‘Introduction’

- Multi-systemic tumor development condition

- Désiré Magloire : « Sclérose tubéreuse des circonvolutions cérébrales: idiotie et épilepsie hémiplégique »

  Archives de Neurologie Paris 1880;1:81-91

- Vogt 1908 : Epilepsy, intellectual disability, adenoma

- Prevalence: 1/6.000

  2/3 sporadic - 1/3 familial

Source:

Natural History

Prenatal **Newborn** **Childhood** **Adult**

- **Cardiac rhabdomyoma**
- Infantile spasms
- Hypomelanotic macules
- *Others cutaneous manifestations*
- *Epilepsy*
- *Cognitive and behaviour difficulties*
- Renal manifestations
- LAM

Davis PE. et al. Pediatrics 2017;140(6)

Clinical criteria - Phenotype

Rationale: indication for gene investigation

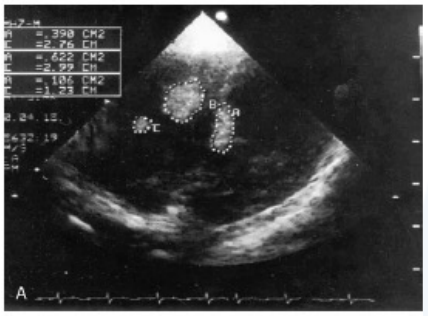
Major features
- Facial angiofibromas or forehead plaque
- Nontraumatic ungual or periungual fibroma
- Hypomelanotic macules (more than three)
- Shagreen patch (connective tissue nevus)
- Cortical tuber*
- Subependymal nodule
- Subependymal giant cell astrocytoma
- Multiple retinal nodular hamartomas
- Cardiac rhabdomyoma, single or multiple
- Lymphangiomyomatosis†
- Renal angiomyolipoma†

Minor features
- Multiple randomly distributed pits in dental enamel
- Hamartomatous rectal polyps†
- Bone cysts‖
- Cerebral white-matter "migration tracts"*‖
- Gingival fibromas
- Nonrenal hamartoma†
- Retinal achromic patch
- "Confetti" skin lesions
- Multiple renal cysts†

Source: *Roach J Child Neurol 2004;19:643-649*

Natural History – Prenatal

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]

Wide Prevalence rates: **47% to 67%**
86% Harding et al Am J Med Genet 1990
87% Davis et al Ped 2017

Mutation identification : *TSC2* (54%)
TSC1 (20%)

Natural History – Cutaneous

'Penetrance' = 'presence of clinical signs'

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)

Roach J Child Neurol 2004;19:643-649

Source:

Penetrance – Frequency related to age

Table 6. Diagnostic Criteria of Tuberous Sclerosis Complex According to Their Frequency in Children Aged 2 to 5 Years

Diagnostic Criteria	*%*	*Significance According to NTSA Classification (1992)*	*Significance According to the Latest Classification (1998)*
Subependymal nodules on neuroimaging	97.3	Primary	Major
Hypomelanotic macules	95.3	Tertiary	Major*
Epilepsy	91.5	Tertiary†	—
Facial angiofibromas	63.2	Primary	Major
Renal angiomyolipomas	41.7	Secondary	Major
Cardiac rhabdomyoma	21.4	Secondary	Major
Shagreen patch	20.8	Secondary	Major
Retinal hamartoma	17.1	Secondary	Major‡
Forehead plaque	9.3	Secondary	Major

Roach J Child Neurol 2004;19:643-649

Penetrance – related to age

Table 8. Diagnostic Criteria of Tuberous Sclerosis Complex According to Their Frequency in Children Aged 9 to 14 Years

Diagnostic Criteria	%	*Significance According to NTSA Classification (1992)*	*Significance According to the Latest Classification (1998)*
Subependymal nodules on neuroimaging	100	Primary	Major
Hypomelanotic macules	97.2	Tertiary	Major*
Epilepsy	96.2	Tertiary†	—
Facial angiofibromas	77.4	Primary	Major
Renal angiomyolipomas	64.7	Secondary	Major
Shagreen patch	47.2	Secondary	Major
Cardiac rhabdomyoma	41.2	Secondary	Major
Liver angiomyolipomas	23.5	—	Minor
Forehead plaque	18.9	Secondary	Major
Retinal hamartoma	17.2	Secondary	Major‡
Periungual fibromas	11.3	Primary	Major

Roach J Child Neurol 2004;19:643-649

Source:

Penetrance – Age related

Table 9. Diagnostic Criteria of Tuberous Sclerosis Complex According to Their Frequency in Children Aged 14 to 18 Years

Diagnostic Criteria	*%*	*Significance According to NTSA Classification (1992)*	*Significance According to the Latest Classification (1998)*
Subependymal nodules on neuroimaging	100	Primary	Major
Cortical and subcortical tubers on magnetic resonance imaging	100*	Secondary	Major
Hypomelanotic macules	97.2	Tertiary	Major†
Epilepsy	96.2	Tertiary†	—
Renal angiomyolipomas	92.3	Secondary	Major
Facial angiofibromas	77.4	Primary	Major
Cardiac rhabdomyoma	75.0*	Secondary	Major
Shagreen patch	48.1	Secondary	Major
Liver angiomyolipomas	46.2	—	Minor
Retinal hamartoma	31.3	Secondary	Major‡
Forehead plaque	18.9	Secondary	Major
Periungual fibromas	15.1	Primary	Major

Roach J Child Neurol 2004;19:643-649

Source:

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

TSC1 encoding for the protein Hamartin 21 coding

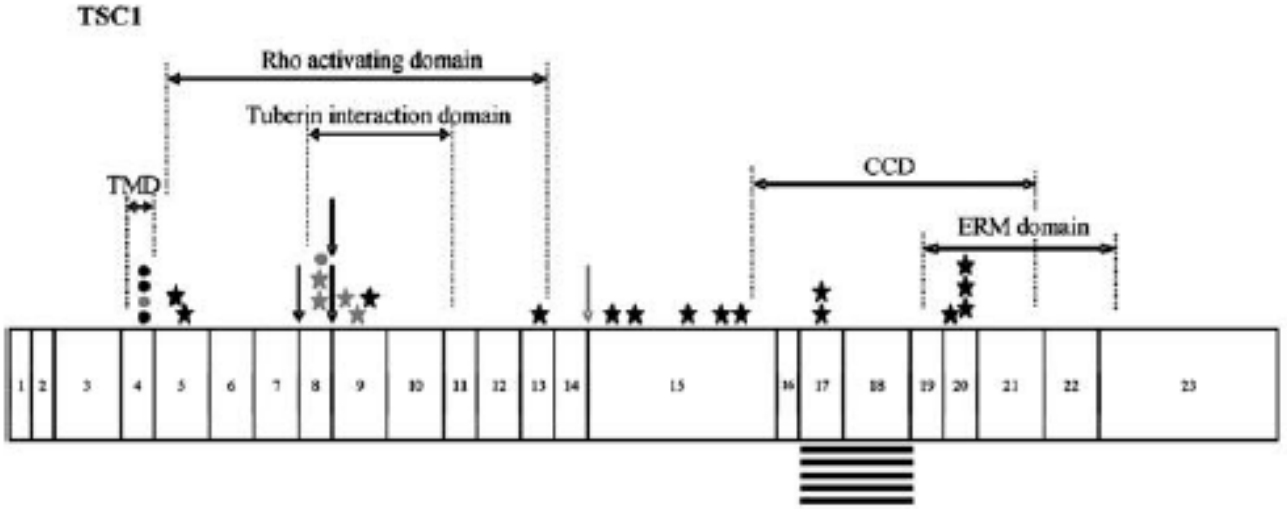


Fig. 4. Predicted amino acid sequence of the *TSC1* protein, hamartin. A potential transmembrane domain (amino acids 127 to 144) and a coiled-coil domain (amino acids 730 to 965) are underlined. The *TSC1* genomic sequence and the cDNA sequence have been deposited in GenBank (accession numbers AC002096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

```
MAQQANVGEL LAMLDSPMLG VRDDVTAVFK ENLNSDRGPM LVNTLVDYYL ETSSQPALHI   60
LTTLQEPHDK HLLDRINEYV GKAATRLSIL SLLGHVIRLQ PSWKHKLSQA PLLPSLLKCL  120
KMDTDVVVLT TGVLVLITML PMIPQSGKQH LLDFFDIFGR LSSWCLKKPG HVAEVYLVHL  180
HASVYALFHR LYGMYPCNFV SFLRSHYSMK ENLETFEEVV KPMMEHVRIH PELVTGSKDH  240
ELDPRRWKRL ETHDVVIECA KISLDPTEAS YEDGYSVSHQ ISARFPHRSA DVTTSPYADT  300
QNSYGCATST PYSTSRLMLL NMPGQLPQTL SSPSTRLITE PPQATLWSPS MVCGMTTPPT  360
SPGNVPPDLS HPYSKVFGTT AGGKGTPLGT PATSPPPAPL CHSDDYVHIS LPQATVTPPR  420
KEERMDSARP CLHRQHHLLN DRGSEEPPGS KGSVTLSDLP GFLGDLASEE DSIEKDKEEA  480
AISRELSEIT TAEAEPVVPR GGFDSPFYRD SLPGSQRKTH SAASSSQGAS VNPEPLHSSL  540
DKLGPDTPKQ AFTPIDLPCG SADESPAGDR ECQTSLETSI FTPSPCKIPP PTRVGFGSGQ  600
PPPYDHLFEV ALPKTAHHFV IRKTEELLKK AKGNTEEDGV PSTSPMEVLD RLIQQGADAH  660
SKELNKLPLP SKSVDWTHFG GSPPSDEIRT LRDQLLLLHN QLLYERFKRQ QHALRNRRLL  720
RKVIKAAALE EHNAAMKDQL KLQEKDIQMW KVSLQKEQAR YNQLQEQRDT MVTKLHSQIR  780
QLQHDREEFY 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 PPHQRAGPFS SRWETTMGEA SASIPTTVGS 1080
LPSSKSFLGM KARELFRNKS ESQCDEDGMT SSLSESLKTE LGKDLGVEAK IPLNLDGPHP 1140
SPPTPDSVGQ LHIMDYNETH HEHS                                        1164
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Slentghorst Science 1997;277:855-858

Gene - ‘hard’ware

TSC2 gene encoding for Tuberin 42 exons

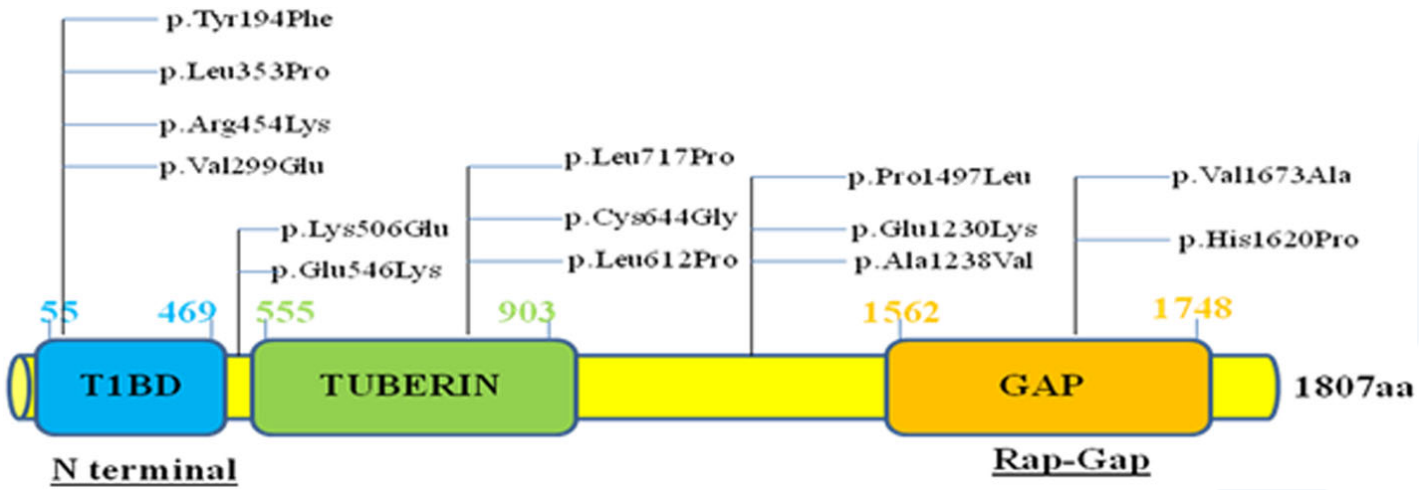
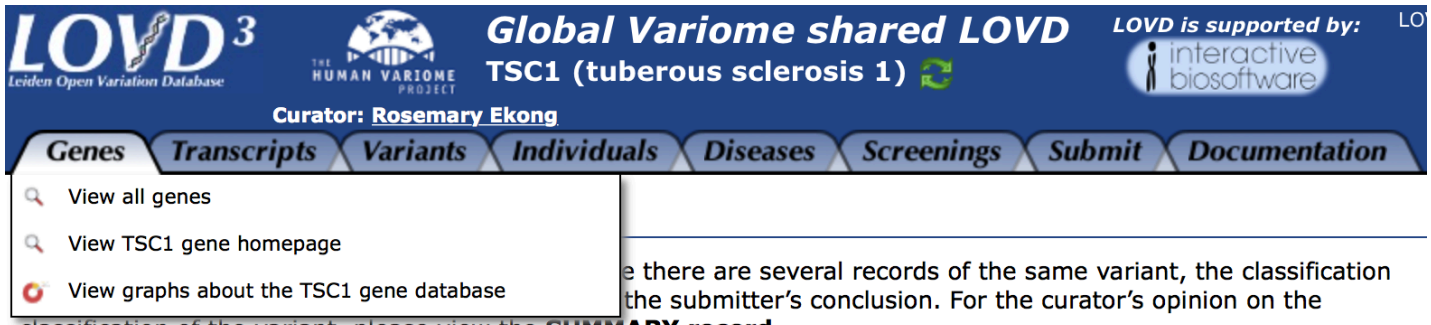


Fig. 1 Schematic representation of 13 novel *TSC2* missense variants identified in respective domains of *TSC2* protein

‘Genotype’ – ‘genetic message - ‘hardware’ and tools

Classification of nucleotidic changes in *TSC1* and *TSC2 gene*

Leiden Open Variation Databases publicly



e there are several records of the same variant, the classification
the submitter’s conclusion. For the curator’s opinion on the
classification of the variant, please view the **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	FCORD-2, ID, LAM, TSC, TSC-1
Citation reference(s)	-
Refseq URL	Genomic reference sequence
Curators (1)	**Rosemary Ekong**
Total number of public variants reported	3446
Unique public DNA variants reported	1078
Individuals with public variants	3136
Hidden variants	151
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.

Support on genotype knowledge

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 **curation of this database** is supported by the TSC Alliance and the TSC Association. 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 we have a backlog of uncurated data, so do contact us.

Support on genotype knowledge

Liability -

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.
Database submitters are required to adhere to their institution's rules for data sharing, and local and national laws. Submitters retain the rights to use their data and can update their data by contacting the curators. Database curators may curate data to ensure that database formatting and quality standards are met. They may also share submitted data with external parties for research purposes or for sharing with other databases.

Date created February 22, 2005
Date last updated October 26, 2019
Version TSC1:191026

One condition – 2 genes

- Inheritance : Autosomal dominant
- 2 genes : *TSC1* and *TSC2*

80% detection of a mutation or a deletion

Mutation/deletion effect: TSC1 or TSC2 inactivation

20% no identified anomaly

somatic / mosaïc

heterogeneity ?

other mechanism ?

Limitation related to absence of genotype identification – 20%

- Impair precise genetic counselling to relatives and future pregnancies for parents

  'probably 'de novo' /not familial

- Impair precise reproductive choices

  dominant condition - if one parent affected

  50% risk

  how to conduct prenatal diagnosis : ultrasound…

State of the art in 2016: Sanger sequencing and deletion identification MLPA

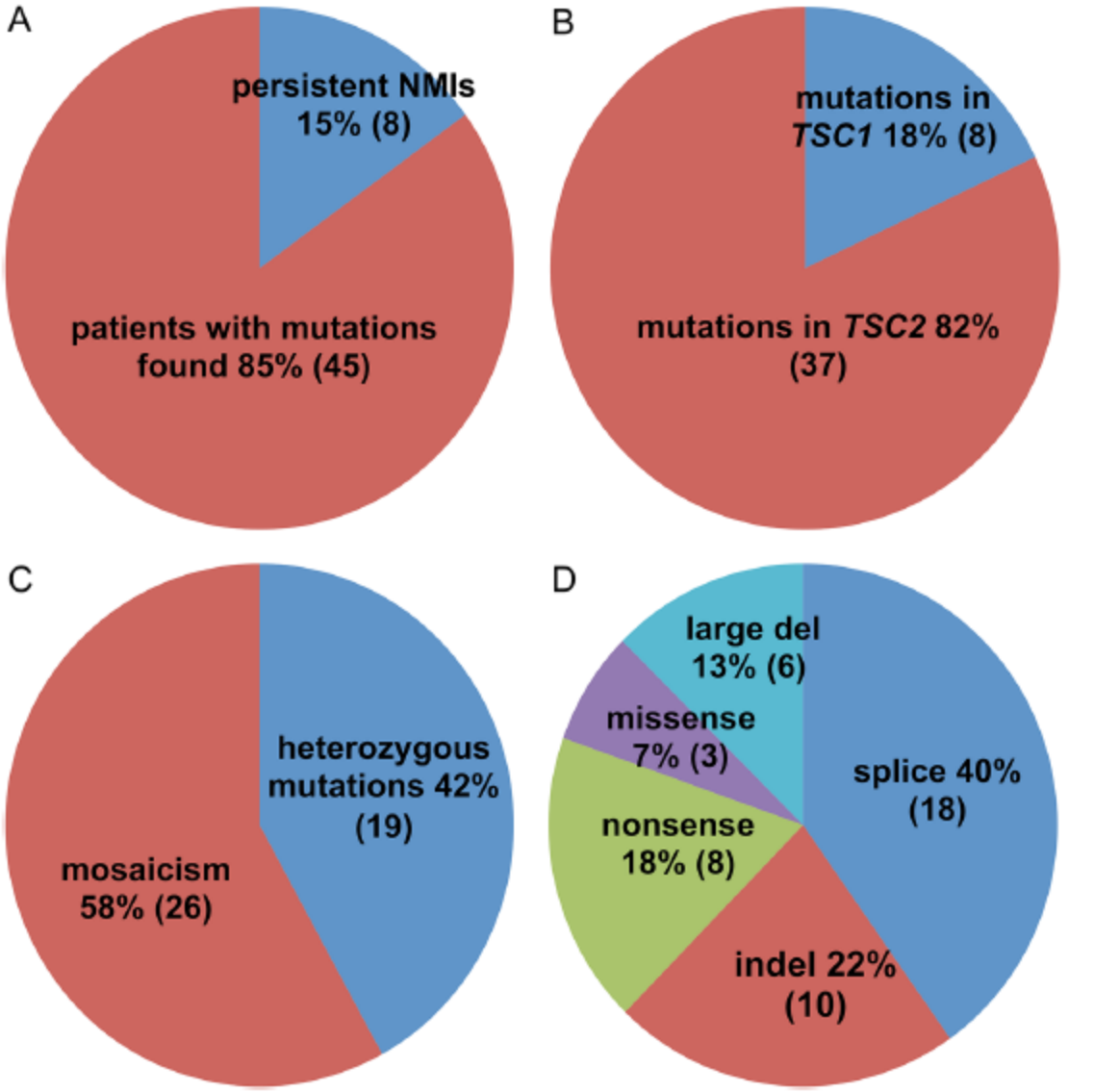
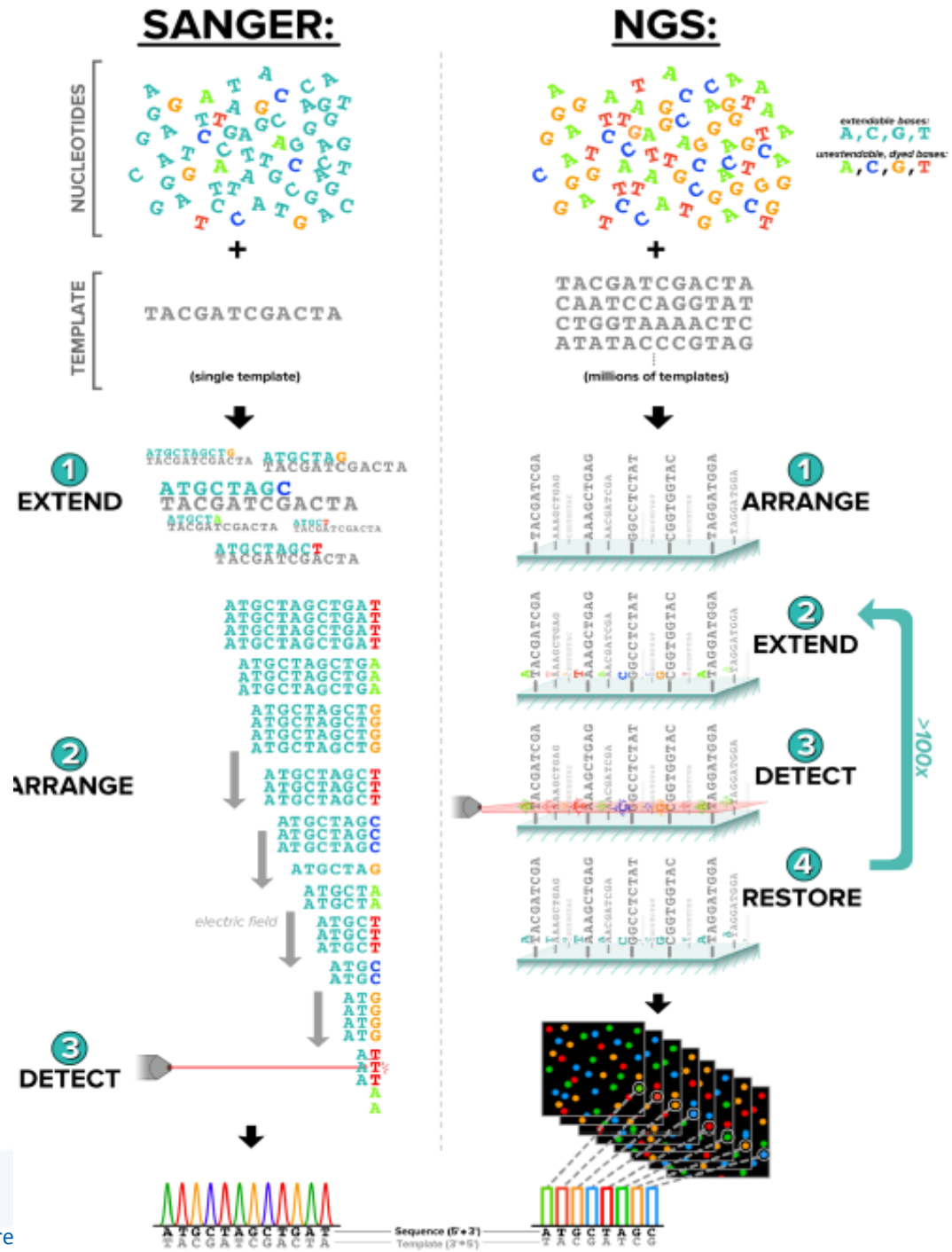


Fig 1. Pie charts displaying the mutation types and frequencies in 53 TSC NMI subjects. (A) Proportion of subjects with mutations identified vs. remaining as persistent NMI. (B) Proportion of mutations in *TSC1* vs. *TSC2*. (C) Proportion of heterozygous vs. mosaic mutations. (D) Different types of identified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

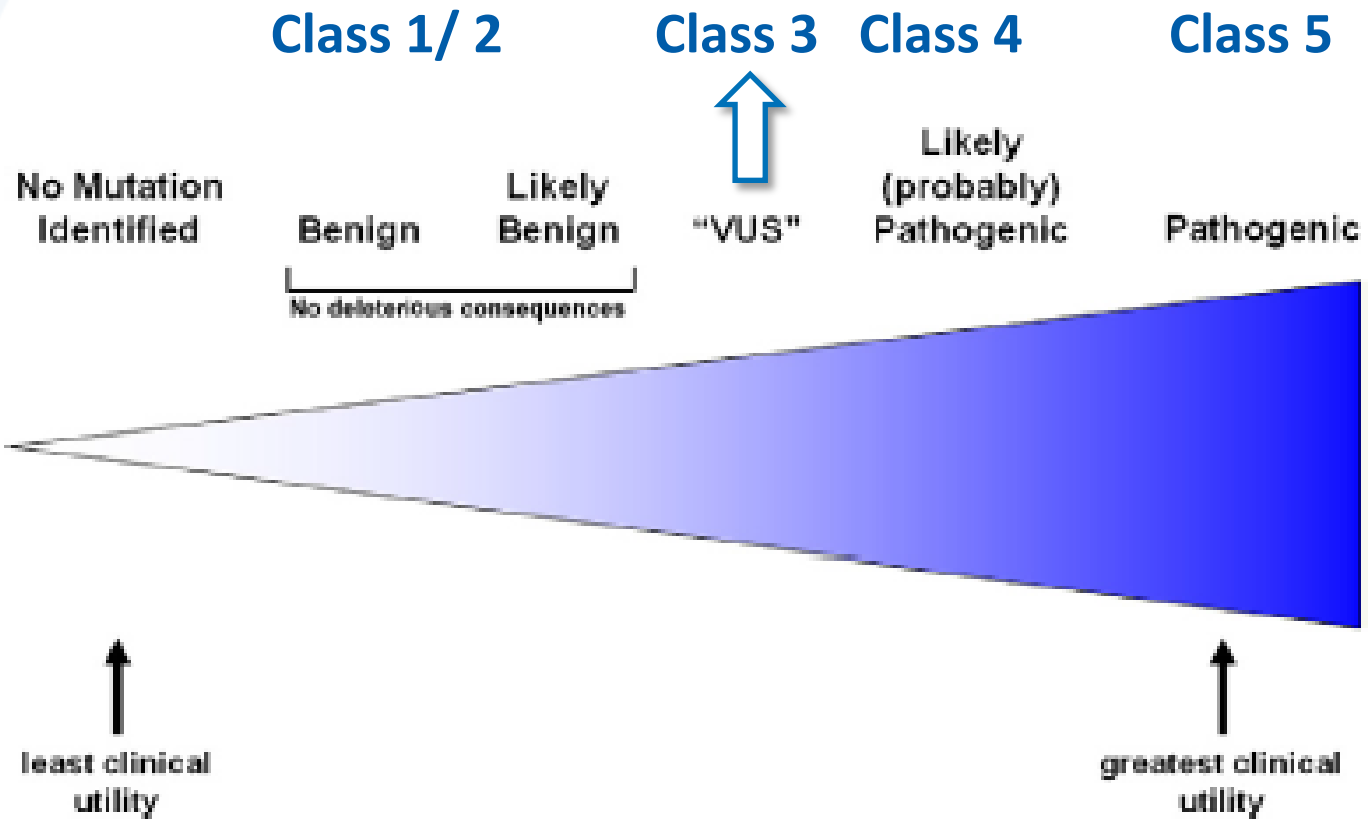
More recent improvement in genotype identification thanks to technology

NGS

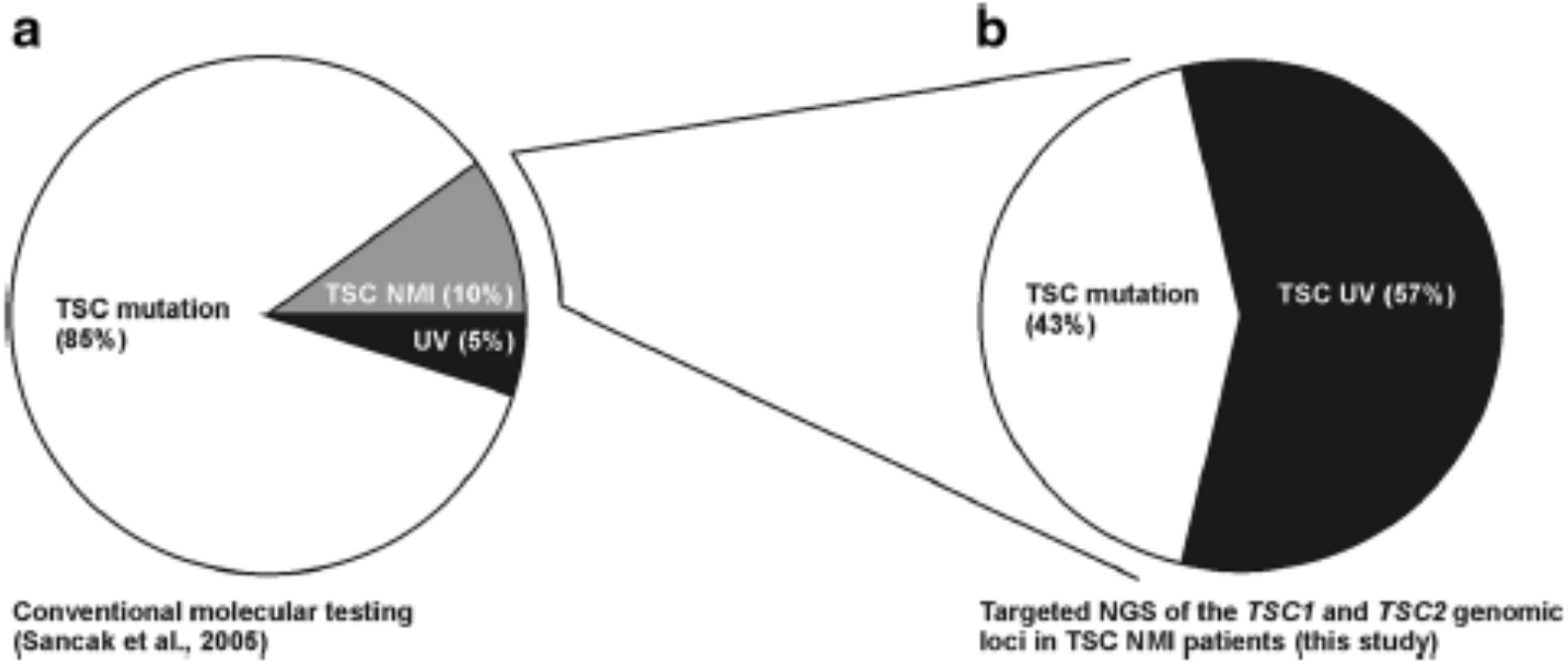
= **N**ext **G**eneration **S**equencing



Standardized nucleotide substitution nomenclature – medical report



Evidence - Up to date state of the art



Nellist et al. BMC 2015

Rationale

Why to complete precise genetic diagnosis ?

phenotype – description of clinical signs /features

and

genotype – description of the genetic code anomaly

Rationale

Why to complete precise genetic diagnosis ?

… to provide precise genetic counselling

for index patient – individual

for parents and sibship

for reproductive options

Pre implantory Genetic Diagnosis - PGD

Prenatal diagnosis

Rationale

Why to complete precise genetic diagnosis ?

… to provide precise genetic counselling

for index patient – at individual level

Numerous genotype – phenotype correlation studies

Au K; et al. Am J Hum Genet 1998;62:286-294: 240 patients

Van Slengthorst J Med Genet 1999;36:285-289 - 225 patients

Niida et al. Hum Mut 1999;42:412-422 -124 patients

Jones Am J Hum Genet 1999;64:1305-1315 – 170 patients

Sancak O. et al. Eur J Hum Genet 2005;13:731-741 - 490 patients

Example of genotype phenotype findings

Table 1 Overview of the TSC patient cohort

	Total	*Mutation identified*	*Unclassified variant*	*No mutation identified*	*TSC1 mutation*	*TSC2 mutation*	*De novo*	*Familial*
Patients with clinical information								
Def. TSC	276	235	14	27	53	182	92	50
Insuf. information	15	4	1	10	1	3	1	1
Total	291	239	15	37	54	185	93	51
No clinical information	199	123	14	62	28	95	44	12
Total	490	362	29	99	82	280	137	63

Groups with a definite diagnosis of TSC (Def. TSC), insufficient clinical information for a definite diagnosis (Insuf. information) and without any clinical information (No clinical information) are shown. For each group, patients with mutations in the *TSC1* and *TSC2* genes in addition to patients with nucleotide changes that could not yet be classified as pathogenic (unclassified variant) are depicted. The number of patients with *de novo* mutations and of patients of familial cases (based on molecular analysis) are also shown.

European Journal of Human Genetics (2005) 13, 731–741
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More in-depth on genotype phenotype correlation

Table 3a Comparison of the clinical features in TSC patients with and without mutations

	TSC1						*TSC2*						*Mutations*						*No Mutation Identified*	
	Total		*Familial*		*De novo*		*Total*		*Familial*		*De novo*		*Total*		*Familial*		*De novo*			
	P/N	*%*	*P/N*	*%*	*P/N*	*%*	*P/N*	*%*	*P/N*	*%*	*P/N*	*%*	*P/N*	*%*	*P/N*	*%*	*P/N*	*%*	*P/N*	*%*
Mental retardation	19/39	49	8/12	67	7/12	58	129/156	83	20/27	74	53/63	84	148/195	76	28/39	72	60/75	80	8/22	36
Seizures	42/46	91	13/13	100	13/15	87	159/175	91	28/34	82	69/71	97	201/221	91	41/47	87	82/86	95	17/25	68
Cortical tubers	20/24	83	6/6	100	7/8	88	93/104	89	14/18	78	37/39	95	113/128	88	20/24	83	44/47	94	14/18	78
SEN	34/37	92	10/11	91	11/12	92	125/138	91	16/22	73	58/58	100	159/175	91	26/33	79	69/70	99	18/22	82
SEGA	5/13	39	2/3	67	1/4	25	17/58	29	2/6	33	7/19	37	22/71	31	4/9	44	8/23	35	1/14	7
Renal AML	2/27	7	0/5	0	0/11	0	57/114	50	10/23	44	19/51	37	59/141	42	10/28	36	19/62	31	9/16	56
Renal cysts	3/28	11	1/5	20	0/11	0	27/98	28	5/20	25	12/44	27	30/126	24	6/25	24	12/55	22	5/16	31
Shagreen patch	22/31	71	7/9	78	5/9	56	42/85	49	3/11	27	22/39	56	64/116	55	10/20	50	27/48	56	3/16	19
Hypomelanotic macules	41/44	93	12/12	100	15/17	88	134/146	92	30/30	100	60/66	91	175/190	92	42/42	100	75/83	90	17/23	74
Forehead plaque	6/22	27	2/8	25	2/6	33	35/78	45	3/13	23	15/31	48	41/100	41	5/21	24	17/37	46	2/15	13
Facial angiofibromas	27/38	71	8/12	67	4/8	50	111/135	82	17/22	77	43/55	78	138/173	80	25/34	74	47/63	75	14/22	64
Ungual fibroma	14/31	45	2/8	25	1/6	17	30/89	34	4/15	27	15/39	39	44/120	37	6/23	26	16/45	36	4/15	27
Retinal phakoma	3/30	10	0/9	0	2/11	18	34/92	37	4/21	19	16/41	39	37/122	30	4/30	13	18/52	35	2/16	13
Retinal depigmentation	0/8	0	0/1	0	0/2	0	11/35	31	1/4	25	6/17	35	11/43	26	1/5	20	6/19	32	1/10	10
Gingival fibroma	5/10	50	2/2	100	1/2	50	10/36	28	0/1	0	6/19	32	15/46	33	2/3	67	7/21	33	2/5	40
Dental pits	10/19	53	2/4	50	2/5	40	27/54	50	4/7	57	14/27	52	37/73	51	6/11	55	16/32	50	1/4	25
Cardiac rhabdomyoma	10/24	42	1/3	33	6/11	55	37/89	42	7/17	41	17/39	44	47/113	42	8/20	40	23/50	46	5/15	33

Familial represents the index patients of familial cases. For each group, the first column shows the number of patients with the feature (*P*) and the total number of patients examined (*N*) and the second column shows the frequency of each clinical feature (%). The median age was 14.0 for TSC1 familial, 4.0 for TSC1 *de novo*, 15.0 for TSC1 total, 11.0 for TSC2 familial, 10.0 for TSC2 *de novo*, 12.0 for TSC2 total, 13.0 for mutations total, 9.5 for *de novo*, 11.5 for familial and 12.0 for no mutation identified groups (SEN, subependymal nodules; SEGA, subependymal giant cell astrocytoma; AML, angiomyolipoma).

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More in-depth on genotype phenotype correlation

Table 3b Significance (*P*) values of the comparisons made between the different groups depicted in table 3a

	TSC1 vs TSC2	*Familial vs de novo*	*TSC2 familial vs TSC2 de novo*	*TSC1 de novo vs TSC2 de novo*	*TSC1 familial vs TSC2 familial*	*TSC1 familial vs TSC1 de novo*	*No mutation vs mutations total*	*No mutation vs TSC1 total*
Mental retardation	<0.001*	0.322	0.203	0.055	0.456	0.500	<0.001*	0.351
Seizures	0.595	0.090	0.013*	0.139	0.125	0.780	0.001*	0.016*
Cortical tubers	0.299	0.169	0.072	0.436	0.288	0.571	0.188	0.473
SEN	0.550	0.001*	<0.001*	0.171	0.232	0.739	0.168	0.227
SEGA	0.368	0.454	0.637	0.565	0.405	0.371	0.066	0.067
Renal AML	<0.001*	0.634	0.399	0.011*	0.087	**	0.270	0.001*
Renal cysts	0.050*	0.829	0.553	0.048*	0.657	0.313	0.515	0.100
Shagreen patch	0.031*	0.637	0.085	0.623	0.035*	0.310	0.006*	0.001*
Hypomelanotic macules	0.527	0.034*	0.098	0.519	**	0.335	0.006*	0.037*
Forehead plaque	0.107	0.095	0.110	0.413	0.656	0.594	0.033*	0.277
Facial angiofibromas	0.102	0.908	0.576	0.104	0.390	0.388	0.086	0.552
Ungual fibroma	0.178	0.430	0.315	0.292	0.666	0.615	0.324	0.190
Retinal phakoma	0.003*	0.030*	0.094	0.177	0.218	0.289	0.113	0.576
Retinal depigmentation	0.073	0.538	0.593	0.456	0.800	**	0.273	0.556
Gingival fibroma	0.172	0.308	0.700	0.567	0.333	0.500	0.546	0.573
Dental pits	0.528	0.795	0.571	0.500	0.652	0.643	0.317	0.329
Cardiac rhabdomyoma	0.586	0.648	0.552	0.380	0.656	0.500	0.541	0.603
Median age	0.321	0.226	0.921	0.364	0.119	0.027*	0.795	0.484

*Indicates statistically significant values. Familial represents the index patients of familial cases (SEN, subependymal nodules; SEGA, subependymal giant cell astrocytoma; AML, angiomyolipoma). **Indicates where statistical analysis could not be performed, since the patients in both groups were either all positive or all negative for the analysed feature.

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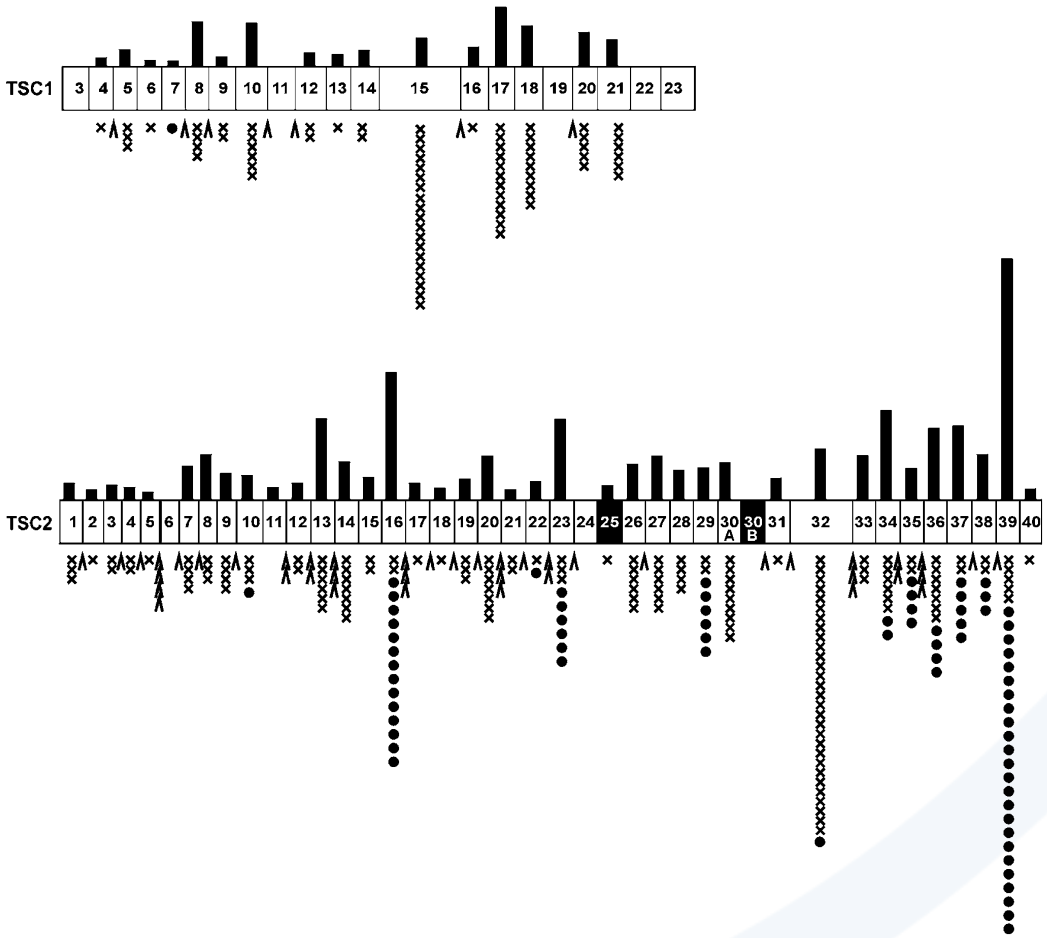
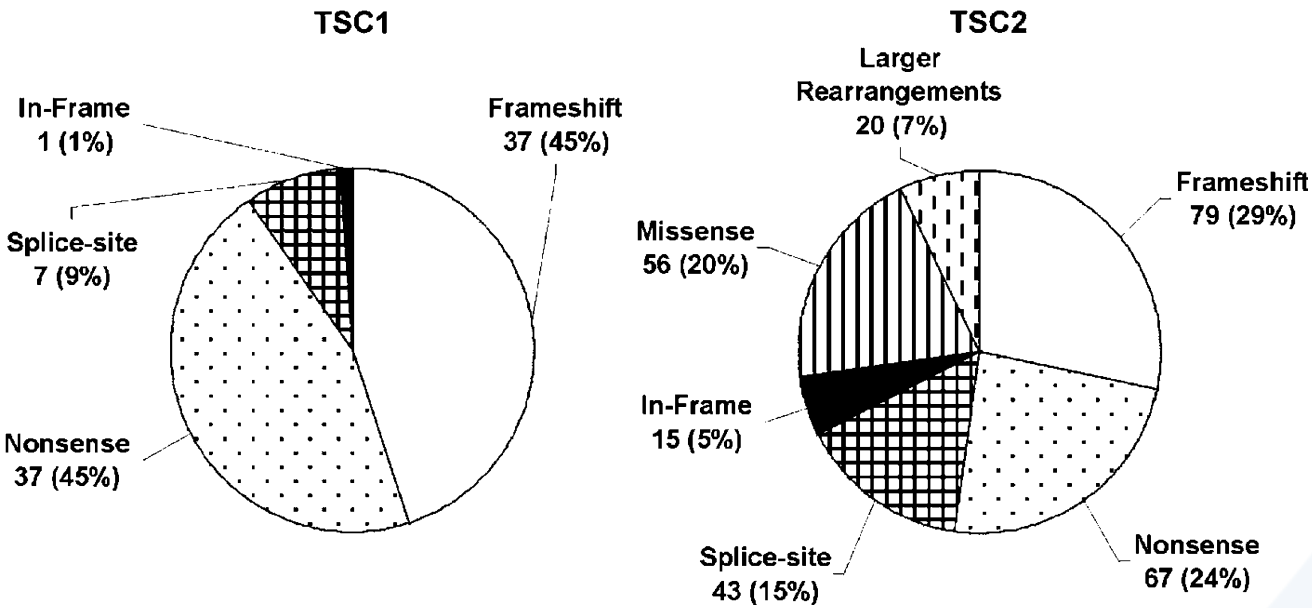
Example of genotype findings



European Journal of Human Genetics (2005) 13, 731–741

More in depth on genotype findings: nature of substitution



European Journal of Human Genetics (2005) 13, 731–741

Genotype finding implication

RESEARCH ARTICLE

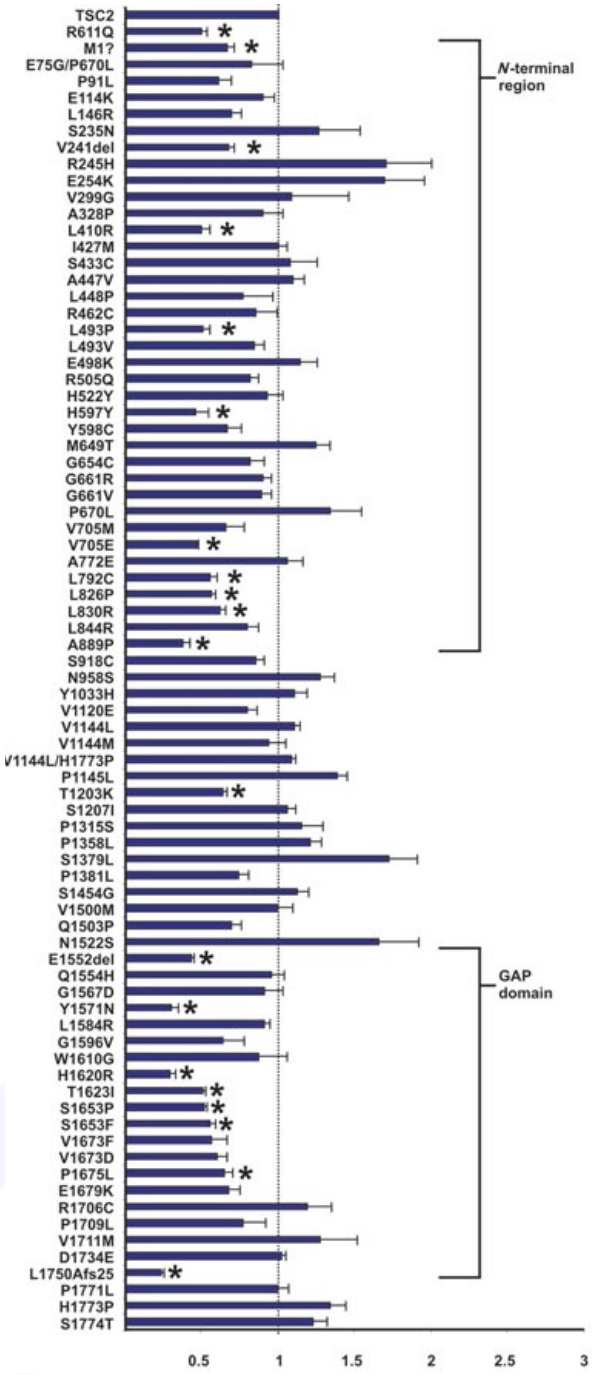
Human Mutation
OFFICIAL JOURNAL
HGVS
HUMAN GENOME VARIATION SOCIETY
www.hgvs.org

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

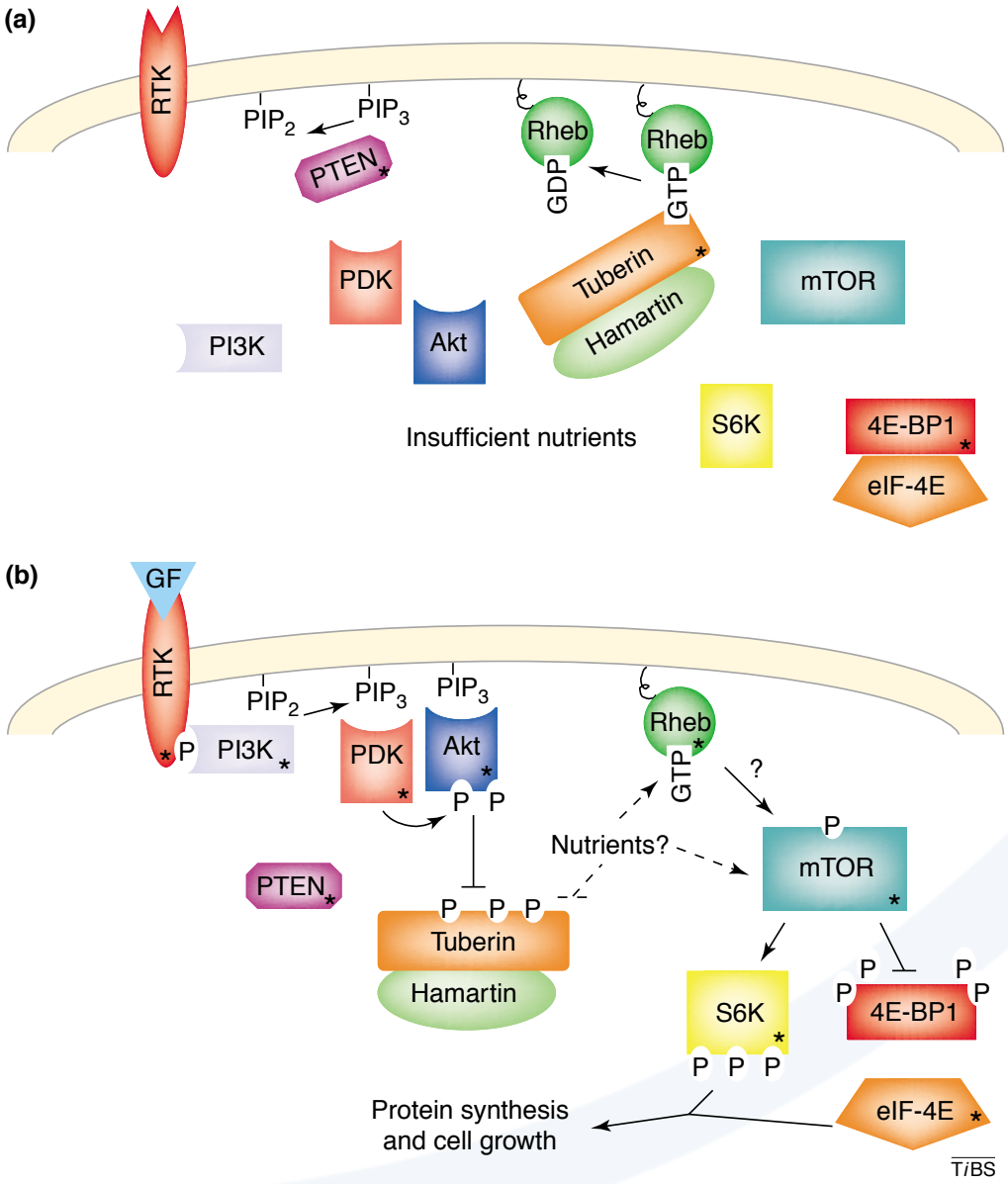
Marianne Hoogeveen-Westerveld,[1] Rosemary Ekong,[2] Sue Povey,[2] Karin Mayer,[3] Nathalie Lannoy,[4] Frances Elmslie,[5] Martina Bebin,[6] Kira Dies,[7] Catherine Thompson,[8] Steven P. Sparagana,[8,9] Peter Davies,[10] Ans van den Ouweland,[1] Dicky Halley,[1] and Mark Nellist[1]*

Functional Assessment of TSC2 Variants identified in individuals with tuberous sclerosis complex

Functional / Expression studies

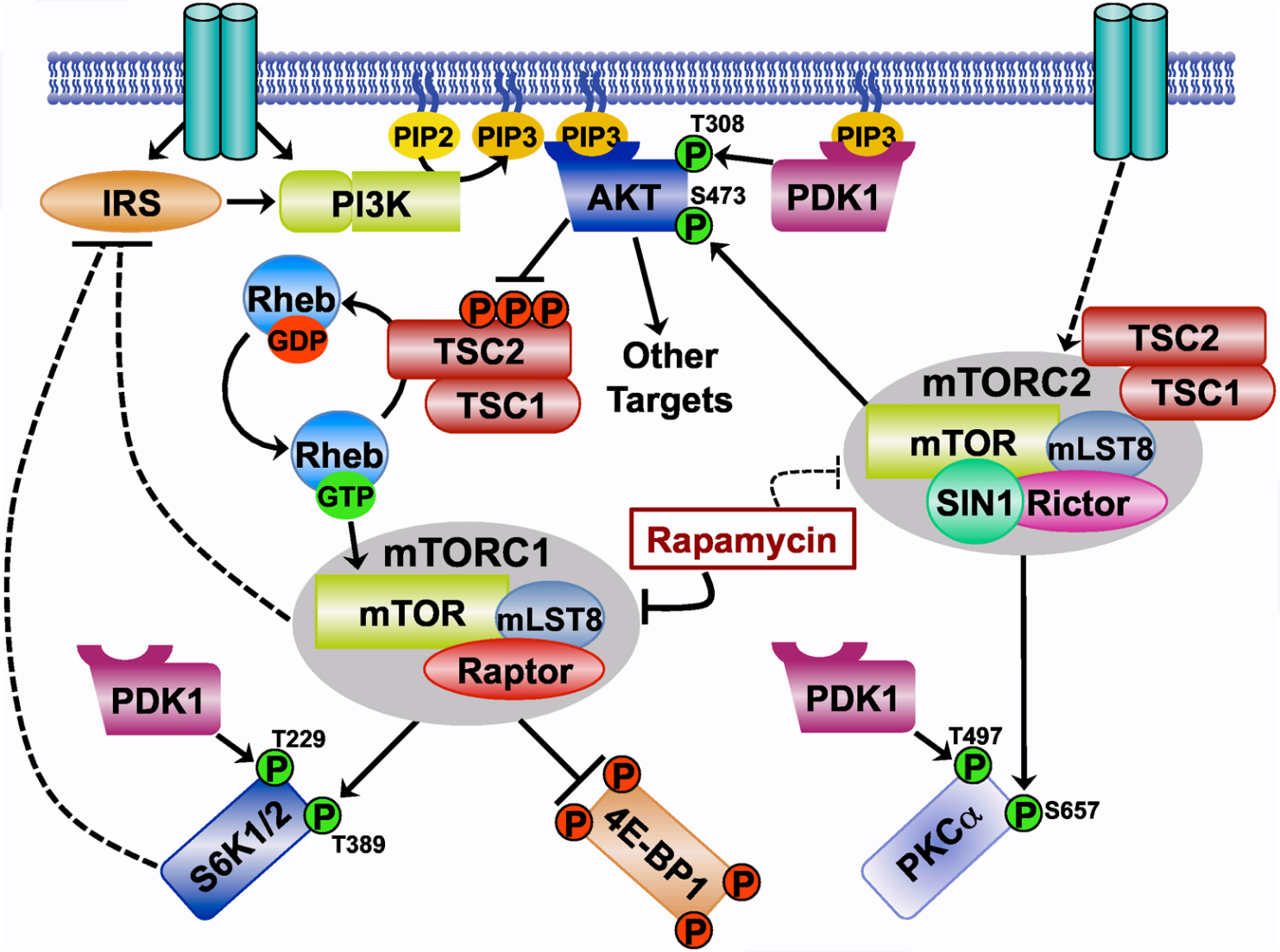


Implication of the Tuberin- Hamartin dimer



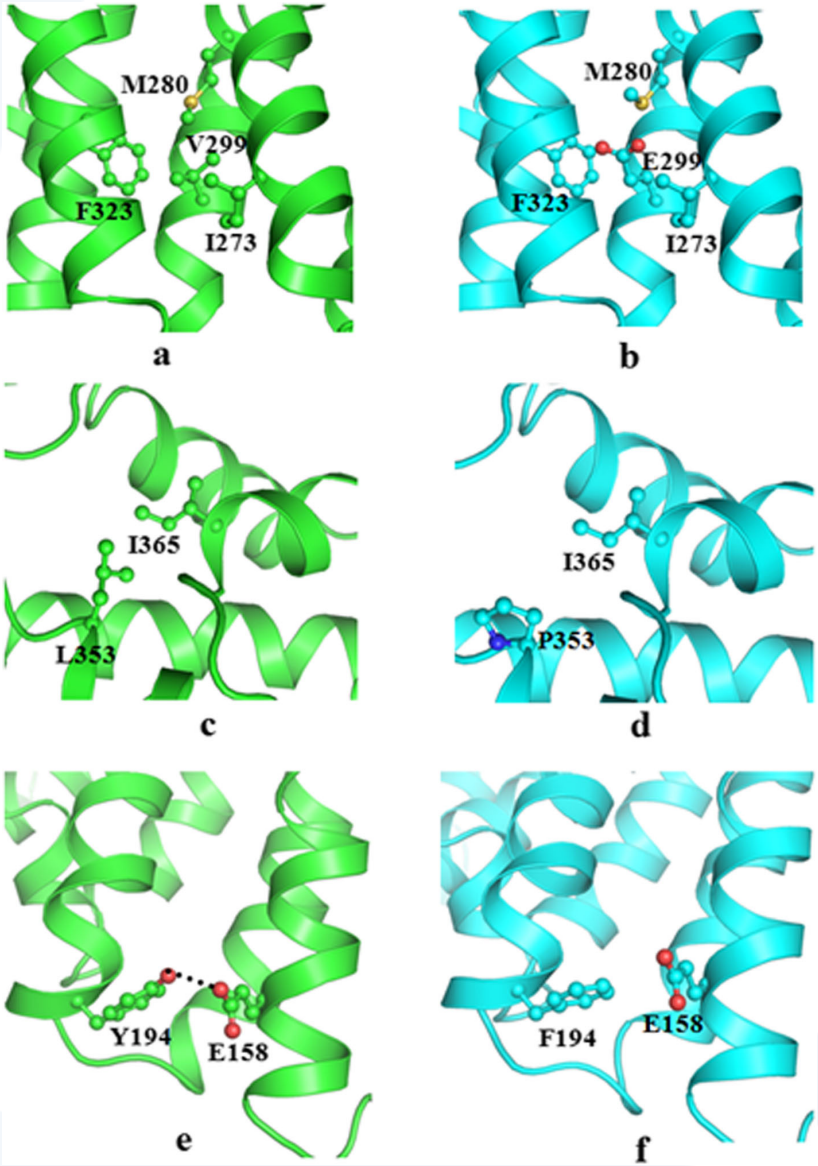
Manning Trends in Bio

Implication of the Tuberin- Hamartin dimers



Huang and Manning Bioch Soc Trans 2009

Modeling of protein conformation change related to substituation



Zudarshan et al. BMC Med Genet 2019;20:164

More in-depth on genotype phenotype correlation

…by involved system: from pathways to cell level

Tuberous Sclerosis and Epilepsy: Role of Astrocytes

ROLE OF GLIAL ABNORMALITIES IN EPILEPTOGENESIS IN TSC

Studies of animal models and human TSC brain tissue have identified a number of histological, cellular, and molecular abnormalities that likely contribute to epileptogenesis in TSC (Wong, 2008). In particular, the TSC1 and TSC2 encoded proteins function as negative regulators of the mammalian target of rapamycin (mTOR) signaling pathway and loss of function *TSC1* or *TSC2* mutations lead to constitutive mTOR activation. Inhibition of mTOR signaling in animal TSC models can alter cellular pathology and prevent seizures suggesting an important link between mTOR and epileptogenesis in TSC. Of central importance, pathological brain lesions of TSC particularly cortical tubers, have been strongly implicated in promoting or causing epilepsy.

More in-depth on genotype phenotype correlation

…by involved system: from path ways to cell level

Tuberous Sclerosis and Epilepsy: Role of Astrocytes

Key summary points	Unresolved questions	Future directions
Increased astrocyte numbers in tubers	What causes increased astrocyte proliferation?	*In vivo* studies of astrocyte division Role of growth factors, e.g., EGF, IGF
Altered astrocyte morphology	Are astrocytes dysmorphic? Are astrocytes gliotic?	Role of mTOR signaling in astrocyte
Increased astrocyte numbers in perituberal regions	What is boundary of tuber?	Define tuber boundary with markers
	Why are there increased	Examine astrocyte proliferation adjacent to tubers
	Astrocytes adjacent to tubers?	Examine astrocyte migration
Altered potassium buffering in astrocytes	Are changes in K+ channels due to TSC mutations?	Define mTOR effects on K+ channels
	Are changes in K+ channels due to mTOR?	Rescue K+ effects with rapamycin
Astrocytes are linked to inflammatory response in tubers	Do astrocytes foster expression of inflammatory cytokines?	Define relation between astrocytes and cytokines *in vivo*
	What is role of inflammation in seizure onset in TSC?	Link between astrocytes and microglia

GLIA 60:1244–1250 (2012)

More in-depth on genotype phenotype correlation

...by investigation to involved system: from pathway to cell level

Wesseling *et al. Molecular Autism* (2017) 8:41
DOI 10.1186/s13229-017-0151-y

Molecular Autism

RESEARCH Open Access

A brain proteomic investigation of rapamycin effects in the *Tsc1*$^{+/-}$ mouse model

Hendrik Wesseling[1], Ype Elgersma[2] and Sabine Bahn[1,2*]

Tsc1$^{+/-}$ mice were generated by replacing exons 6 through to 8 of the *Tsc1 gene* with a selection cassette, as described previously [29]. This leads to the generation of *Tsc1* null embryos which express *Tsc1* transcripts in which exon 5 and 9 are fused, leading to a premature TGA stop codon. Consequently, any protein translated from this allele lacks all of the known functional domains of hamartin including the putative Rho activation domain.

Results

Label-free LC-MS^E proteomic profiling of *Tsc1*$^{+/-}$ mouse brains

We investigated protein abundance changes in the frontal cortex and hippocampus of the *Tsc1*$^{+/-}$ mouse. LC-MS^E analysis resulted in the identification of 522 proteins (7071 peptides) in the frontal cortex and 463 proteins (5149 peptides) in the hippocampus. Of these, the levels of 51 proteins were altered in the frontal cortex (FDR-adjusted $^*p < 0.05$) and 108 in the hippocampus (FDR-adjusted $^*p < 0.05$). In the frontal cortex, 17 of the changed proteins were altered by more than 10%, as were 49 of the 108 changed hippocampal proteins

Knowledge from the studies on genotype-phenotype correlation

TSC2

To keep in mind « Large » deletion extending to *PKD1 gene*

'contiguous genes syndrome'

…Not enough precise correlation to allow for monitoring targeted follow up at individual bedside

No link to diagnostic category

…Additional complexity

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.

Volume 340 Number 9 · 705

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 ''

Knowledge from the studies on genotype

Hum Genet (2016) 135:61–68
DOI 10.1007/s00439-015-1611-0

CrossMark

ORIGINAL INVESTIGATION

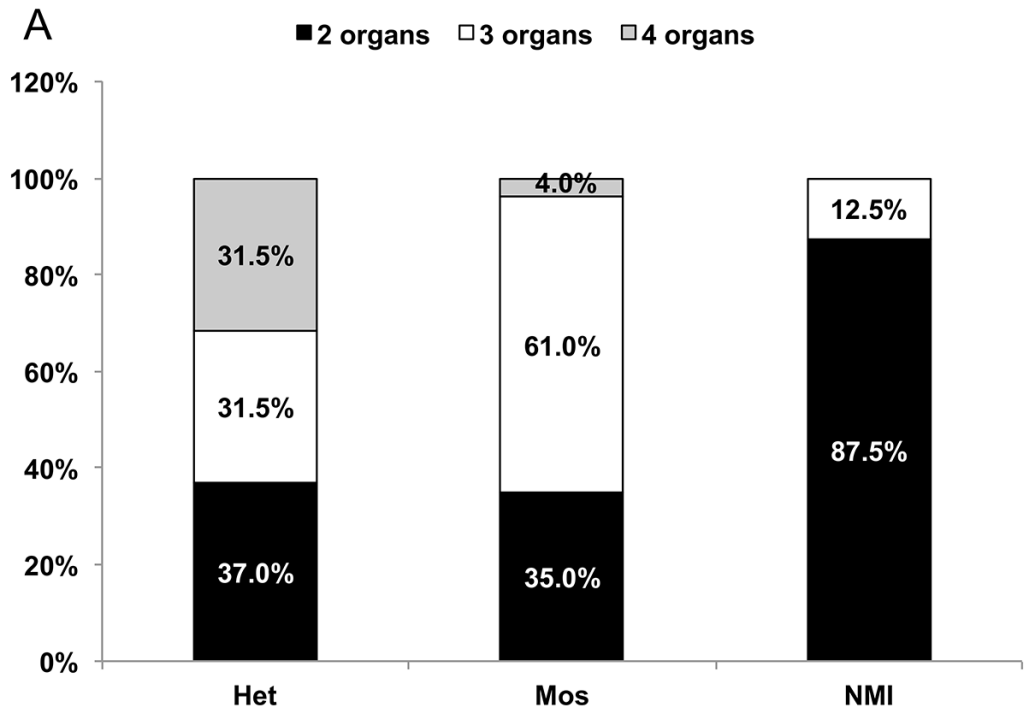
Detection of low-prevalence somatic *TSC2* mutations in sporadic pulmonary lymphangioleiomyomatosis tissues by deep sequencing

Atsushi Fujita[1] · Katsutoshi Ando[2] · Etsuko Kobayashi[2] · Keiko Mitani[3] · Koji Okudera[4] · Mitsuko Nakashima[1] · Satoko Miyatake[1] · Yoshinori Tsurusaki[1] · Hirotomo Saitsu[1] · Kuniaki Seyama[2] · Noriko Miyake[1] · Naomichi Matsumoto[1]

NGS studies on genotype reaveled even more complex biology

Mosaic and Intronic Mutations in *TSC1/TSC2* Explain the Majority of TSC Patients with No Mutation Identified by Conventional Testing

Magdalena E. Tyburczy[1], Kira A. Dies[2], Jennifer Glass[3], Susana Camposano[4], Yvonne Chekaluk[1], Aaron R. Thorner[5], Ling Lin[5], Darcy Krueger[6], David N. Franz[6], Elizabeth A. Thiele[4], Mustafa Sahin[2], David J. Kwiatkowski[1]*



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Mosaicism and analysis of skin biopsies

Mutations were mosaic in 26 of 45 (58%) subjects in whom mutations were identified, ranging from 0.21% to 34% mutant allele frequency (AF) (Table 1 and Fig 1 and S2 Table). Seventeen subjects had mosaicism with very low mutant AF, < 5%. These mutations consisted of five indel, five nonsense, two splice site, two missense, and three genomic deletion mutations.

Tyburczy et al. PLOS Genet 2015

NGS studies on genotype reaveled even more complex biology

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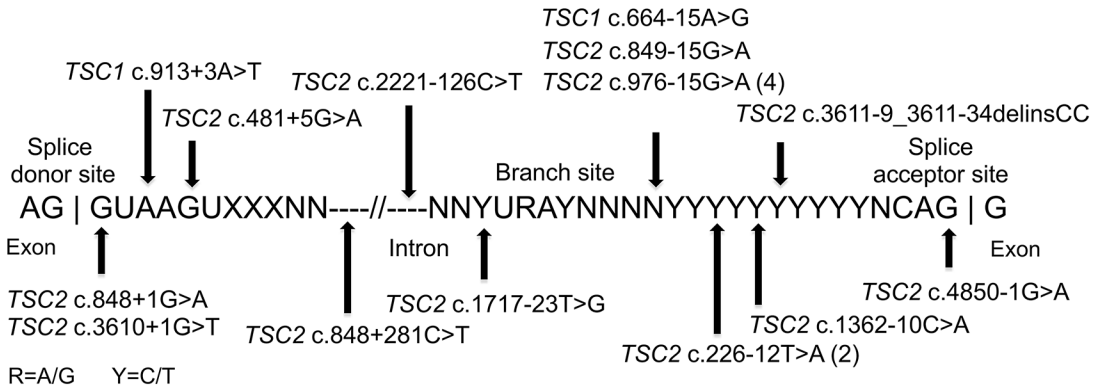
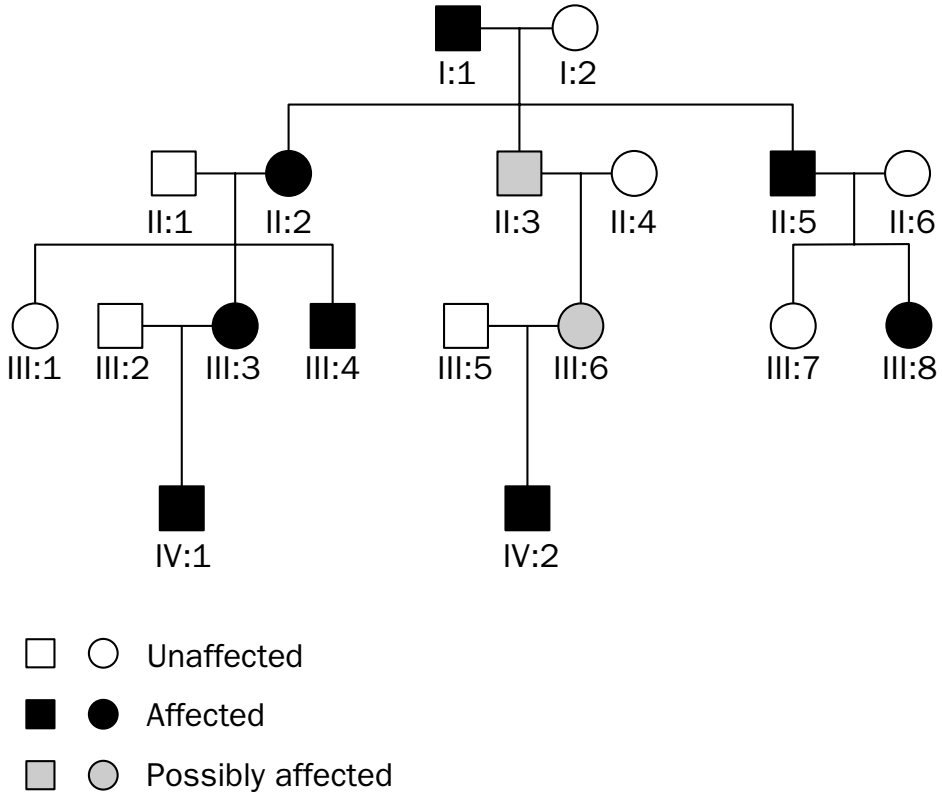


Fig 2. Intronic mutations in 18 TSC NMI subjects. The locations of 18 splice site mutations identified are shown relative to the canonical consensus sequences present at the 3' exon region, the branch site, and the 5' exon region.

.. Still not solved/understood

1- Intra familial variability (phenotype)

2- Absence of any clinical sign = 'Non penetrance'



Osborne Lancet 2000;355:1698

To Sum up

1- NGS technology: detection mutation rate = 100%

If not found: other diagnosis or somatic ?

2- Inheritance /Transmission

parent with identified mutation/deletion:

1- 50% risk - dominant - for any coming pregnancy BUT limitation on prediction for coming phenotype severity

2- option available for pre implantory genetic diagnosis - PGD

3- option for prenatal diagnosis at 11 weeks
- Chorionic villous sample (CVS)

Thank you for your attention

To Ana Jansen

For parents, children and relatives

