TUBEROUS SCLEROSIS COMPLEX (TSC)

Pathologic and Molecular Aspects
(with an emphasis on *P13K-AKT-mTOR*
in the Central Nervous System)

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H. Vinters DISCLOSURES:

- The ***Harry V. Vinters trust*** owns shares in, & receives significant dividends from stock holdings in pharmaceutical companies, makers of medical equipment & devices, diagnostics companies
- ***These investments have no conflict with the content of this talk***
- ***Grant support from NIH (various) and the UC Pediatric Neuropathology Consortium/ Multi-Campus (UC) Research Program***
- ***Receive book royalties from Elsevier-Mosby***

TUBEROUS SCLEROSIS COMPLEX

--NEUROCUTANEOUS/MULTISYSTEM DISORDER IN WHICH

EPILEPSY (RELATED TO NEOCORTICAL TUBERS?)

IS A PROMINENT MANIFESTATION—TUBER *resections*…

--RESULTS FROM *MUTATION IN ONE of TWO GENES*....

TSC2 on 16p13.3--5.5kbase transcript--TUBERIN

TSC1 on 9q34------8.6 kbase transcript--HAMARTIN

.....'*GROWTH SUPPRESSOR GENES*'

Acta Neuropathol (2013) 125:317–332
DOI 10.1007/s00401-013-1085-x

REVIEW

Evolving neurobiology of tuberous sclerosis complex

Peter B. Crino

Received: 15 November 2012 / Revised: 14 January 2013 / Accepted: 19 January 2013 / Published online: 6 February 2013

Diagnostic criteria for TSC—revised, 2012

- A. Genetic diagnostic criteria: either a *TSC1* or *TSC2* pathogenic mutation is diagnostic (*pathogenic* mutation = one that *inactivates* function of *hamartin/tuberin*)

- B. Clinical diagnostic criteria:
- MAJOR:

  Cortical *dysplasias* (tubers)

  Subependymal nodules (SENs)

  Subependymal giant cell *astrocytoma* (SEGA)

H. Northrup et al, Pediatr Neurol 49: 243, 2013

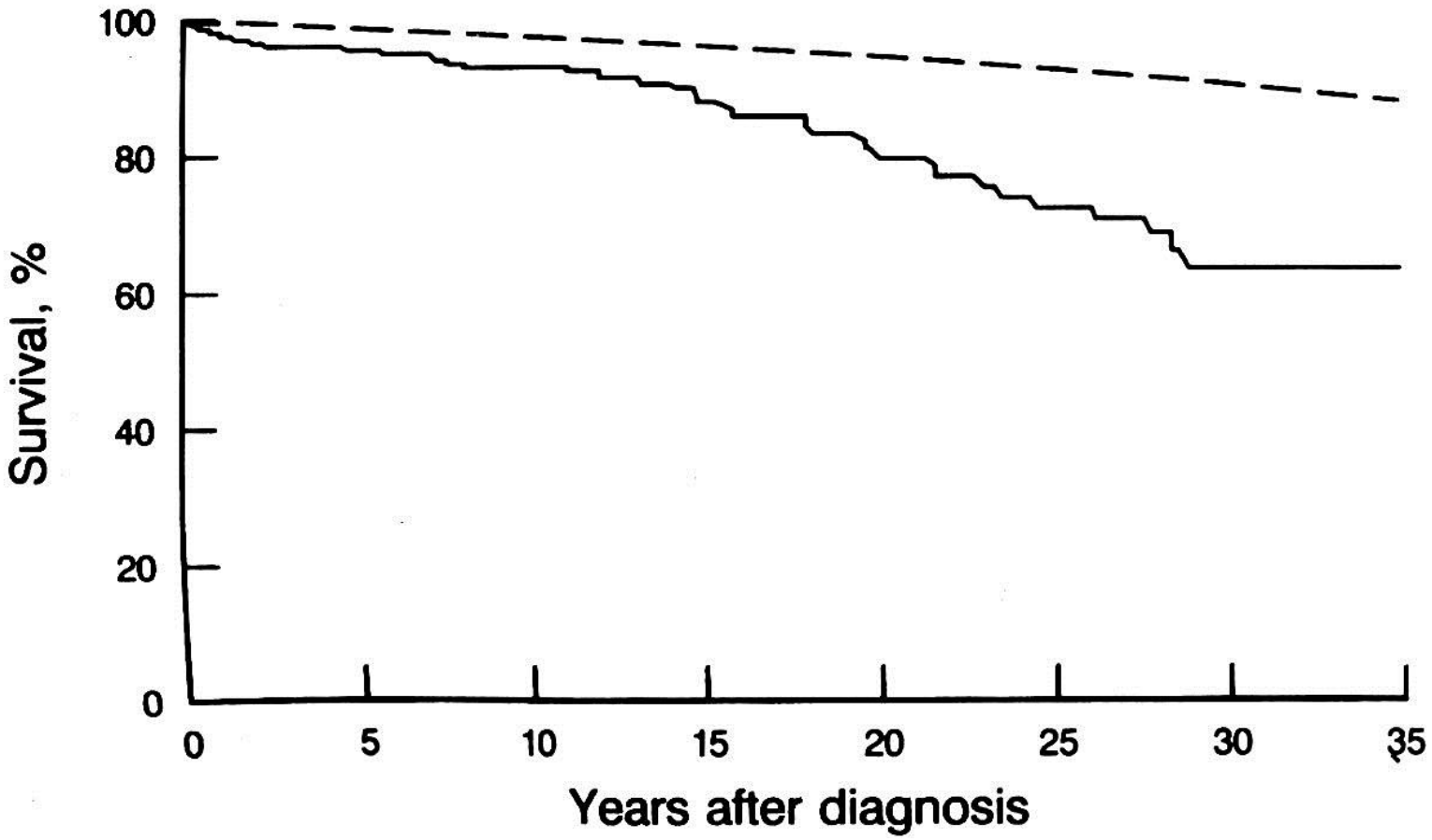


Fig. 3. Kaplan-Meier survival curves for patients with tuberous sclerosis complex (*solid line*) and for the white population of the United States in 1970 (*broken line*).

Shepherd, *et al*, *Mayo Clinic Proceedings*, 1991

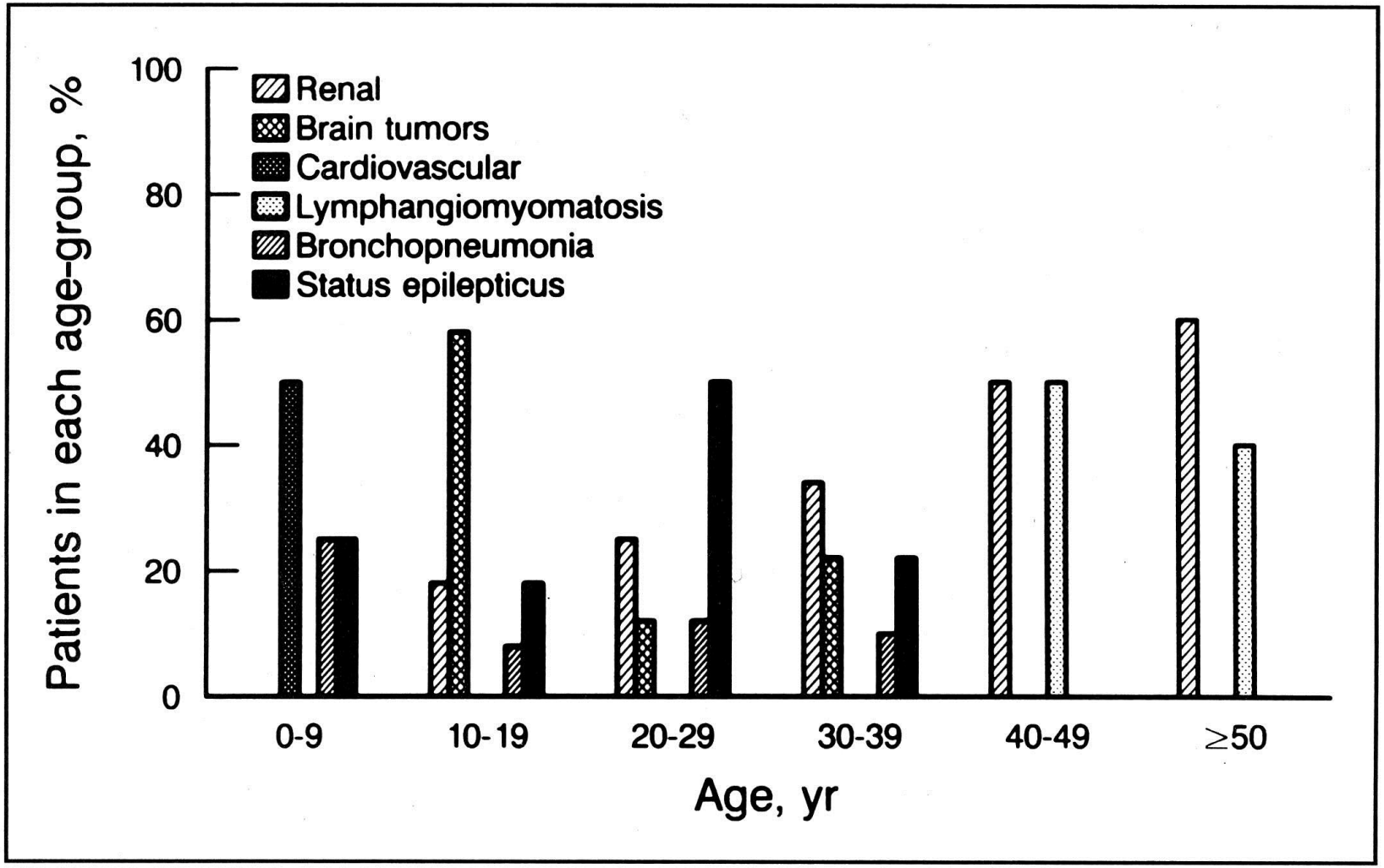
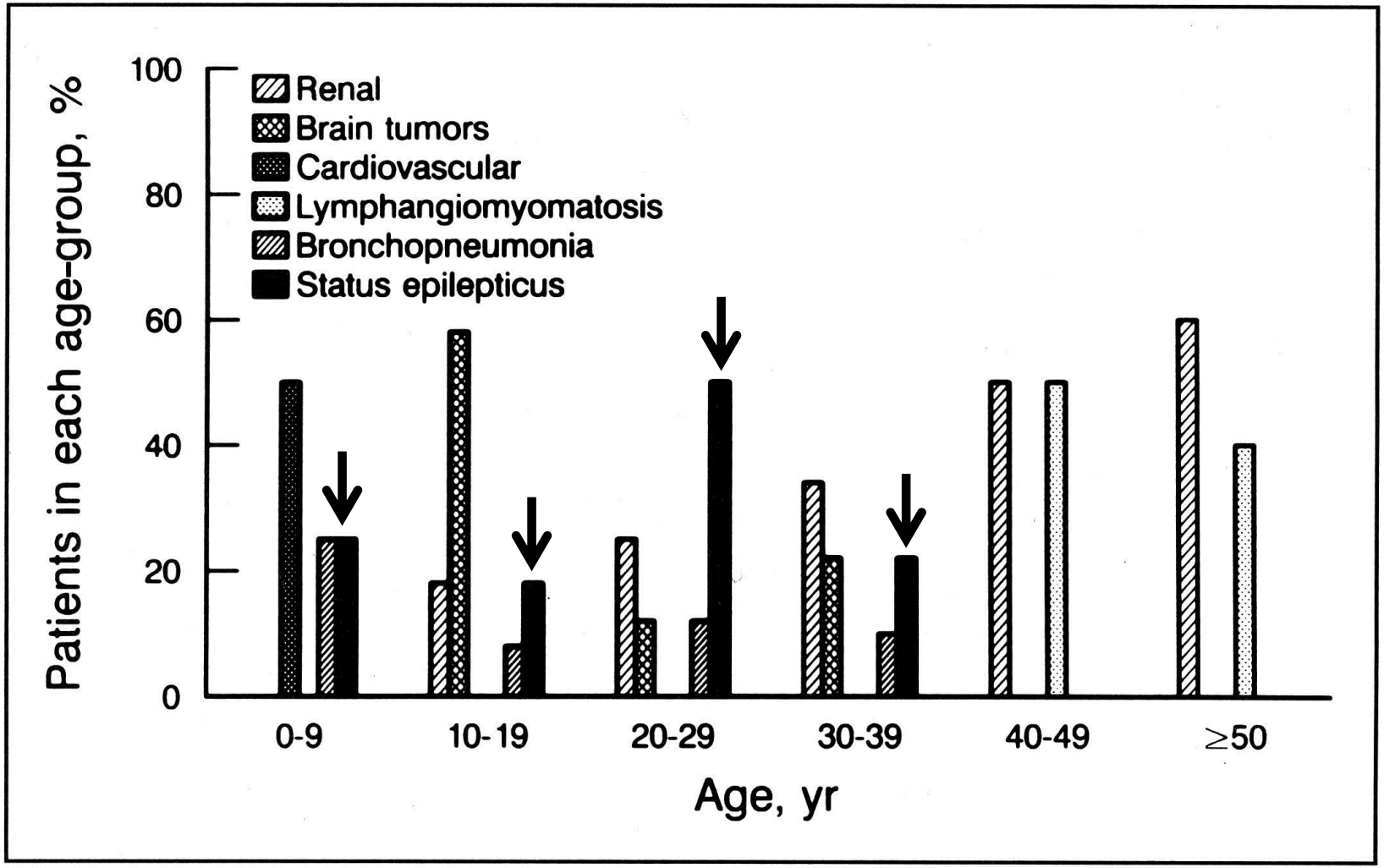


Fig. 2. Distribution of causes of death and ages of patients at time of death from tuberous sclerosis complex.

Shepherd, *et al*, *Mayo Clinic Proceedings*, 1991



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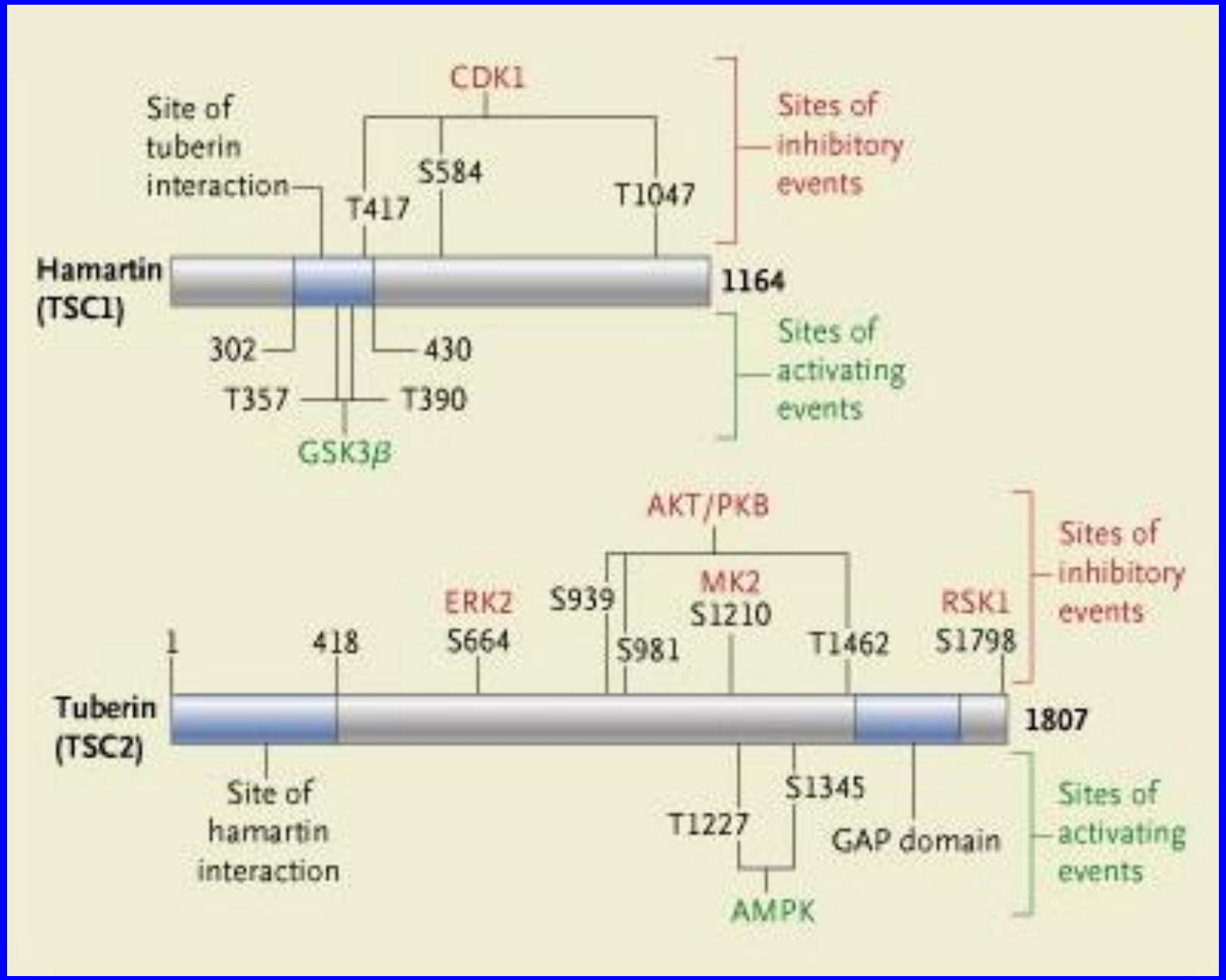
Shepherd, *et al*, *Mayo Clinic Proceedings*, 1991



GSK3B=glycogen synthase 3 beta
CDK-1=cyclin-dependent kinase 1

Crino PB *et al. N Engl J Med* 2006;355:1345-1356.

TSC Genetics……important factoids:

80%+ of TSC cases result from sporadic *TSC1/2* mutations

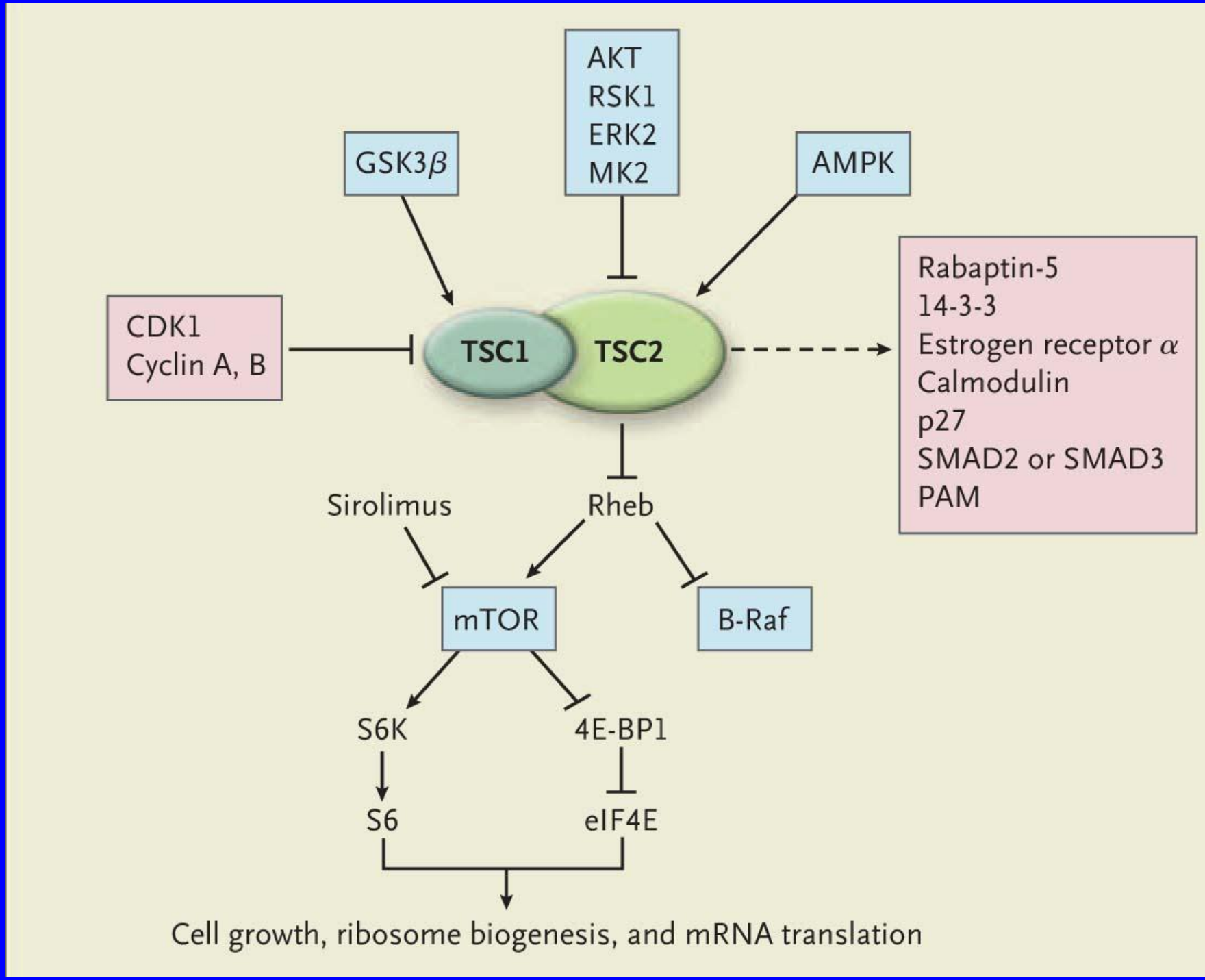
20% of cases have an inherited *TSC1/2* mutation

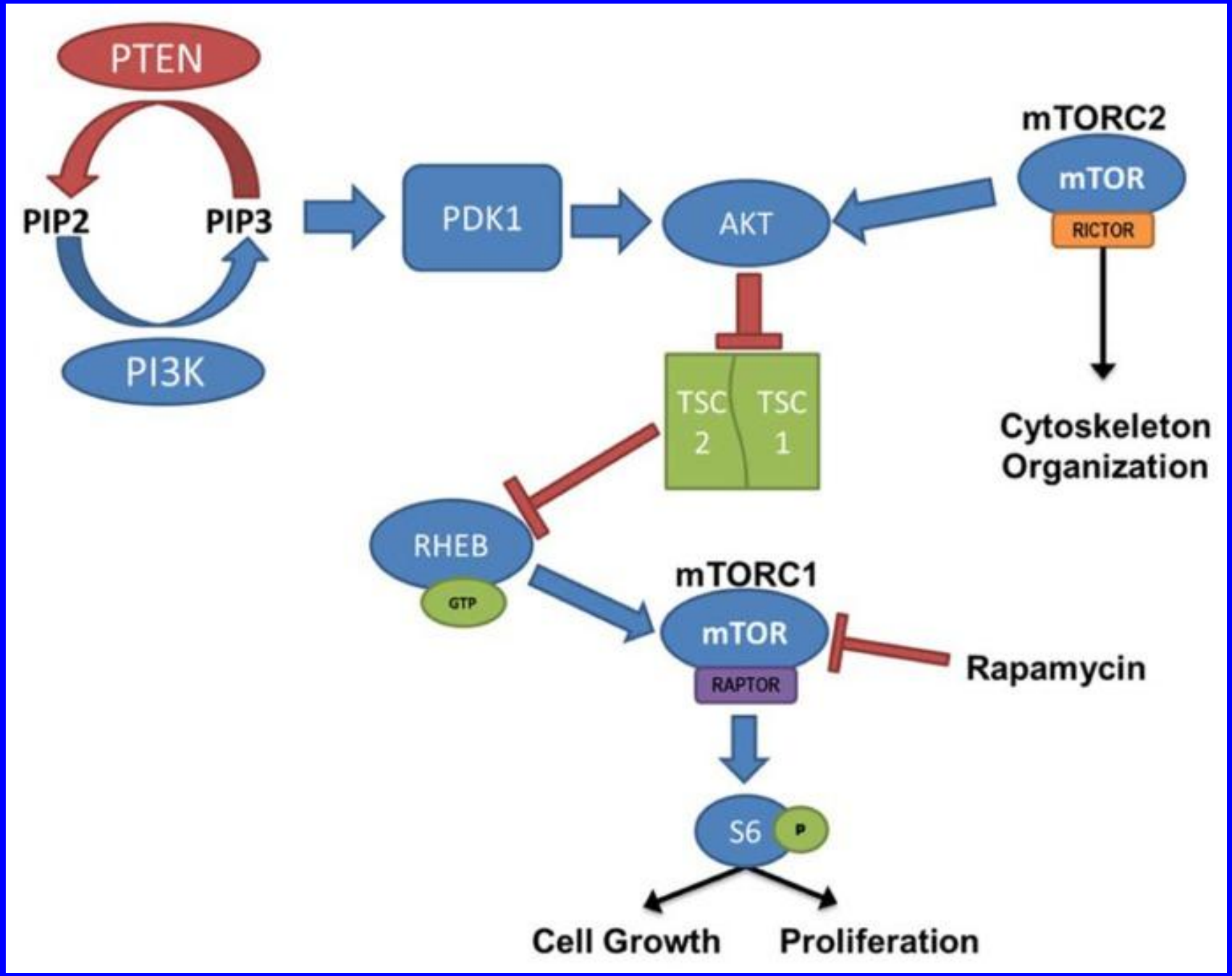
1000+ unique allelic variations of *TSC1/2 (mis-/nonsense, insertions, deletions)*

60% of patients have *TSC2* mutations, 30% *TSC1*

10-15% of clinically defined subjects have no mut'n (NMI)—low-level somatic mosaicism or a (?) putative *TSC3* gene

Genotype does not predict *phenotype*


AKT
RSK1
ERK2
MK2
GSK3β
AMPK
CDK1
Cyclin A, B
TSC1
TSC2
Rabaptin-5
14-3-3
Estrogen receptor α
Calmodulin
p27
SMAD2 or SMAD3
PAM
Sirolimus
Rheb
mTOR
B-Raf
S6K
4E-BP1
S6
eIF4E
Cell growth, ribosome biogenesis, and mRNA translation



LaSarge *et al*, *Front* Mol Neurosci 2014:7:18

mTOR in TSC

-- Ubiquitous serine/threonine kinase

-- Integrates signals from growth factors (e.g. IGF-1), nutrients, energy & stresses

-- Regulates multiple fundamental cell processes, e.g. growth, transcription, translation, autophagy

-- mTOR found in two distinct protein complexes:

a. mTOR C1 (mTOR, reg'y assoc'd protein of mTOR/ raptor, & PRAS40)

b. mTOR C2 (mTOR, rapamy-insensit component of mTOR/rictor), Protor 1/2

-- TSC2 acts as GTPase-activating protein towards Rheb

Neuropathologic lesions in TSC

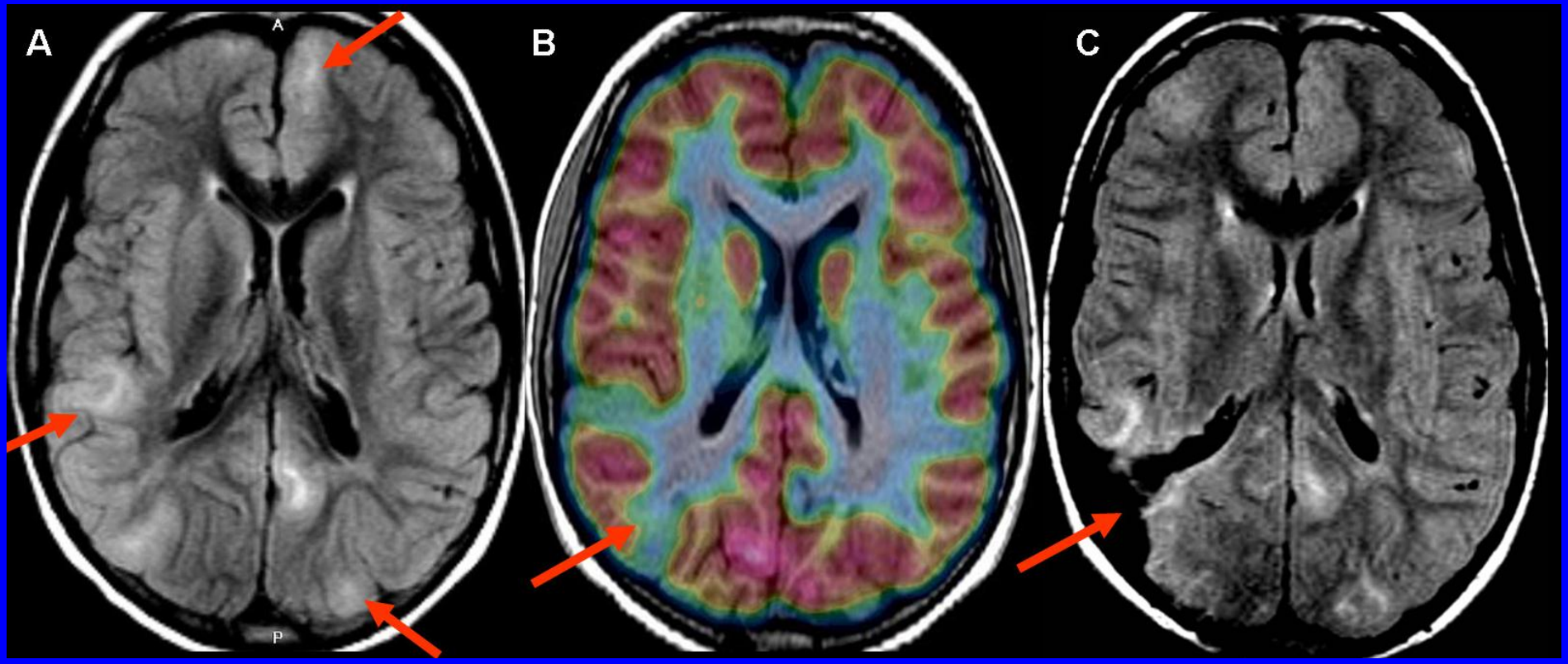
- Subependymal nodules/subependymal giant cell *astrocytoma* (SEGA)
- Hemimegalencephaly (rare)
- *Tubers and cortical disorganization*

J Child Neurol. 1997 Feb;12(2):85-90.

Cortical tuber count: a biomarker indicating neurologic severity of tuberous sclerosis complex

Goodman M, Lamm, SH, Engel A, Shepherd CW, Houser OW, Gomex MR
Johns Hopkins University School of Public Health, Baltimore, MD, USA.

Abstract
The relationship between the number of cortical tubers observed by magnetic resonance imaging (MRI) and the severity of cerebral dysfunction of tuberous sclerosis patients has been examined in a meta-analysis of the published literature. The literature review has identified five independent studies for examining the association. These studies consistently reveal that the cortical tuber count detected on MRI scans is increased among those with more severe cerebral disease. Severity of the cerebral dysfunction is measured by the seizure status and its control and by the developmental status and the level of mental retardation. Meta-analysis demonstrates that within a study population, the MRI-detected cortical tuber count is six times more likely to be above the median count for tuberous sclerosis patients with severe cerebral dysfunction (poor seizure control or moderate-severe retardation or both) than more mildly affected tuberous sclerosis patients. Similarly, across studies, moderately to severely affected patients are five times more likely to have greater than seven MRI-detected cortical tubers than those more mildly affected. These associations are both statistically significant and strong. The cortical tuber count is a biomarker that reasonably predicts the severity of cerebral dysfunction of tuberous sclerosis. Cortical tubers of tuberous sclerosis form in the early gestational period. The embryologic disruption determining the clinical severity of the cortical dysfunction of tuberous sclerosis is set in the early gestational period.



Pre-operative

Post-operative

Tubers---*MRI and PET* scans co-registered (courtesy Noriko Salamon)

Childs Nerv Syst (2008) 24:1437–1445
DOI 10.1007/s00381-008-0679-4

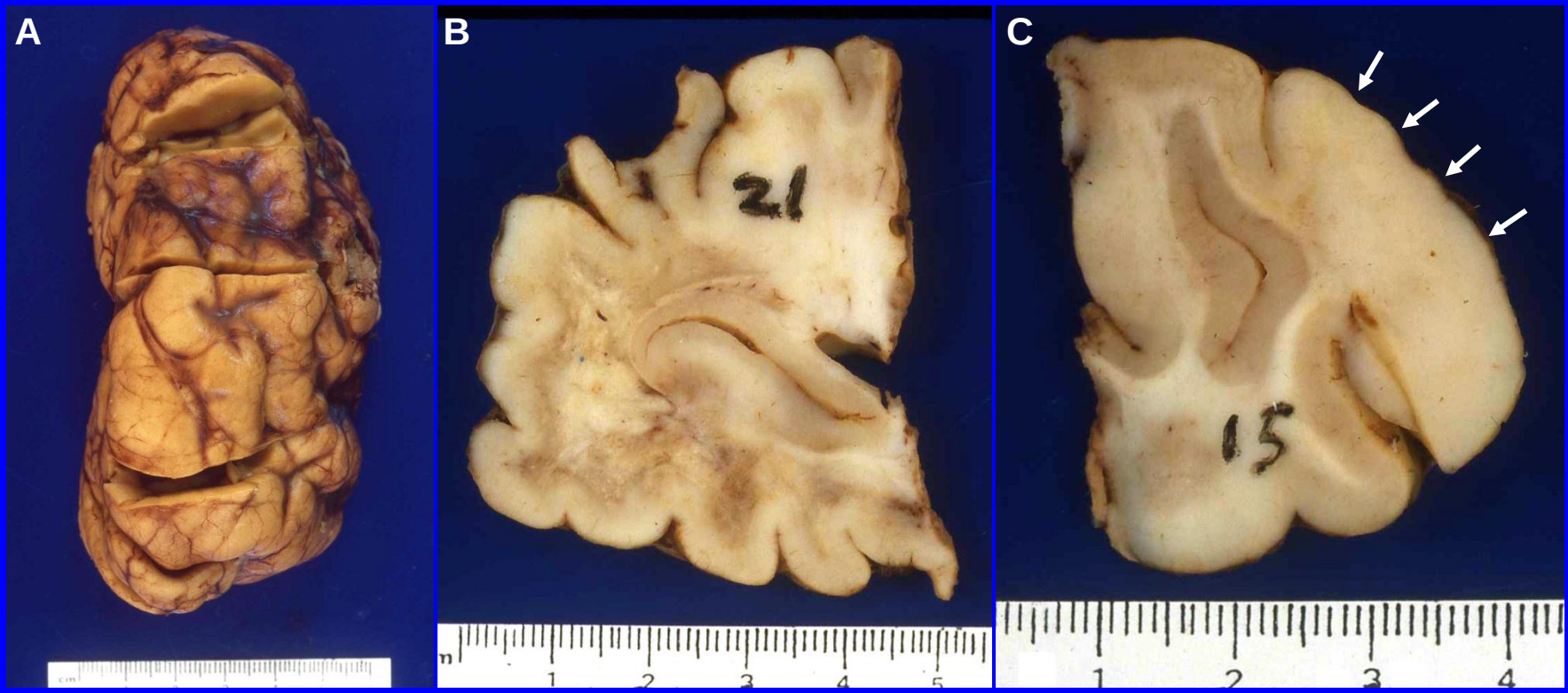
ORIGINAL PAPER

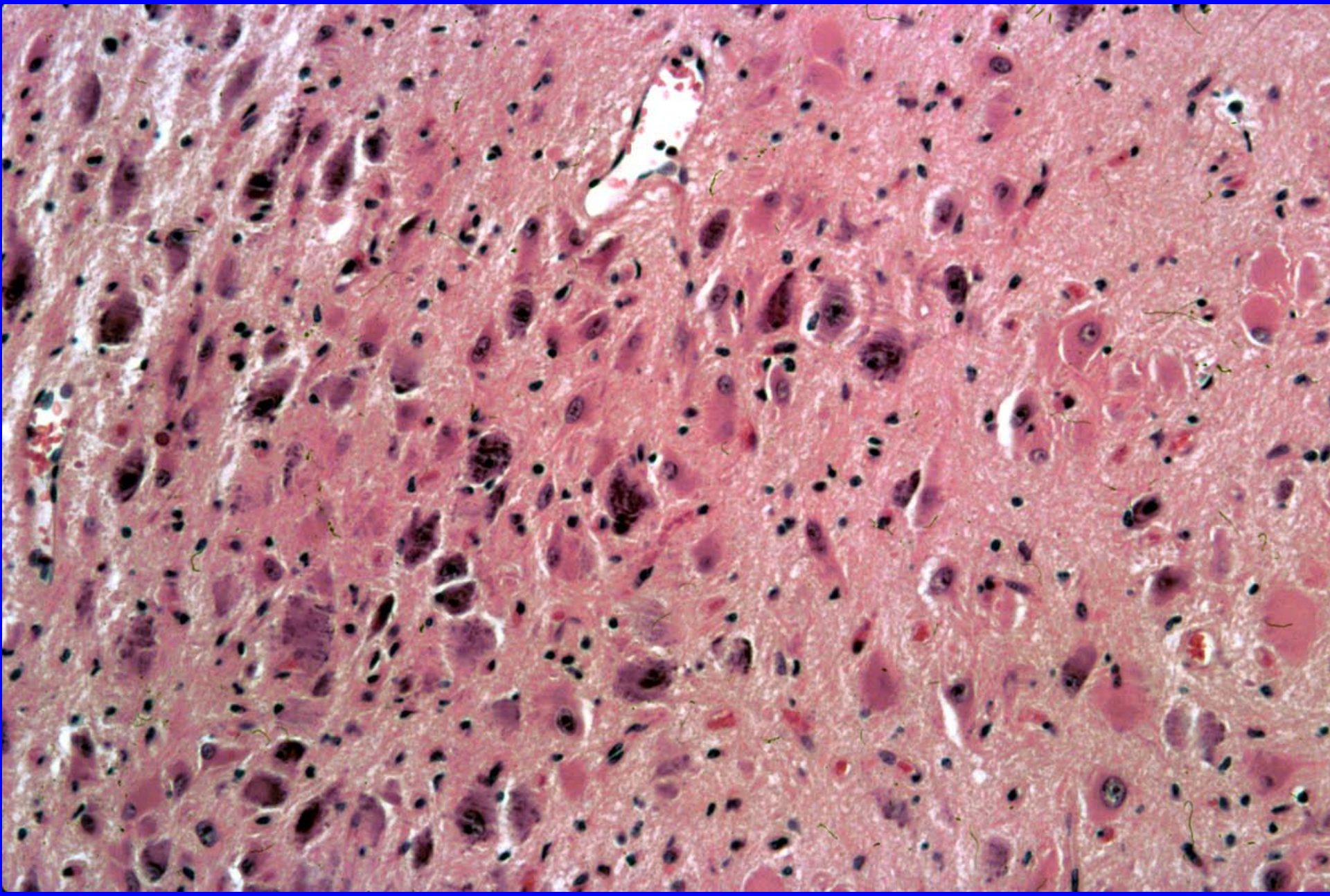
Epilepsy surgery in tuberous sclerosis complex: early predictive elements and outcome

F. Teutonico · R. Mai · O. Devinsky · G. Lo Russo · H. L. Weiner · P. Borrelli · U. Balottin · P. Veggiotti

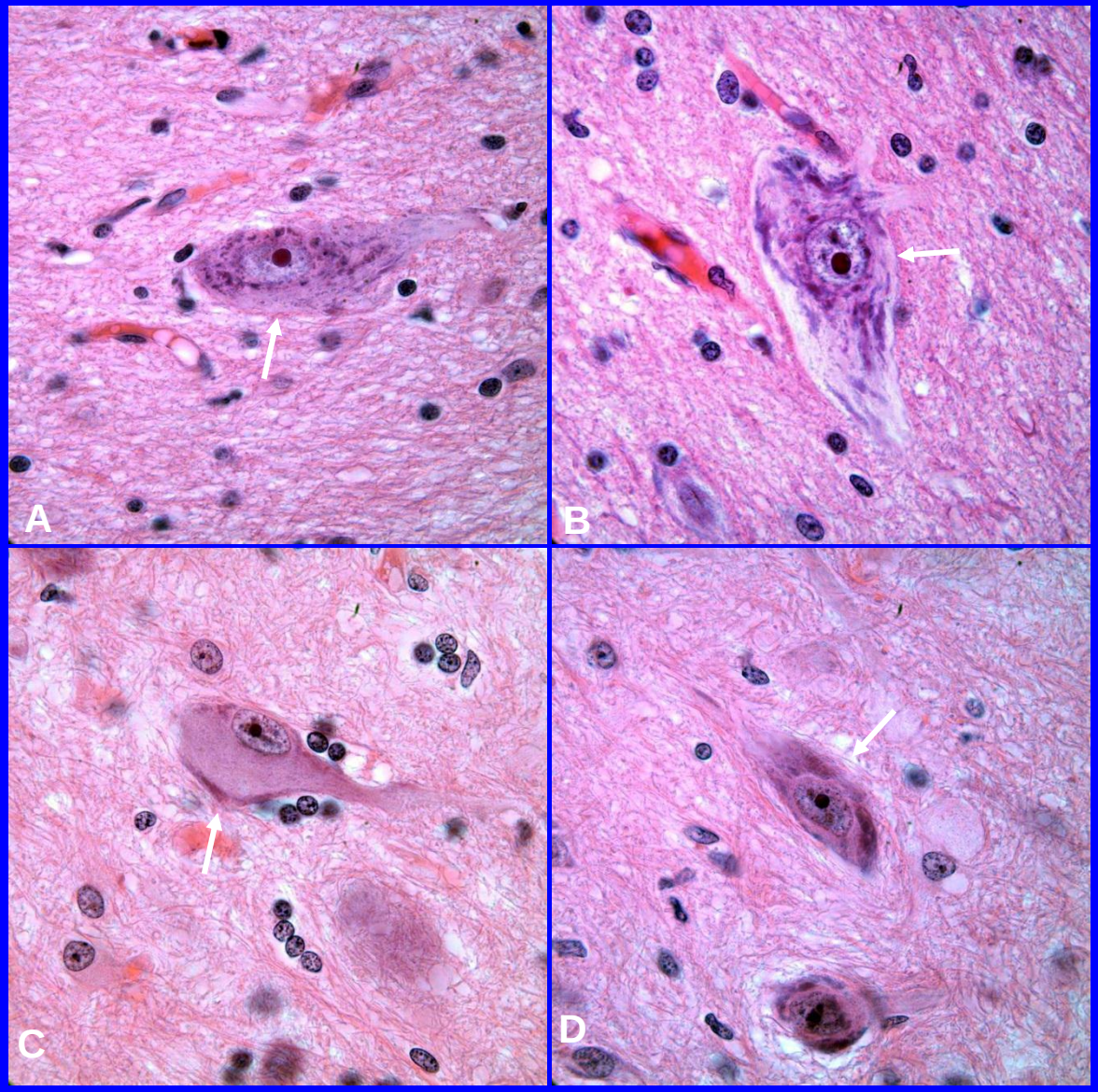
Received: 26 April 2008 / Revised: 10 June 2008 / Published online: 14 August 2008

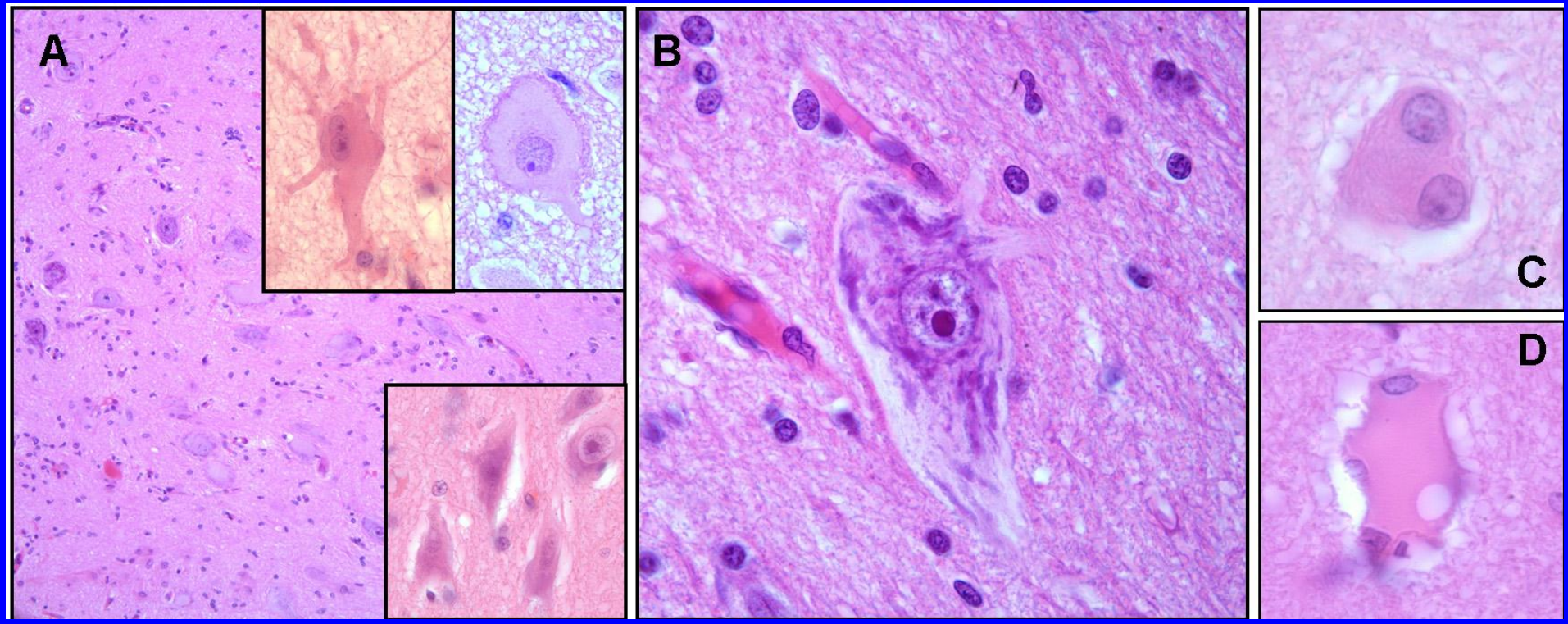
‘*Tuberectomy*’ now routine in epilepsy surgery…..



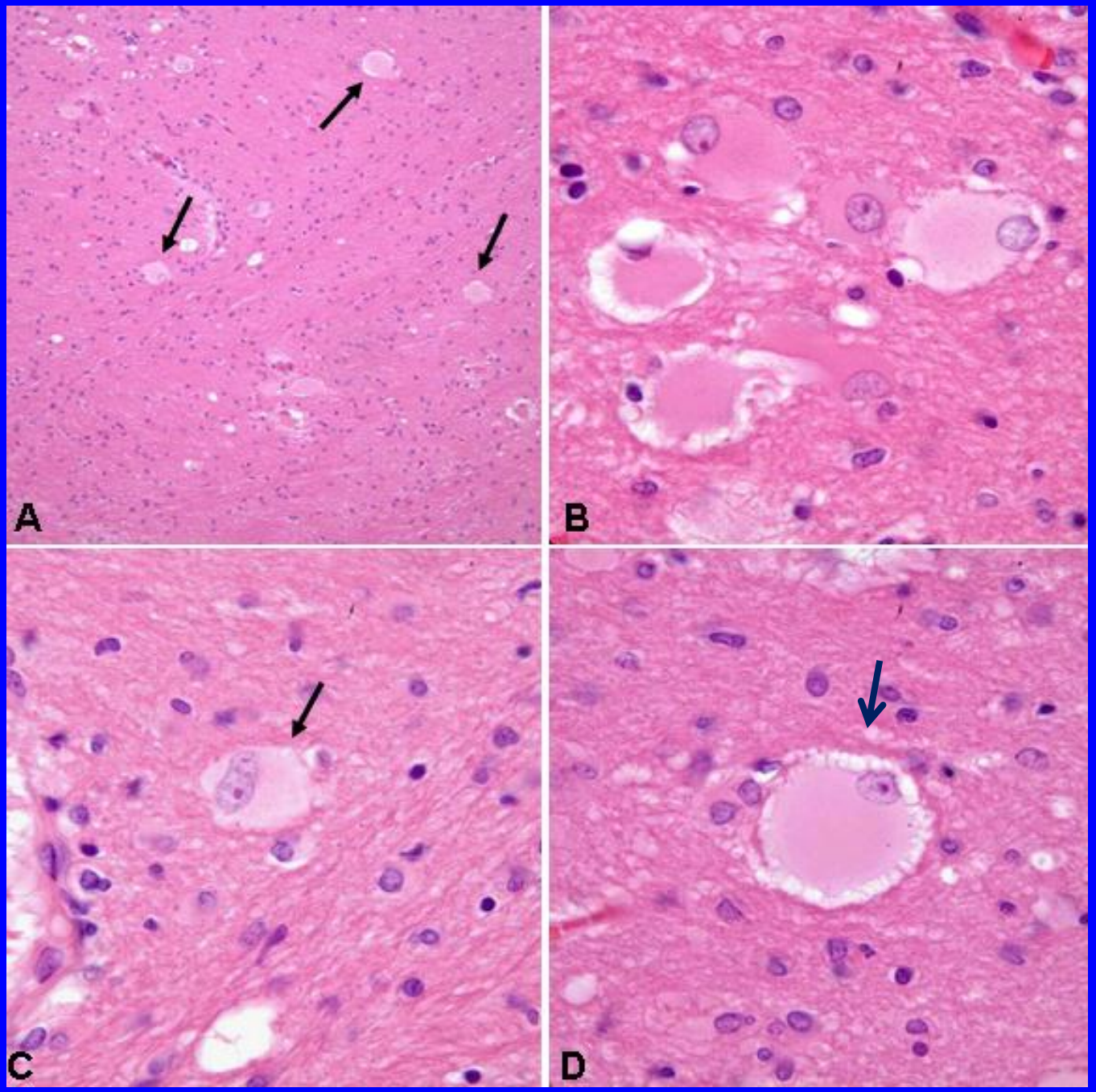


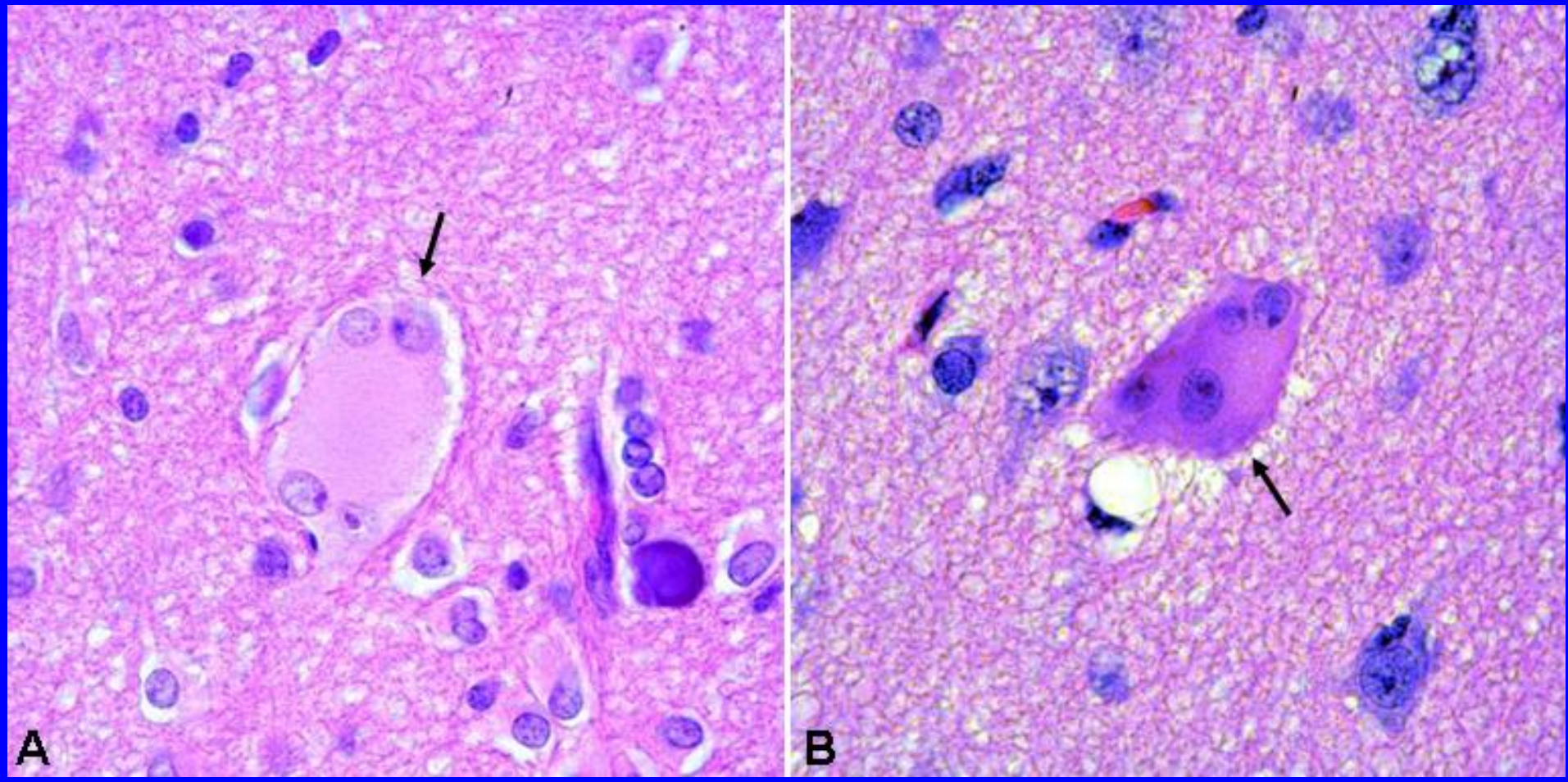
Neuronal cytologic abnormalities in tubers…..



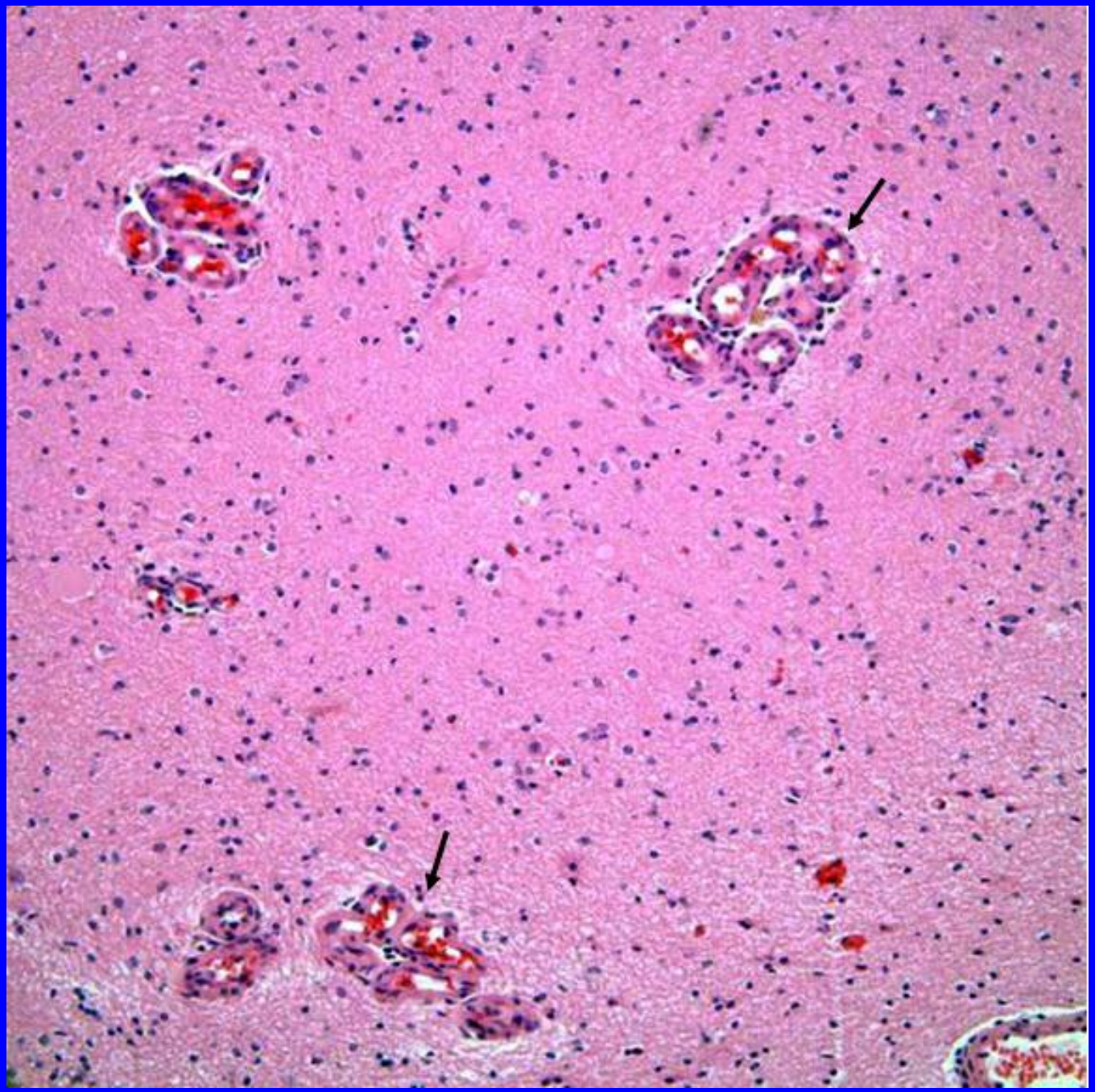

A
B
C
D

BCs—similar to FCD IIB

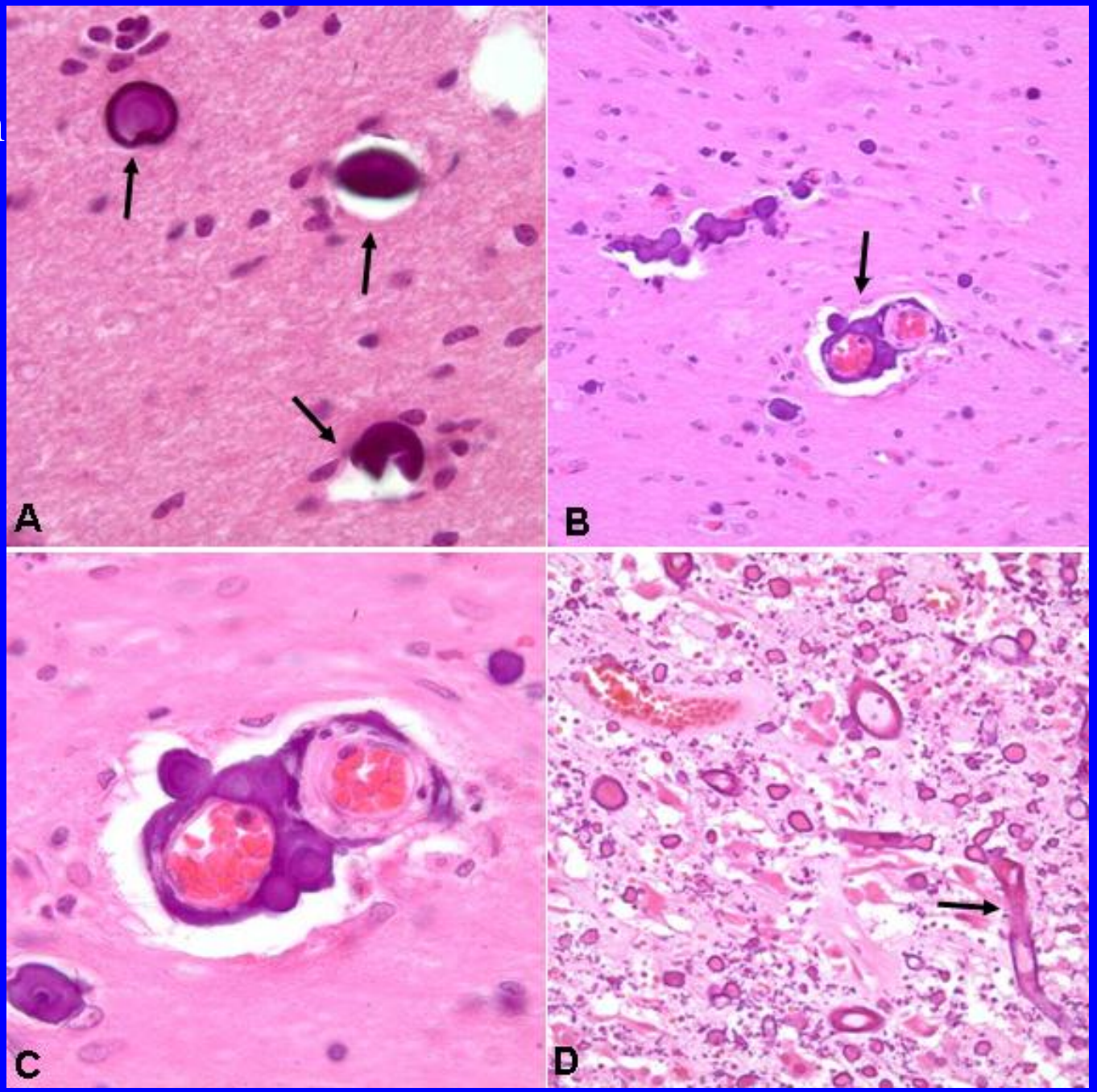



A
B

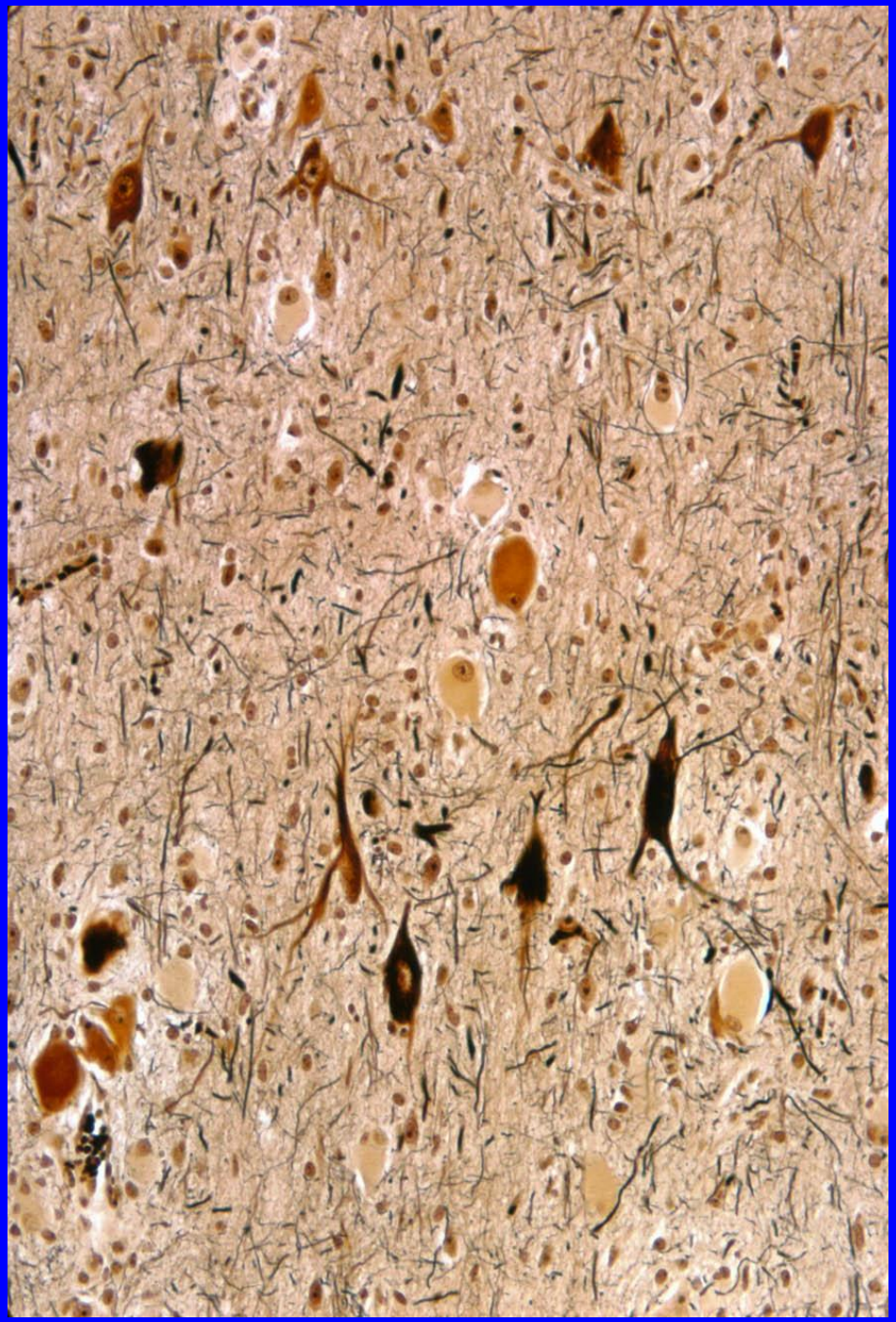
Vascular changes



Calcific’n

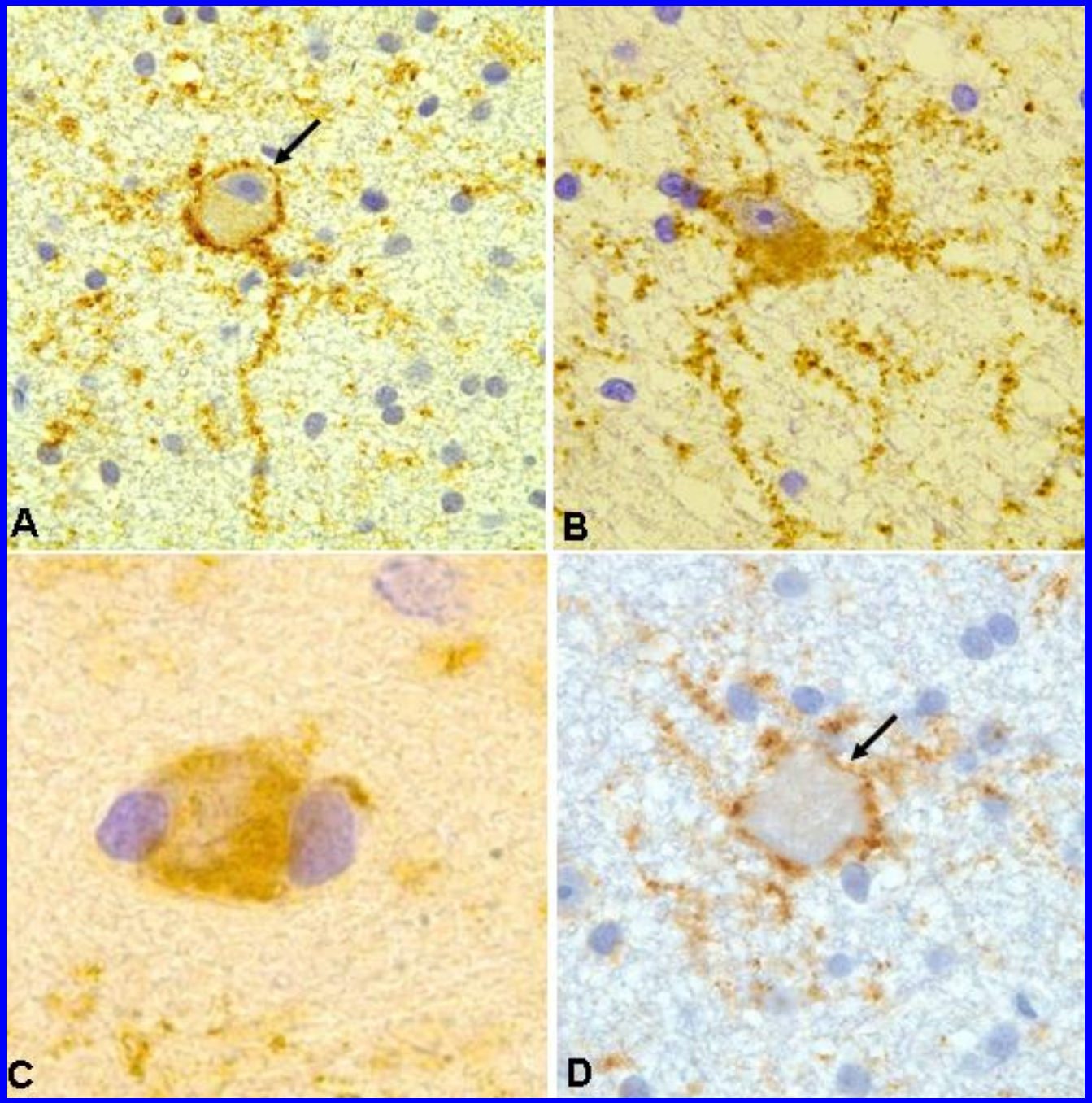


Cytoskeletal changes (silver stain)

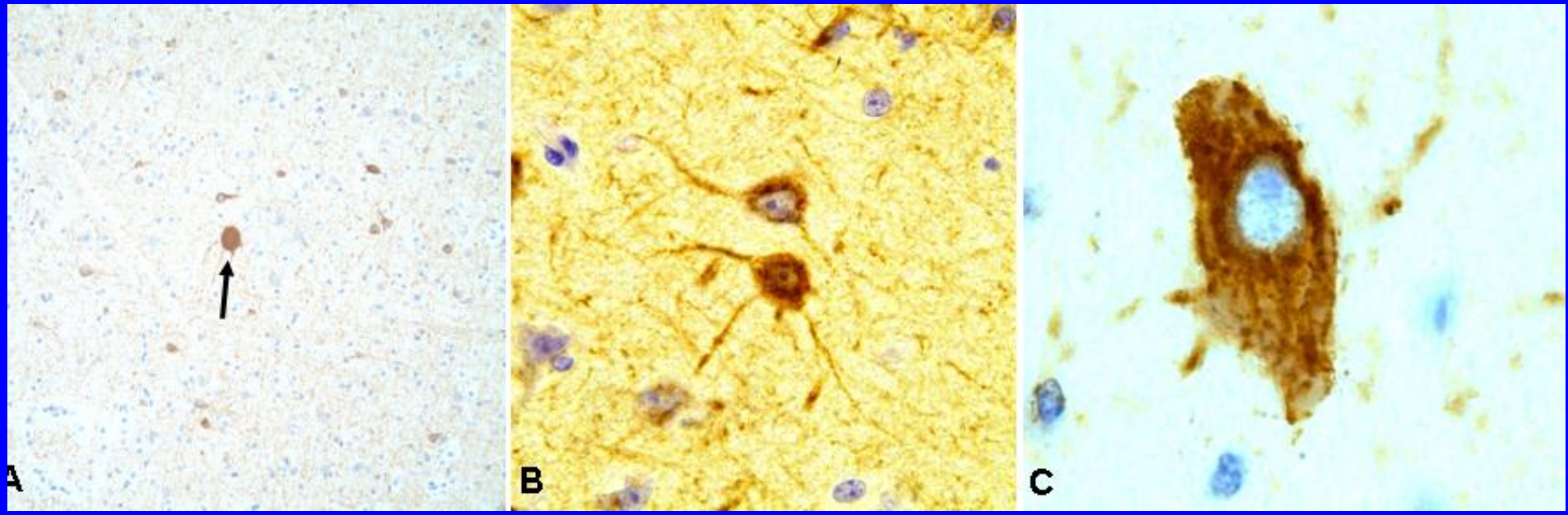


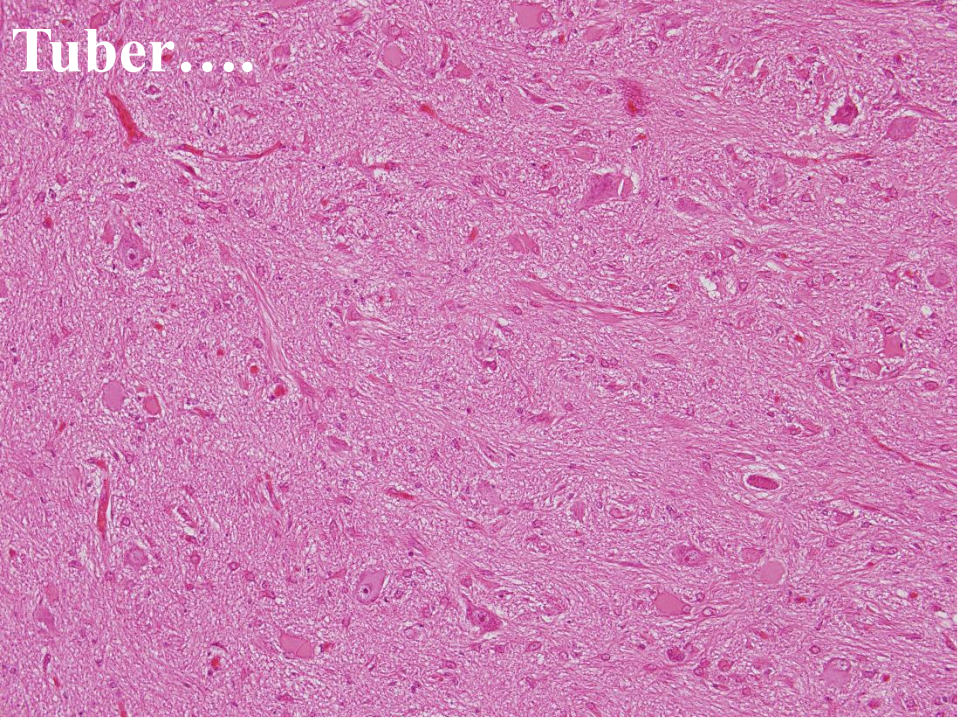
Synapto

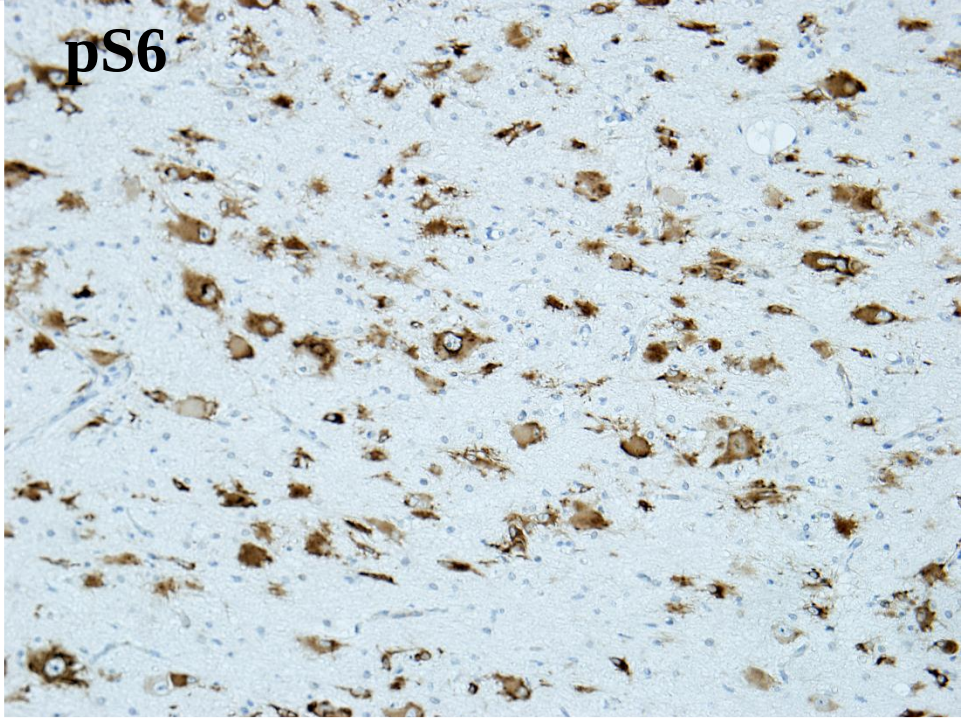
IHC

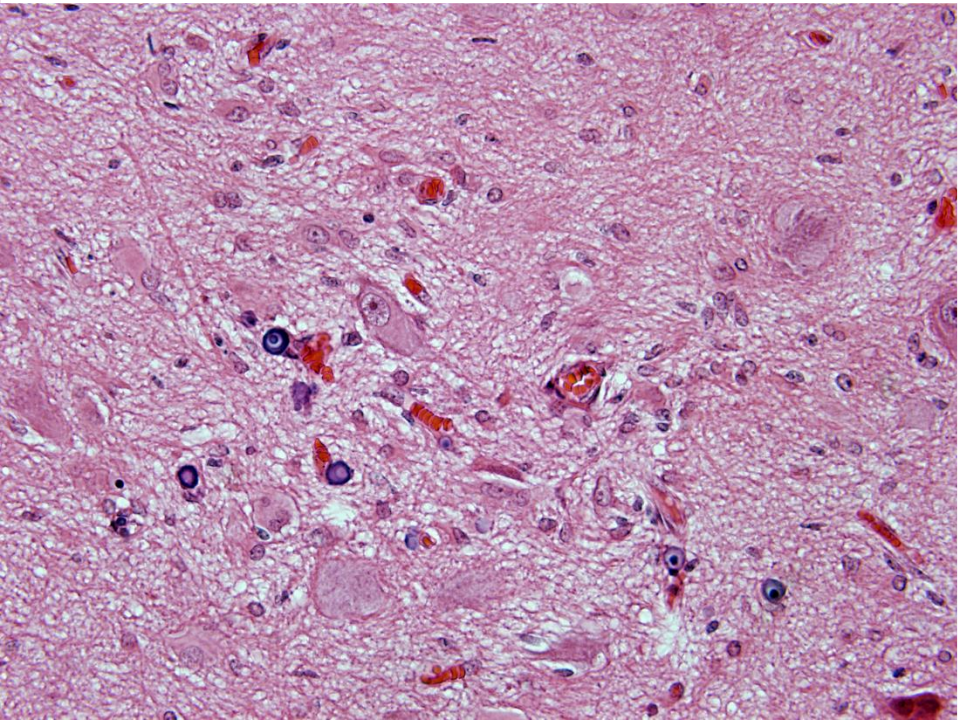


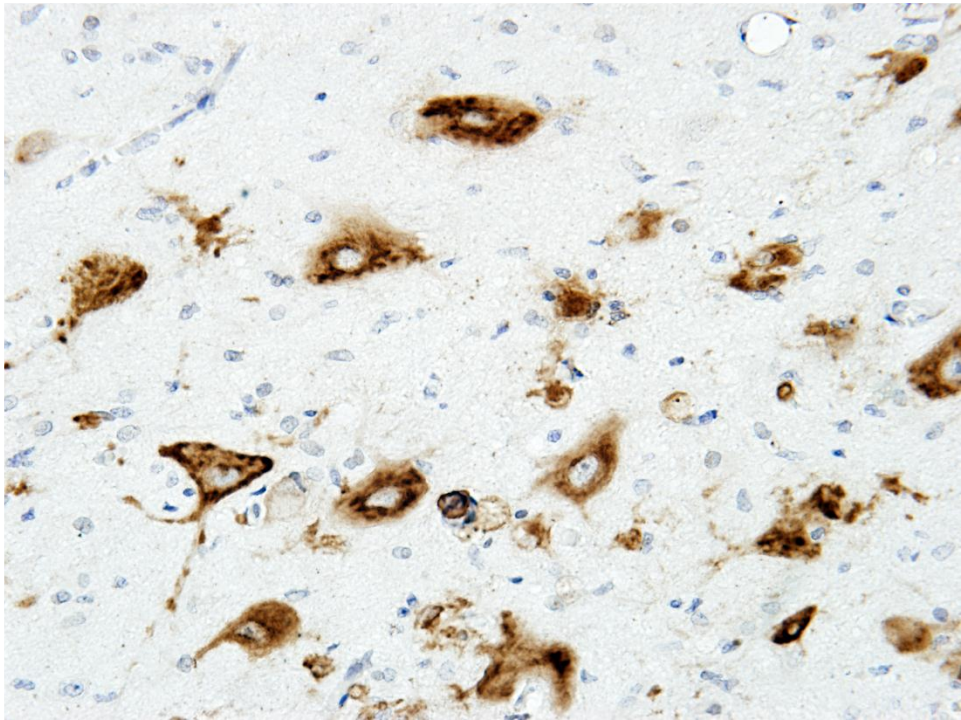
Neurofilament….




Tuber….


pS6





Focal cortical dysplasia (MCD) vs tuber/TSC

- Severe FCD (Palmini IIB) and a tuber of TSC are essentially *indistinguishable* without appropriate history of a *TSC1/TSC2* mutation or other major/minor diagnostic criteria in support of a Dx of TSC

- Both can be *multifocal*, *multilobar*, *bihemispheric*

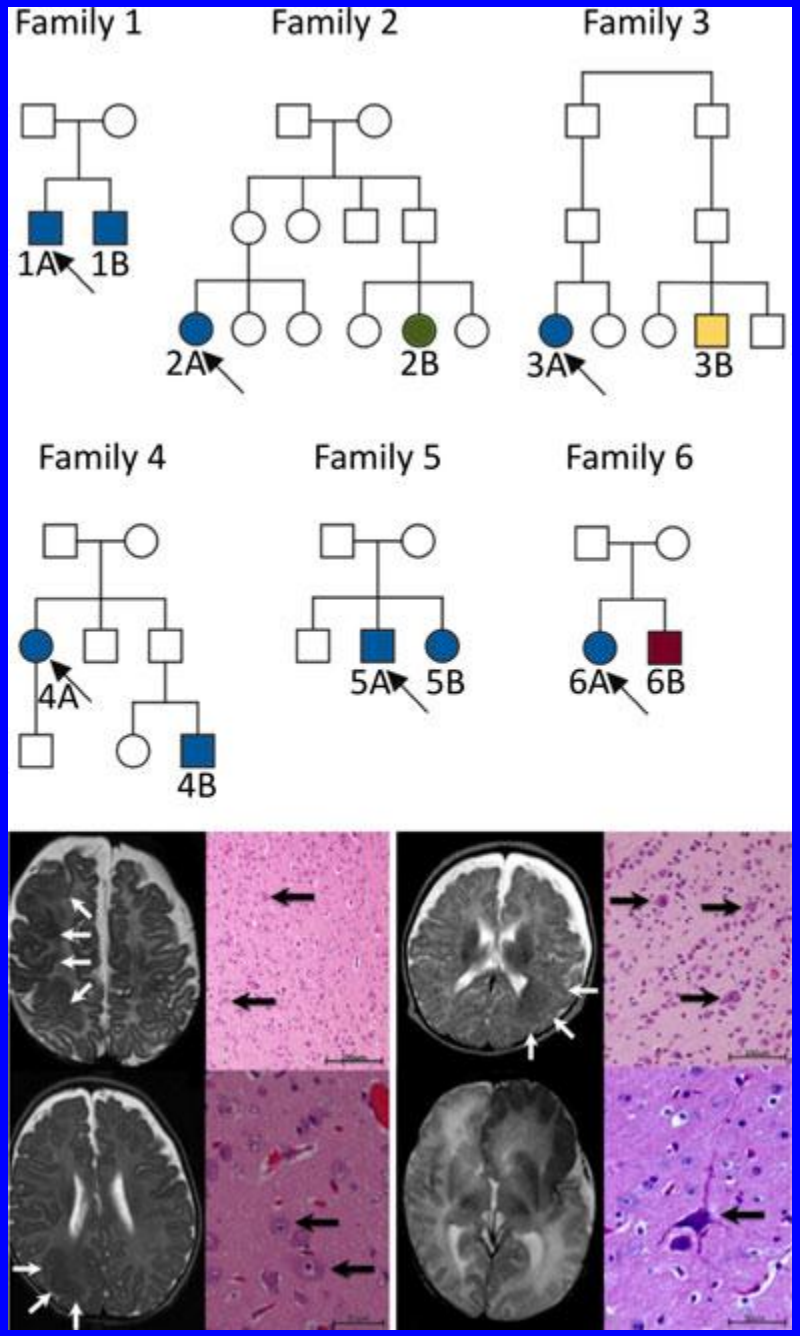
- *Similar mechanisms of formation?*

- *Tuberin/hamartin IHC not helpful to distinguish*

Is focal cortical dysplasia sporadic? Family evidence for genetic susceptibility

*†‡[1]Richard J. Leventer, §¶[1]Floor E. Jansen, **††Simone A. Mandelstam, ‡‡Alice Ho, §§Ismail Mohamed, ¶¶Harvey B. Sarnat, ##Mitsuhiro Kato, ***Tatsuya Fukasawa, †††Hirotomo Saitsu, †††Naomichi Matsumoto, ‡‡‡Masayuki Itoh, §§§¶¶¶Renate M. Kalnins, ‡###Chung W. Chow, *†‡A. Simon Harvey, ¶**Graeme D. Jackson, ****Peter B. Crino, ¶Samuel F. Berkovic, and *‡¶**Ingrid E. Scheffer

Epilepsia, 55(3):e22–e26, 2014
doi: 10.1111/epi.12533



RJ Leventer *et al*, Epilepsia, 55(3):e22–e26, 2014

Epilepsia, 50(6):1396–1408, 2009
doi: 10.1111/j.1528-1167.2008.01979.x

FULL-LENGTH ORIGINAL RESEARCH

Focal cortical dysplasia: A genotype–phenotype analysis of polymorphisms and mutations in the TSC genes

*Christoph Gumbinger, *Constantin B. Rohsbach, *Andreas Schulze-Bonhage, †Rudolf Korinthenberg, ‡Josef Zentner, *Monika Häffner, and *Susanne Fauser

*Epilepsy Center, University of Freiburg, Freiburg, Germany; †Department of Neuropediatrics and Muscular Diseases, University of Freiburg, Freiburg, Germany; and ‡Department of Neurosurgery, University of Freiburg, Freiburg, Germany

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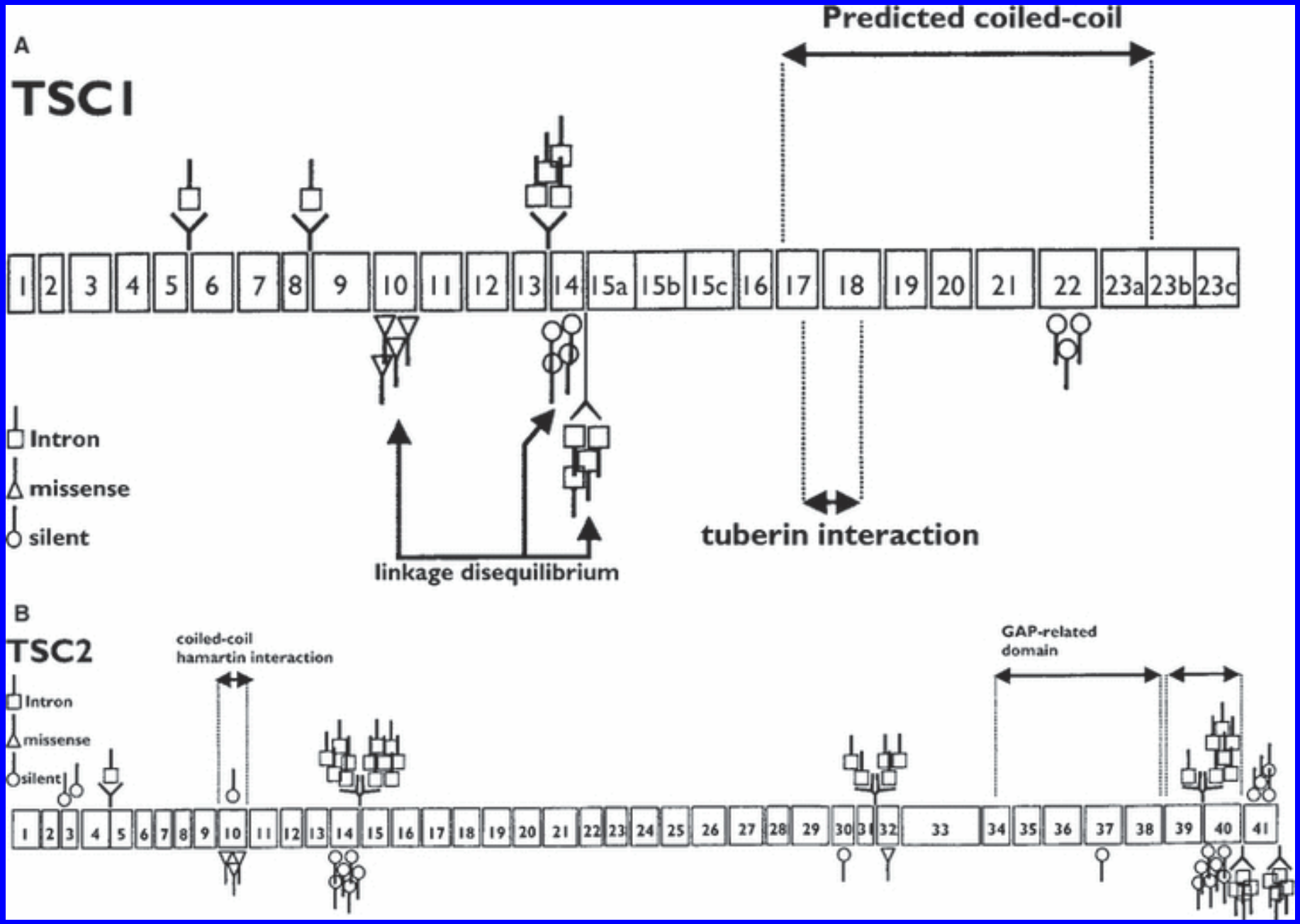
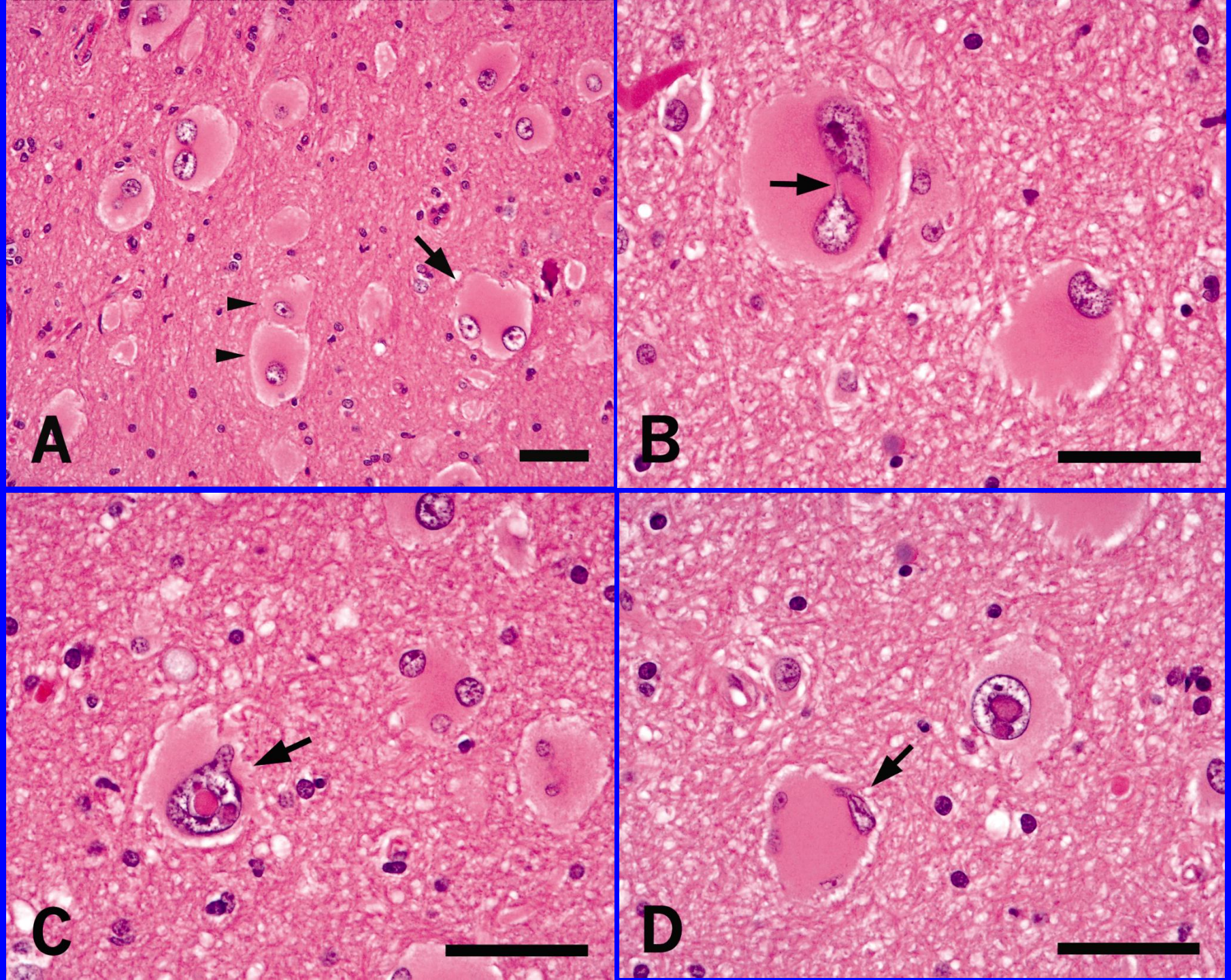
Discussion: This study shows that FCD—even multifocal FCD—is not caused by mutations in the TSC genes and seems not to be promoted by polymorphisms in the TSC genes. Therefore, FCD cannot be regarded as a "forme fruste" of TSC.



Gumbinger *et al*, Epilepsia June 2009


A
B
C
D

Neuropathologic lesions in TSC

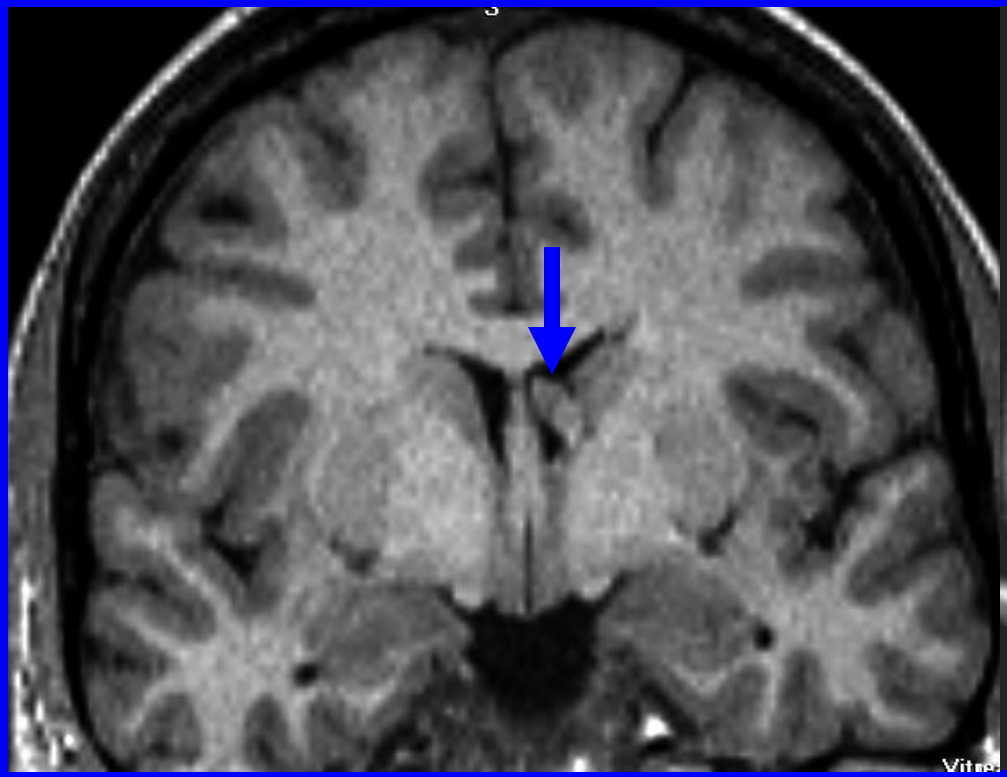
- *Subependymal nodules/subependymal giant cell astrocytoma (SEGA)*

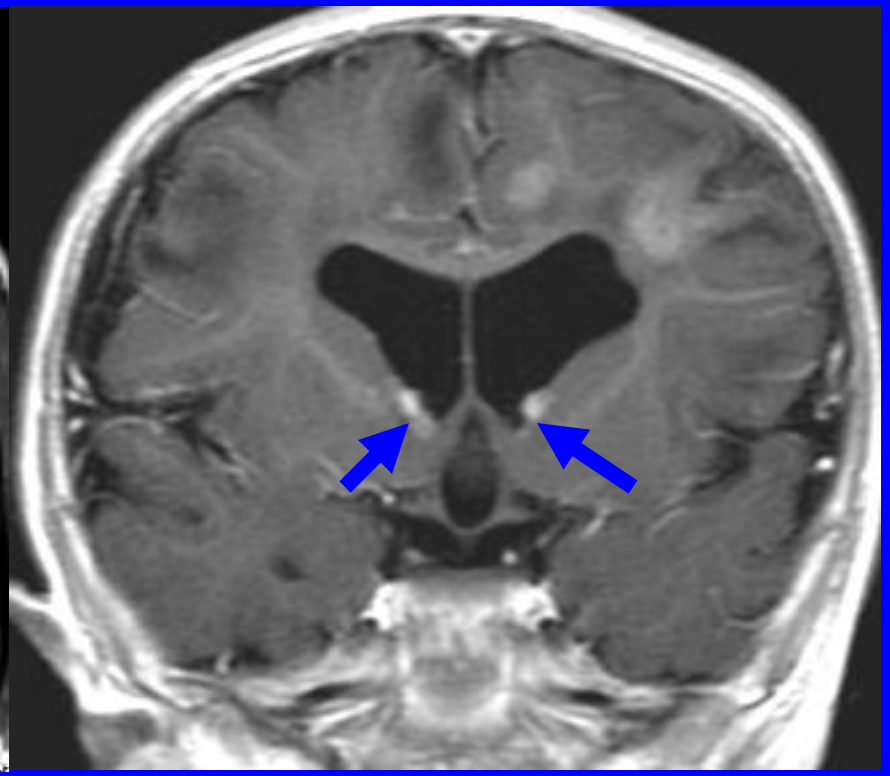
  [Regression documented with *Rapamycin/ Sirolimus*]

- Hemimegalencephaly (rare)

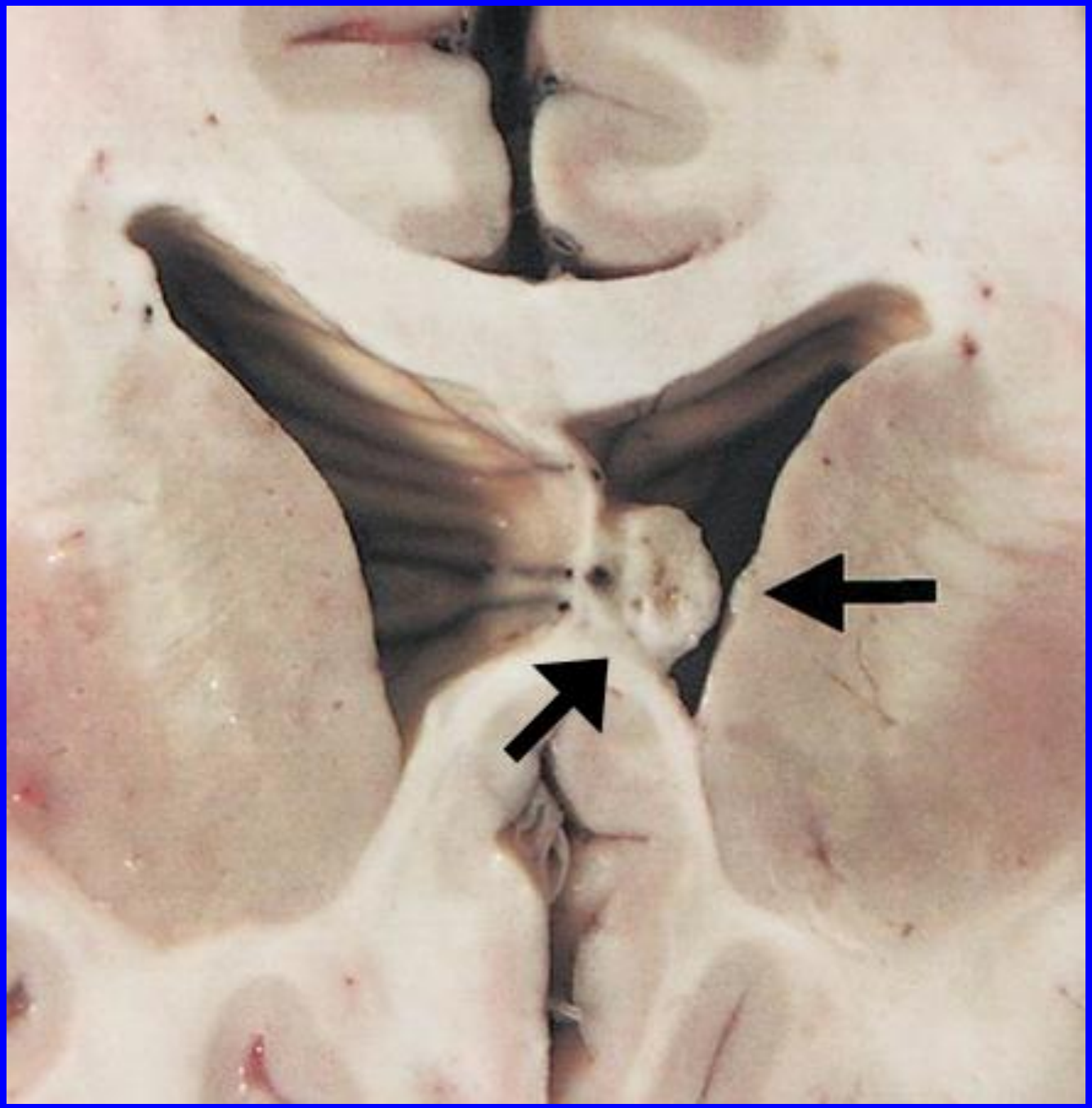
- Tubers and cortical disorganization

Subependymal nodules

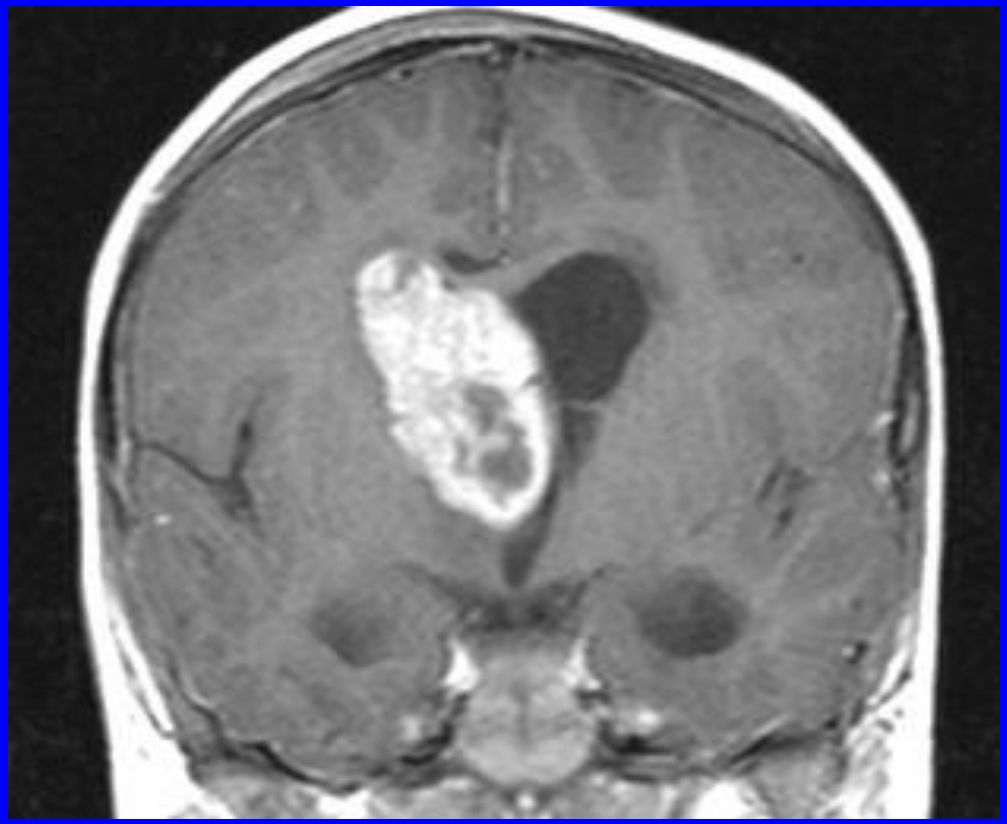




SEN….



SEGA



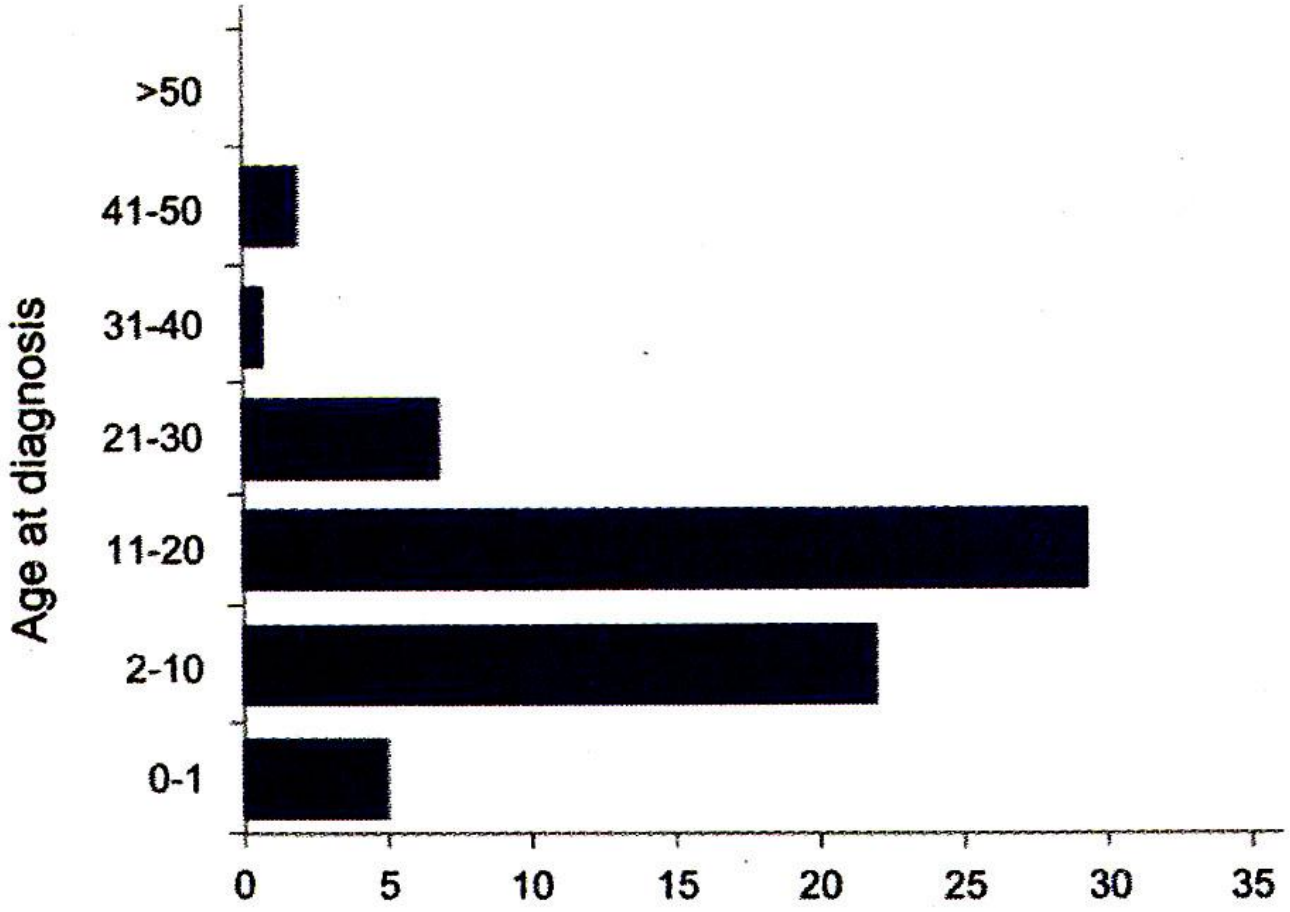
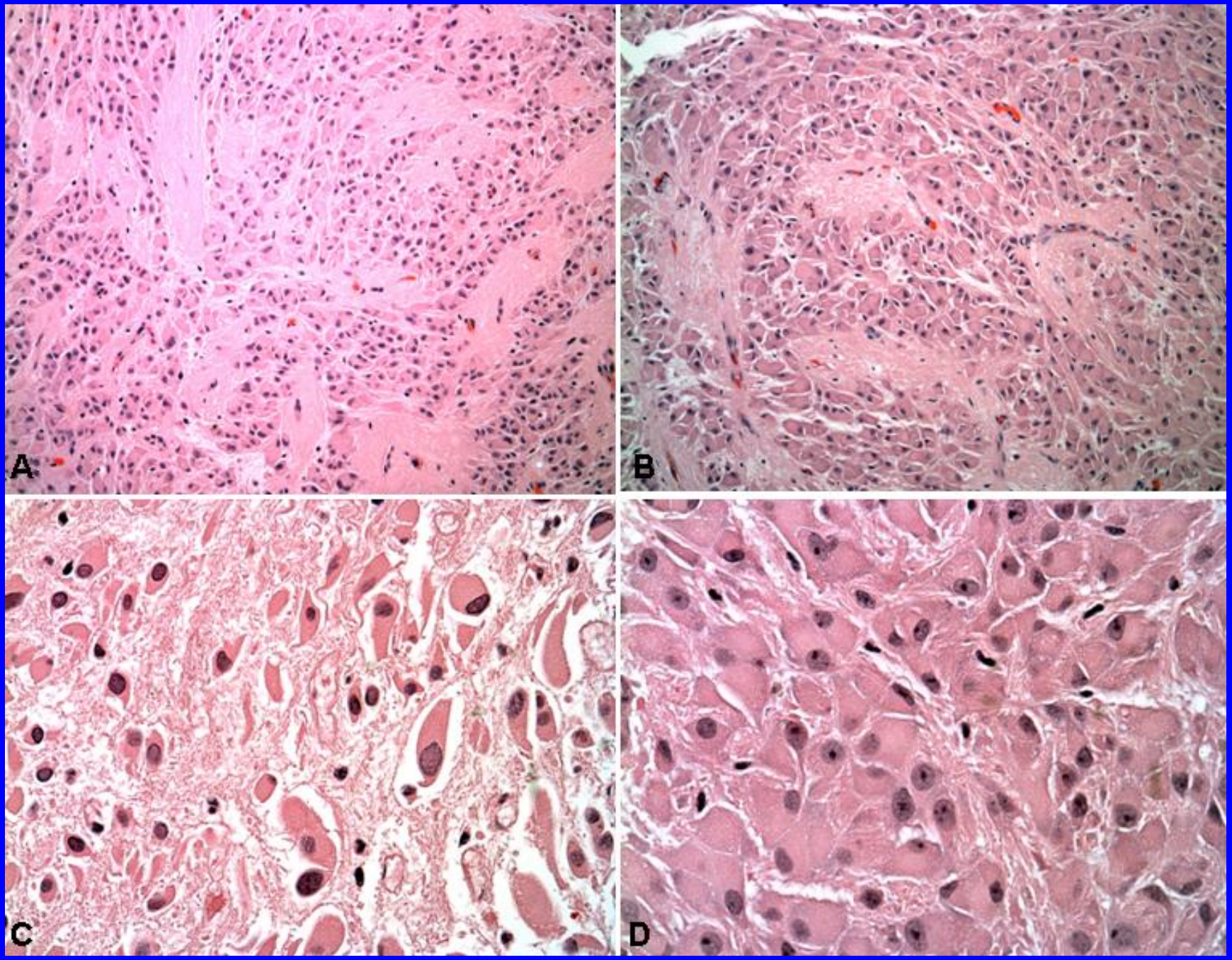


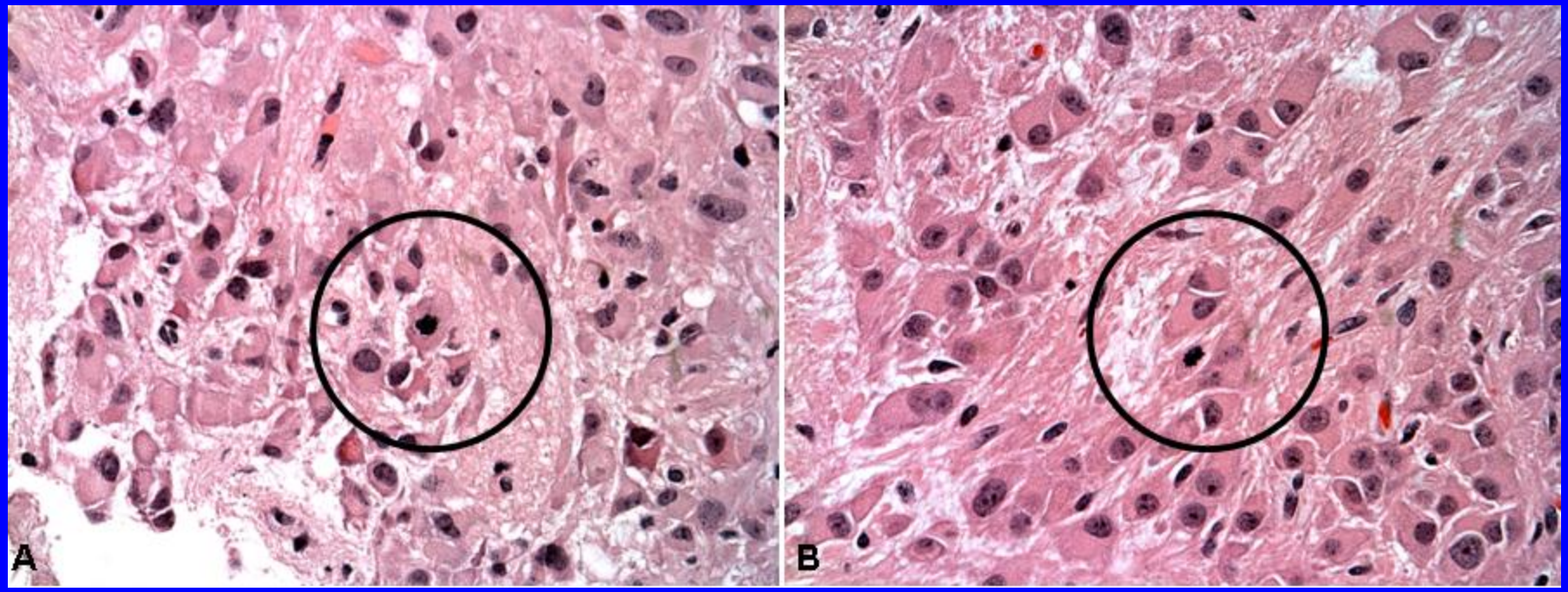
Fig. 13.19 Age distribution of subependymal giant cell astrocytoma (SEGA) at the time of clinical manifestation. Combined data for male and female patients.

Lopes *et al*, WHO CNS, ed. DN Louis, *et al, 2007*

SEGA….

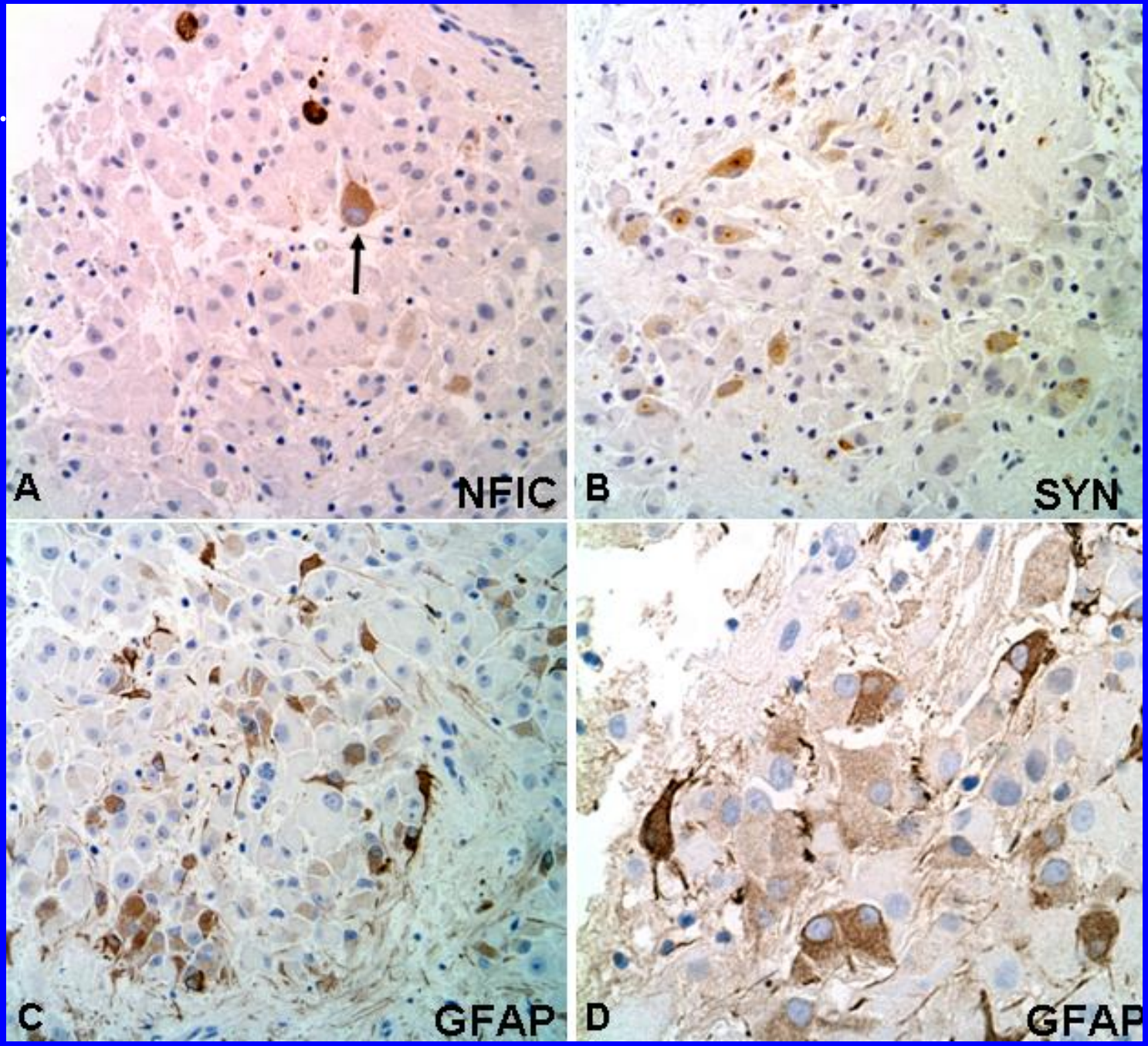


SEGA….mitoses (rare)



SEGA…

IHC



SUBEPENDYMAL GIANT CELL ASTROCYTOMA (SEGA)

Review of 15 cases (Cuccia et al, *Child's Nerv System* 19: 232, 2003)

Age range at surgery 55-228 months

Usually long history of seizures

1-10 tumor nodules/ Solid/cystic

Diameter at surgery 8-46 mm

Ki-67 LI *2.1* to *14.1*

Neuropathology. 2009 Feb;29(1):25-30. Epub 2008 Jun 17.

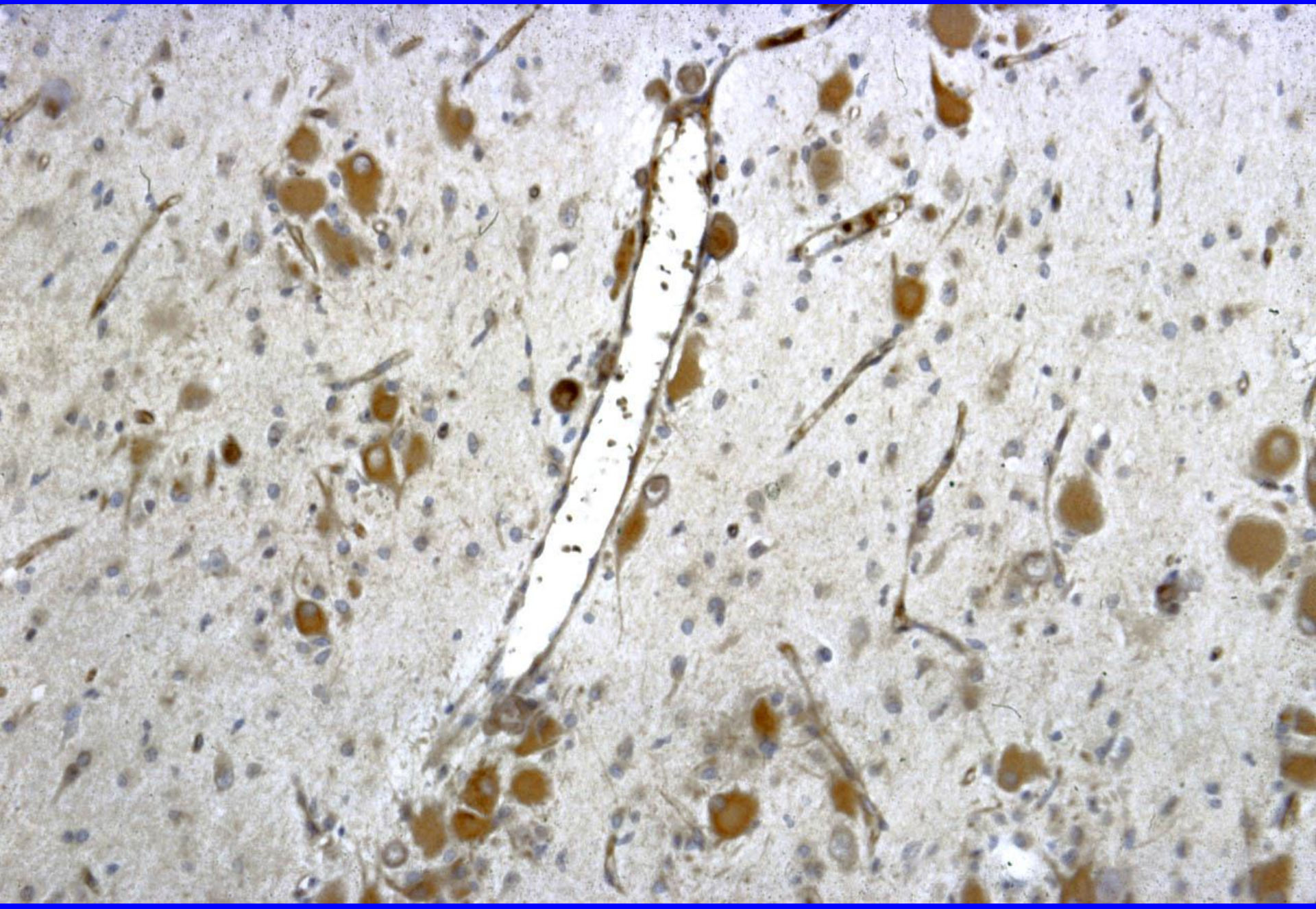
Subependymal giant cell astrocytoma (SEGA): Is it an astrocytoma? Morphological, immunohistochemical and ultrastructural study

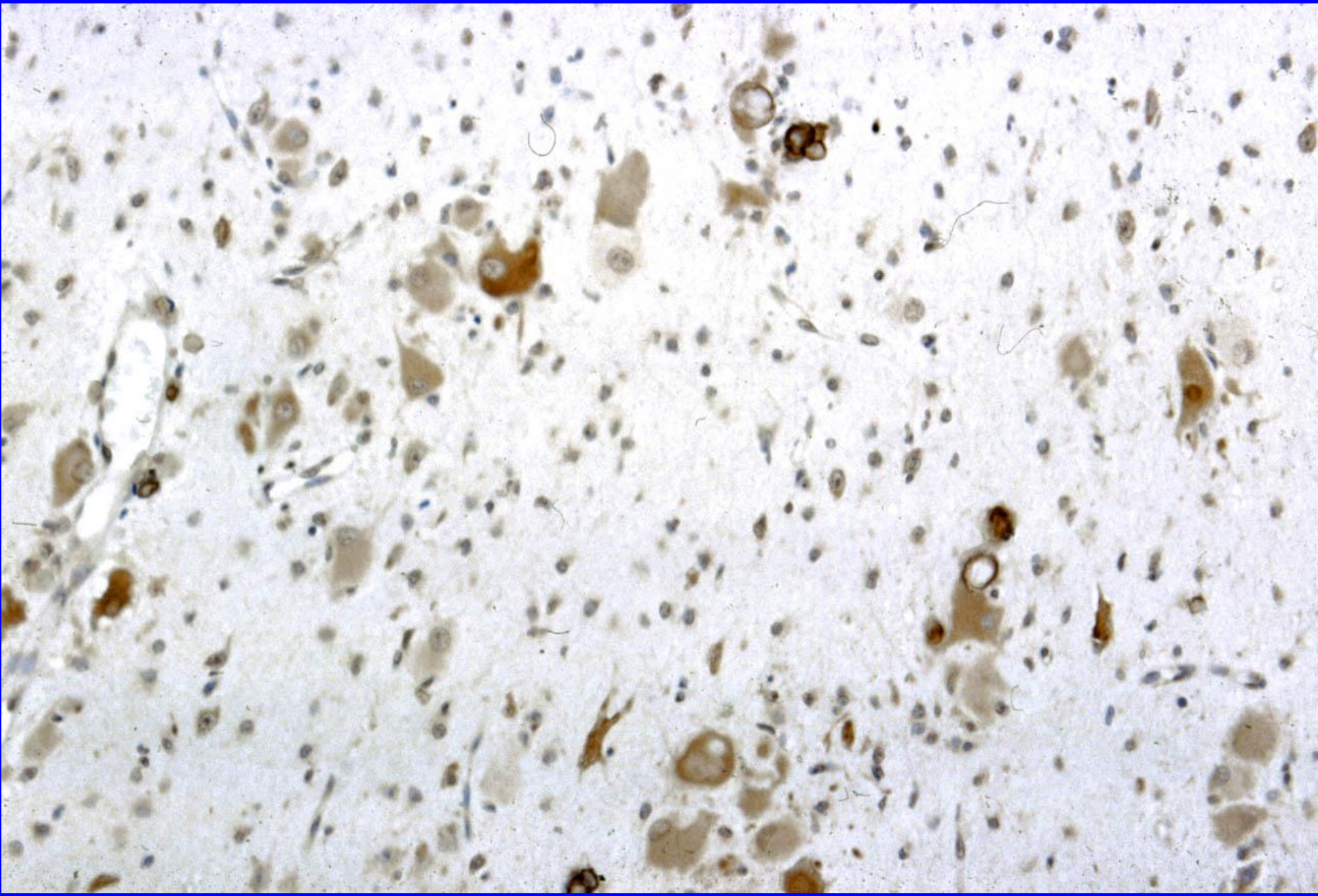
Buccoliero AM, Franchi A, Castiglione F, Gheri CF, Mussa F, Giordano F, Genitori L, Taddei GL

Department of Human Pathology and Oncology, University of Florence, Italy. ambuccoliero@unifi.it

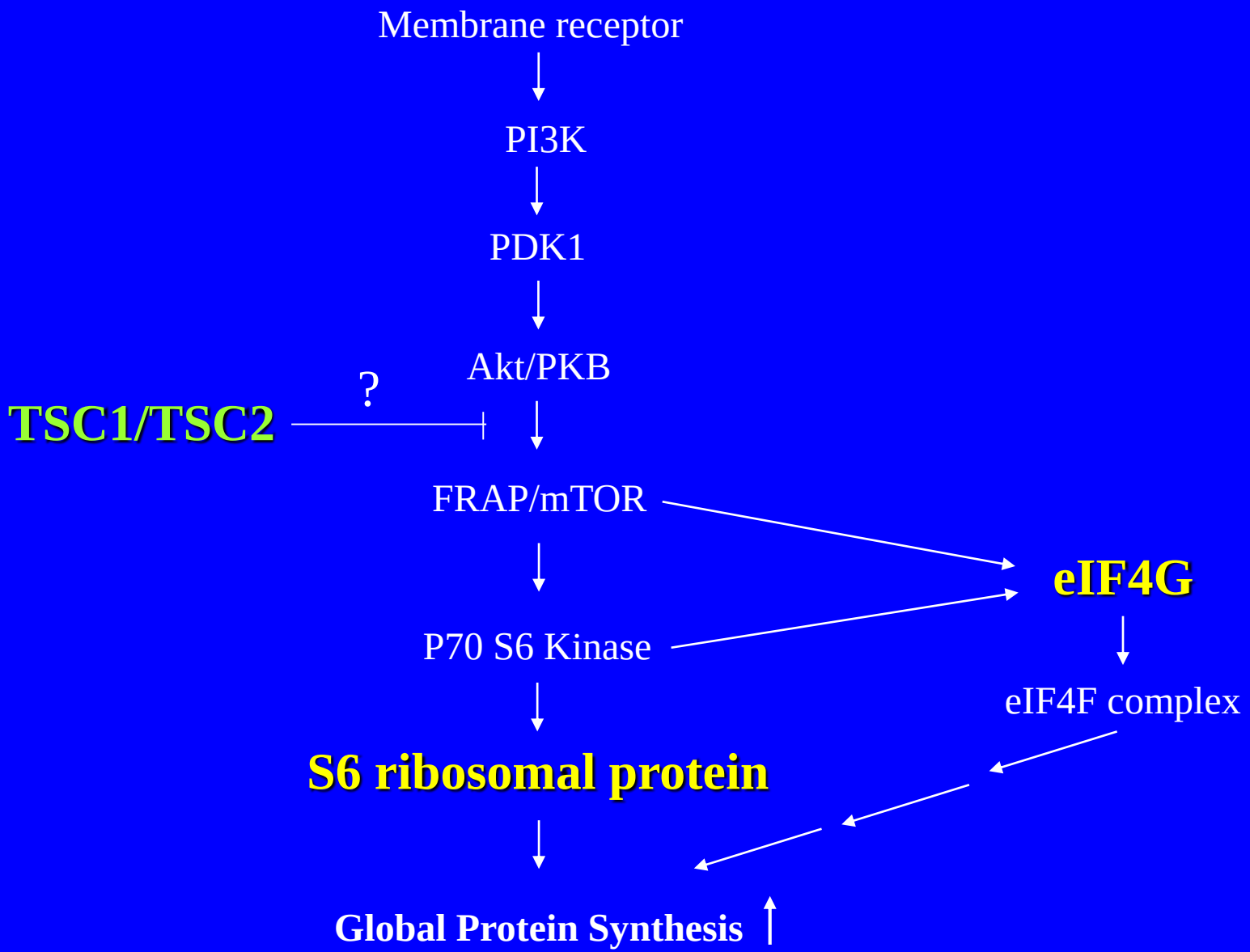
TSC lesions—Expression Profiling

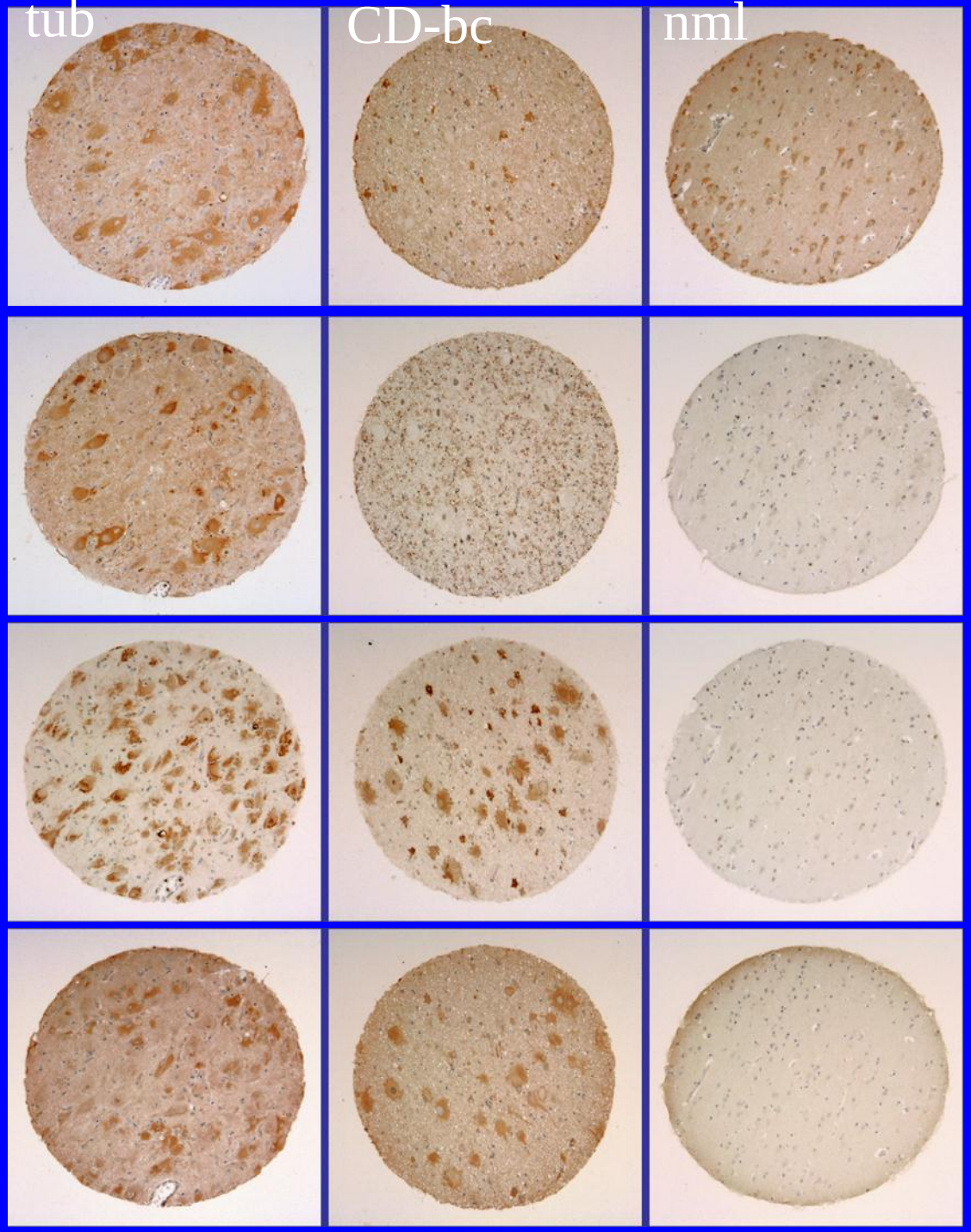
- Does *tuberin/hamartin* IHC help in making a diagnosis of ‘tuber’?
- Unique insulin-signaling pathway modulation in TSC vs. FCD?
- Distinctive *electrophysiologic* properties of TSC tubers that render them ‘*epileptogenic*’?



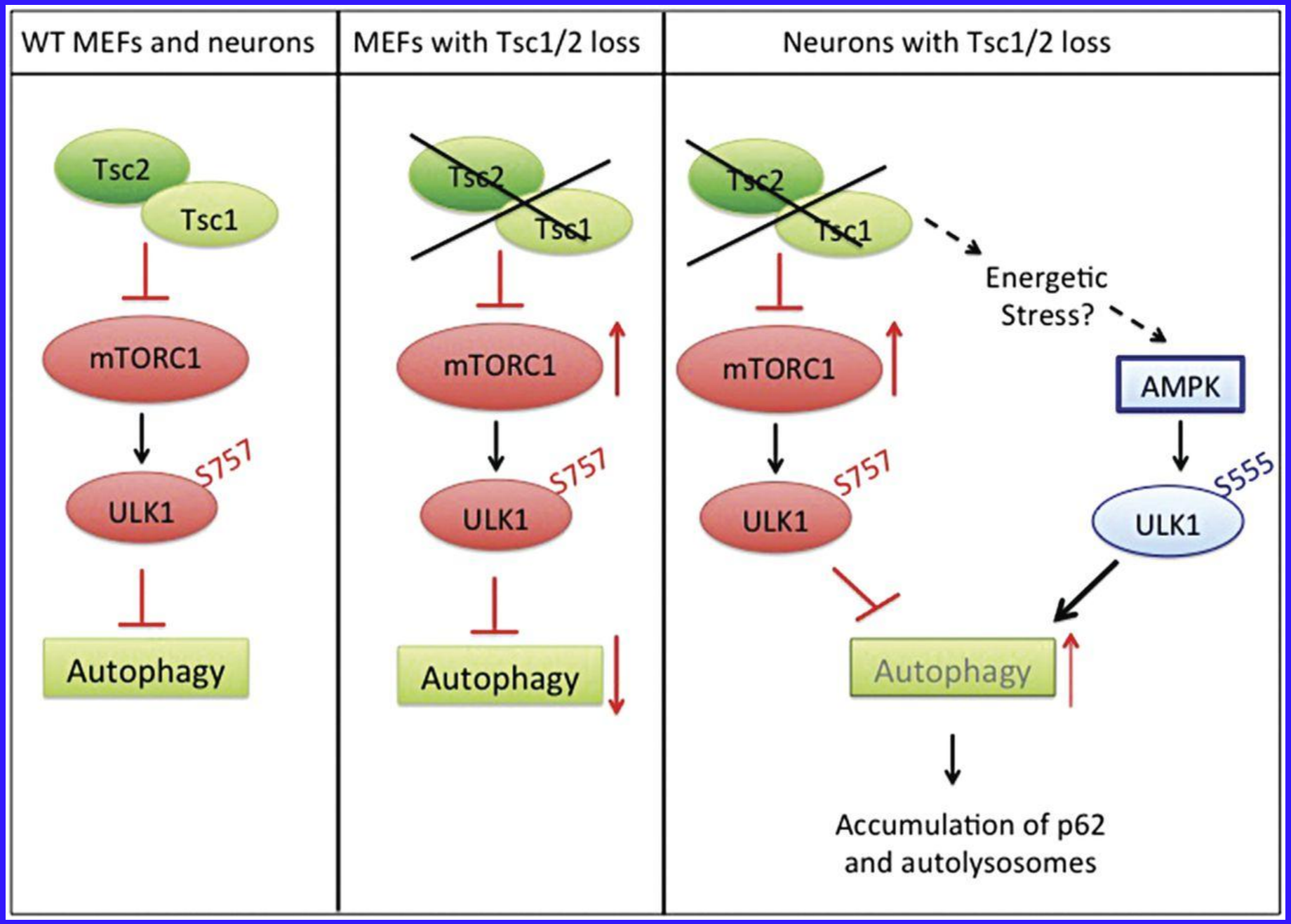


Insulin Signaling Pathway





Miyata et al, *Annals Neurol.*, 2004



Di Nardo A *et al*, Hum. Mol. Genet. 2014

Brain Pathology ISSN 1015-6305, vol 23 (2013)

RESEARCH A R T I C L E

Fetal Brain Lesions in Tuberous Sclerosis Complex: TORC1 Activation and Inflammation

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Fetal Brain mTOR Signaling Activation in Tuberous Sclerosis Complex

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TSC1 and TSC2 in TUBERS of TSC

Genomic deletions very rare in tubers

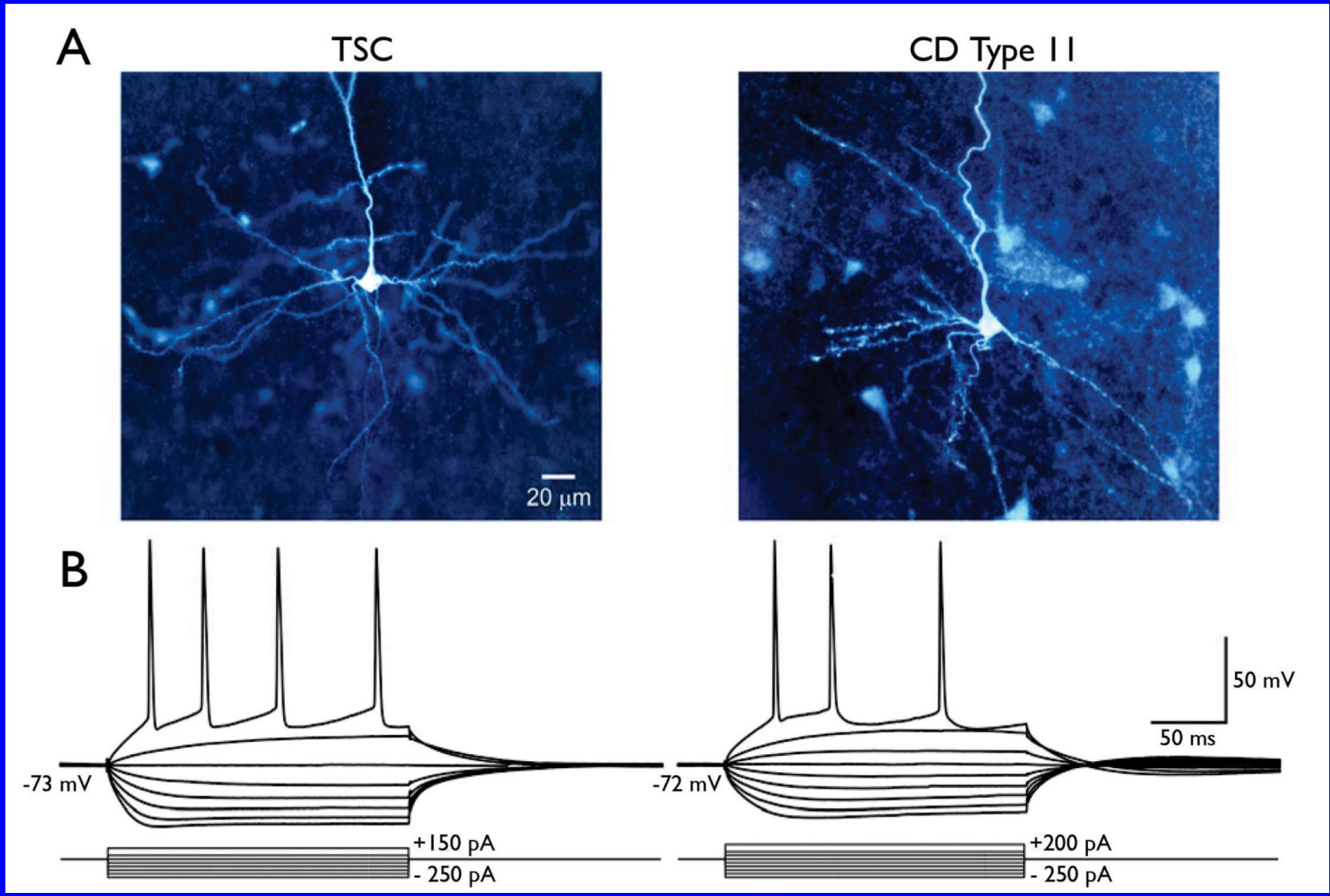
"*Deep sequencing*" to assess all coding exons of *TSC1* and *TSC2*

Germline heterozygous mutations found in 81% of tubers

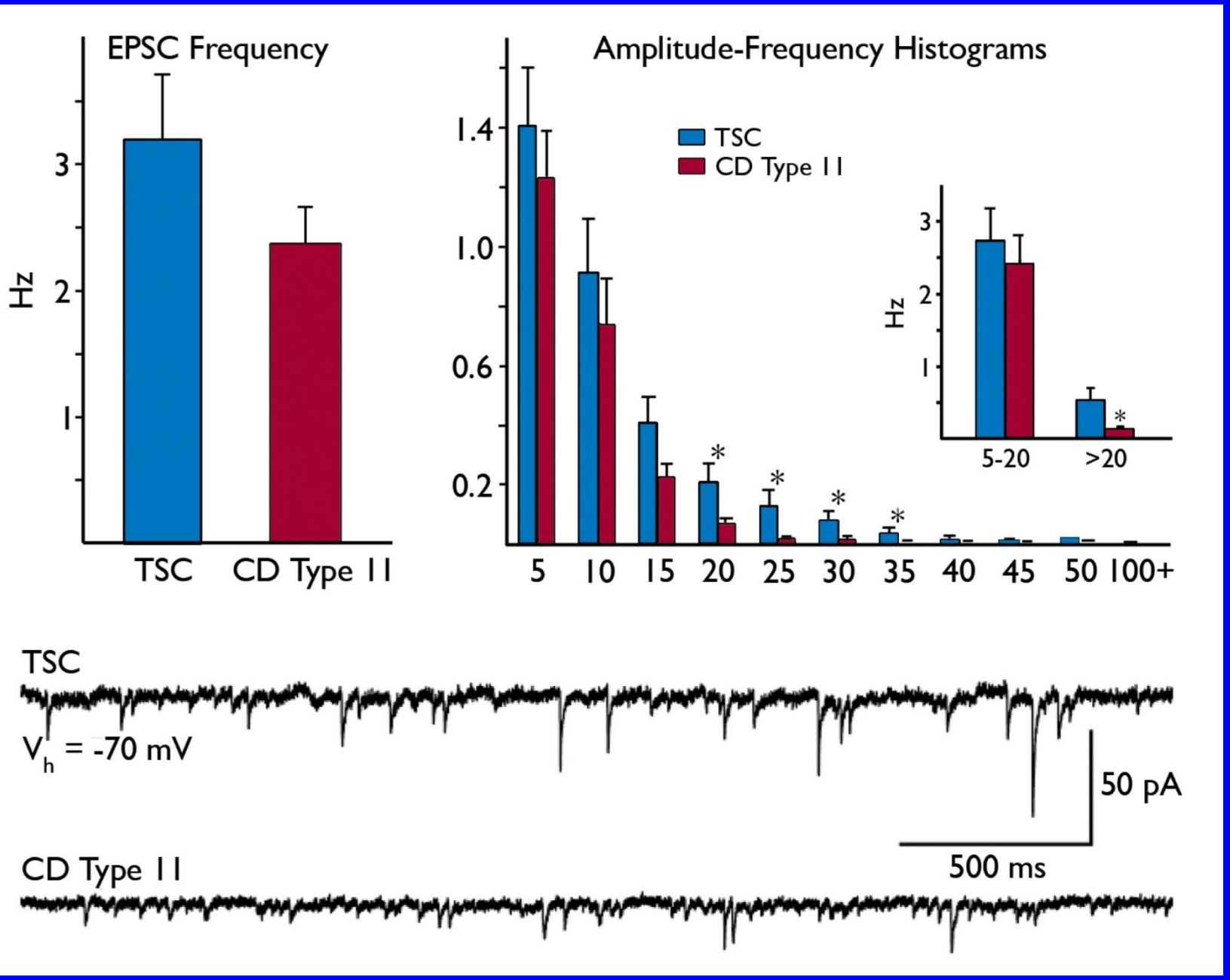
Secondary mutation in *TSC2* found in 6 tubers from 1/41 subjects

***CONCLUSION:* *Second hit* mutations rare in TSC tubers**

(W. *Qin et al, Brain Pathology, v. 20, 2010*)



Cepeda, *et al*, Epilepsia, 2010



Cepeda, *et al*, WONOEP, *Epilepsia*, 2010

Regulable neural progenitor-specific *Tsc1* loss yields giant cells with organellar dysfunction in a model of tuberous sclerosis complex

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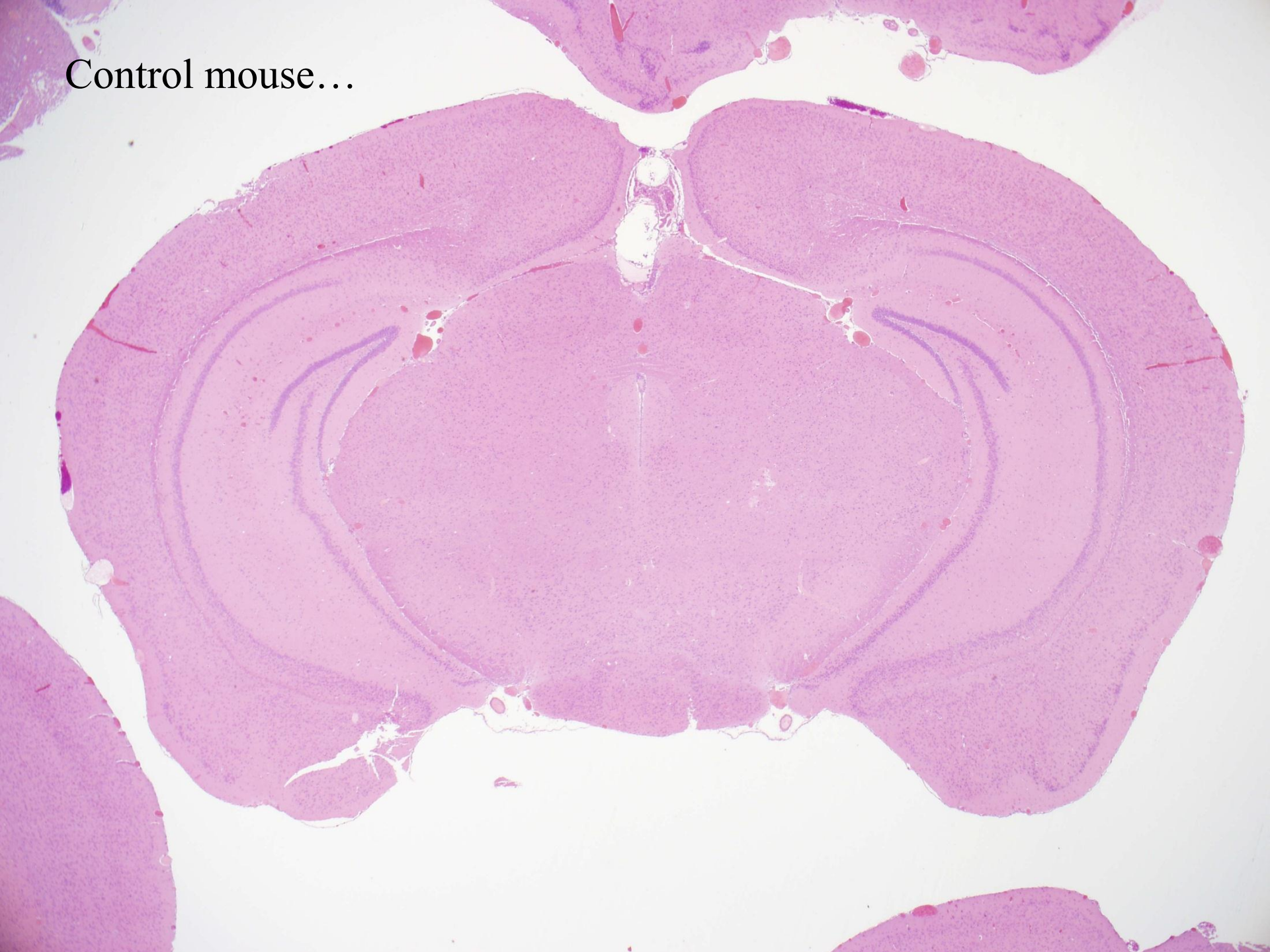
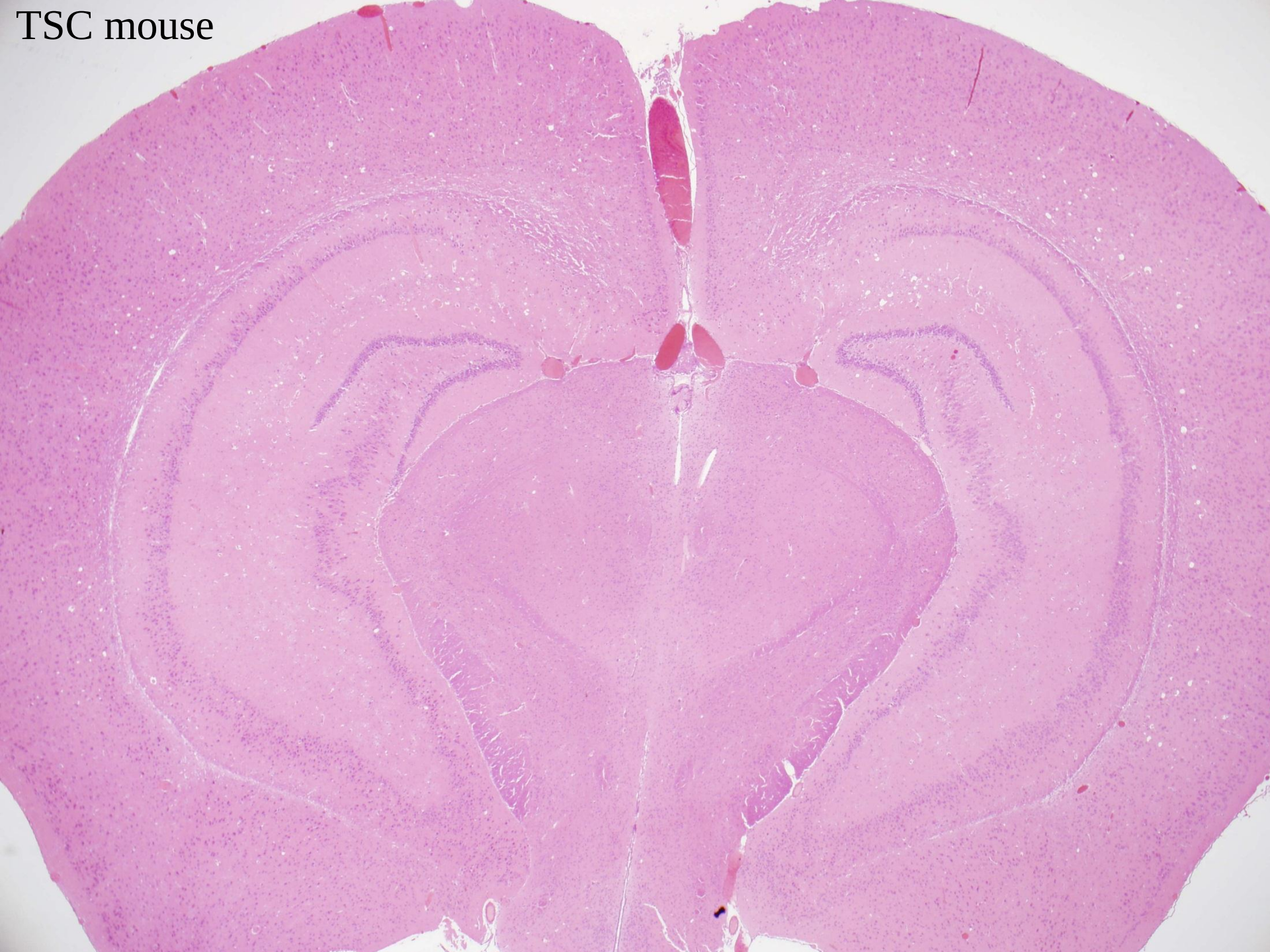
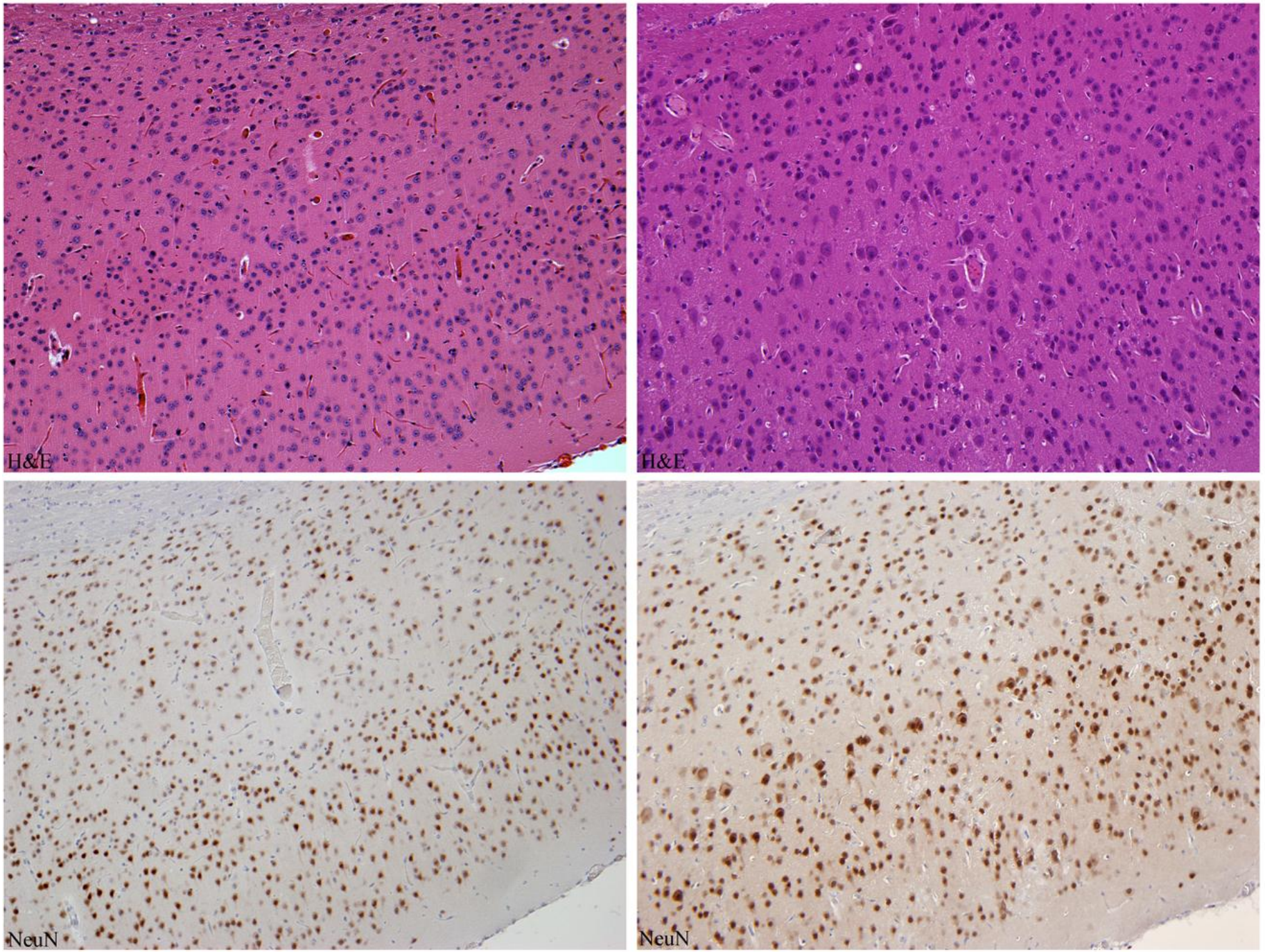
Mosaic induction of *Tsc1* loss in embryonic neural progenitor cells
Tsc1-null neural progenitor cells develop into enlarged vacuolated giant cells
Vacuolated G-cells show +++ evidence of organelle dysfunction (mitochondria, giant cells)

Control mouse…



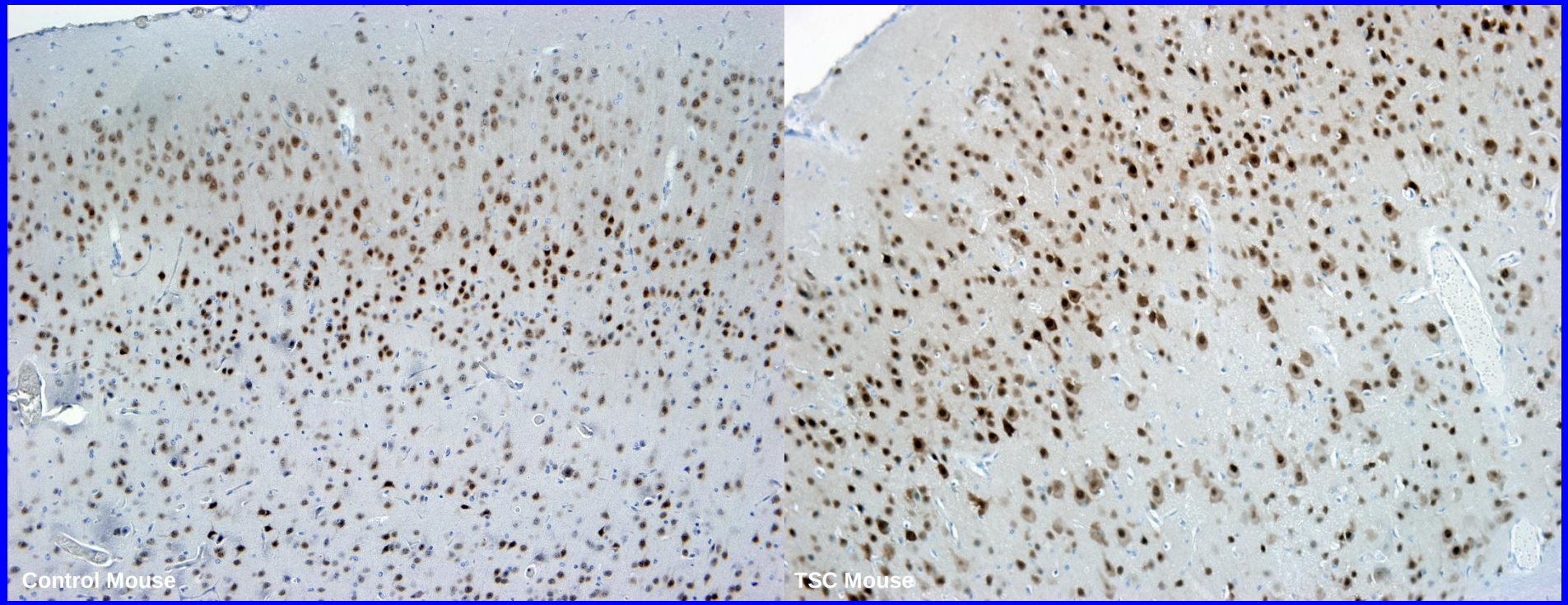

TSC mouse


Control Mouse
Tuberous Sclerosis Mouse
H&E
H&E
NeuN
NeuN

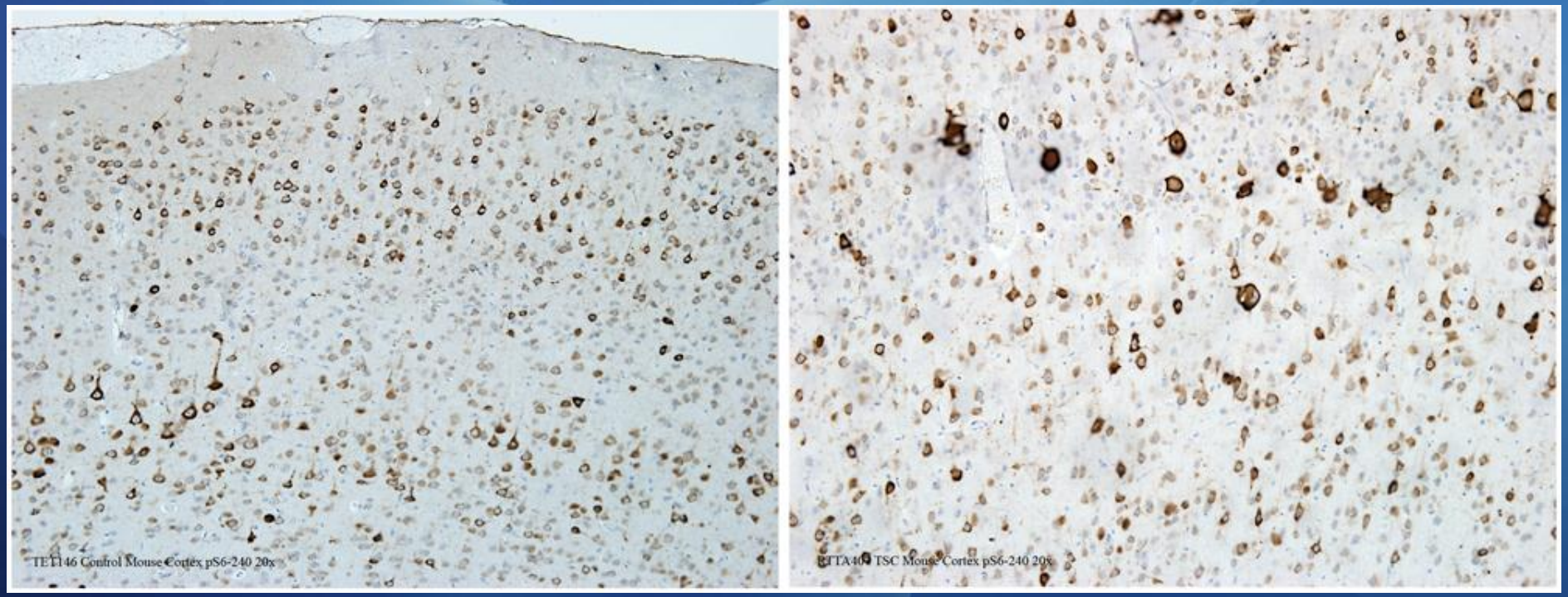
NeuN

Control

TSC mouse

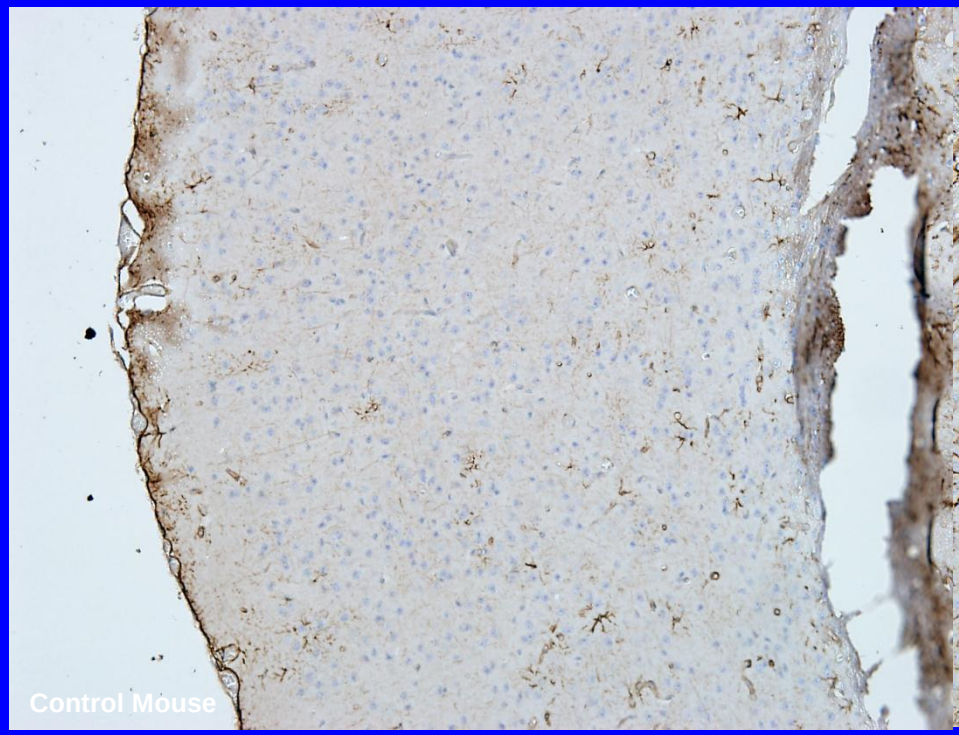


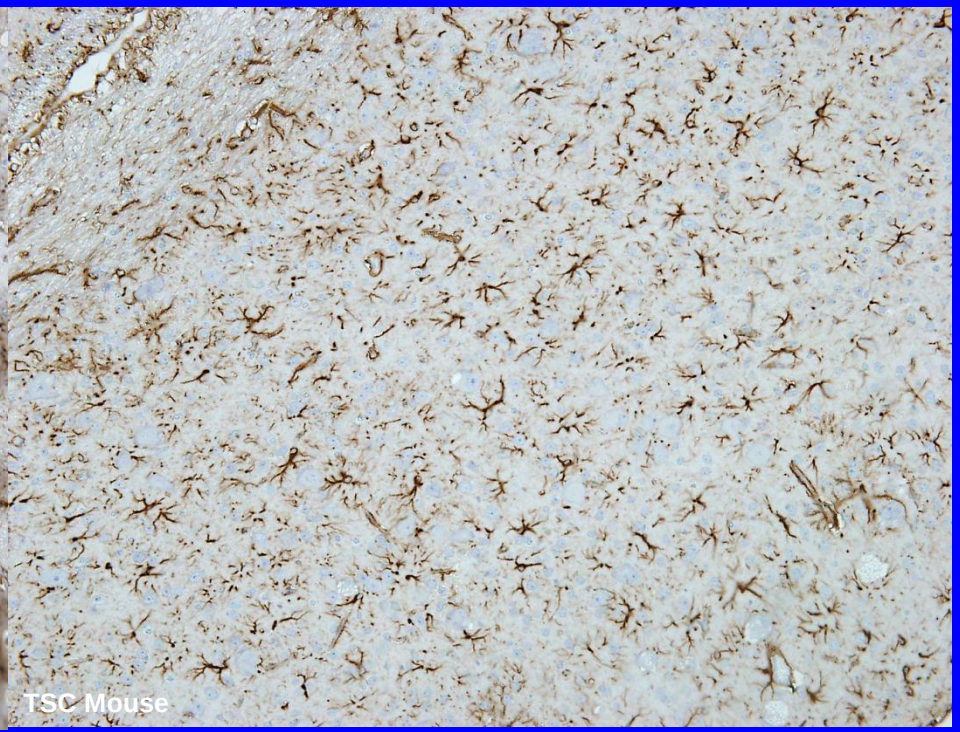
Anti- pS6 (240/244) Reactivity



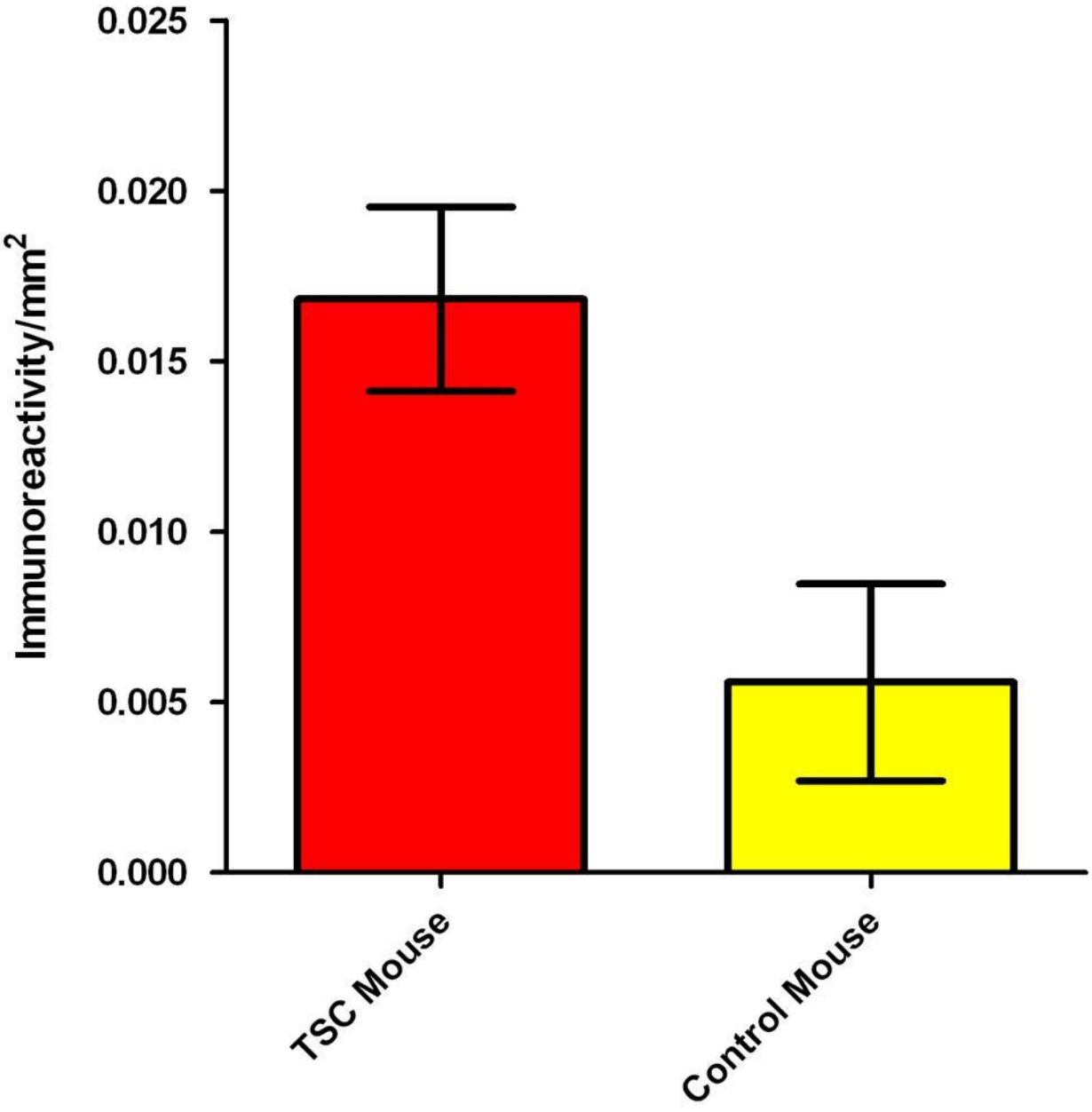
Control

TSC mouse



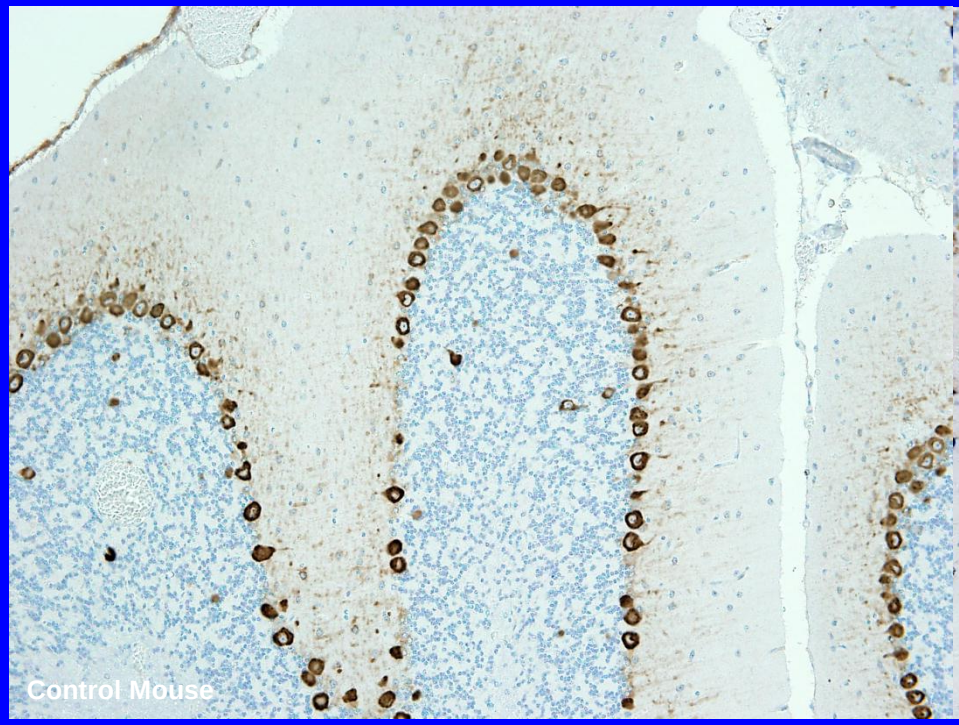


GFAP

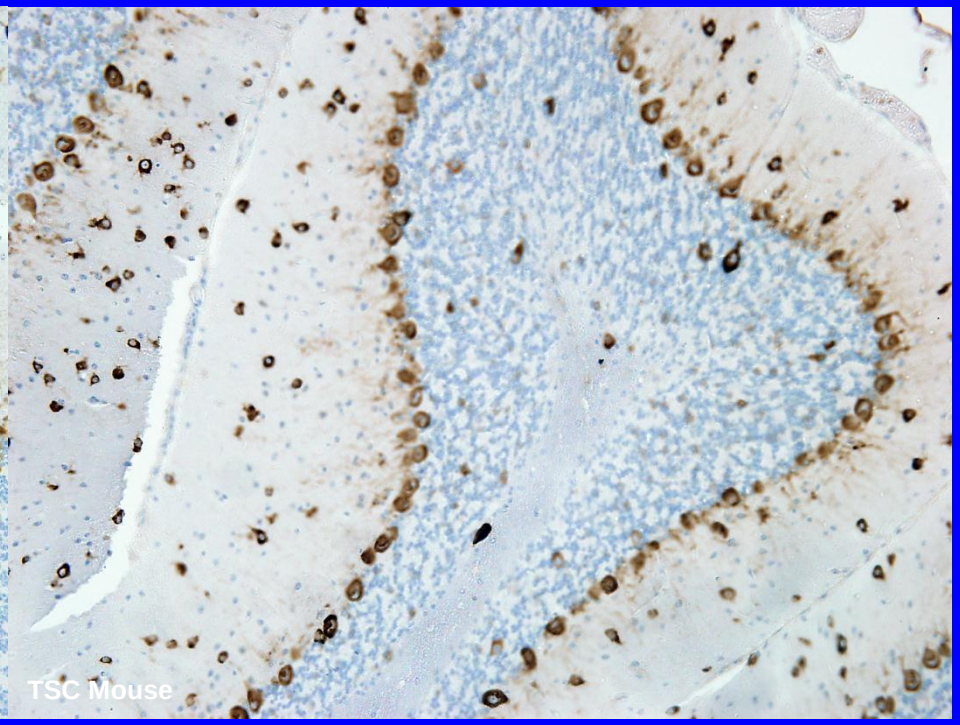


Mean GFAP Immunoreactivity of TSC Mouse Cortex Vs. Control Mouse Cortex

pS6 IHC—cerebellum/ 6 months



Control



TSC mouse

Vinters Lab

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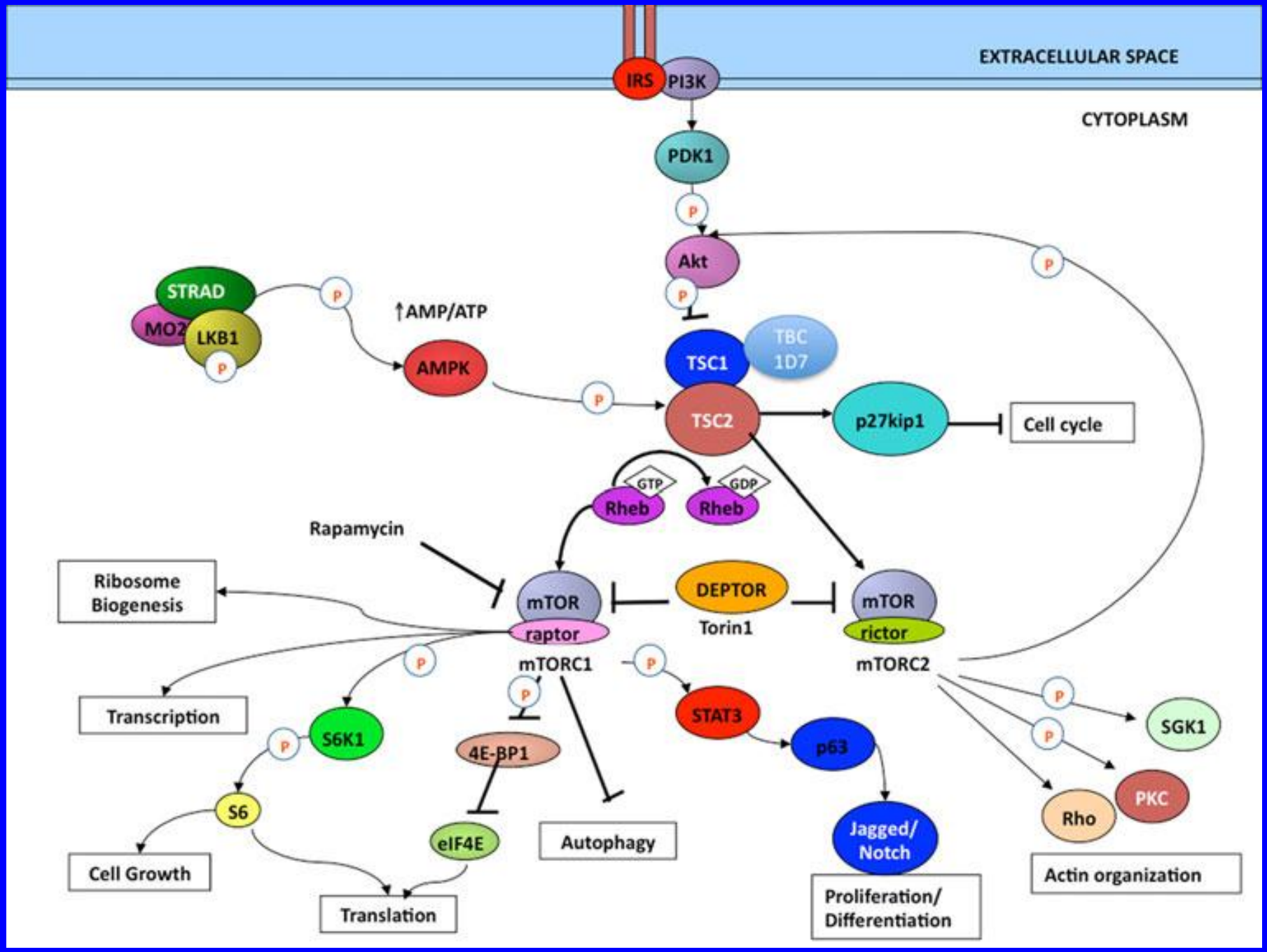
Julia Chang

Rupal Mehta

Founders of the UCLA Pediatric Epilepsy Surgery Program…

Donald Shields

Warwick Peacock



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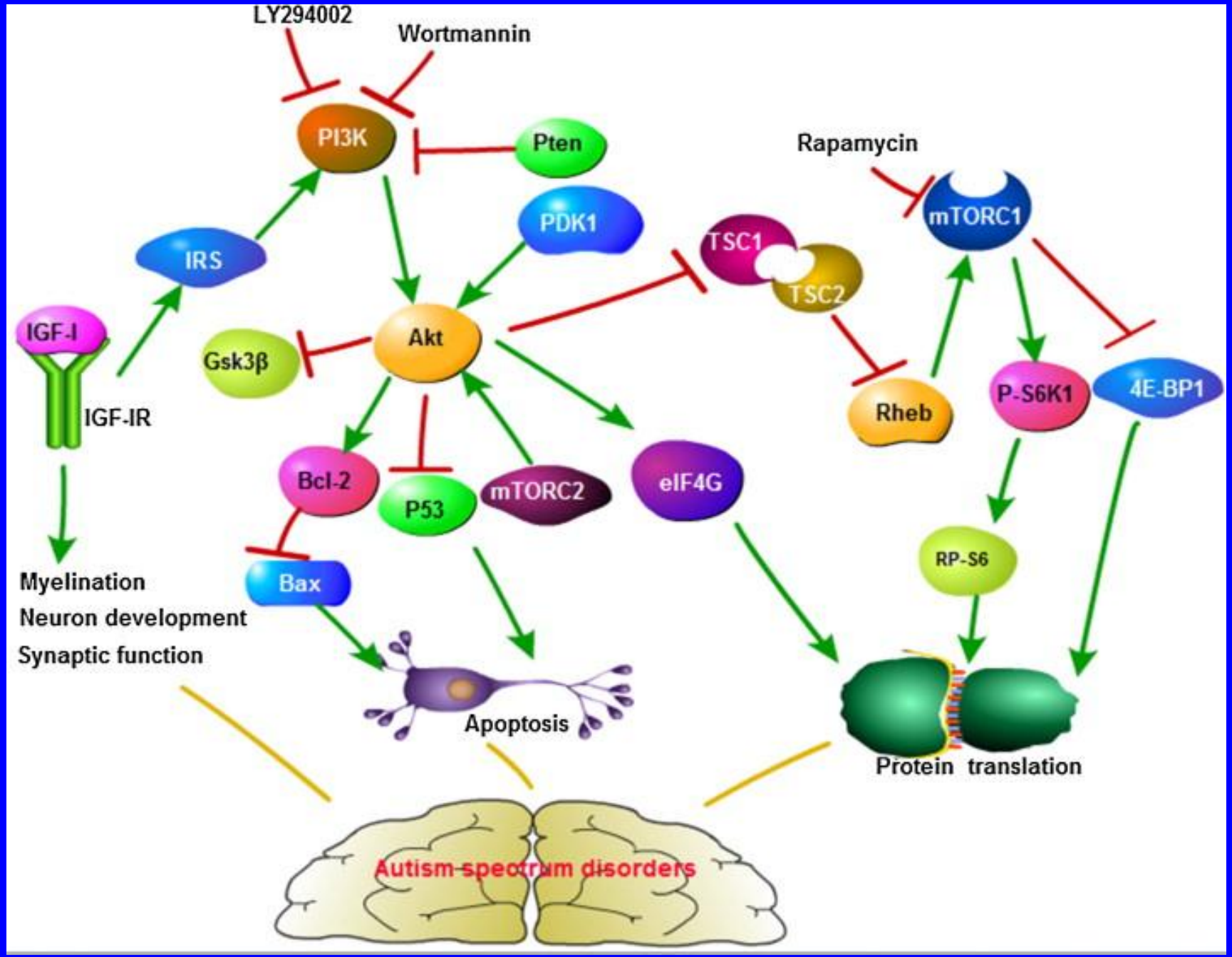

Chen, *et al*, Int. J. Devl Neuroscience 35 (2014)