Post-graduate course on genetics

Tuberous sclerosis of Bourneville and related conditions

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Plan

- Introduction
- Natural history - timeline
- Systematic phenotype description
- Genotype identification
  from Sanger sequencing to NGS
- Genotype - Phenotype correlation
- « Unusual genotype »
  from non penetrance to mosaïc
- Therapeutic approach
- Dedicated multidisciplinary clinic
- Patient association

Learning objectives

- ***Learn the clinical presentations*** *(of the groups of hereditary skin disorders and vascular malformations)*
- ***Understand molecular genetic diagnosis*** *(of hereditary skin disorders and vascular malformations)*
- ***How to collaborate*** *with dermatologists (genodermatoses consultation)* ***and neurologists, nephrologists, pneumologists, ophthalmologists – multi disciplinary***
- *To be aware of rare skin syndromes/vascular malformations and* ***evolution in experimental/targeted treatment***

Introduction

Original description in adult patients

« Tuberous Sclerosis of cerebral circonvolutions, idiotism and hemiplegic epilepsy »

Désiré Magloire Bourneville Archives Neurologie Paris 1880;1:81-91

Triad of features « mental retardation » , seizure and facial, phakoma/ angiofibroma

Vogt H. Zur Diagnostik der tubereusen sclerose 1908;2:1-16

‘Epidemiology’

- Multi-systemic condition
- Multiple Hamartoma

- Prevalence: 1/6.000 - 1/10.000

  2/3 sporadic 1/3 familial

Source:

Natural history – *adapted from Davis PE. et al. Pediatrics 2017;140(6)*

- Cardiac rhabdomyoma

- Infantile spasms
- Hypomelanotic macules
- *Others cutaneous manifestations*
- *Epilepsy*
- *Cognitive and behavioural difficulties*
- *TAND 'tuberous sclerosis associated neuro-developpemental disorder'*

- Renal manifestations
- LAM

Features prevalence related to age

Table 5. Diagnostic Criteria of Tuberous Sclerosis Complex According to Their Frequency in Children Below 2 Years of Age

Diagnostic Criteria	*%*	*Significance According to NTSA Classification (1992)*	*Significance According to the Latest Classification (1998)*
Hypomelanotic macules	89.6	Tertiary	Major*
Cardiac rhabdomyoma	83.3	Secondary	Major
Epilepsy, usually infantile spasms	83.0	Tertiary†	—
Subependymal nodules on neuroimaging	82.9	Primary	Major
Renal angiomyolipomas	16.7	Secondary	Major
Facial angiofibromas	10.4	Primary	Major
Retinal hamartoma	8.2	Secondary	Major†

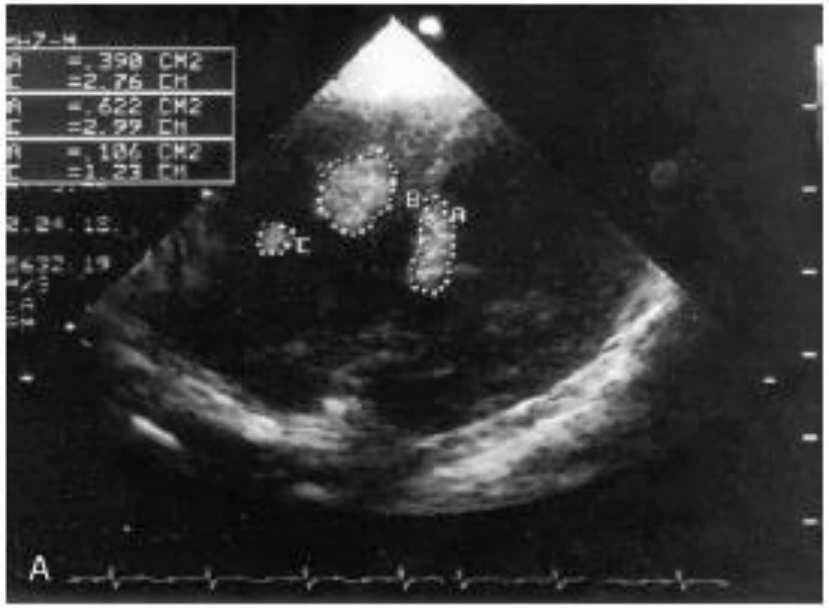
Natural history - Prenatal presentation

Cardiac rhabdomyomas - CR

Clinical and Genotype Studies of Cardiac Tumors in 154 Patients With Tuberous Sclerosis Complex

Sergiusz Jóźwiak, MD, PhD[a], Katarzyna Kotulska, MD, PhD[a], Jolanta Kasprzyk-Obara, MD[a], Dorota Domańska-Pakieła, MD, PhD[a], Małgorzata Tomyn-Drabik, MD, PhD[b], Penelope Roberts, PhD[c], David Kwiatkowski, MD, PhD[c]

Most frequent findings
Prenatal (if sporadic)
22nd weeks: Intra cardiac auricular and/or right ventricular mass (es) (tumor)
Appearance may lead to the diagnosis of **rhabdomyoma**



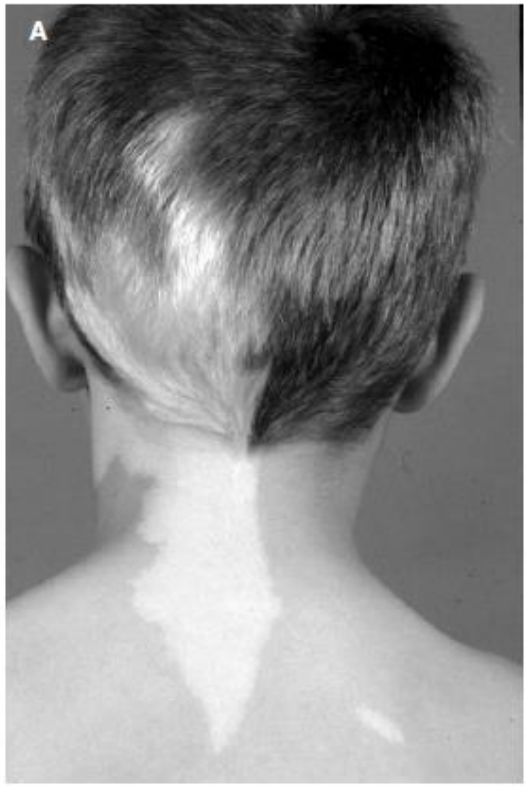
Jozwiak S. Ped 2006;118:1148

« Range of prevalence »

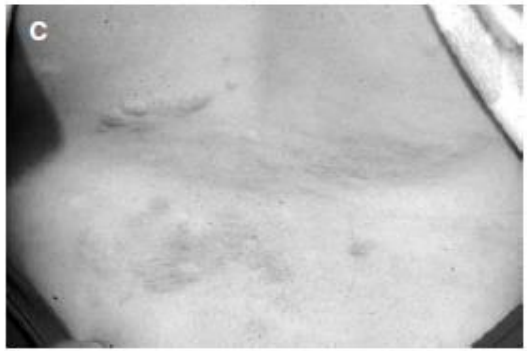
50 to 90% Jozwiak 2006
86% Harding et al. Am J Med Genet 1990
87% Davis et al. Ped 2017

if present - postulate inside 'diagnostic setting' - 'TSC unless proven otherwise'
look for presence of criteria brain, kidney

Dermatologic involvement

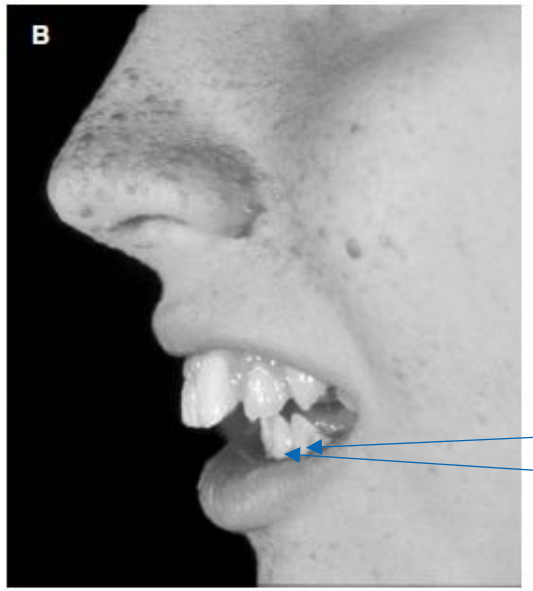


Achromic lesion
('ash leaf shape')



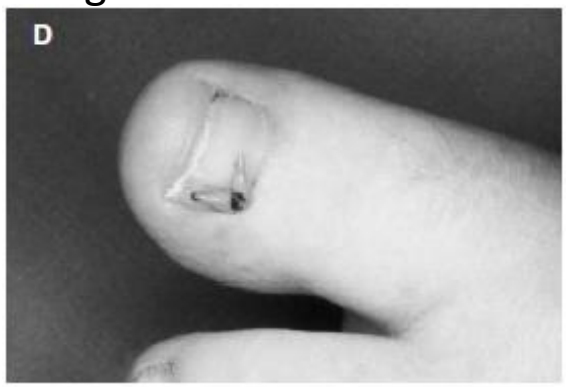
Kuehne lesion
Shagreen plaque

Angiofibroma of alae nasi or nails area



Look for
- Gingival fibroma
- Enamel pit(s)

Angiofibroma of nails area



Roach J Child Neurol 2004;19:643-649

Skin phenotype - Penetrance - Evolving phenotype

Table 3. Age at Appearance of Skin Lesions in Patients With Tuberous Sclerosis Complex

Age at Appearance (yr)	*Hypomelanotic Macules*	*"Confetti-like" Lesions*	*Facial Angiofibromas*	*Shagreen Patches*	*Molluscum Pendulum*	*Forehead Fibrous Plaque*	*Periunqual Fibromas*
>0–2	95	—	8	6	—	6	—
>2–5	6	—	56	16	4	4	1
>5–9	2	2	11	15	5	7	3
>9–14	—	—	4	13	10	3	8
>14–18	—	1	—	1	5	—	3
>18	—	—	—	—	—	—	1
Total (%)	103 (97.2)	3 (2.8)	79 (74.5)	51 (48.1)	24 (22.6)	20 (18.9)	16 (15.1)

Natural history - Neurologic features

Seizures: up to 85% of patients

Onset: during first year of life

Presentation: Focal epilepsy, Infantile spasms/West

Leading to wide range of cognitive and development delay

The largest natural history study of TSC to date – the TOSCA (**T**uber**O**us **S**clerosis registry to increase disease **A**wareness) study is a multi-centre, international disease registry designed with the aim of **providing deeper insights into the manifestations of TSC and its management**

Example: Epilepsy in tuberous sclerosis complex:

TABLE 3 Type of epilepsy and treatment outcomes in overall epilepsy cohort and in patients diagnosed at <2 years at baseline

Characteristics	Overall epilepsy cohort (N = 1852), n (%)	Early onset seizure group, (N = 1461), n (%)
Epilepsy type		
Focal seizures[a]	1250 (67.5)	984 (67.4)
Infantile spasms[a]	720 (38.9)	684 (46.8)
Focal seizures only	765 (41.3)	530 (36.3)
Infantile spasms only	246 (13.3)	221 (15.1)
Co-occurrence of infantile spasms and focal seizures	380 (20.5)	375 (25.7)

TABLE 4 Characteristics of epilepsy according to mutation type

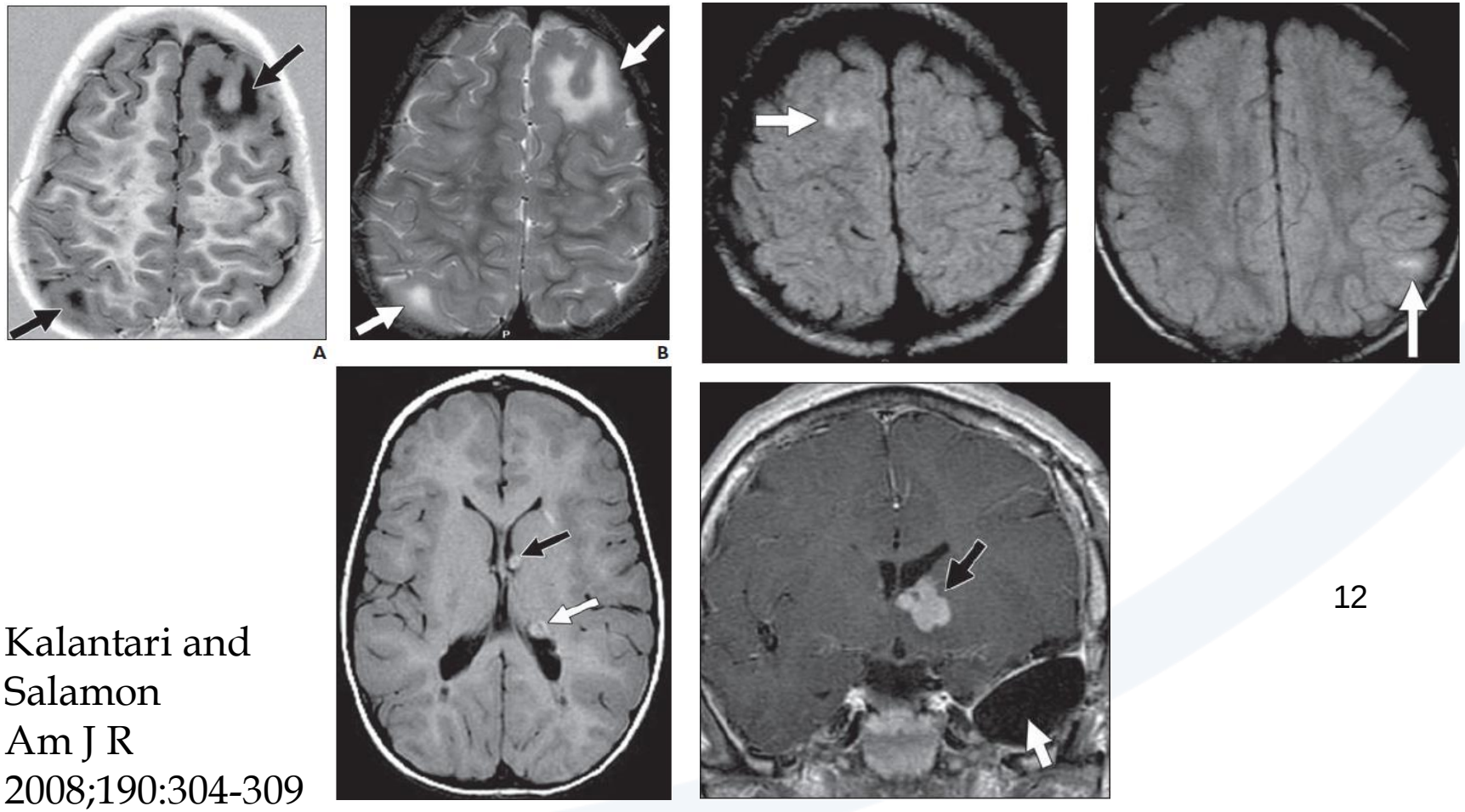
Characteristics	Overall epilepsy cohort with molecular testing		Early onset seizure group with molecular testing	
	TSC1 mutation (N = 152), n (%)	*TSC2* mutation (N = 569), n (%)	*TSC1* mutation (N = 98), n (%)	*TSC2* mutation (N = 489), n (%)
Epilepsy type				
Focal seizures[a]	113 (74.3)	409 (71.9)	75 (76.5)	350 (71.6)
Infantile spasms[a]	35 (23)	269 (47.3)	34 (34.7)	260 (53.2)
Infantile spasms only	12 (7.9)	67 (11.8)	11 (11.2)	61 (12.5)
Focal seizures only	88 (57.9)	220 (38.7)	52 (53.1)	168 (34.4)
Concomitant infantile spasms and focal seizures	21 (13.8)	163 (28.6)	21 (21.4)	161 (32.9)
Age at diagnosis, years				
Focal seizures				
Mean	3.7	2.2	1.1	0.9
Median	2.0	<1	1	<1
Range	<1-47	<1-59	<1-14	<1-16
Infantile Spasms				
Mean	0.3	0.3	0.3	0.2
Median	<1	<1	<1	<1
Range	<1-6	<1-5	<1-6	<1-4

Narbbout R. et al Epilepsia Open 2019;4:73–84

NEUROLOGIC FEATURES

Secondarily to brain lesions

Typically: « tubers », sub ependymal giant astrocytic lesion(s) (SEGA)



Kalantari and Salamon Am J R 2008;190:304-309

Distinctive neuro – psychiatric phenotype

In spite of the high rates and burden of neuropsychiatric manifestations in individuals with TSC, a 2010 study from the UK reported that only 18% of all families had ever received any of the recommended evaluations or treatments for the range of neuropsychiatric manifestations

TSC-associated neuropsychiatric disorders (TAND)is an umbrella term coined by the Neuropsychiatry Panel of the 2012 International Consensus Conference for TSC and encompasses a range of neuropsychiatric manifestations across various levels of investigation

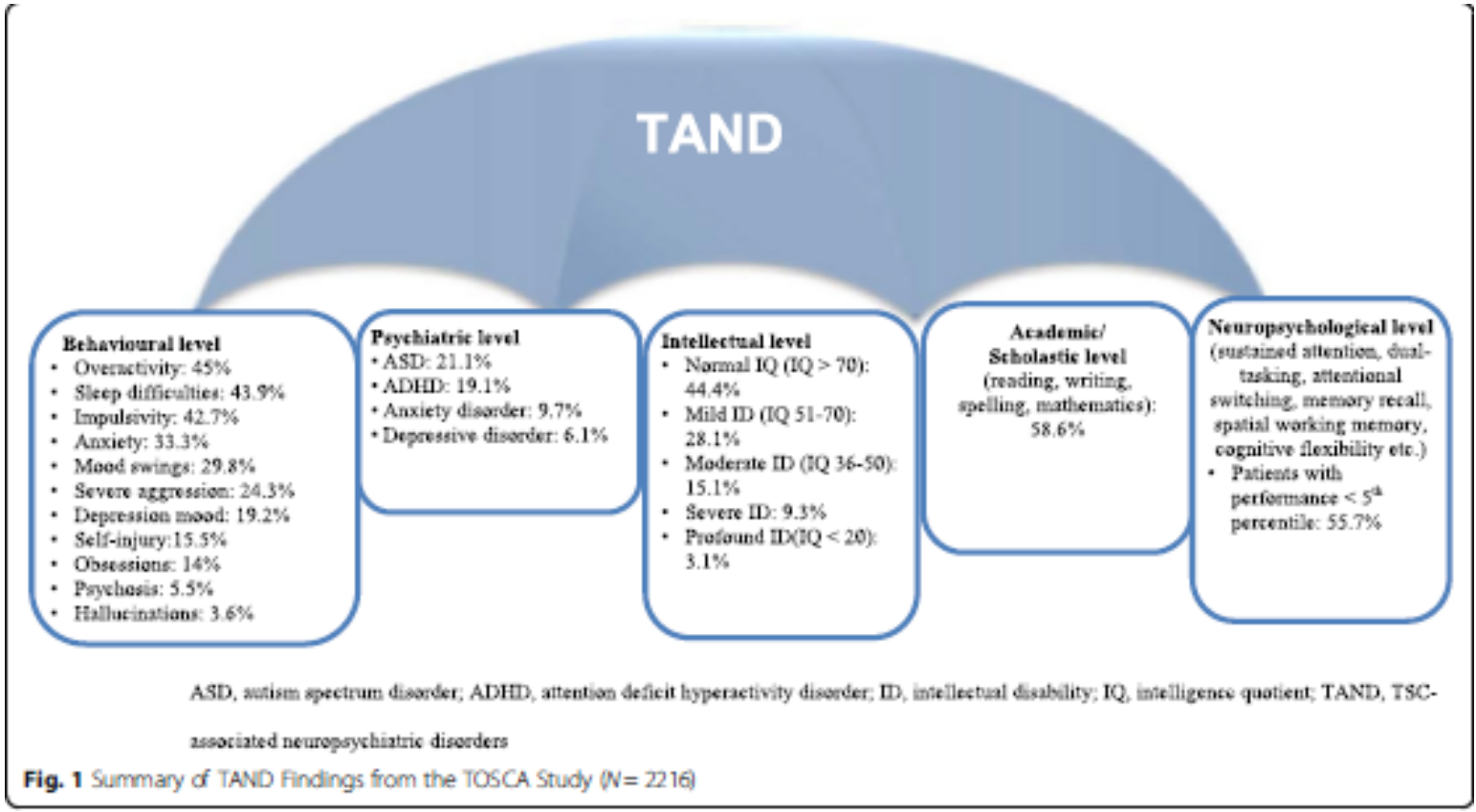


Fig. 1 Summary of TAND Findings from the TOSCA Study (N = 2216)

In a previous publication outlining baseline findings from the TOSCA cohort of 2093 individuals, we presented topline findings of TAND features in the largest TSC cohort reported globally to date [28]. Results showed that ID was observed in 54% of the evaluated participants and suggested that psychiatric disorders were typically diagnosed late. We also identified significant non-reported or missing data, which suggested that even in expert TSC centres around the globe, TAND may be underdiagnosed and therefore under-treated

Genotype - phenotype

TAND variables into 6 clusters: a scholastic cluster (reading, writing, spelling, mathematics, visuo-spatial difficulties, disorientation), a hyperactive/impulsive cluster (hyperactivity, impulsivity, self-injurious behavior), a mood/anxiety cluster (anxiety, depressed mood, sleep difficulties, shyness), a neuropsychological cluster (attention/concentration difficulties, memory, attention, dual/multi-tasking, executive skills deficits), a dysregulated behavior cluster (mood swings, aggressive outbursts, temper tantrums), and an autism spectrum disorder (ASD)-like cluster (delayed language, poor eye contact, repetitive behaviors, unusual use of language, inflexibility, difficulties associated with eating)

de Vries et al. Journal of Neurodevelopmental Disorders 2020:24(12):1-13

To more recent

Approximately 90% of people with TSC evidence TAND manifestations at some point in their lives and TAND has been identified by families as the **greatest clinical burden of the disorder**

Vanclooster *et al.*
Journal of Neurodevelopmental Disorders (2022) 14:13
https://doi.org/10.1186/s11689-022-09423-3

Journal of Neurodevelopmental Disorders

REVIEW Open Access

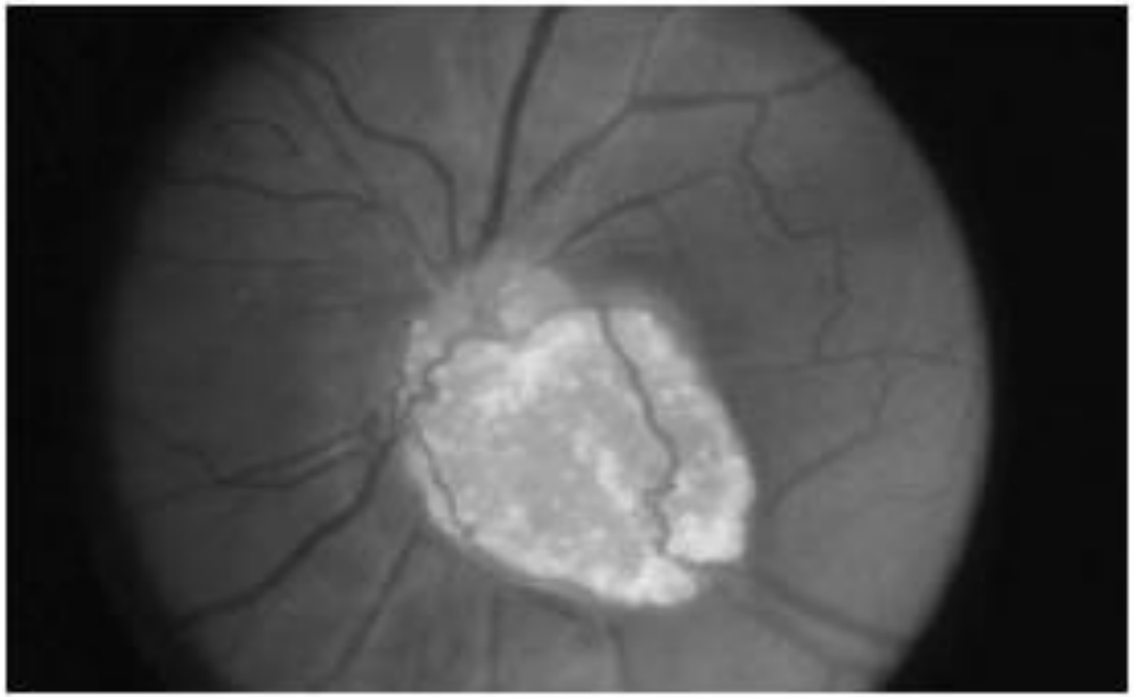
The research landscape of tuberous sclerosis complex–associated neuropsychiatric disorders (TAND)—a comprehensive scoping review

Stephanie Vanclooster[1†], Stacey Bissell[2†], Agnies M. van Eeghen[3,4], Nola Chambers[5], Liesbeth De Waele[6,7], Anna W. Byars[8], Jamie K. Capal[9], Sebastián Cukier[10], Peter Davis[11], Jennifer Flinn[12], Sugnet Gardner-Lubbe[13], Tanjala Gipson[14,15], Tosca-Marie Heunis[1], Dena Hook[16], J. Christopher Kingswood[17,18], Darcy A. Krueger[19,20], Aubrey J. Kumm[5], Mustafa Sahin[21], Eva Schoeters[22], Catherine Smith[16], Shoba Srivastava[5,23], Megumi Takei[24], Robert Waltereit[25], Anna C. Jansen[1,26] and Petrus J. de Vries[5*]

Table 3 The seven natural TAND clusters and their items

TAND clusters	TAND items
1. Scholastic	Reading, writing, spelling, mathematics
2. Neuropsychological	Memory, disorientation, attention deficits (behavioural and neuropsychological), visuo-spatial deficits, dual-task deficits, executive function deficits
3. Dysregulated behaviour	Aggressive outbursts, temper tantrums, self-injury
4. Overactive/impulsive	Overactivity, impulsivity, restlessness
5. Eat/sleep	Eating difficulties, sleep difficulties
6. Mood/anxiety	Anxiety, depressed mood, extreme shyness, mood swings
7. Autism spectrum disorder–like	Inflexibility, unusual language, delayed language, repetitive behaviours, poor eye contact, peer difficulties

Ophthalmic phenotype



Retinal hamartoma

Diagnostic Criteria

'certain' IF: 2 major criteria
1 major + 2 minor criteria

'probable' IF : 1 major + 1 minor

'possible' IF : 1 major

	Major criteria	Minor criteria
Dermatology	1) Facial angiofibroma or frontal plaque 2) Fibroma of nail (non traumatic) 3) ≥3 hypomelanic macules (amelanotic spots) 4) "Shagreen" plaque	Ski lesions "in confetti"
Ophthalmology	Multiple retinal nodular hamartoma	Amelanotic retinal pellet
Cerebral	1) Cortical tuber 2) Subependymal nodule 3) Giant cells astrocytoma (SEGA)	Abnormal migration of white matter (neuronal migration anomaly)
Cardiac	Rhabdomyoma (single or multiple)	
Renal	Renal angiomyolipoma or lymphangiomyomatosis	Multiple cyst
Teeth / oral cavity		1) Enamel tooth anomalies (multiple pits) 2) gingival fibroma
Digestif		Colon hamartomatous polyposis
Bone		Bone cyst
Vascular		Hamartoma (other than renal)

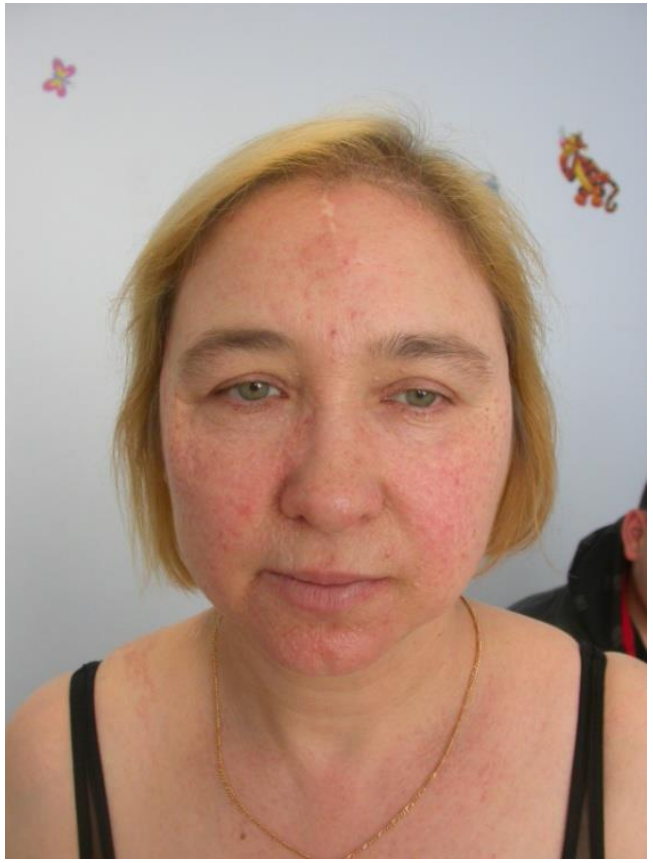
Roach et al. 1998

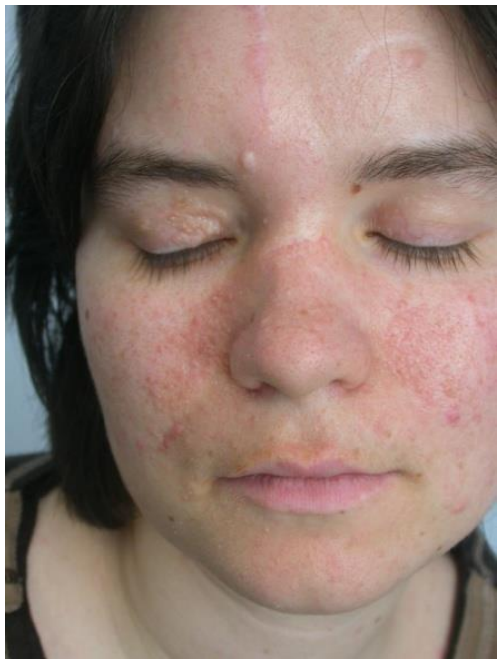
Synthesis…

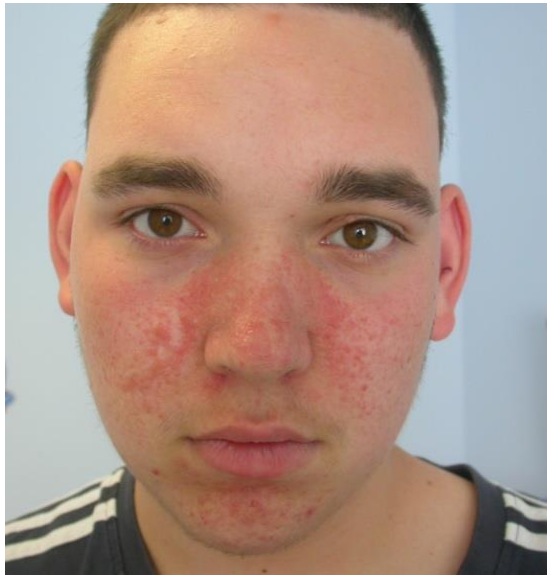
Table 1 Diagnostic criteria for tuberous sclerosis

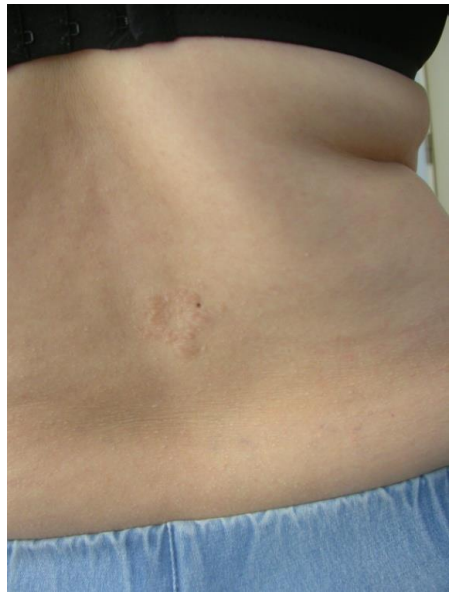
Type of criteria	Description
Definite diagnosis	Two major features OR one major feature with two or more minor features OR a pathogenic variant in *TSC1* or *TSC2*
Possible diagnosis	One major feature OR two or more minor features
Genetic diagnostic criteria	A pathogenic variant in *TSC1* or *TSC2* identified in DNA from normal tissue, where a pathogenic variant (105, 106) is defined as a variant that inactivates the function of TSC1 or TSC2 [i.e., a frameshift (insertion or deletion) or nonsense variant], a variant that prevents protein synthesis (i.e., a large deletion), or a missense variant that has been shown by a functional study to affect the function of TSC1 or TSC2
Clinical diagnostic criteria	Major features: 1. At least three hypomelanotic macules that are at least 5 mm in diameter 2. At least three angiofibromas or fibrous cephalic plaque 3. At least two ungual fibromas 4. Shagreen patch 5. Multiple retinal hamartomas 6. Cortical dysplasias (tubers and cerebral white matter radial migration lines) 7. Subependymal nodules 8. Subependymal giant cell astrocytoma 9. Cardiac rhabdomyoma 10. Lymphangioleiomyomatosis[a] 11. At least two angiomyolipomas[a] Minor features: 1. “Confetti” skin lesions 2. At least three dental enamel pits 3. At least two intraoral fibromas 4. Retinal achromic patch 5. Multiple renal cysts 6. Nonrenal hamartomas

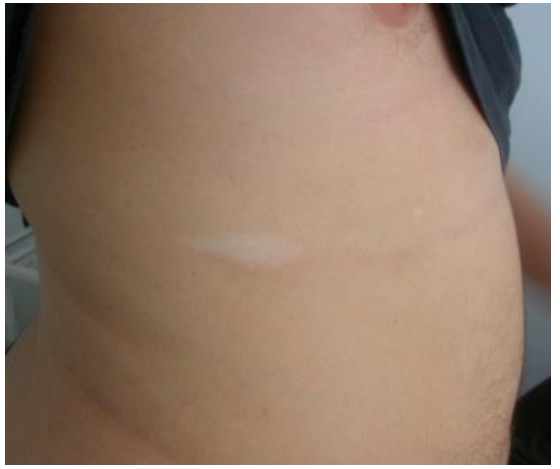
Intra familial variability

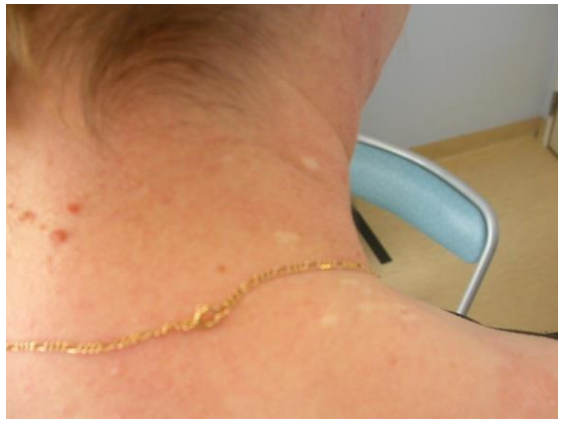












Genotype, on the way

J Med Genet 1993; **30:** 224–227

Identification of markers flanking the tuberous sclerosis locus on chromosome 9 (*TSC1*)

M Nellist, P T Brook-Carter, J M Connor, D J Kwiatkowski, P Johnson, J R Sampson

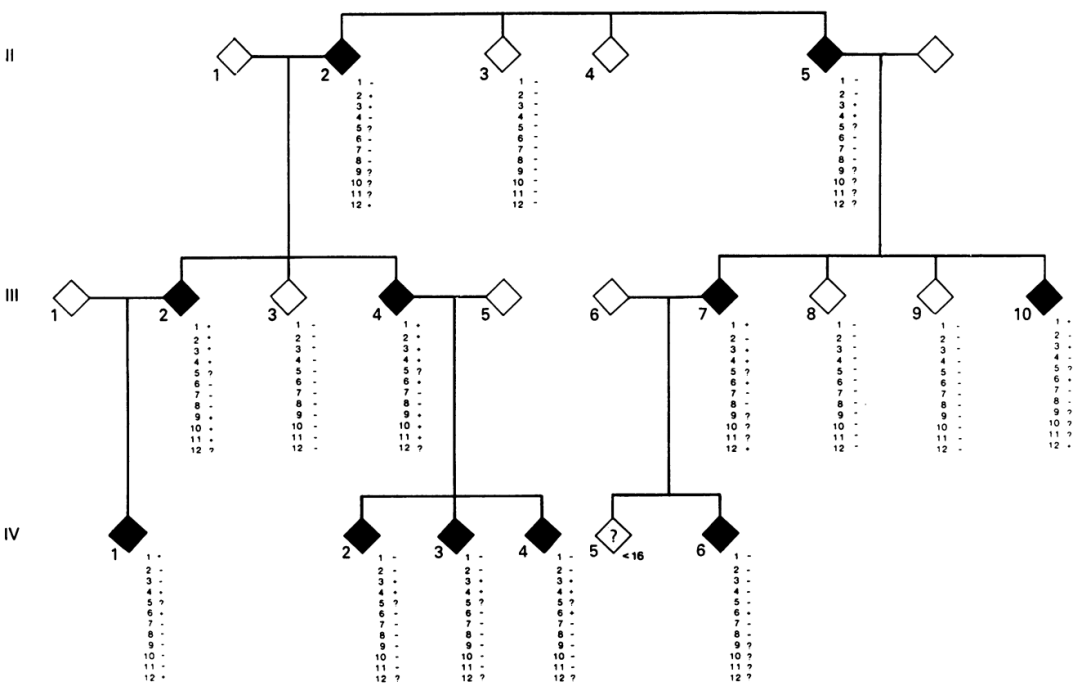
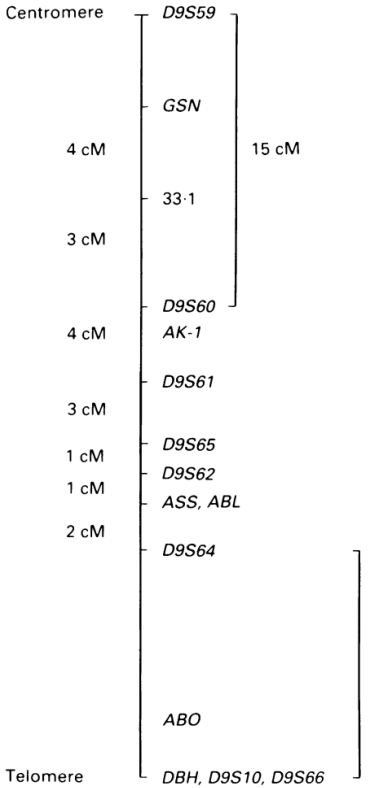


Figure 1 Chromosome 9 linked tuberous sclerosis pedigree. Affected subjects are represented by solid diamonds and +/− refers to the diagnostic signs as follows. (1) Adenoma sebaceum. (2) Periungual fibroma(s). (3) Shagreen patch(es). (4) Hypopigmented macules. (5) Retinal phakoma(ta). (6) Seizures. (7) Mental retardation. (8) Brain tumour. (9) Renal cysts. (10) Renal angiomyolipoma(ta). (11) Renal adenocarcinoma. (12) Periventricular calcification on brain CT scan.



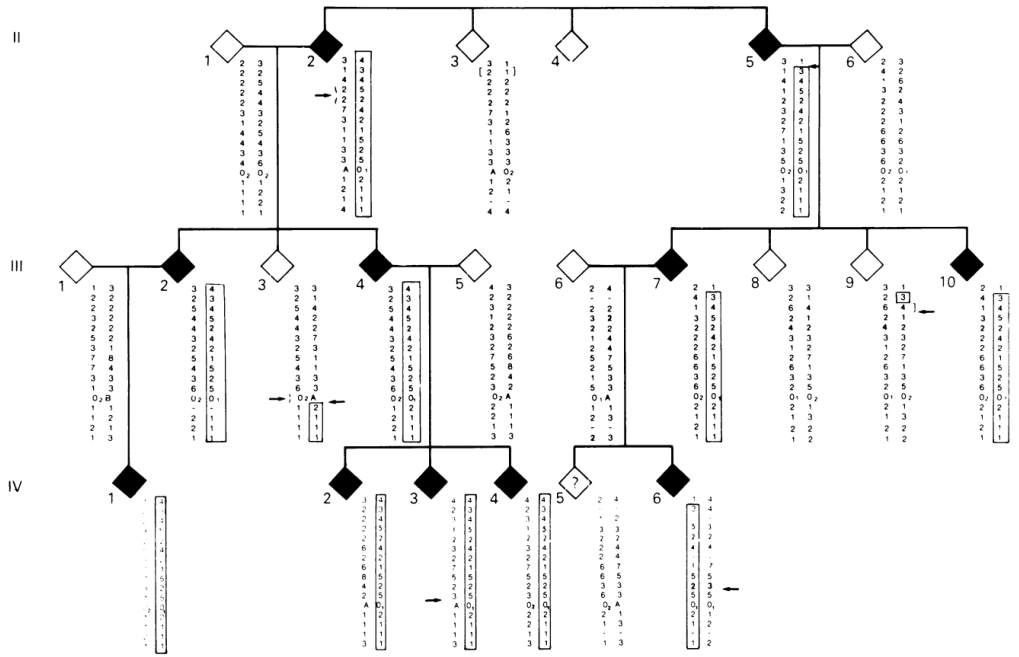


Figure 3 Haplotypes for markers from distal 9q in a chromosome 9 linked tuberous sclerosis pedigree. Affected subjects are represented by solid diamonds and recombination events are arrowed. Markers are listed proximal (top) to distal (bottom) as follows: D9S59, GSN, 33.1, D9S60, AK-1, D9S61, D9S65, D9S62, ASS, ABL, D9S64, ABO, DBH dinucleotide repeat, DBH deletion polymorphism, D9S10, D9S66.

Genotype identification on the way...

Genomics
Volume 41, Issue 3, 1 May 1997, Pages 385-389

Regular Article

A 1.7-Megabase Sequence-Ready Cosmid Contig Covering the TSC1 Candidate Region in 9q34

N. Hornigold [a], M. van Slegtenhorst [b], J. Nahmias [c], R. Ekong [c], S. Rousseaux [c], C. Hermans [b], D. Halley [b], S. Povey [c], J. Wolfe [a, 1]

We have used fingerprinting methods and hybridization to produce a 1.7-Mb overlapping clone map covering the TSC1 candidate region, with a single gap of 20 kb. We have localized 12 previously cloned genes and 17 genetic markers on this map and have confirmed the order of the genetic map

This deep set of overlapping clones is now ready to be used for candidate gene isolation, for transcription studies, or for sequencing

Genotype, on the way (ct'd)

Cliniques universitaires SAINT-LUC UCLouvain BRUXELLES

9q34 loss of heterozygosity in a tuberous sclerosis astrocytoma suggests a growth suppressor-like activity also for the TSC1 gene

Get access >

Caterina Carbonara, Lucia Longa, Enrico Grosso, Carla Borrone, Maria Grazia Garré, Massimo Brisigotti, Nicola Migone ✉

Human Molecular Genetics, Volume 3, Issue 10, October 1994, Pages 1829–1832,

Genotype identification on the way…

…The*TSC1* gene was identified from a 900-kilobase region containing at least 30 genes

The 8.6-kilobase *TSC1* transcript is widely expressed and encodes a protein of 130 kilodaltons – **hamartin** - that has homology to a putative yeast protein of unknown function

In one of these six: a somatic mutation in the wild-type allele was found in a TSC-associated renal carcinoma, which suggests that hamartin **acts as a tumor suppressor**

Genotype evidence

Identification of the Tuberous Sclerosis Gene *TSC1* on Chromosome 9q34

TSC1 encoding for the protein **Hamartin** - 21 exons

Fig. 4. Predicted amino acid sequence of the *TSC1* protein, hamartin. A potential transmembrane domain (amino acids 127 to 144) and a coiled-coil domain (amino acids 730 to 965) are underlined. The *TSC1* genomic sequence and the cDNA sequence have been deposited in GenBank (accession numbers AC002096 and AF013168, respectively)

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MAQQANVGEL LAMLDSPMLG VRDDVTAVFK ENLNSDRGPM LVNTLVDYYL ETSSQPALHI   60
LTTLQEPHDK HLLDRINEYV GKAATRLSIL SLGGHVIRLQ PSWKHKLSQA PLLPSLLKCL  120
KMDTDVVVLT TGVLVLITML PMIPQSGKQH LLDFFDIFGR LSSWCLKKPG HVAEVYLVHL  180
HASVYALSHR LYGMYPCNFV SPGRSHYSMK ENLETFEEVV KPMMEHVRIH PELVTGSKDH  240
ELDPRRWKRL ETHDVVIECA KISLDPTEAS YEDGYSVSHQ ISARFPHRSA DVTTSPYADT  300
QNSYGCATST PYSTSRLMLL NMPGQLPQTL SSPSYRLITE PPQATLWSPS MVCGMTTPPT  360
SPGNVPPDLS HPYSKVFGTT AGGKSTPLGT PATSPPPAPL CHSDDYVHIS LPQATVTPPR  420
KEERMDSARP CLHRQHELLN DRGSEEPPQS KGSVTLSDLP GFLGDLASEE DSLEKDKEEA  480
AISRELSEIT TAEAEPVVPR GGFDSPFYRD SLPGSQRKTH SAASSSQGAS VNPEPLHSSL  540
DKLGPDTPKQ AFTPIDLPCG SADESPAGDR ECQTSLETSI FTPSPCKIPP PTRVGFGSGQ  600
PPPYDHLFEV ALPKTAHHFV IRKTPELLKK AKGNTEEDGV PSTSPMEVLD RLIQQGADAH  660
SKELNKLPLP SKSVDWTHFG GSPPSDEIRT LRDQLLLLHN QLLYERFKRQ QHALRNRRLL  720
RKVIKAAALE EHNAAMKDQL KLQEKDIQMW KVSLQKEQAR YNQLQEQRDT MVTKLHSQIR  780
QLQHDREEFV NQSQELQTKL EDCRNMIAEL RIELKKANNK VCHTELLLSQ VSQKLSNSES  840
VQQQMEFLNR QLLVLGEVNE LYLEQLQNKH SDTTKEVEMM KAAYRKELEK NRSHVLQQTQ  900
RLDTSQKRIL ELESHLAKKD HLLLEQKKYL EDVKLQARGQ LQAAESRYEA QKRITQVFEL  960
EILDLYGRLE KDGLLKKLEE EKAEAAEAAE ERLDCCNDGC SDSMVGHNEE ASGHNGETKT 1020
PRPSSARGSS GSRGGGGSSS SSSELSTPEK PPHQRAGPES SRWETTMGEA SASTPTTVGS 1080
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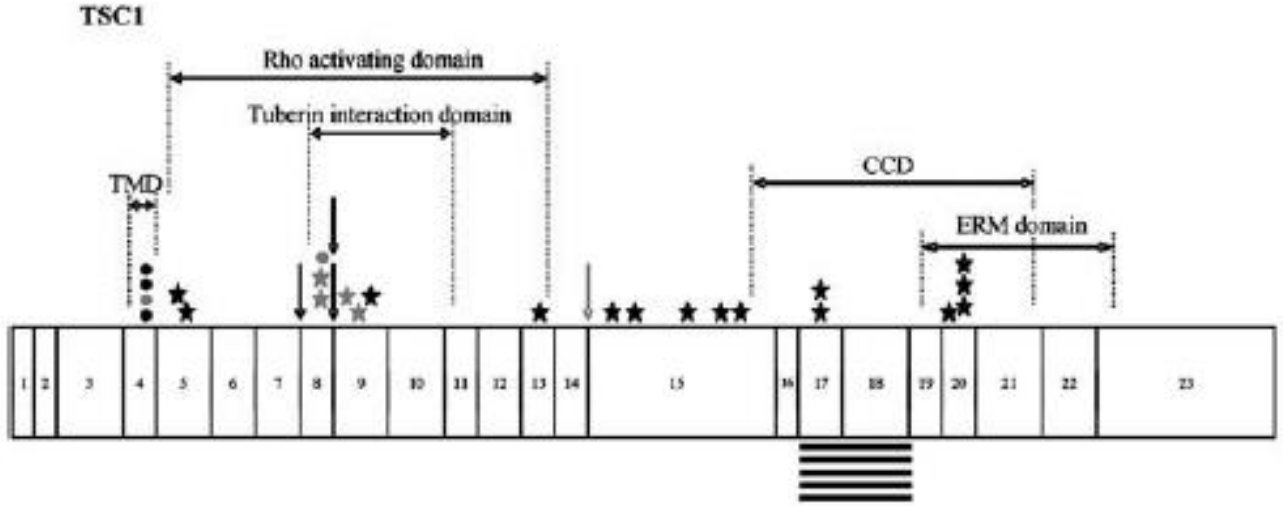


Slentghorst Science 1997;277:855-858

Genotype identification on the way

ELSEVIER

Genomics

Volume 8, Issue 2, October 1990, Pages 237-242

Genetic heterogeneity in tuberous sclerosis

L.A.J. Janssen [*, a, b], L.A. Sandkuyl [*, †, a, b], E.C. Merkens [*, a, b], J.A. Maat-Kievit [*, a, b], J.R. Sampson [†], P. Fleury [‡], R.C.M. Hennekam [§], G.C. Grosveld [*, a, b], D. Lindhout [*, a, b], D.J.J. Halley [*, a, b]

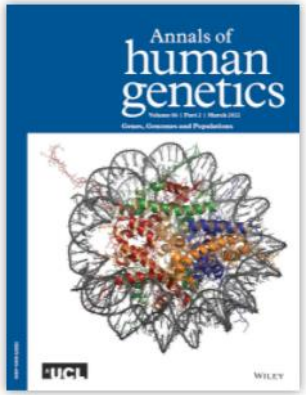
Our results support a model with two different loci independently causing the disease :

- one locus (TSC1) maps in the vicinity of the Abelson oncogene (ABO) at 9q34

a second locus (TSC2) maps in the region of the anonymous DNA marker Lam L7 and the dopamine D2 receptor gene at 11q23

Genotype, on the way (ct'd)

Annals of human genetics



Volume 58, Issue 2
May 1994
Pages 107-127

Two loci for Tuberous Sclerosis: one on 9q34 and one on 16p13

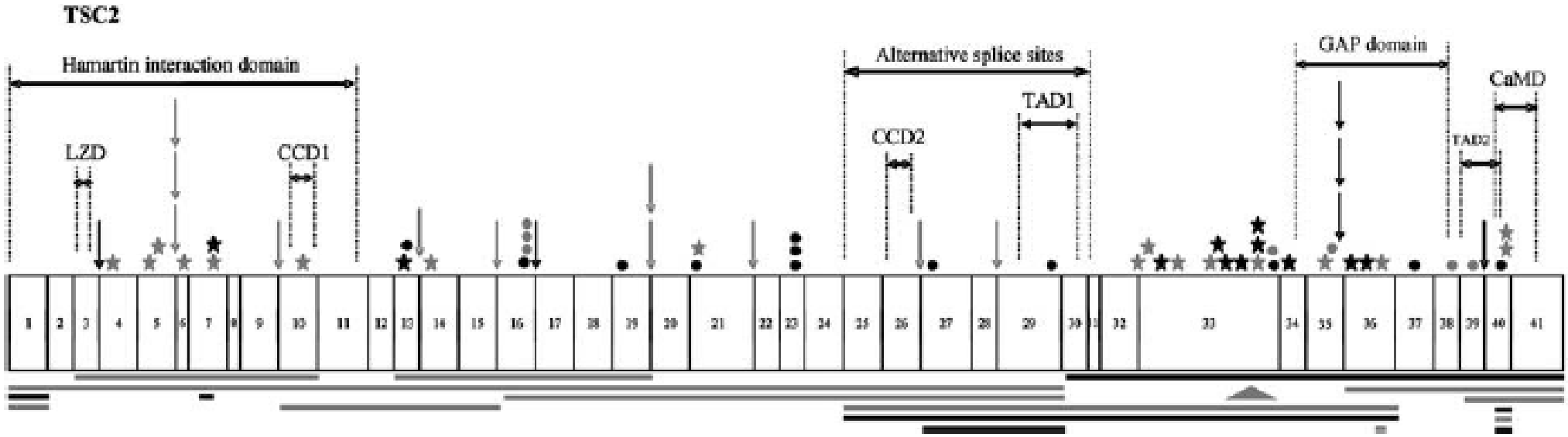
S. POVEY, M. W. BURLEY, J. ATTWOOD, F. BENHAM, D. HUNT, S. J. JEREMIAH, D. FRANKLIN, G. GILLETT, S. MALAS, E. B. ROBSON, P. TIPPETT, J. H. EDWARDS, D. J. KWIATKOWSKI, M. SUPER, R. MUELLER, A. FRYER, A. CLARKE, D. WEBB, J. OSBORNE ... See fewer authors

The secund locus

Cell 1993;75(7):1305-15

Identification and characterization of the tuberous sclerosis gene on chromosome 16

European Chromosome 16 Tuberous Sclerosis Consortium. PMID: 8269512



'igure 1 *TSC1* and *TSC2* gene exon map, depicting mutation types of patients with and without MR. CaMD, calmodulin-binding domain; CCD, coil–coil lomain; ERM, ezrin–radixin–moesin; GAP, GTPase-activating protein; LZD, leucine zipper domain; TAD, transcription-activating domain; TMD, ransmembrane domain.

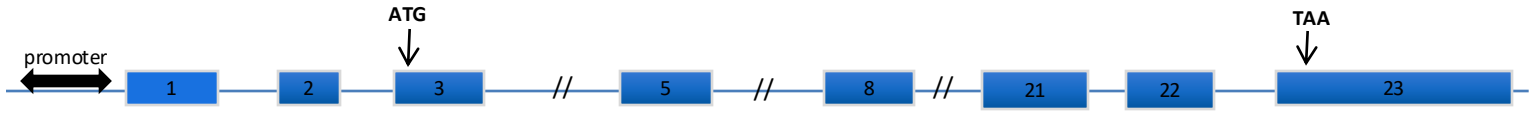
TSC2 - GenBank NG 005895.1 GI:125662814 – 42 exons

http://ftp.ebi.edu.au/pub/ databases/lrgex/LRG_487.xml]

TWO genes - ONE disease

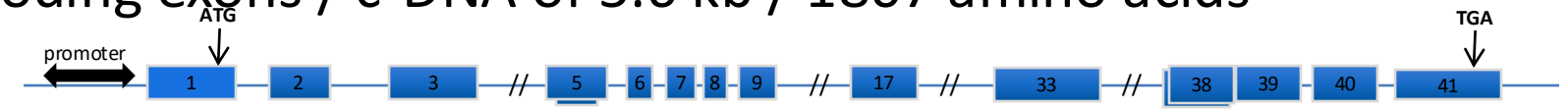
- TSC1 encoding for Hamartin on chromosome 9q34

  21 coding exons / c-DNA of 3.4kb/ 1164 amino acids



- TSC 2 encoding for Tuberin on chromosome 16p13.3

  41 coding exons / c-DNA of 5.6 kb / 1807 amino acids



Functions of the TSC1/TSC2 gene products

- Hamartin and Tuberin are ubiquitously expressed
- Expression: predominantly localized in cells with a rapid mitotic rate and turnover, e.g., epithelia, **lymphocytes**, heart, skin, kidney, lung, eyes

functions as a tumor/growth suppressor

Evidence : TSC1/TSC2 gene products

- Tuberin and Hamartin expression: reduced in Subependymal Giant Cell Astrocytomas (SEGA) by immunohistochemistry

  Sergiusz J *et al.* Journal of Child Neurology 2004; **19**:102-106

- Loss of heterozygosity (LOH) of both TSC1 and TSC2 in hamartomas from TS patients

  van Slegtenhorst *et al.* Science 1997; **277:805-808**

- Loss of a single TSC gene allele is sufficient to disrupt neuronal morphology and function in mouse models

  Tavazoie SF et al, Nat Neurosci. 2005; **8**:1727–1734

- High frequency of LOH in renal angiomyolipoma, cardiac rabdomyoma and lymphangioleiomyomatosis but low frequency in cortical tubers

  Henske *et al.* Am J Pathol. 1997; 151:1639-1647 / Chan JA et al. J Neuropathol Exp Neurol. 2004 **63**:1236-42

- Methylation of promotor described in renal angiomyolipoma

  Lesma E *et al.* Am J Pathol. 2009; **174**:2150–2159

Expression studies of hamartin - tuberin

Evidence for population variation in *TSC1* and *TSC2* gene expression

Jentarra GM *et al.* BMC Med Genet. 2011; 12: 29

first evidence that *TSC1* and *TSC2* genes exhibit allele-specific differences in mRNA expression in blood leukocytes isolated from normal individuals

difference between 10 to 20% for TSC1 and TSC2 mRNA expression

Data interpretation

- Database on nucleotidic changes in *TSC1* and *TSC2 genes*

Leiden **O**pen **V**ariation **D**atabases publicly (2006)

https://databases.lovd.nl/shared/genes/TSC1
https://databases.lovd.nl/shared/genes/TSC2

Ekong et al. Hum Mutation 2016

Genotype deciphering

LOVD 3 Leiden Open Variation Database — THE HUMAN VARIOME PROJECT

Global Variome shared LOVD

TSC1 (tuberous sclerosis 1)

Curator: Rosemary Ekong

Genes | Transcripts | Variants | Individuals | Diseases | Screenings | Submit | Documenta

The TSC1 gene homepage

Classification of variants: please note that where there are several records of the same variant, the classific of that variant may be contradictory depending on the submitter's conclusion. For the curator's opinion on the classification of the variant, please view a **SUMMARY record**.

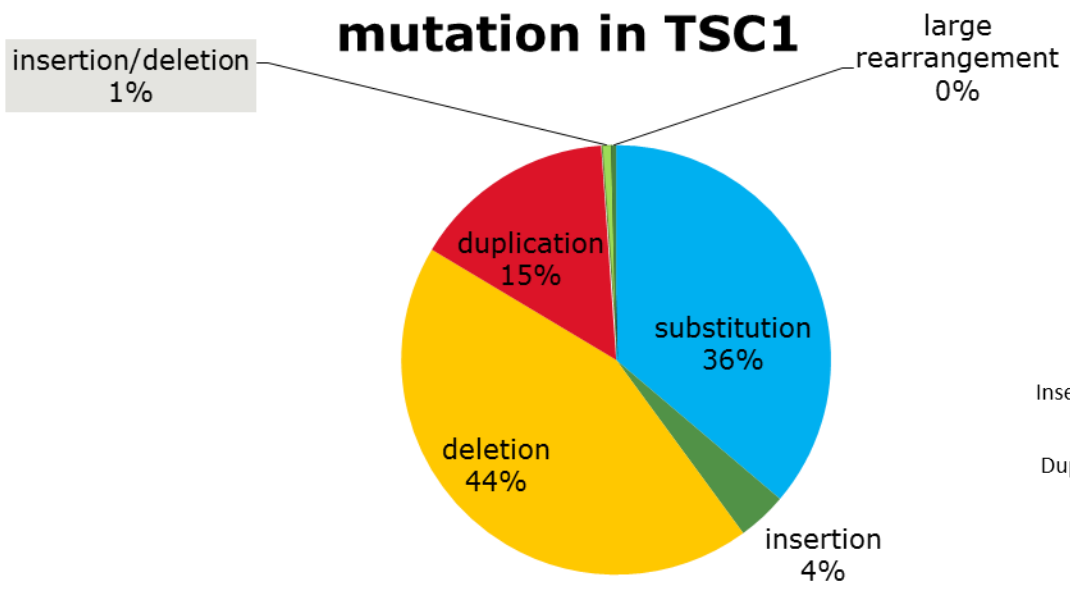
General information	
Gene symbol	TSC1
Gene name	tuberous sclerosis 1
Chromosome	9
Chromosomal band	q34
Imprinted	Unknown
Genomic reference	LRG_486
Transcript reference	NM_000368.4
Exon/intron information	NM_000368.4 exon/intron table
Associated with diseases	FCORD2, ID, LAM, TSC, TSC1
Citation reference(s)	-
Refseq URL	Genomic reference sequence
Curators (1)	**Rosemary Ekong**
Total number of public variants reported	4466
Unique public DNA variants reported	1356
Individuals with public variants	3918
Hidden variants	122
Notes	The **Purpose of the Database** is to provide information to help diagnostic laboratories and clinicians interpret the results of genetic testing for tuberous sclerosis (TSC). It can be difficult to decide whether a change found in the DNA of one of the TSC genes is the change that is causing TSC or a harmless variation which can be ignored. The database allows scientists and clinicians to check whether the change has been reported by other laboratories and whether it is thought to be a cause of TSC or a normal variation. All the information in the database is anonymous and care has been taken to exclude any information which might lead to patients being identified. In supporting genetic testing for TSC the database provides a valuable service for patients and their families. **Variants** listed here are collated from The Cardiff-Rotterdam Tuberous Sclerosis Mutation Database, David Kwiatkowski's Tuberous Sclerosis Project, publications and submissions to the database. Should you notice that there are variants not in the database or that there are errors, please inform us. We gratefully acknowledge the key role of ***Sue Povey*** in establishing the TSC databases and their curation (2005-2019). She always gave thoughtful and careful consideration to the classification of each variant because there was always a patient involved.

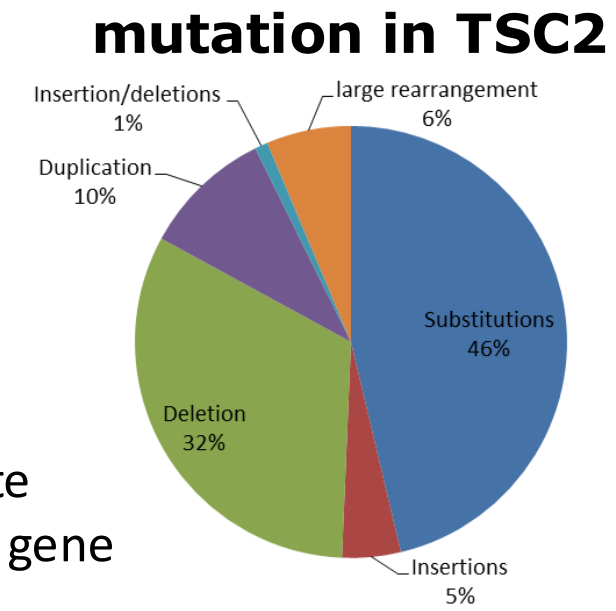
The TSC2 gene homepage

Classification of variants: please note that where there are several records of the same va of that variant may be contradictory depending on the submitter's conclusion. For the curato classification of the variant, please view a **SUMMARY record**.

General information	
Gene symbol	TSC2
Gene name	tuberous sclerosis 2
Chromosome	16
Chromosomal band	p13.3
Imprinted	Not imprinted
Genomic reference	LRG_487
Transcript reference	NM_000548.3
Exon/intron information	NM_000548.3 exon/intron table
Associated with diseases	FCORD2, ID, LAM, TSC, TSC2
Citation reference(s)	-
Refseq URL	Genomic reference sequence
Curators (1)	**Rosemary Ekong**
Total number of public variants reported	12582
Unique public DNA variants reported	4069
Individuals with public variants	8722
Hidden variants	456

Genetic testing: number of mutation reported for Tuberous Sclerosis in LOVD (Leiden Open Variation Database)





No hotspot for the two genes:
<u>Substitutions</u> represents nonsense, splice site mutations and misssence mutations in *TSC2* gene

Result - detection yield

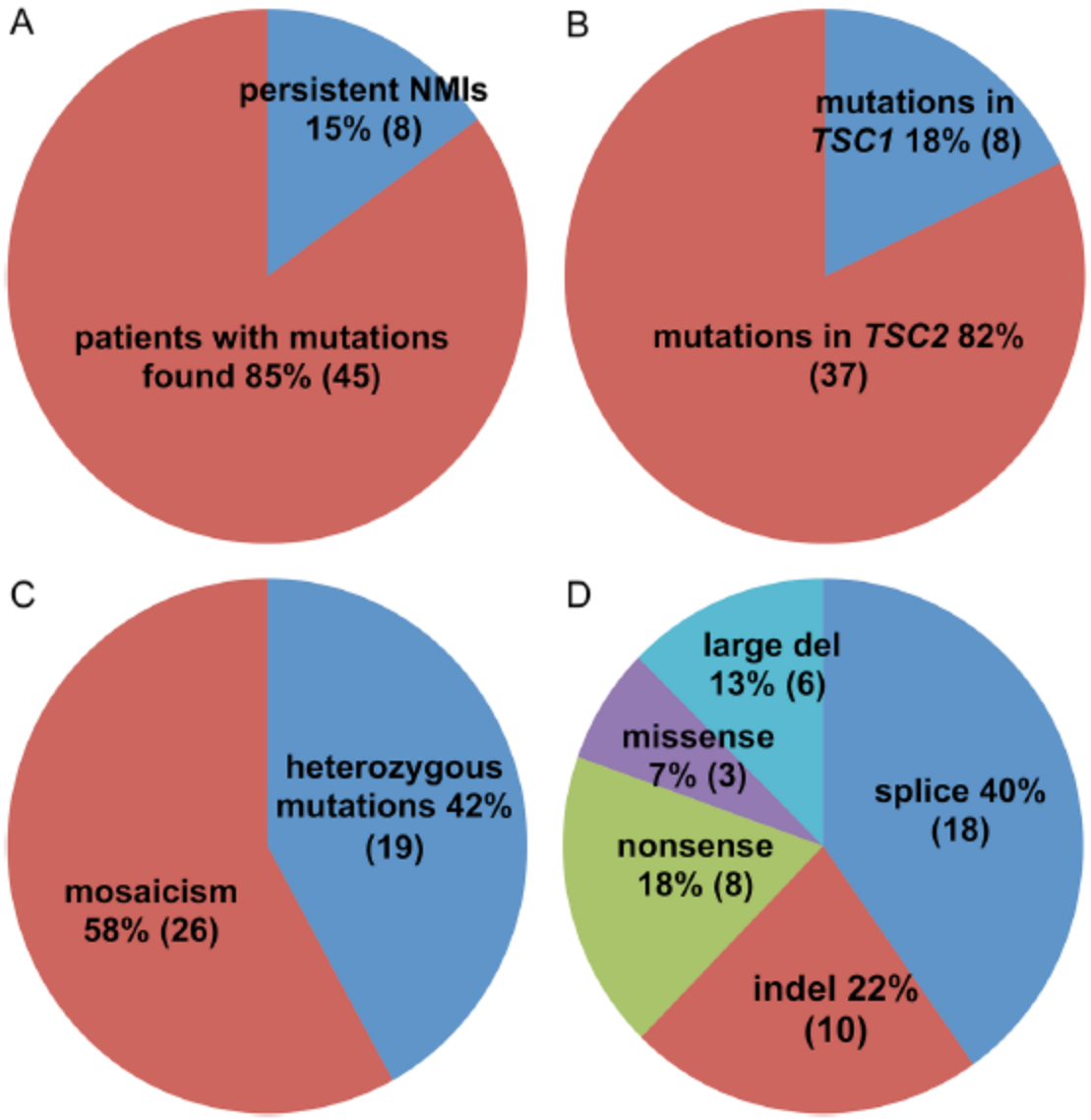
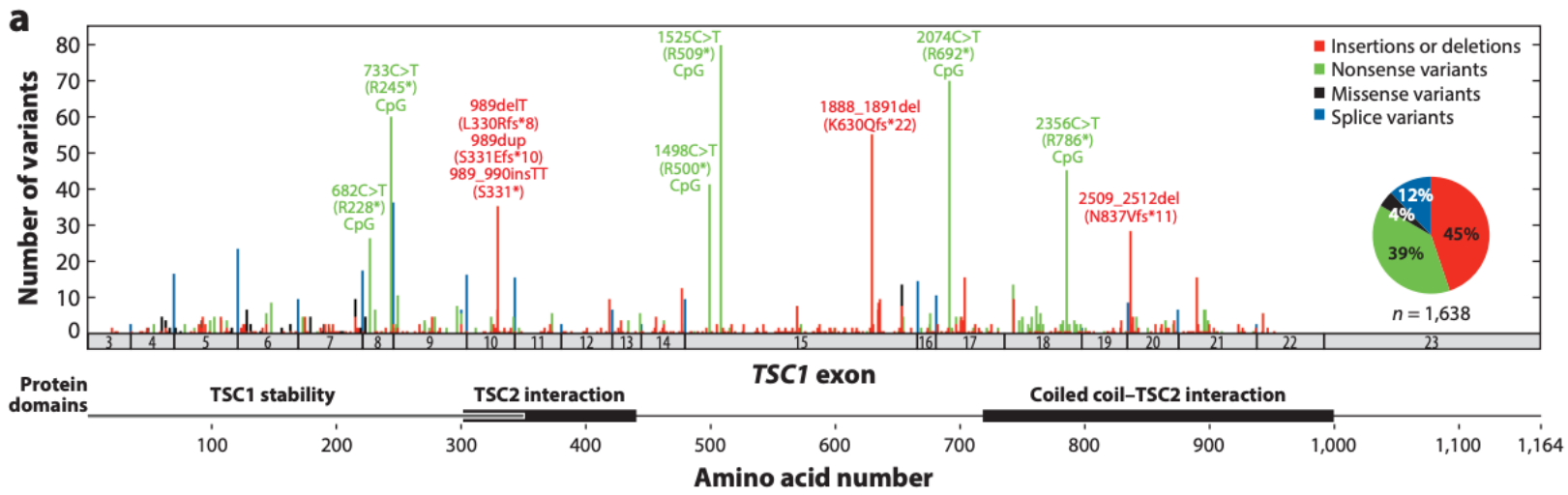
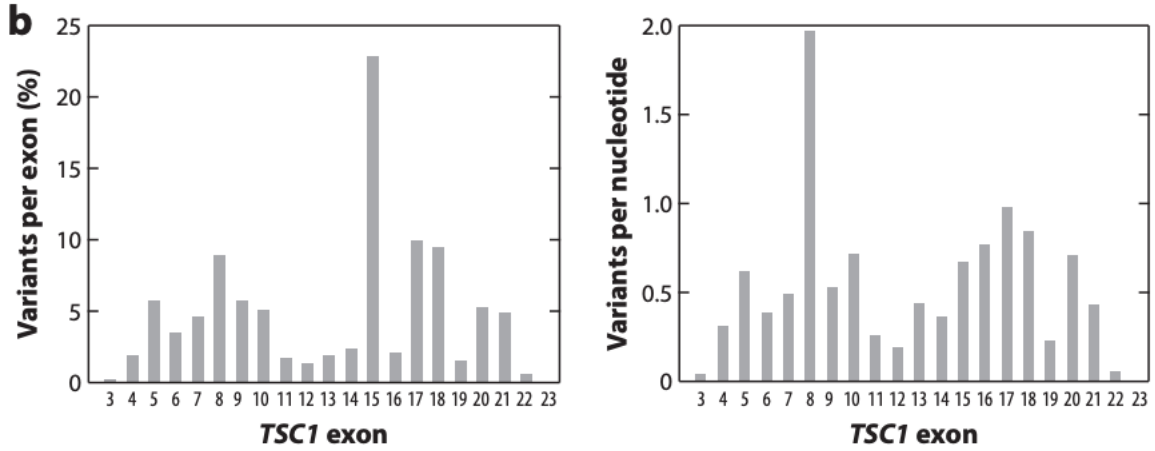


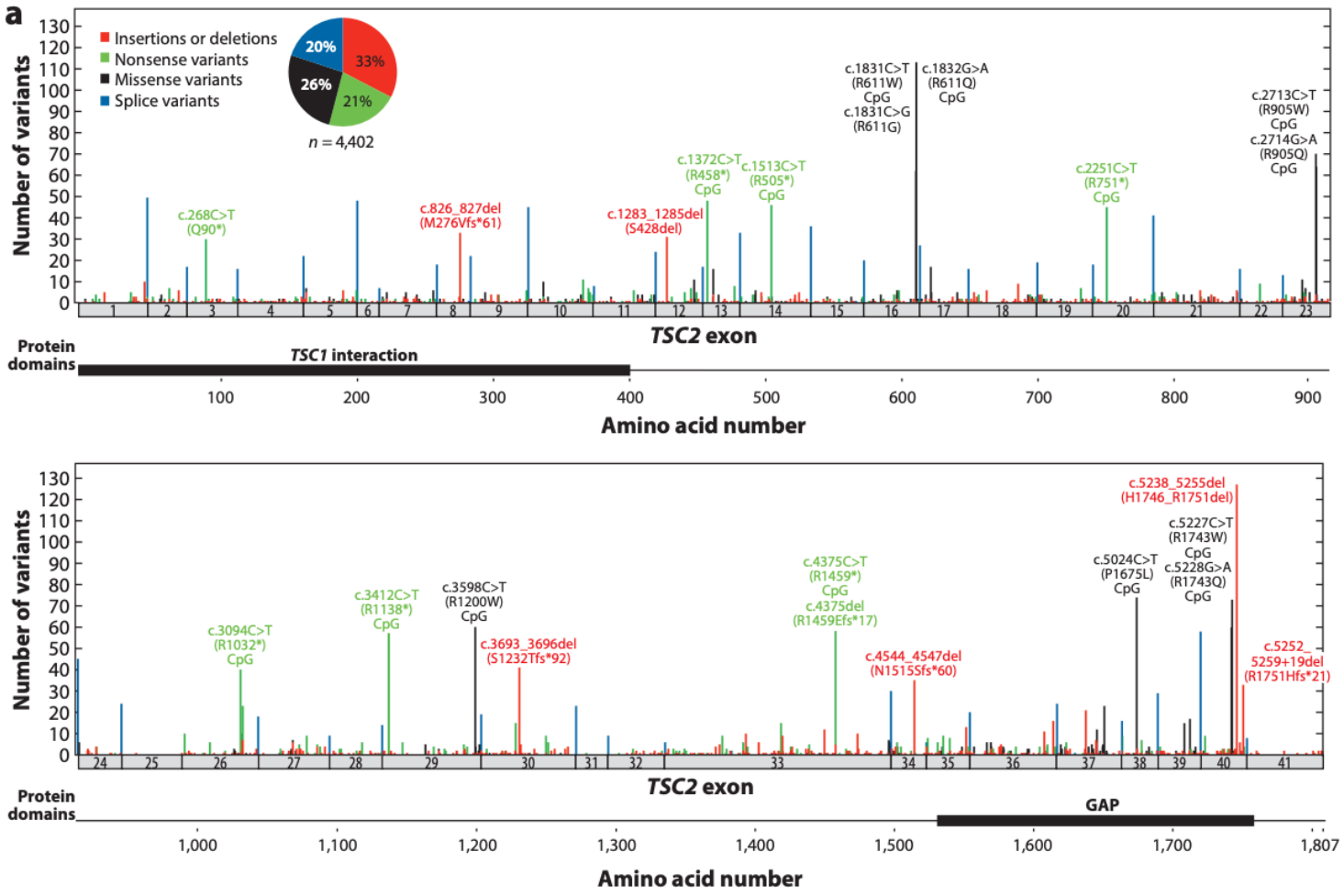
Fig 1. Pie charts displaying the mutation types and frequencies in 53 TSC NMI subjects. (A) Proportion of subjects with mutations identified vs. remaining as persistent NMI. (B) Proportion of mutations in *TSC1* vs. *TSC2*. (C) Proportion of heterozygous vs. mosaic mutations. (D) Different types of identified mutations.

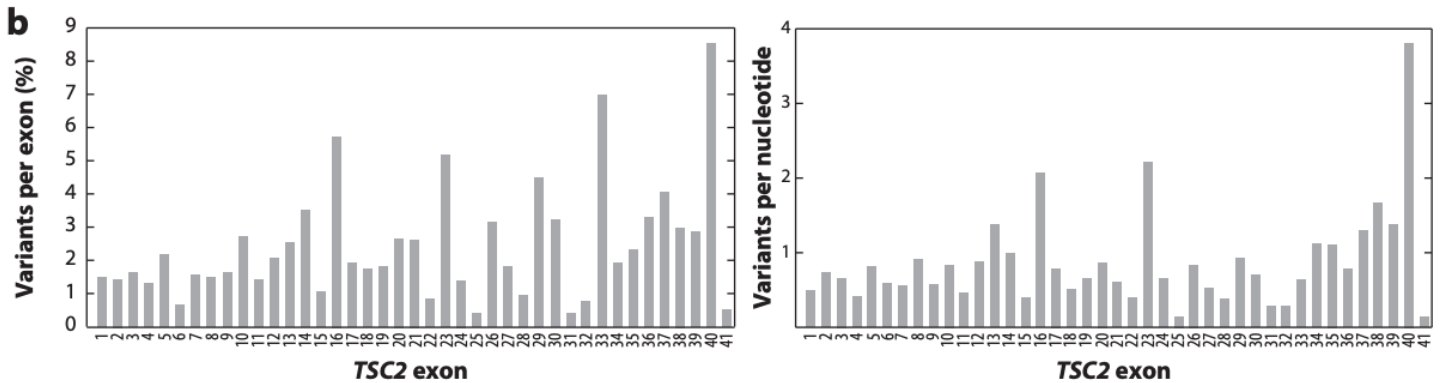
Database...


a
Number of variants
80
70
60
50
40
30
20
10
0
733C>T (R245*) CpG
682C>T (R228*) CpG
989delT (L330Rfs*8)
989dup (S331Efs*10)
989_990insTT (S331*)
1525C>T (R509*) CpG
1498C>T (R500*) CpG
1888_1891del (K630Qfs*22)
2074C>T (R692*) CpG
2356C>T (R786*) CpG
2509_2512del (N837Vfs*11)
Insertions or deletions
Nonsense variants
Missense variants
Splice variants
12%
4%
39%
45%
n = 1,638
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
TSC1 exon
Protein domains
TSC1 stability
TSC2 interaction
Coiled coil–TSC2 interaction
100 200 300 400 500 600 700 800 900 1,000 1,100 1,164
Amino acid number


b
Variants per exon (%)
25
20
15
10
5
0
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
TSC1 exon
Variants per nucleotide
2.0
1.5
1.0
0.5
0
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
TSC1 exon

Database...


a
Insertions or deletions
Nonsense variants
Missense variants
Splice variants
20%
33%
26%
21%
n = 4,402
Number of variants
c.268C>T (Q90*)
c.826_827del (M276Vfs*61)
c.1283_1285del (S428del)
c.1372C>T (R458*) CpG
c.1513C>T (R505*) CpG
c.1831C>T (R611W) CpG
c.1831C>G (R611G)
c.1832G>A (R611Q) CpG
c.2251C>T (R751*) CpG
c.2713C>T (R905W) CpG
c.2714G>A (R905Q) CpG
TSC2 exon
Protein domains
TSC1 interaction
Amino acid number
c.3094C>T (R1032*) CpG
c.3412C>T (R1138*) CpG
c.3598C>T (R1200W) CpG
c.3693_3696del (S1232Tfs*92)
c.4375C>T (R1459*) CpG
c.4375del (R1459Efs*17)
c.4544_4547del (N1515Sfs*60)
c.5024C>T (P1675L) CpG
c.5227C>T (R1743W) CpG
c.5228G>A (R1743Q) CpG
c.5238_5255del (H1746_R1751del)
c.5252_5259+19del (R1751Hfs*21)
GAP


b
Variants per exon (%)
TSC2 exon
Variants per nucleotide
TSC2 exon

Genotype – Phenotype Correlation ?

First large cohort studies

Jones et al. (1997) 9/24 familial and only 13/147: sporadic

Kwiatkowska et al. (1998) 13 families link to *TSC1* gene – 126 sporadic

22 'limited' families without haplotype linkage to *TSC1 gene*

Jones et al. (1999) 150 unrelated patients

120/150 (80%) mutation : 22 in *TSC1* gene - 98 in *TSC2 gene*

Ali et al. (1998) 83 unrelated - link to *TSC1* gene: mutations 16/ 83 - 19% 'ins or small del'

Genotype – Phenotype Correlation ?

First large cohort studies

Mayer et al. (1999) all identified mutations (TSC1 ; TSC2) responsible for truncated protein (either hamartine or tuberin) PTT

Niida et al. (1999) 126 patients unrelated

(40 familial and 86 sporadic): mutation detection rate: 59%

Cheadle et al. (2000) 154 patients identified carrier of a mutations in *TSC1* gene

292 in *TSC2* gene

47% TSC1: single-base substitutions - 82% ‘nonsense’ mutations

Genotype – Phenotype Correlation ?

First large cohort studies

Dabora et al. (2001) 224 index patients :

mutations in 186 (83%): 138 small *TSC2* gene mutations

20 large *TSC2* gene mutations and 28 small *TSC1* gene mutations

Both germline and somatic mutations appear to be less common in TSC1 gene (vs TSC2)

Au et al. (2007) 325 individuals

72% (199/257) *'de novo'* and 77% (53/68) familial

17% of mutations in the *TSC1* gene

50% in the *TSC2* gene

Patients with *TSC2* gene mutations: more frequent: ***hypomelanosis - learning disability***

Correlation...PHENOTYPE AND GENOTYPE IN TUBEROUS SCLEROSIS COMPLEX

- *TSC2* gene mutations are more frequently identified in prenatal setting when Rhabdomyoma is noted on ultrasound 54% vs 20% for *TSC1* gene

Genotype - phenotype

At the psychiatric level, ASD was observed at significantly higher frequency in participants with *TSC2* gene than those with *TSC1 gene* mutations (28.6% vs 12.2%, $P < 0.001$)

ADHD, anxiety disorder and depressive disorder were not significantly different between the two genotypes, but it was interesting to observe that all three showed higher absolute frequencies in association with *TSC1* rather than *TSC2* (ADHD
TSC1 = 17.6%; TSC2 = 16%, $P = 0.6881$)

More individuals with *TSC2* gene mutation had neuropsychological performance scores falling below the 5th percentile compared to those with *TSC1* gene mutation (63% vs 38.8%, $P = 0.0024$)

Correlation…PHENOTYPE AND GENOTYPE IN TUBEROUS SCLEROSIS COMPLEX

- Large phenotypic diversity - high variability in the clinical manifestations among patients with same pathogenic variant

Grossly : « pathogenic variants in *TSC2* gene responsible for a more severe phenotype (than variants in *TSC1* gene) »

- Neurologic manifestations
  - *TSC2* gene variants are associated with
    - earlier onset of seizures
    - seizures are often more refractory and harder to treat (than those of patients with TSC1 variants)
    - a higher percentage with infantile spasms then with *TSC1* gene
    - one study has shown that missense variants in exons 23-33 decrease the risk for development of infantile spasms (to be confirmed)
    - a higher rate of ASD as well as intellectual disability

Correlation...PHENOTYPE AND GENOTYPE IN TUBEROUS SCLEROSIS COMPLEX

The differences in phenotype between *TSC1 and TSC2* genes variants seem to result from two main effects

- First as germline pathogenic variants are less common in *TSC1* than *TSC2* gene, the frequencies of second-hit events - that are crucial for the development of tumors and cortical tubers in TSC - are less common in TSC1 than TSC2. Indeed, tumor in multiple organs and cortical tuber counts are lower in TSC1 than they are in TSC2 disease

- « Second it appears that loss of a single allele of TSC1 has less effect on the functional activity of the TSC protein complex in the cell than does loss of an allele of TSC2 ». Although there is a difference in phenotype in population cohorts, it is not a major distinction, such that **one cannot guarantee that a TSC1 phenotype will be milder than that of TSC2**. Hence, *consensus guidelines for surveillance of developmental issues and tumor development in TSC do not depend on the gene or variant identified or level of mosaicism*

To keep in mind !

LARGE DELETIONS IN TSC1 or TSC2 gene

- **will not be picked up by sequencing**
- **MLPA/Shallow sequencing**

- accounting for

2.8% of all *TSC1* gene disease causing variants
6.4% of all *TSC2* gene disease-causing variants

…**often extend into adjacent genes**

deletions at the 3 end of *TSC2* gene often extend to the closely adjacent *PKD1* gene (**contiguous gene syndrome) :** cause a unique clinical phenotype of accelerated polycystic kidney disease: Such individuals may have multiple renal cysts identified at birth (early onset and 'severe') - tend to progress to renal failure by the teenage years

Lead to …precise genetic counselling

- autosomal dominant trait – if parent carrier of a mutation
  - transmission risk 50%

80% **yield for the detection of a mutation inside one of the 2 genes**

Mutation/deletion: inactivation of TSC1 or TSC2

15% - 20% **somatic/ mosaïc**
genetic heterogeneity?
other ?

UNDETECTABLE MUTATION ALL AROUND THE STUDIES

Molecular genetic testing of *TSC1* and *TSC2* by sequence and deletion/duplication analysis identifies a causal mutation in approximately 85% of individuals with a **definite** diagnosis of TSC.

Approximately **15%** of persons with TSC have no mutation identified – NMI

Limited genetic counselling

no access to reproductive choices – PGD

no access to Prenatal Diagnosis

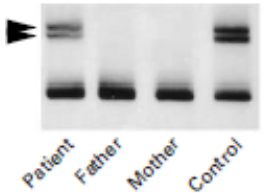
Hypothesis for ‘inability to detect molecular mechanism’

MOSAICISM IN TUBEROUS SCLEROSIS AS A POTENTIAL CAUSE OF THE FAILURE OF MOLECULAR DIAGNOSIS

JOLANTA KWIATKOWSKA, PH.D., JADWIGA WIGOWSKA-SOWINSKA, M.D., DOBRAWA NAPIERALA, M.S., RYSZARD SLOMSKI, PH.D., AND DAVID J. KWIATKOWSKI, M.D., PH.D.

New Engl J Med 1**999**

‘’ We describe a patient with severe tuberous sclerosis in whom a mutated *TSC1* allele was present in only one third of leukocytes and in different proportion in other tissues ’’



Three novel types of splicing aberrations in the tuberous sclerosis TSC2 gene caused by mutations apart from splice consensus sequences
Karin et al Biochimica et Biophysica Acta 1502 (2000) 495-507

Splice site mutations
IVS38-18A-->G
IVS8+281C-->T
IVS9-15G-->A

Mosaïc...

BRIEF COMMUNICATION | Genetics inMedicine

Volume 21 | Number 11 | 2019

Cliniques universitaires SAINT-LUC UCLouvain BRUXELLES

Low-level mosaicism in tuberous sclerosis complex: prevalence, clinical features, and risk of disease transmission

Krinio Giannikou, PhD[1], Kathryn D. Lasseter, BA[1], Joannes M. Grevelink, MD, PhD[2], Magdalena E. Tyburczy, PhD[1], Kira A. Dies, ScM, CGC[3], Zachary Zhu[1], Lana Hamieh, MD[1], Bruce M. Wollison, MSc[4], Aaron R. Thorner, PhD[4], Stephen J. Ruoss, MD[5], Elizabeth A. Thiele, MD, PhD[6], Mustafa Sahin, MD, PhD[3] and David J. Kwiatkowski, MD, PhD[1]

Interestingly, TSC patients from our cohort with the lowest MVAF in normal tissues (<0.63%, lowest) had fewer TSC clinical features in comparison with patients with higher MVAF (0.67–7.2%)

We found that **there is a deficit in the observed number of TSC individuals at very low MVAF,** in comparison with the number predicted by genome-wide studies and theoretical modeling (Fig. 2b, c). **There is a substantial number of individuals in the population with low-level mosaicism for a pathogenic variant in TSC2 who are not recognized clinically due to minimal or no TSC clinical manifestations**

Unrecognized low-level mosaic individuals may contribute to the known recurrence risk for parents of a sporadic TSC child, usually estimated as 1–2%

Mosaïc

Genetics inMedicine | ARTICLE

Phenotypic distinctions between mosaic forms of tuberous sclerosis complex

Alison M. Treichel, BS[1,2], Lana Hamieh, MD[3], Neera R. Nathan, MD, MSHS[1,2], Magdalena E. Tyburczy, PhD[3], Ji-an Wang, AS[1], Oyetewa Oyerinde, MD[1,2], Sorana Raiciulescu, MSc[4], Patricia Julien-Williams, NP[2], Amanda M. Jones, NP[2], Vissaagan Gopalakrishnan, BS[2], Joel Moss, MD, PhD[2], David J. Kwiatkowski, MD, PhD[3] and Thomas N. Darling, MD, PhD[1]

Volume 21 | Number 11 | November 2019

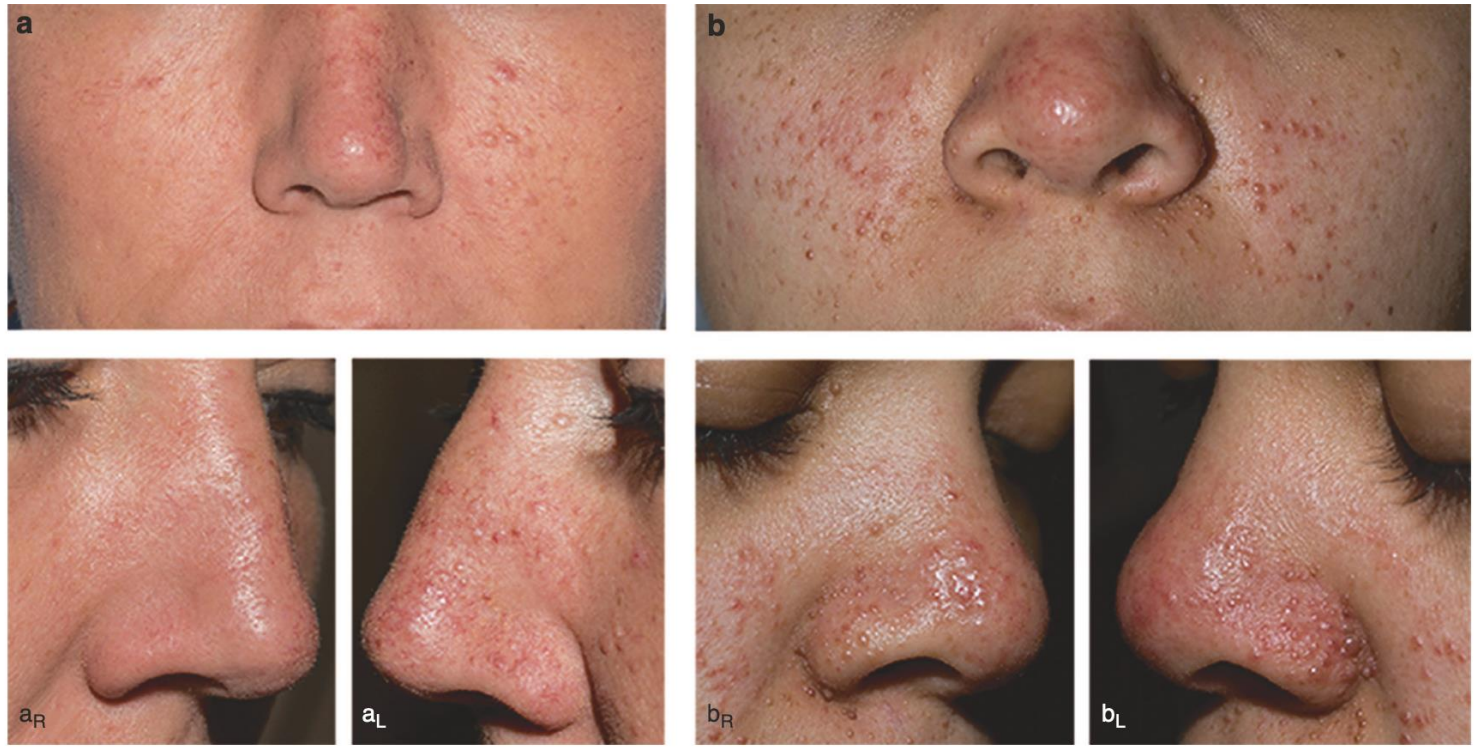


Fig. 1 The clinical picture of mosaicism in tuberous sclerosis complex. a Mosaicism with an asymmetric distribution of angiofibromas (AFs) on the nose and cheeks. $\mathbf{a_R}$,$\mathbf{a_L}$ Right and left lateral views of the nose highlight the left-sided predominance of AFs. **b** Mosaicism with numerous AFs distributed symmetrically on the nose and cheeks, indistinguishable from a patient with germline tuberous sclerosis complex (TSC). $\mathbf{b_R}$,$\mathbf{b_L}$ Right and left lateral views of the nose reveal more numerous and symmetric distribution of AFs.

Mosaïc…

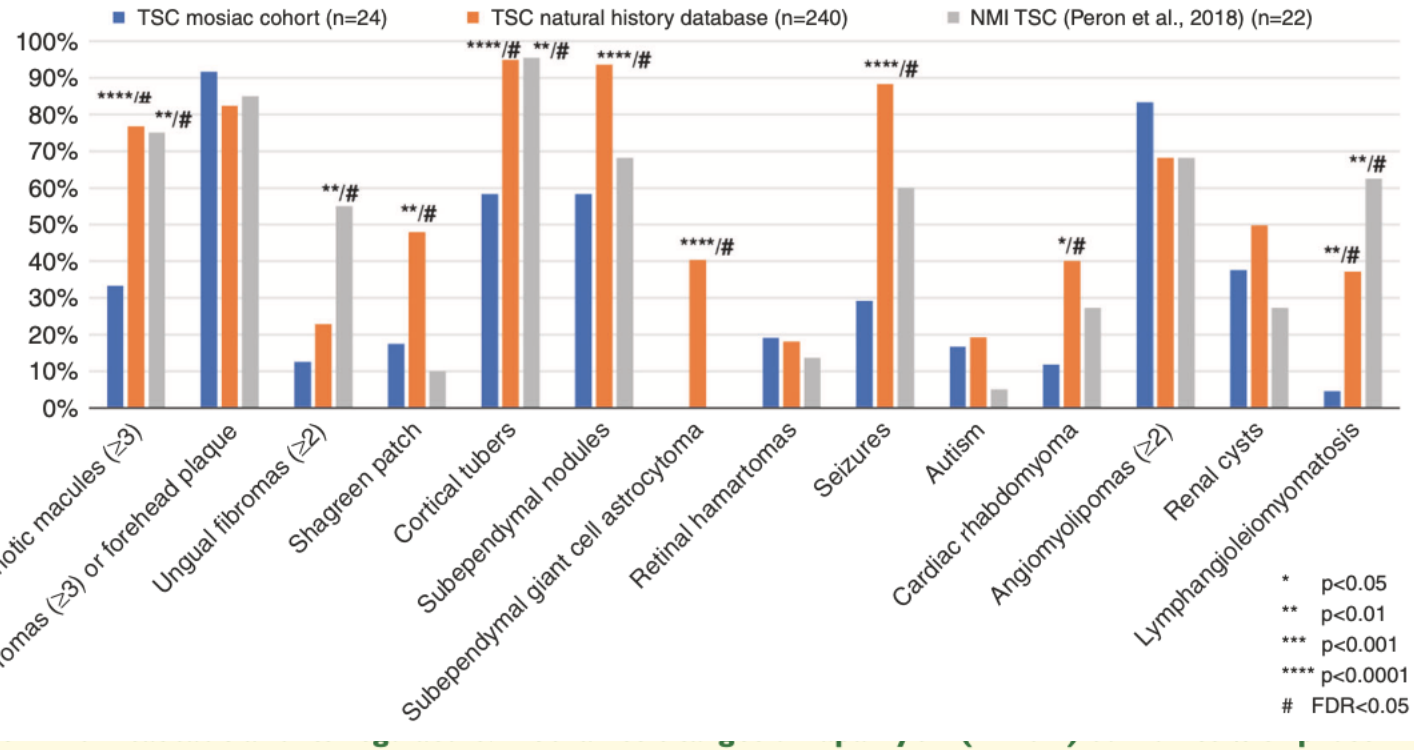


Fig … binding partners to form mTOR complex 1 (mTORC1). The downstream substrates of mTORC1 signalling include ribosomal S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein-1 (4E-BP1). Rapamycin inhibits mTORC1 signalling by binding with FK506 binding protein 1 A 12 kDa (FKBP12). Three pathways converge to regulate mTORC1 signalling. Hamartin (TSC1), tuberin (TSC2) and TBC1 domain family member 7 (TBC1D7) form a protein complex that indirectly inhibits mTORC1 signalling via Ras homologue enriched in brain (Rheb). (**A**) Growth factors stimulate phosphoinositide 3-kinase (PI3K) to trigger phosphoinositide-dependent kinase 1 (PDK1) to phosphorylate and activate Akt. TSC2 is repressed by Akt activation, which has a disinhibitory effect on mTORC1 signalling. Phosphatase and tensin homologue (PTEN) is a negative regulator of the PI3K-Akt pathway. (**B**) The energy-sensing arm is regulated by the STE20-related kinase adaptor alpha (STRADα) and liver kinase B (LKB) complex. In response to depleted ATP, the STRADα/LKB complex inhibits mTORC1 signalling by activating TSC2 via phosphorylation of adenosine monophosphate-activated kinase (AMPK). (**C**) The amino acid-sensing pathway is regulated by GTPase-activating protein (GAP) activity towards Rags 1 complex (GATOR1). GATOR1 is composed of three subunits: Dishevelled, Egl-10, and Pleckstrin domain-containing protein 5 (DEPDC5); nitrogen permease regulator-like 2 and 3 (NPRL2, NPRL3). The GATOR2 complex inhibits GATOR1 in response to increasing amino acid levels, resulting in mTORC1 disinhibition, facilitating pathways for cell growth. When amino acid levels are low GATOR1 directly inhibits mTORC1 activity. The KICSTOR (KPTN, ITFG2, C12orf66 and SZT2-containing regulator of mTORC1) complex scaffolds GATOR1 to the lysosomal surface (adapted from Peter B. Crino's review[7] with permission from Springer Nature).

Functional study

Hum Mut 2013;34(1):167–175

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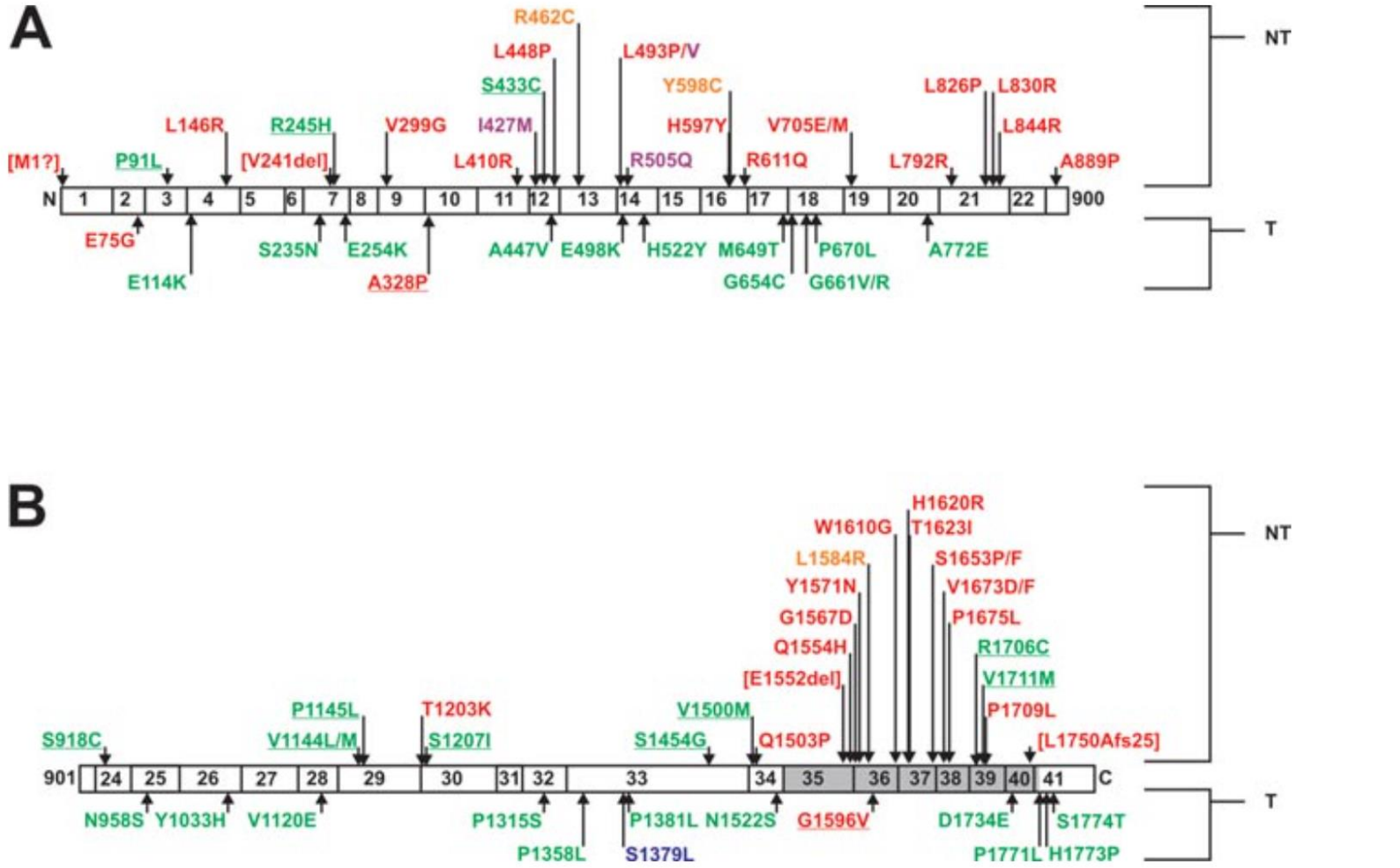
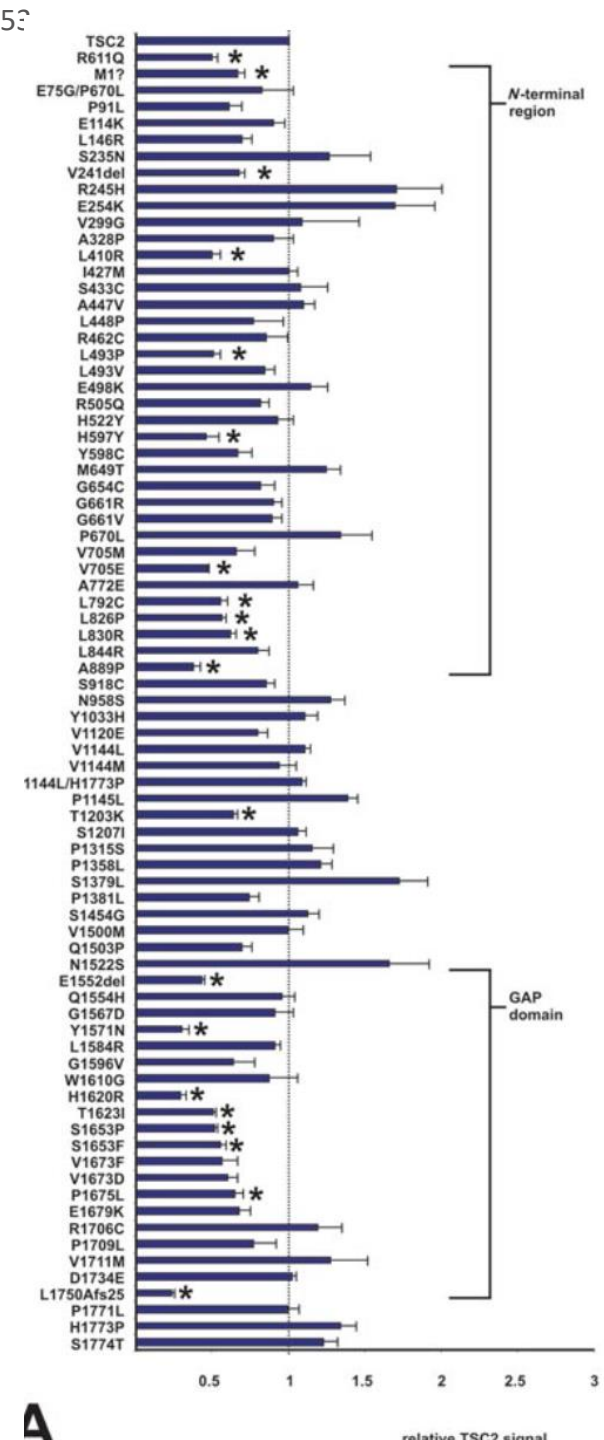
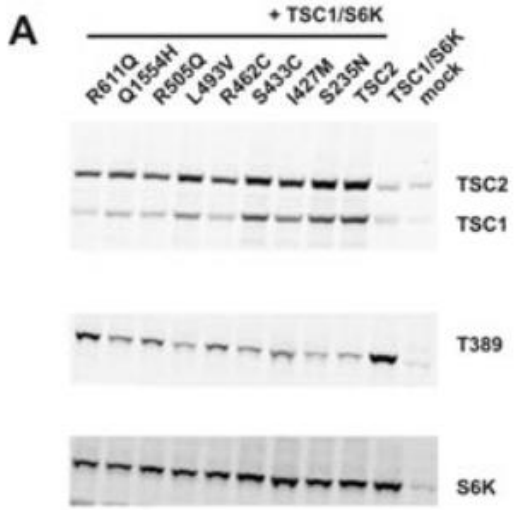


Figure 3. Overview of the functional assessment of the TSC2 variants. The positions of the TSC2 variants analyzed as part of this study are indicated relative to the coding exons of the *TSC2* gene and are numbered according to the *TSC2* LOVD (http://www.lovd.nl/TSC2). Amino acid substitutions listed above the exons were not tolerated (NT) by the SIFT algorithm; substitutions listed below the exons were tolerated (T) by SIFT; variants for which SIFT did not make a prediction are indicated with square brakets above the exons. Variants classified as pathogenic are indicated in red; variants classified as probably pathogenic are indicated in orange; variants classified as possibly pathogenic are indicated in purple; variants classified as unlikely to be pathogenic are indicated in blue; variants classified as probably neutral are indicated in green. Variants for which there was strong disagreeement between our assay and the SIFT prediction are underlined. **A**: TSC2 variants mapping to *TSC2* exons 1–23 (amino acids 1–900). **B**: TSC2 variants mapping to *TSC2* exons 23–41 (amino acids 901–1807). The location of the TSC2 GAP domain is indicated by gray shading.

Functional study





Hum Mut 2013;34(1):167–175

Functional study

RESEARCH ARTICLE

Human Mutation 2013;34(1):167–175

OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

Functional Assessment of *TSC2* Variants Identified in Individuals with Tuberous Sclerosis Complex

Marianne Hoogeveen-Westerveld,[1] Rosemary Ekong,[2] Sue Povey,[2] Karin Mayer,[3] Nathalie Lannoy,[4] Frances Elmslie,[5] Martina Bebin,[6] Kira Dies,[7] Catherine Thompson,[8] Steven P. Sparagana,[8,9] Peter Davies,[10] Ans van den Ouweland,[1] Dicky Halley,[1] and Mark Nellist[1]*

Table 1. Classification of TSC2 Variants

Group	SIFT	TSC2 signal	TSC1 signal	T389/S6K ratio	TSC2 variants
1	NT	Reduced	Reduced	Increased	V241del[4.], L410R, L493P, H597Y, V705E, L792R, L826P, L830R, A889P, T1203K, E1552del[4.], H1620R, S1653P, S1653F
2	NT	–	Reduced	Increased	L146R, V299G, L448P, R462C[1.], Y598C[1.], V705M, L844R
3	NT	Reduced	–	Increased	M1?[4.], Y1571N, T1623I, P1675L, L1750Afs25[4.]
4	NT	–	–	Increased	I427M[2.], L493V[2.], R505Q[2.], Q1503P, Q1554H[1.], G1567D, L1584R[1.], W1610G, V1673F, V1673D, P1709L
5	T	–	–	Increased	E75G/P670L, G1596V, A328P
6	NT	–	–	–	P91L, R245H, S433C, S918C, V1144L, V1144M, V1144L/H1773P, P1145L, S1207I, S1454G, V1500M, R1706C, V1711M
7	T	–	–	–	E114K, S235N, E254K, A447V, E498K, H522Y, M649T, G654C, G661R, G661V, P670L, A772E, N958S, Y1033H, V1120E, P1315S, P1358L, S1379L[3.], P1381L, N1522S, E1679K, D1734E, P1771L, H1773P, S1774T

‘mTOR opathies’ - *related conditions*

The term ***mTOR pathway related malformations*** **-** has been introduced to define a **spectrum of MCDs characterised by altered cortical architecture, abnormal neuronal/glial morphology and intractable seizures as a consequence of a deregulation of the mTOR signalling** - **mTOR is a serine/threonine protein kinase -** hyperactivation of the mTOR signaling cascade

A. Muhlebner J Anat 2019;235:521--542

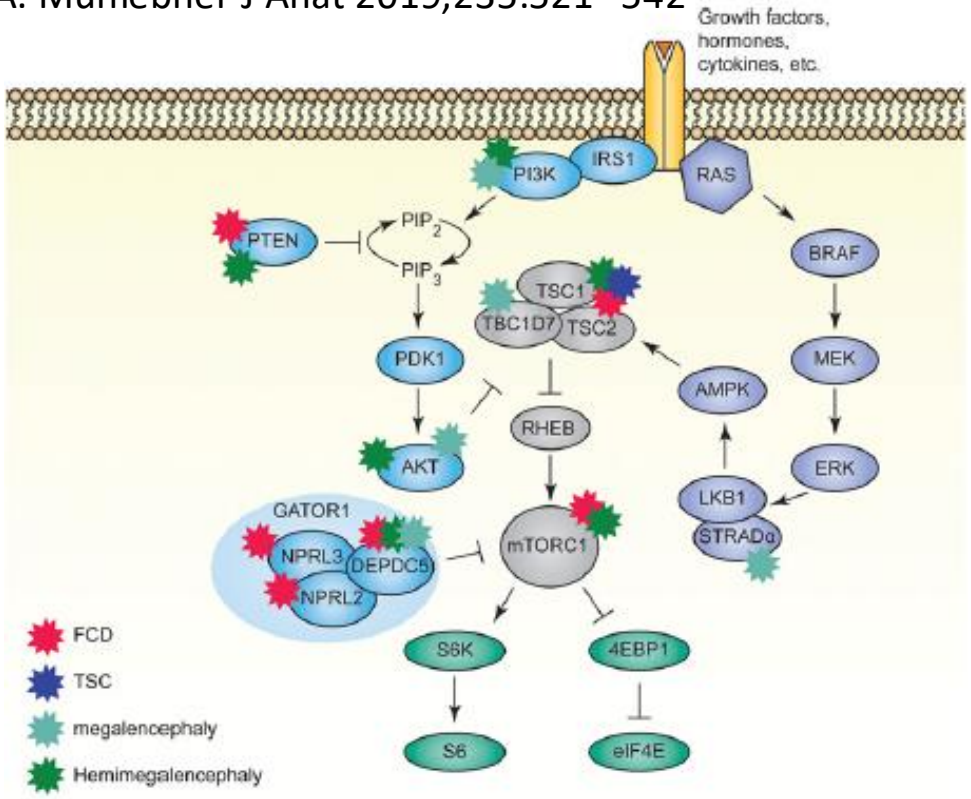


Fig. 1 A schematic overview of the mTOR pathway. A schematic overview of the mTORC1 signalling pathway showing the proteins that are affected by mutations of different mTORpathies (FCD, TSC, megalencephaly and hemimegencephaly) as summarised in Table 1. Mutations related to FCD are indicated with a red star, mutations related to TSC with a blue star, mutations related to megalencephaly with a light green star, and mutations related to hemimegencephaly with a dark green star. IRS1, insulin receptor substrate 1; PI3K, PI3kinase; PDK1, phosphoinositide-dependent kinase-1; PTEN, phosphatase and tensin homologue; AKT, protein kinase B; BRAF, v-raf murine sarcoma viral oncogene homolog B1; MEK, mitogen activated protein kinase; ERK, extracellular signal-regulated kinase; LKB1, tumor suppressor liver kinase B1; STRADα, STE20-related kinase adaptor alpha; AMPK, AMP-activated protein kinase; TBC1D5, TBC1 Domain Family Member 5; RHEB, ras homolog enriched in brain; mTORC1, mammalian target of rapamycin complex 1; DEPDC5, DEP Domain Containing 5; NPRL2, NPR2 Like, GATOR1 Complex Subunit; NPRL3, NPR3 Like, GATOR1 Complex Subunit; GATOR1, Gap Activity TOward Rags 1; S6K1, p70S6kinase; S6, ribosomal S6 protein; 4EBP1, eIF4E-binding protein 1; eIF4E, binding of eukaryotic translation.

mTORopathies - mTOR pathway related conditions

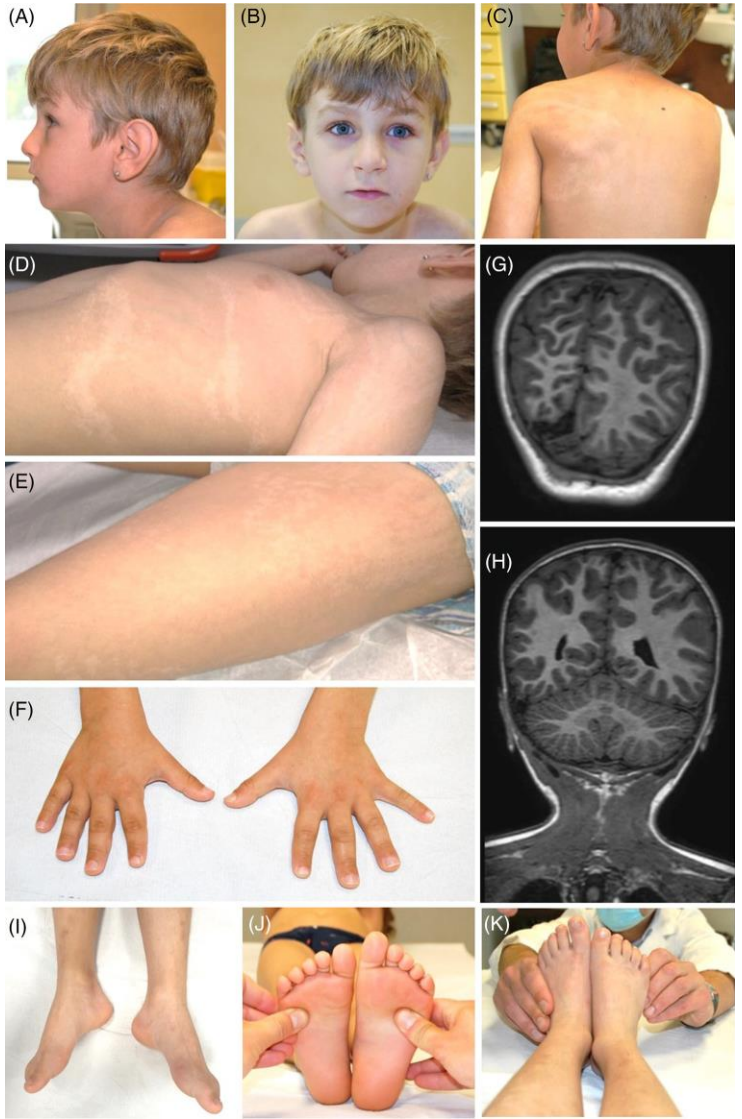
Smith Kingsmore syndrome SKS (OMIM #616638)
macrocephaly, (hemi-)megalencephaly, intellectual disability, seizures, facial dysmorphic features that include curly /wavy hair, frontal bossing, midface hypoplasia, small chin, and hypertelorism

TABLE 1 Features of SKS patients as described in medical literature and in our case report

Features	Our patient	Germline (*n* = 29)	Disseminated mosaicism[a] (*n* = 5)	Total (*n* = 35)
Macrocephaly/megalencephaly	–	26 (90%)	5 (100%)	31 (89%)
Developmental delay/intellectual disability	+	26 (90%)	2 (40%)	29 (83%)
Seizures	+	19 (65.5%)	3 (60%)	23 (66%)
Ventriculomegaly/hydrocephalus	–	12 (41%)	5 (100%)	17 (50%)
Dysmorphic facial features	–	13 (45%)	1 (20%)	14 (40%)
Macrosomia at birth/large for gestational age	–	11 (38%)	1 (20%)	12 (34%)
Curly or wavy hair	–	10 (34%)	0 (0%)	10 (29%)
Hypomelanosis/patchy hypopigmentation of skin	+	3 (10%)	5 (100%)	9 (26%)
Autism	–	8 (28%)	0 (0%)	8 (23%)
Abnormal corpus callosum	–	5 (17%)	2 (40%)	7 (20%)
FCD/polymicrogyria	–	2 (7%)	4 (80%)	6 (17%)
Diastasis recti/umbilical hernias	–	5 (17%)	0 (0%)	5 (14%)
Capillary malformation of the skin/hemangiomas	–	3 (10%)	0 (0%)	3 (9%)
Hemimegalencephaly	+	2 (7%)	0 (0%)	3 (9%)
Neonatal hypoglycemia	–	1 (3%)	1 (20%)	2 (6%)
Lateralized overgrowth	+	0 (0%)	0 (0%)	1 (3%)

Abbreviation: FCD, focal cortical dysplasia.

germinal MTOR variants, mosaicism confined to the brain usually results in isolated focal cortical dysplasia (FCD) without additional SKS features



mTORopathies - mTOR pathway related malformation

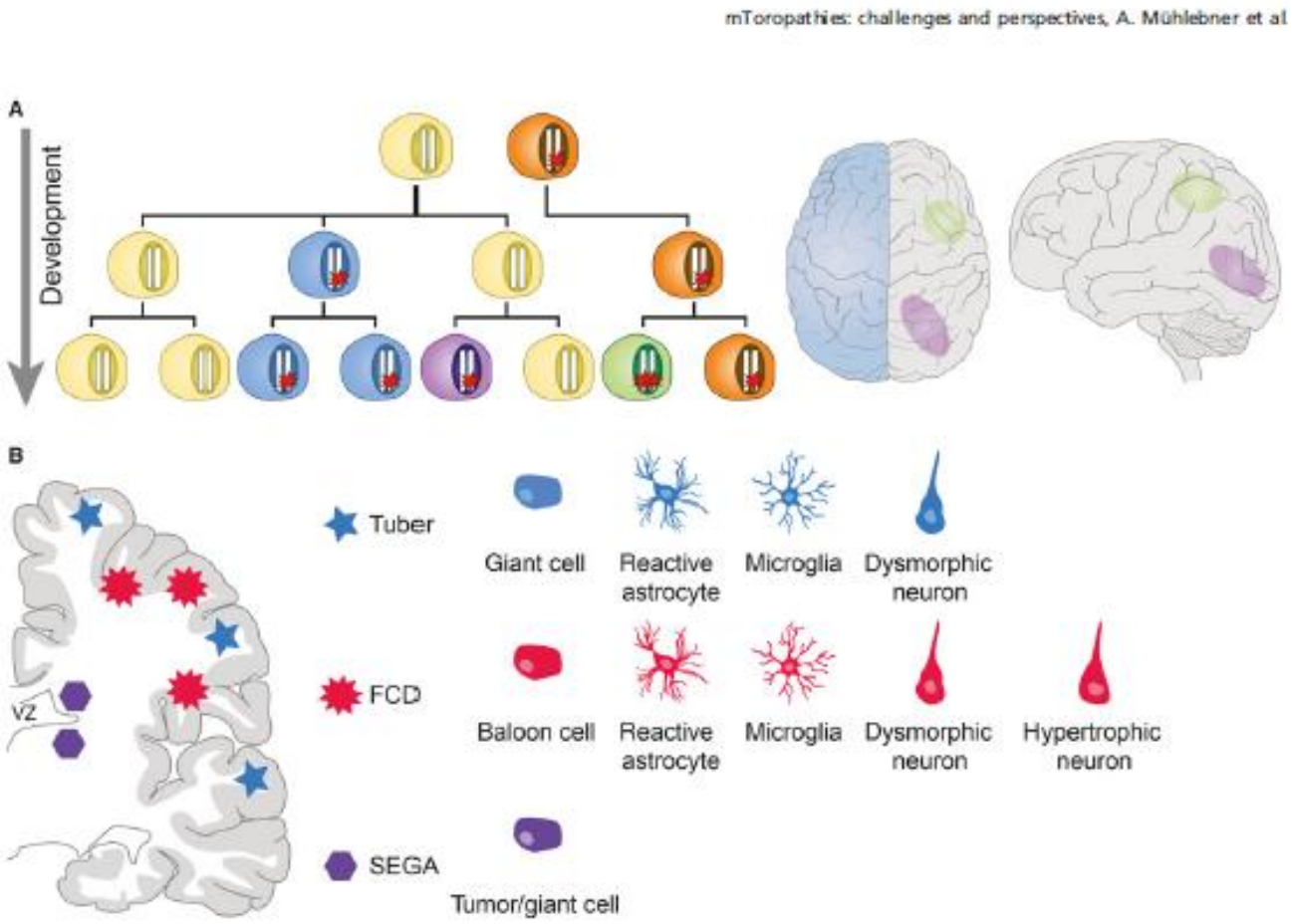
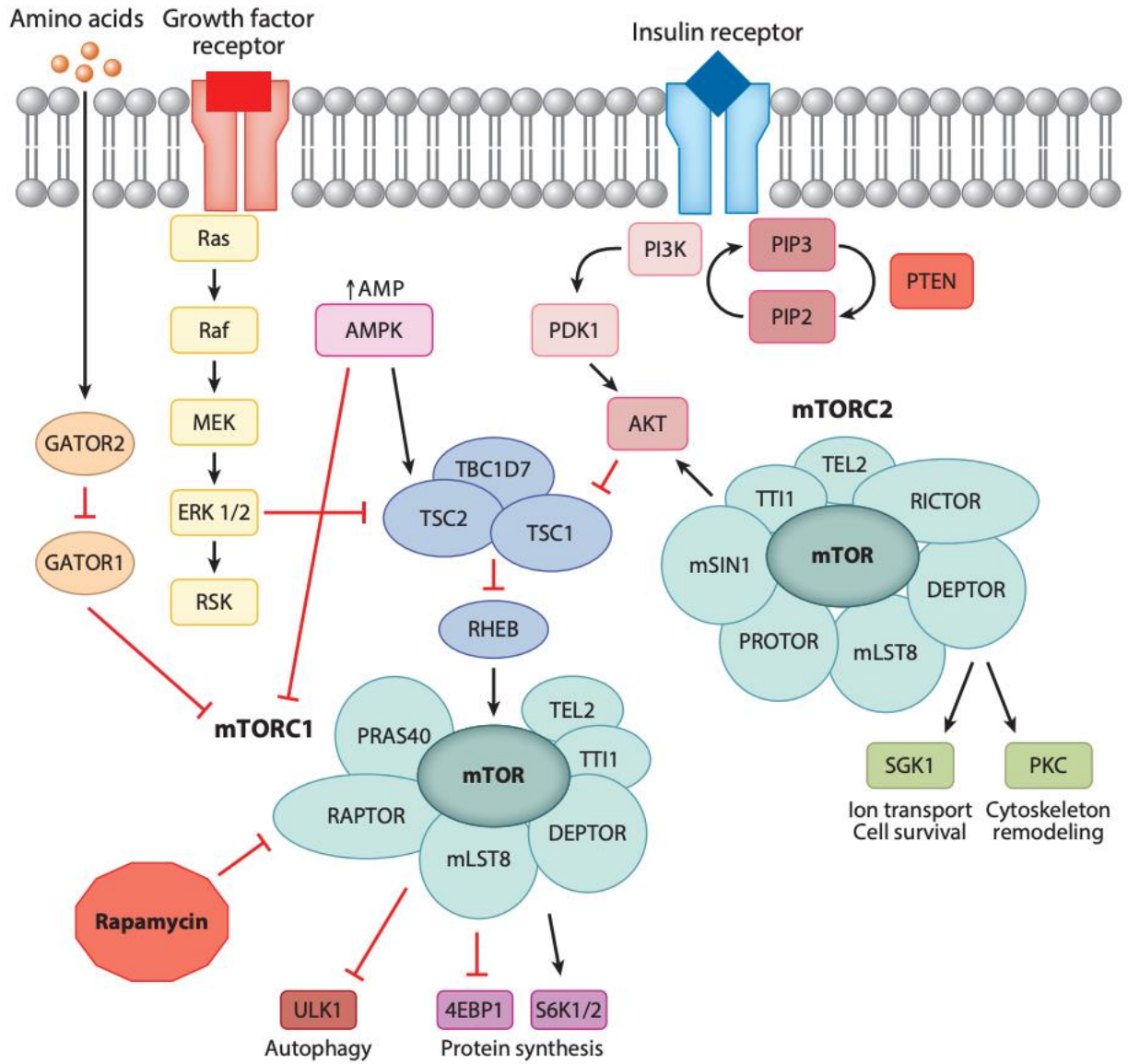


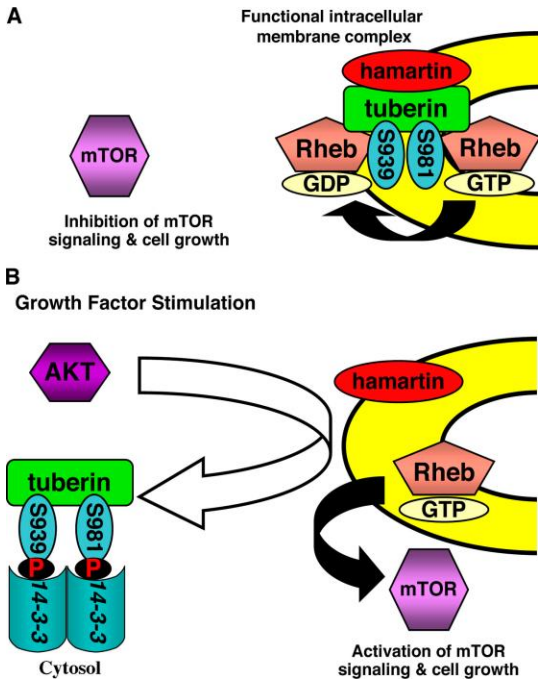
Fig. 2 Lesion differences in mTORopathies. (A) A schematic overview showing the concept of mosaicism in the brain where somatic mutations can occur during development with or without an underlying germline mutation. Somatic mutations that appear during early development (in blue) can lead to hemimegalencephaly (in blue). Somatic mutations that occur later in development (in purple) or second-hit mutations (in green) that occur when a germline mutation is present (in orange) can lead to focal lesions (indicated in purple and green). Both time and location of the somatic mutations can influence the affected region and size of the brain. (B) A schematic overview showing both the location and the histological characteristics of TSC tubers, FCD and SEGAs.

And from cellular pathway…to treatment



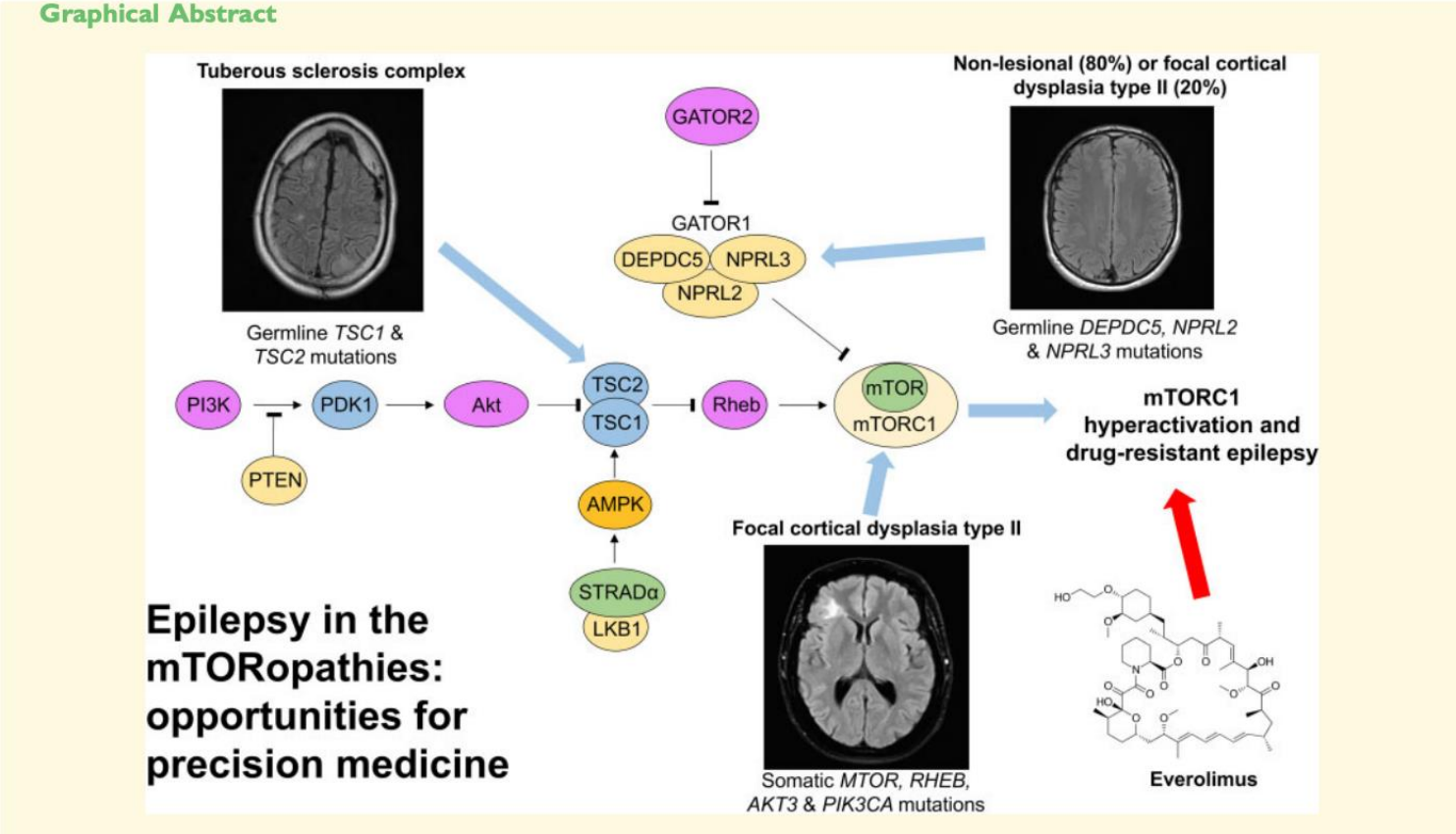
Functions of the TSC1/TSC2 gene products

Negative regulators of the mTOR signaling pathway



Model for regulation of tuberin under conditions of mitogenic sufficiency.
(A) Tuberin normally functions as a GAP for Rheb within an intracellular membrane compartment, inhibiting Rheb and mTOR signaling, which suppresses cell growth.
(B) Upon stimulation by growth factors, AKT is activated, leading to phosphorylation and cytosolic sequestration of tuberin by 14-3-3 proteins. This cytosolic translocation relieves tuberin repression of the Rheb-mTOR signaling, stimulating cell growth.

From pathway to treatment...



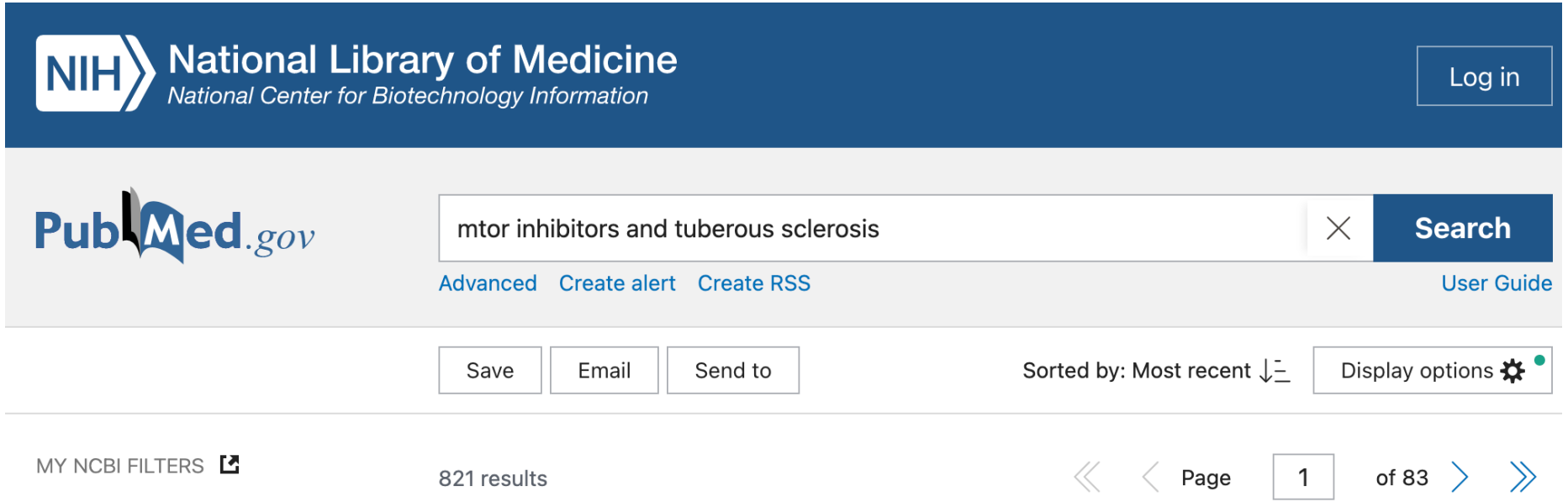
Perfect match: mTOR inhibitors and tuberous sclerosis complex.

Luo C, Ye WR, Shi W, Yin P, Chen C, He YB, Chen MF, Zu XB, Cai Y.

Orphanet J Rare Dis. 2022 Mar 4;17(1):106. doi: 10.1186/s13023-022-02266-0.

PMID: 35246210 Review.

From gene to treatment

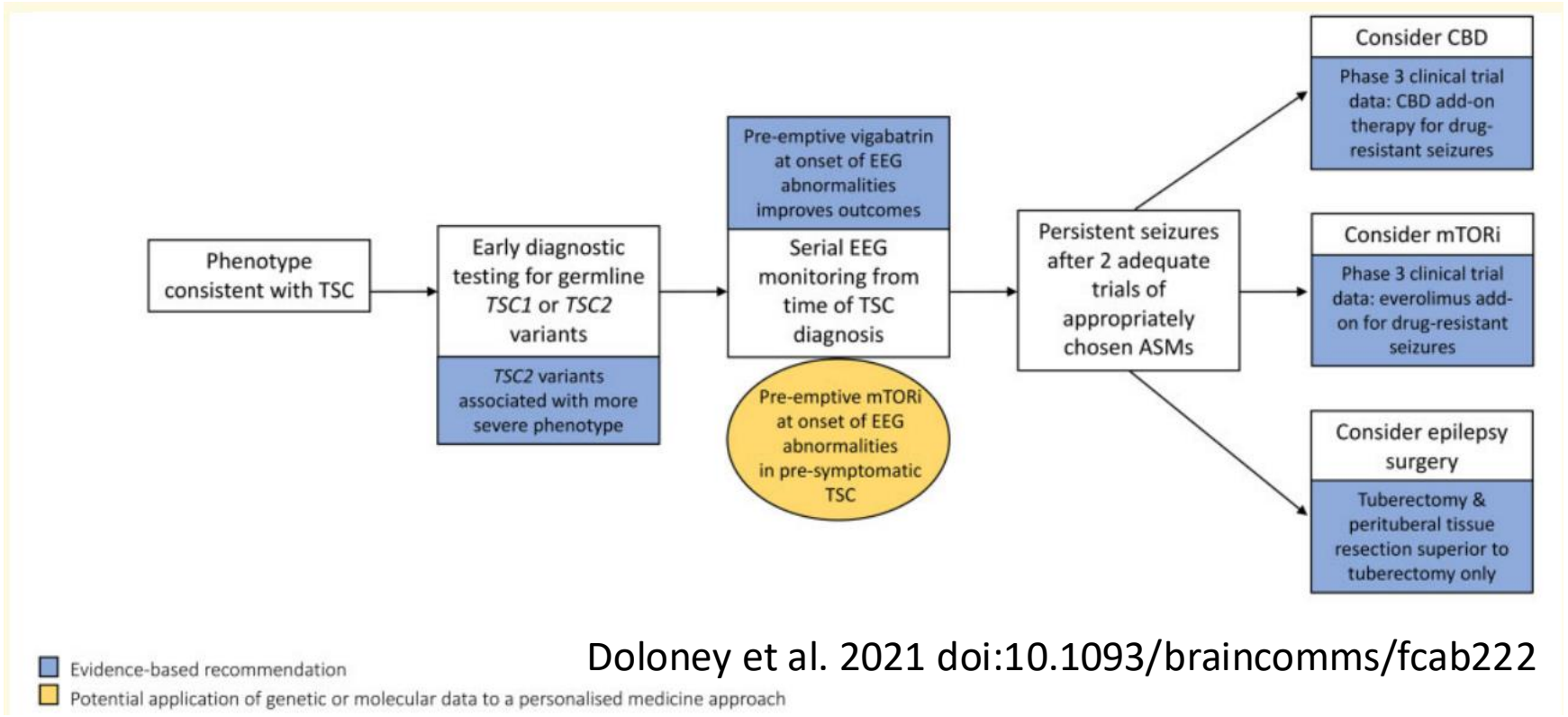


Review

Treatment of Cardiac Rhabdomyomas with mTOR Inhibitors in Children with Tuberous Sclerosis Complex—A Systematic Review

Monika Sugalska [1], Anna Tomik [2], Sergiusz Jóźwiak [1,*] and Bożena Werner [2]

Synthesis…



Doloney et al. 2021 doi:10.1093/braincomms/fcab222

Figure 2 A personalised medicine approach to the management of tuberous sclerosis complex-related epilepsy. This figure outlines a therapeutic and prognostic framework, utilizing genetic and molecular data for the management of TSC-related epilepsy. Early genetic testing for *TSC1* or *TSC2* mutations is recommended for infants with phenotypic features of TSC. *TSC2* mutations are associated with a more severe neurological phenotype. In pre-symptomatic TSC, serial EEG monitoring is recommended, as pre-emptive vigabatrin at the onset of epileptiform abnormalities is associated with better long-term epilepsy outcomes. Evidence-based treatment options for TSC-related DRE include everolimus, CBD and tuberectomy with resection of surrounding perituberal tissue. Early treatment with everolimus in seizure-naïve TSC patients may improve long-term epilepsy and cognitive outcomes. Evidence-based recommendations are highlighted in blue and potential future applications are highlighted in gold. ASM, anti-seizure medication; CBD, cannabidiol; DRE, drug-resistant epilepsy; mTORi, mechanistic target of rapamycin inhibitor; TSC, tuberous sclerosis complex.

http://www.institutdesmaladiesrares.be

- Altogether > 20.000 patients
- One dedicated clinic for Tuberous sclerosis patients (CMNCC)
- 1) referal to confirm the phenotype
- 2) molecular genotype identification
  sequencing and MLPA since 1997… as more recent NGS technology (somatic)
- offer Genetic counselling
- Work in progress: Guidelines - Genetic Centers - Marije Meuwissen - Mark Nellist

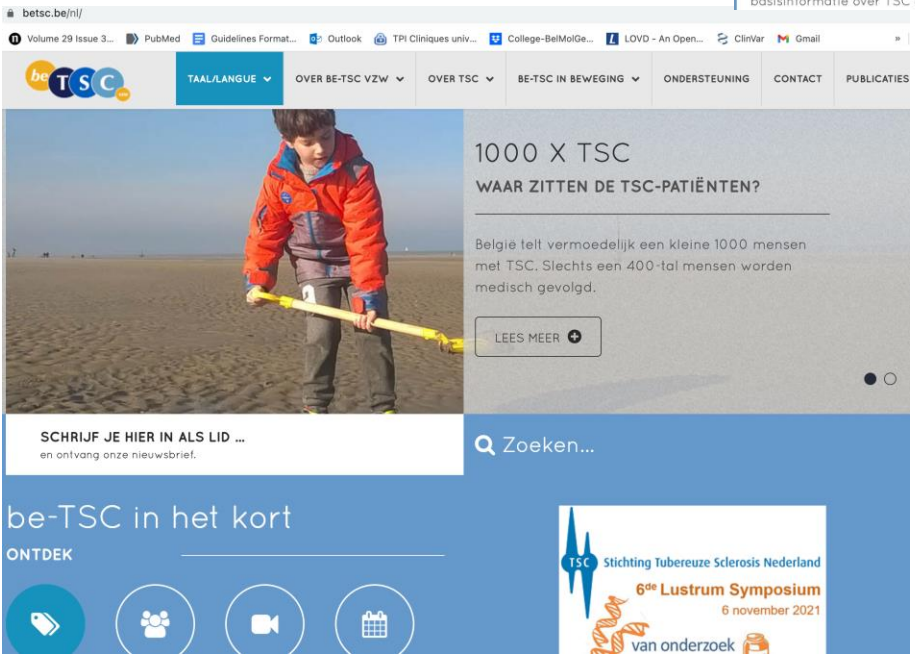
European Reference Networks

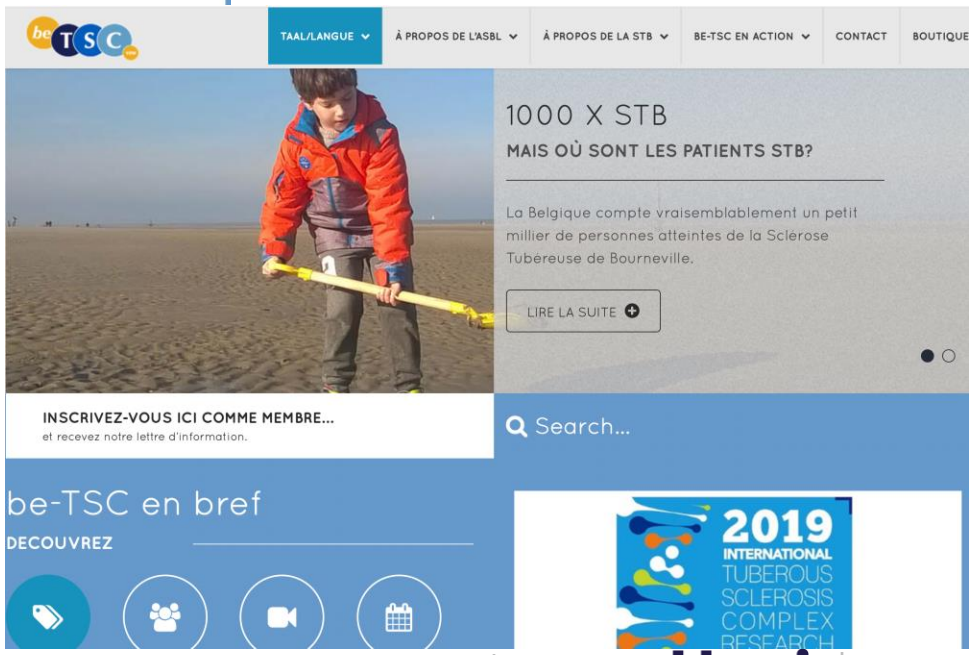
Reference Genodermatosis (‘CMNCC – centre des maladies cutanées congénitales’)

Laboratoire de référence
reening prénatal

National Patient's association

https://www.betsc.be





The **curation of this database** was supported (2005-2020) by the Tuberous Sclerosis Alliance and the tsa tuberous sclerosis association DONATE.

The database is kindly hosted by Global Variome, and the TSC curators are based at University College London.

Uncurated Data - If you have a **Clinical problem** with a variant that is not visible in the database, please note that some variants are not publicly viewable, so do contact us.

Liability - All contents of this database are protected by local and international copyright laws. The information is submitted for the purpose of sharing genetic and clinical information. Genetic variants listed may or may not have a causal association with disease phenotypes, irrespective of stated classifications or other information presented in the database. All information in this database, including variant classifications, is subject to change and there is no warranty, express or implied, as to its accuracy, completeness, or fitness for a particular purpose. Use of this database and information is subject to User responsibility and discretion. Clinical decisions regarding individual patient care should be carried out in conjunction with a healthcare professional with expertise in the relevant genes and diseases. We (the TS Alliance, the Tuberous Sclerosis Association, University College London, Leiden University Medical Centre or any of their employees or agents) do not accept any liability for any injury, loss or damage incurred by use of or reliance on the information provided by this database.

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Date created	February 22, 2005
Date last updated	February 10, 2022
Version	**TSC1:220210**

Synthesis…

Learning objectives

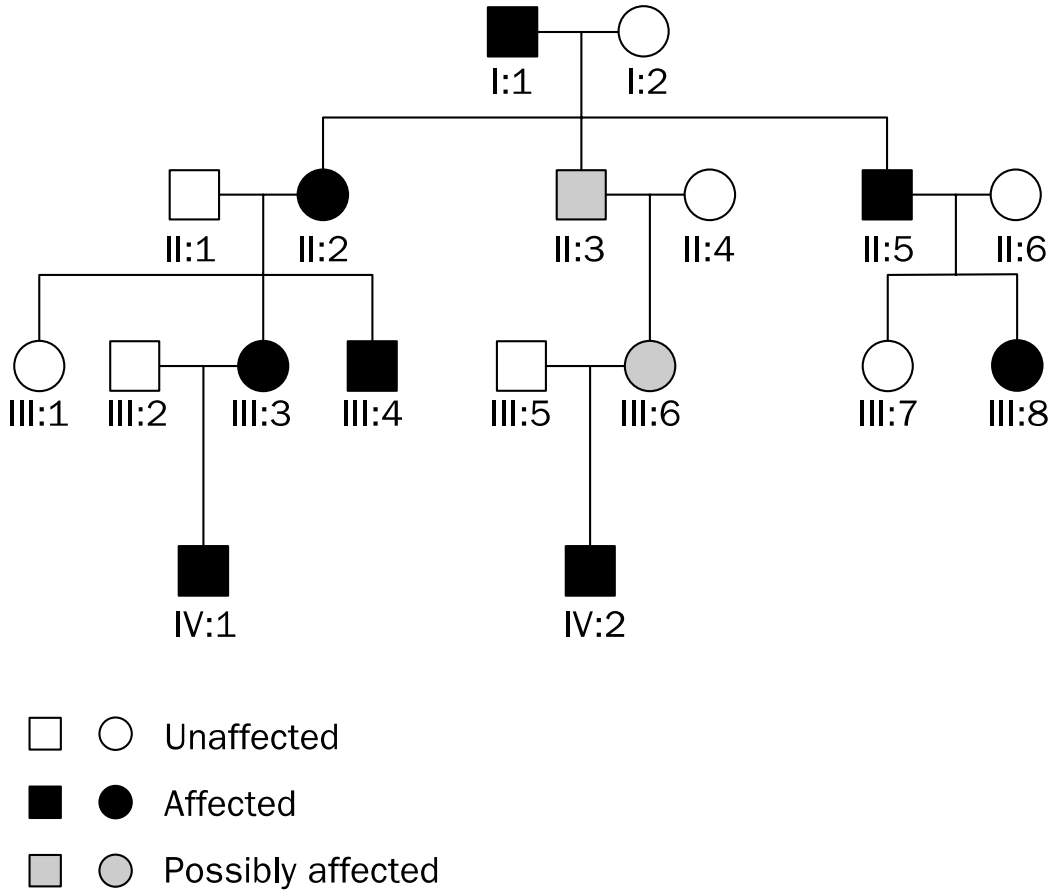
- ***Learn the clinical presentations*** of the groups of hereditary skin disorders and vascular malformations
- ***Understand molecular genetic diagnosis*** of hereditary skin disorders and vascular malformations
- **How to collaborate** with dermatologists (genodermatoses consultation) **and neurologists, nephrologists, pneumologists, ophthalmologists – multi disciplinary**
- Understand **guidelines for diagnosis and surveillance** of the relevant conditions: 7 **workgroups - in progress Coordinator Peter Janssens - UZ Brussel**
- To be aware of rare skin syndromes/vascular malformations and evolution in experimental/targeted treatment

Thank you for your attention

Open to your question

yves.sznajer@uclouvain.be

Complexity Non penetrance



Osborne Lancet 2000;355:1698