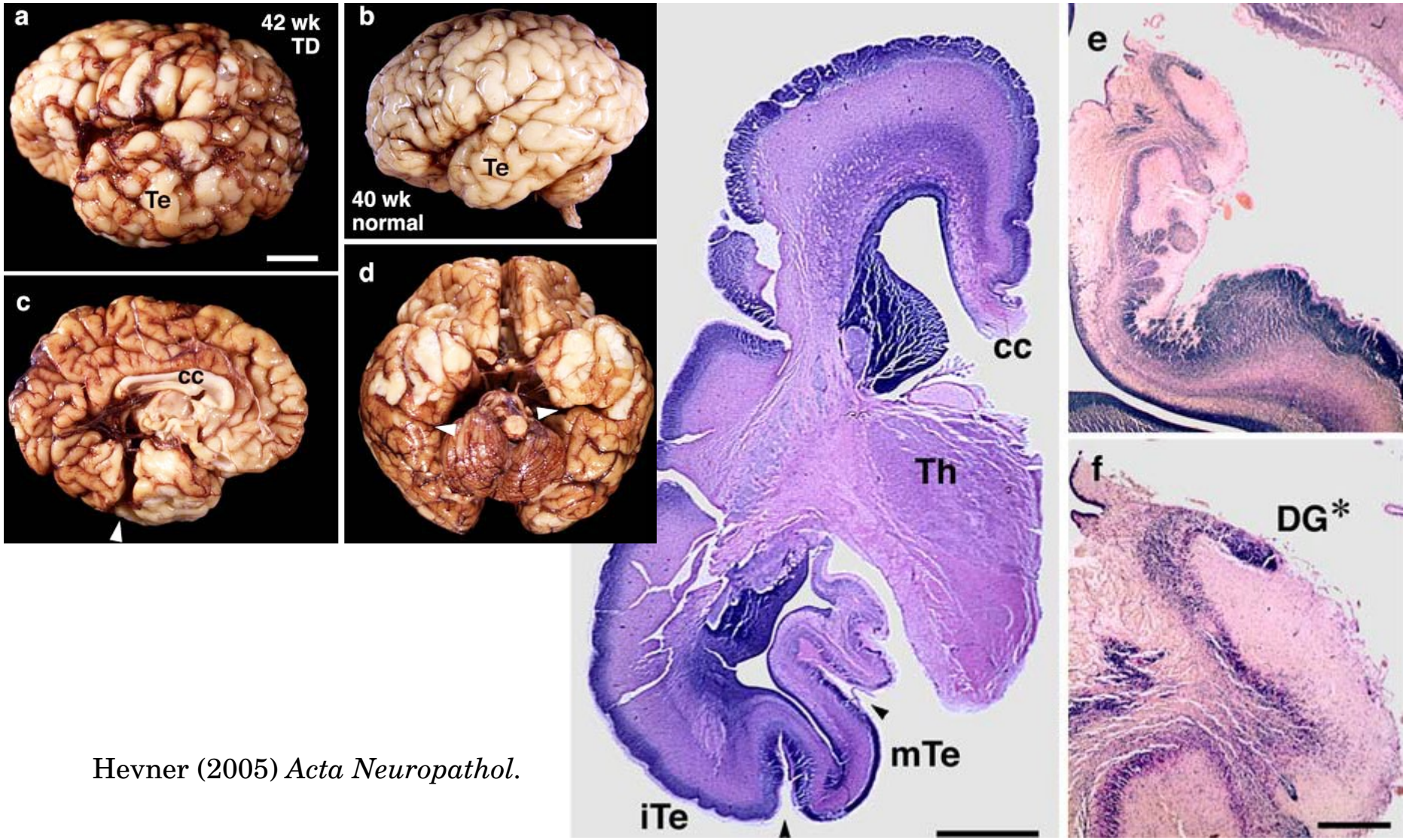
Summary of *Akt3*^{D219V} Mice

- Developmental trajectory reveals an early overproduction of cortical neurons
- Seizure development may result from aberrant migration of not only neurons, but also progenitor cells
- The hippocampus shows multiple migration defects, not only in DG but also CA fields

Ongoing Questions

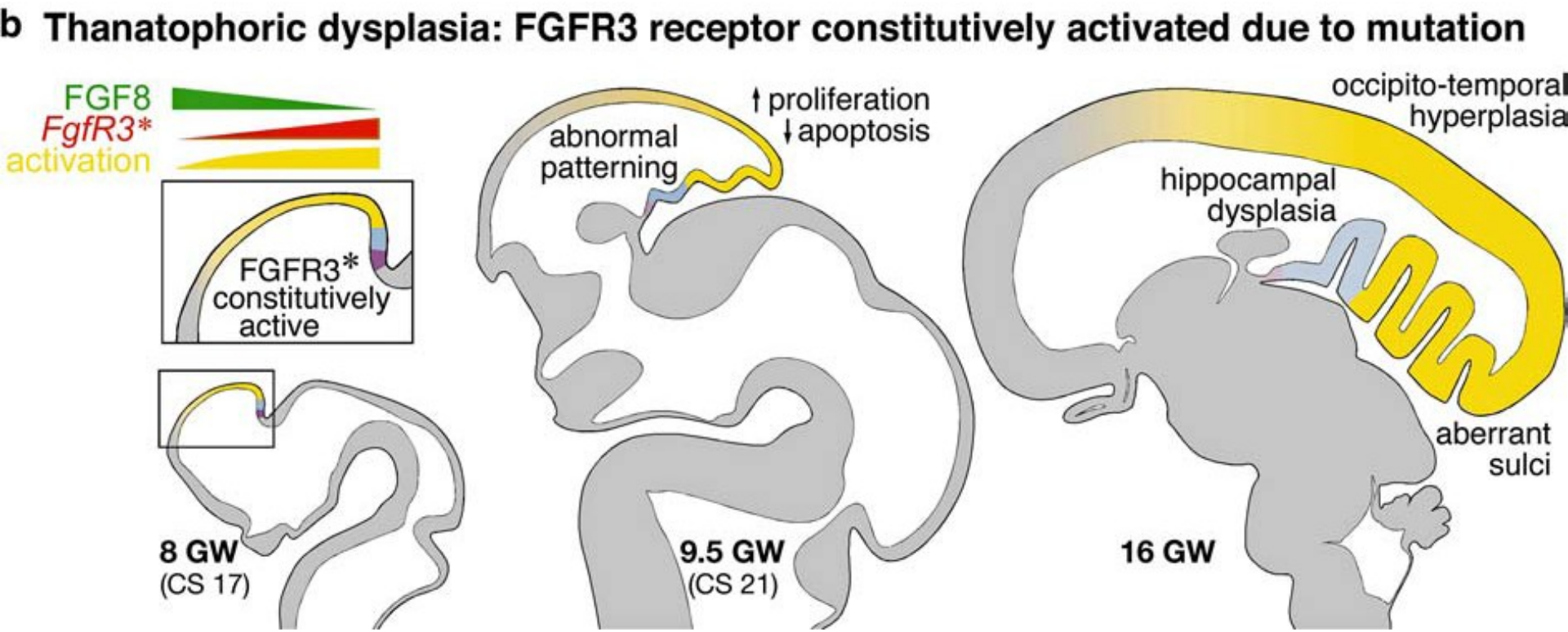
- What's upstream of PI3K-AKT-mTOR?
  - Growth factors: FGF signaling?
- What is the relation to FCD?
  - Differences of distribution?
  - On the same spectrum?

Megalencephaly and Abnormal DG in Thanatophoric Dysplasia (*FGFR3* GOF)



Hevner (2005) *Acta Neuropathol.*

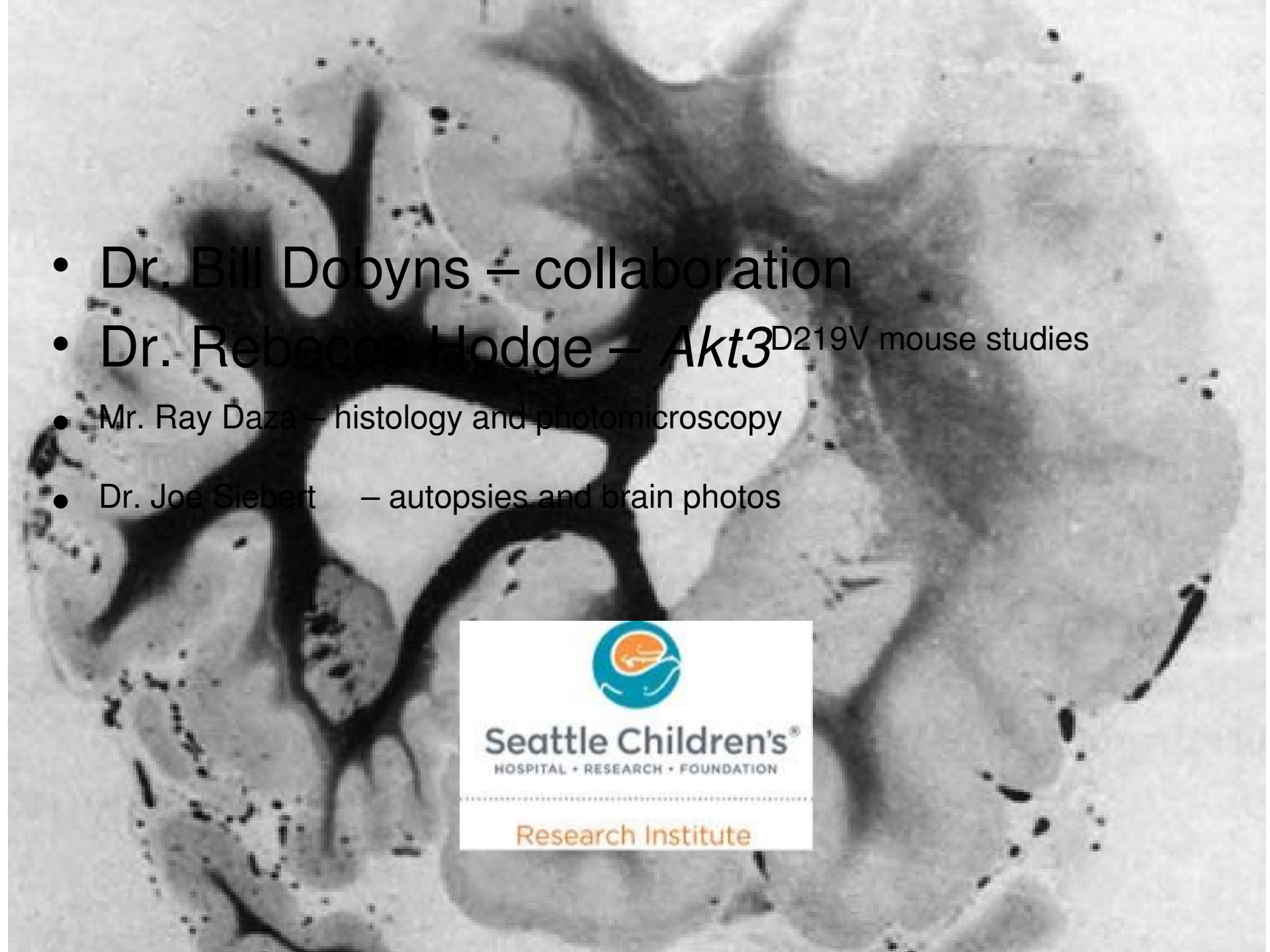
DG Hypoplasia: A Patterning Defect



Hevner (2005) *Acta Neuropathol.*

Summary

- **HME is an extraordinarily diverse pathological entity**. Phenotypes likely depend on causative genes, genetic background, and degree of mosaicism in each case, as well as extrinsic factors
- **Ongoing current challenges:**
  - What is the relation of HME to FCD?
  - What pathways other than mTOR are activated downstream of AKT in developing brain?
  - Can AKT inhibitors, or other drugs affect HME



- Dr. Bill Dobyns – collaboration
- Dr. Rebecca Hodge – *Akt3*^{D219V} mouse studies
- Mr. Ray Daza – histology and photomicroscopy
- Dr. Joe Siebert – autopsies and brain photos

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