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Variations in Clinical Findings of Patients with Identical Tuberous Sclerosis Gene Mutations

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We herein report on 3 nonsense and 1 deletion mutations in TSC1 or TSC2 genes in 10 Japanese individuals with various phenotypes of tuberous sclerosis complex (TSC). Even having identical mutations, some patients suffered from intractable epilepsy and showed severe intellectual and behavioral disabilities, while others were intellectually normal and epilepsy was absent or easily controlled. Review of the data of these and other 196 cases in the literature revealed that certain missense mutations are characteristic in yielding mild phenotype, particularly at the GTPase-activating protein domain of TSC2 gene. Non-truncating mutations in this functionally important domain may tend to cause clinical symptoms, while those in the other regions may remain subclinical and interpreted as polymorphism. On the other hand, many truncating and missense mutations of TSC genes could cause either mild or severe phenotypes. Somatic mosaicism, either in the initial or second-hit mutations, cannot explain the whole feature of this clinical variability. Mutation database with sufficient information of clinical manifestations and family history is necessary to draw reliable conclusion for genetic counseling, as well as to evaluate any modifying factors on the clinical severity other than the TSC gene mutations.

Key words: hamartin; haploinsufficiency; GTPase-activating protein domain; mammalian target of rapamycin; tuberin

Tuberous sclerosis complex (TSC) is a disorder with autosomal dominant inheritance, characterized by development of hamartomatous lesions in various organs and a wide range of neurological abnormalities. Two genes causing TSC have been identified: TSC1 is located at chromosome 9q34 and encodes hamartin, and TSC2 is at chromosome 16p13.3 and encodes tuberin. These proteins interact and form a cytoplasmic heterodimer complex that inhibits the phosphorylation of mammalian target of rapamycin (mTOR) pathway (Swiech et al., 2008), and play a cardinal role in the regulation of differentiation, growth, and proliferation of various cell types.

The disruption of mTOR pathway could explain the extracerebral complications; i.e., an

Abbreviations: CT, computed tomography; GAP, GTPase-activating protein; MR, magnetic resonance; mTOR, mammalian target of rapamycin; TSC, tuberous sclerosis complex

inherited mutation is carried in one allele, and the second mutation in the other allele of TSC genes have been identified in the hamartomatous lesions of TSC patients. However, this second hit has not been identified in the dysplastic cerebral tissues of TSC patients, including cortical tubers (Niida et al., 2001; Ramesh et al., 2003). In addition, studies on the genotype-phenotype correlations in TSC mutants have revealed that mutations in TSC2 gene tend to cause more severe neurological outcome than TSC1 gene mutations (Dabora et al., 2001; Sancak et al., 2005; Au et al., 2007), resulting in lower intelligence quotient and higher prevalence of autistic traits and infantile spasms (Lewis et al., 2004). Variation of severity can be seen even between monozygotic twins with identical TSC gene mutations (Gomez et al., 1982; Martin et al., 2003; Humphrey et al., 2004). These facts have made the pathogenesis of cerebral lesions difficult to understand and the prognosis of individual TSC patients hard to predict.

In order to have a better insight into the clinical variability of TSC patients, particularly regarding neurological complications, we reviewed our Japanese series of TSC gene mutations and compared the clinical features of patients with identical mutations. In addition, we reviewed the around 400 TSC1 and 1,000 TSC2 mutations that have been reported to date*, and identified 5 TSC1 mutations and 33 TSC2 mutations that have been found in different members of a single family or in individuals from different families, for whom clinical data were also provided in the literature (Smalley et al., 1994; Vrtel et al., 1996; Wilson et al., 1996; Jobert et al., 1997; Maheshwar et al., 1997; Au et al., 1998; Beauchamp et al., 1998; Kwiatkowska et al., 1998; Verhoef et al, 1998, 1999; Niida et al., 1999; Zhang et al., 1999; Yamashita et al., 2000; Dabora et al., 2001; Yamamoto et al., 2002; Martin et al., 2003; Feng et al., 2004; Humphrey et al., 2004; Mayer et al., 2004; Ali et al., 2005; Rok et al., 2005; Choi et al., 2006; Hung et al., 2006; Jansen et al., 2006; Lyczkowski et al., 2007). We found that certain mutations were identified in patients with various severities, or otherwise were common in mildly affected patients. Analysis on the mutation types, either truncating or missense, also revealed that missense mutations were clustered in the GTPase-activating protein (GAP) domain of TSC2 gene. Significance of these findings is discussed.

* http://chromium.liacs.nl/LOVD2/TSC/home.php?select_db=TSC1 or db=TSC2

Subjects and Methods

In the series of 140 Japanese patients, clinically suspected with either definite, probable or possible TSC (Roach et al., 1998), mutation analysis of TSC1 and TSC2 genes in blood samples was performed by means of polymerase chain reaction-single strand conformation polymorphism analysis as described in previous reports (Zhang et al., 1999; Yamamoto et al., 2002). We found TSC1 mutation in 20 patients, and TSC2 mutation in 49 patients. Out of the 20 TSC1 mutations, there were 9 missense mutations, 4 nonsense mutations, 4 insertions, 3 deletions. The 49 TSC2 mutations included 25 missense mutations, 5 nonsense mutations, 3 insertions, 9 deletions, and 7 mutations in the intron sequence that were considered to result in splicing errors. Among these, there were 4 sets of patients with the same type of the mutations. These identical mutations were present in 3 patients having the same TSC1 mutations, and 7 patients in 3 sets of TSC2 mutations. Clinical data of these 10 patients were collected in terms of cutaneous, cardiac, renal, and liver involvement, as well as the presence and nature of epilepsy, intellectual assessment, autistic traits, and findings on neuroimaging (computed tomography (CT) in 9, and magnetic resonance (MR) imaging in 6 patients). Some data of patients 1, 2, 5, 6 and 7 have been published previously (Zhang et al., 1999; Feng et al., 2004).

Results

TSC1 c.1746C>T (p.R509X) mutation (Table 1)

Patient 1: This patient, a 41-year-old woman, suffered from infantile spasms when she was 2 months old. Adrenocorticotropin was effective

Table 1. Clinical features of patients with *TSC1* c.1746C>T (p.R509X) mutation

Symptom	Patient 1	Patient 2	Patient 3
Age examined (yr)	41	27	62
Sex	Female	Male	Male
Heredity	Sporadic	Familial	Sporadic
Type of epileptic seizures	Infantile spasms → complex partial seizures	(No seizures)	Complex partial seizures
Onset of seizure	2 mo	Not applicable	School age
Control of seizure	Good	Not applicable	Good
Mental retardation	Severe (DQ < 10)	–	–
Autism	–	–	–
Impaired social interaction	–	–	–
Stereotypical behavior	–	–	–
Other autistic behaviors	–	–	–
Hypomelanotic macule	–	+	+
Facial angiofibroma	–	–	+
Shagreen patch	–	Not described	+
Renal angiomyolipoma	+	+	+
Periventricular calcification on brain CT	+	+	+
Cortical tuber	+	+	+
Other symptoms	Triplegia, mastoadenoma		Liver cyst, adrenal nodule (1.5 mm), ungual fibroma

–, absent; +, present; DQ, developmental quotient; mo, months; yr, years.

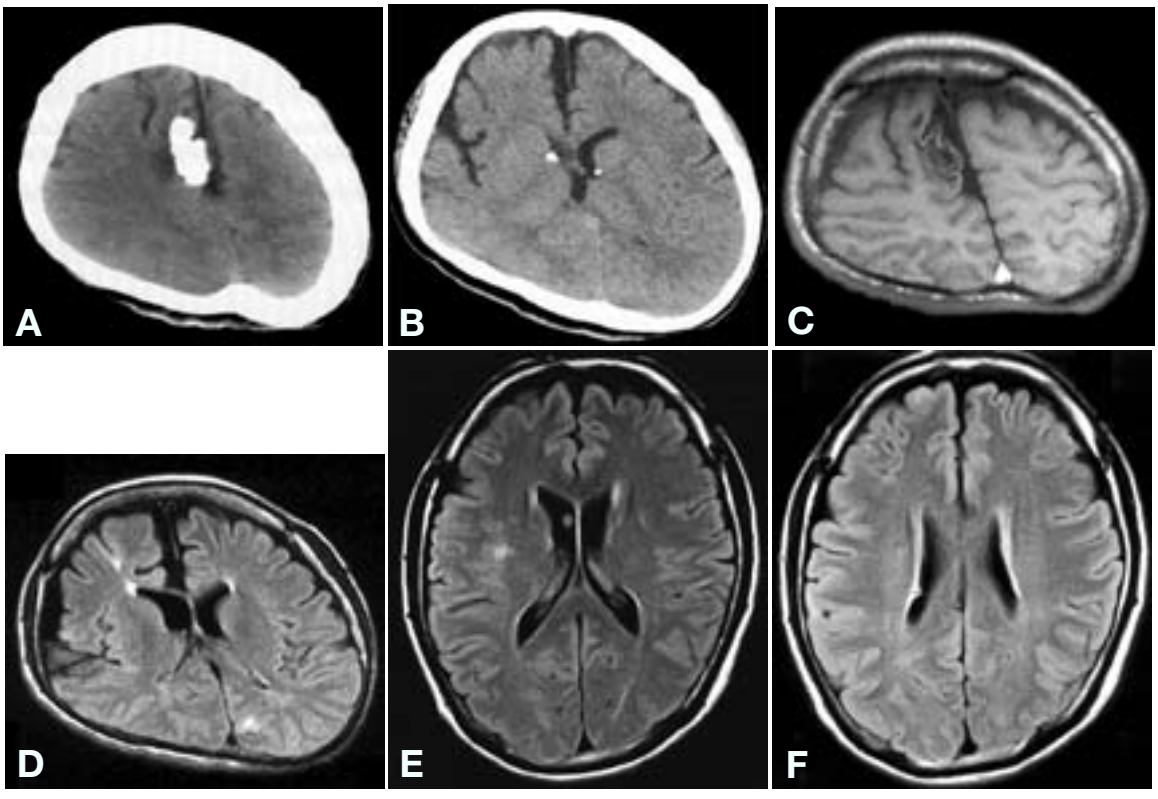


Fig. 1. Neuroimaging of patients with *TSC1* p.R509X mutation. **A** to **D**: Patient 1. **E** and **F**: Patient 2. **A** and **B**: computed tomography (CT). **C** to **F**: magnetic resonance (MR) imaging [**C**: T1 weighted image, **D** to **F**: Fluid-attenuation recovery images].

Table 2. Clinical features of patients with *TSC2* c. 3355C>T (p.Q1119X) mutation

Symptom	Patient 4	Patient 5
Age examined (yr)	8	23
Heredity	Sporadic	Sporadic
Type of epileptic seizures	Infantile spasms	Right hemiconvulsion
Onset of seizure	6 mo	13 mo (with fever)
Control of seizure	Good	Only once
Mental retardation	Severe (DQ 17)	Normal intelligence
Autism	+	–
Impaired social interaction	+ (disobedience)	–
Stereotypical behavior	++	–
Other autistic behaviors	Panic, self injury	–
Hypomelanotic macule	+	–
Facial angiofibroma	+	–
Renal angiomyolipoma	–	2 (diameter 1 cm, 19 yr)
Periventricular calcification on brain CT	8	4
Cortical tuber	+	Not available
Other symptoms	Cardiac rhabdomyoma, Wolff-Parkinson-White syndrome	Renal cystic dysplasia with cancer-like lesions (3 mo), angiomyolipoma of liver (15 yr)

–, absent; +, present; ++, marked; DQ, developmental quotient; mo, months; yr, years.

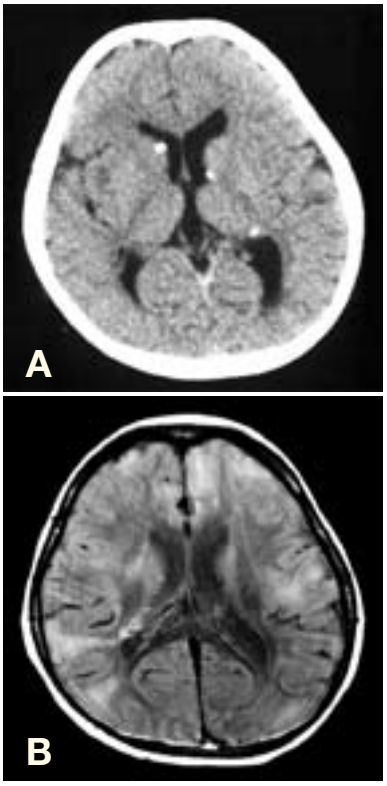


Fig. 2. Neuroimaging of Patient 4 with *TSC2* p.Q1119X mutation.
A: CT image.
B: Fluid-attenuation recovery MR image.

in treatment of her epilepsy, and subsequent complex partial seizures have been controlled well. Paraparesis and left hemiparesis have been noted, and a large cortical tuber with calcification at the medial part of right frontal lobe has been revealed on neuroimaging (Figs. 1A to D).

Patient 2: The patient is a 27-year-old man, who has hypomelanotic macules, but has not shown intellectual disability or epileptic seizures. A small number of subependymal nodules and cortical tubers are noted (Figs. 1E and 1F), but less evident compared to Patient 1. Clinical data on other family members were not available.

Patient 3: This 62-year-old man is intellectually normal. He suffered from epilepsy in his school years, which was well controlled by antiepileptics thereafter.

TSC2 c.3355C>T (p.Q1119X) mutation (Table 2)

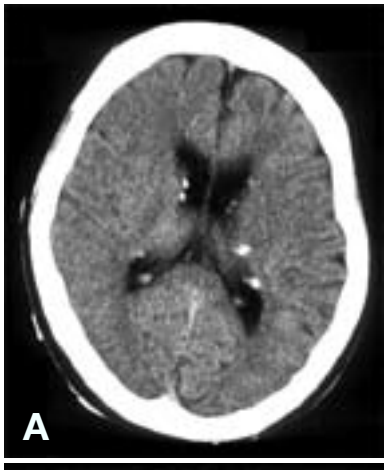
Patient 4: This 8-year-old girl suffered from infantile spasms at age 6 months. Residual seizures are currently controlled by potassium bromide, but she shows severe intellectual disability and autistic behavior including panic and self injury. Numerous cortical tubers and subependymal nodules are noted on neuroimaging (Fig. 2).

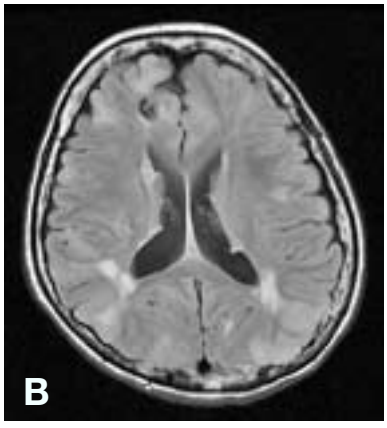
Patient 5: A large renal tumor, histologically identified as cystic dysplasia with renal cancer, was resected when this girl was 3 months old. She suffered from febrile hemiconvulsion at age 13 months, but seizure never recurred thereafter, and electroencephalography remained normal. Intracranial calcifications and hepatic angiomyolipoma were revealed at the age of 15 years. She shows normal intelligence and has graduated from college.

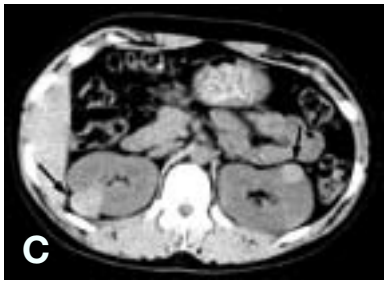
Table 3. Clinical features of patients with *TSC2* c. 4393 C>T (p. R1459X) mutation

Symptom	Patient 6	Patient 7
Age of examined patient (yr)	18	19
Sex	Male	Female
Heredity	Familial	Sporadic
Type of epileptic seizures	Complex partial seizures	Complex partial seizures
Onset of seizure	3 yr and 2 mo	1 yr
Focus in electroencephalography	Right fronto-parietal, left frontal	Right frontal
Control of seizure	Fair (→ twice a year since 12 yr)	Poor → excision of right frontal lobe (17 yr) → good
Mental retardation	IQ 33	IQ 41
Autism	+	+
Impaired social interaction	+	+ (likes to play alone)
Stereotypical behavior	Preoccupation with special events	Compulsive
Other autistic behaviors	Difficult to adapt to changes	Anxious, easily excited
Hypomelanotic macule	Not available	+
Facial angiofibroma	Not available	+
Renal angiomyolipoma	+	many → embolization
Periventricular calcification on brain CT	+	several, 1 in right frontal
Brain MRI	Cortical tubers, bilateral subependymal nodules	Cortical tubers

+, present; IQ: intelligence quotient; mo, months; yr, years.







TSC2 c.4393C>T (p.R1459X) mutation (Table 3)

Patient 6: This patient suffered from epilepsy with complex partial seizures since age 3. He is now 18 years old, and seizures still appear twice per year despite treatment. Moderate intellectual disability and autistic traits have been evident. Multiple subependymal nodules (Fig. 3A) and cortical tubers are noted. Data on other family members with TSC were not available.

Patient 7: This girl suffered from intractable, complex partial seizures since 1 year of age, which disappeared after resection of the right frontal lobe at age 17. She has moderate intellectual disability, likes to play on her own, and shows anxious and compulsive behaviors. Numerous cortical tubers (Fig. 3B) are noted on MR imaging. Renal angiomyolipoma (Fig. 3C) were treated with arterial embolization at age 17 years.

Fig. 3. Radiological findings of patients with *TSC2* p.R1459X mutation.
A: brain CT of Patient 6.
B: brain MR imaging of Patient 7.
C: abdominal CT image of Patient 7.
Arrows in **C** indicate the angiomyolipoma in bilateral kidneys.

Table 4. Clinical features of patients with *TSC2* c.5238–5255del (p. del 1746 HIKRLR) mutation

Symptom	Patient 8	Patient 9	Patient 10
Age of examined patient (yr)	3	6	13
Sex	Male	Female	Male
Heredity	Sporadic	Sporadic	Sporadic
Type of epileptic seizures	Infantile spasms	CPS, infantile spasms	Infantile spasms
Onset of seizure	4 mo	6 mo	5 mo
Focus in electroencephalography		Right front-parietal, right frontal, right central, left parieto-temporal?	
Control of seizure	Good (since 2 yr)	Fair (5–10 times/day)	Once in 3 mo
Mental retardation	Moderate–severe	Severe (DQ 35 at 2 yr)	Moderate (IQ 45–50)
Autism	–	+	–
Impaired social interaction	–	+ (lack of social reciprocity, failure to use eye-to-eye gaze)	+ (until 2–3 yr) → – (since 5 yr)
Communication disturbance	–	+ (nonverbal)	–
Stereotypical behavior	–	Not available	+ (mild)
Other autistic behaviors	–		
Hypomelanotic macule	+	+	+
Facial angiofibroma	+	–	–
Renal angiomyolipoma	–	–	–
Other renal lesions			One renal cyst in the right kidney, bilateral lipoma
Periventricular calcification on brain CT	+	+	+
Brain MRI	Not available	+	Subependymal nodules, multiple subcortical hypomyelination
Other symptoms	Cardiac rhabdomyoma, fibroma on the occiput		

–, absent; +, present; CPS, complex partial seizures; DQ, developmental quotient; IQ, intelligence quotient; mo, months; yr, years.

TSC2 c.5238–5255del (p.del 1746HIKRLR) mutation (Table 4)

Patient 8: This patient, a severely retarded but not autistic boy, suffered from infantile spasms at age 4 months, which evolved into localization-related epilepsy and remained intractable.

Patient 9: This patient also developed infantile spasms and complex partial seizures at age 5 months. The seizures have been intractable, and she shows severe intellectual disability and autism.

Patient 10: This boy developed infantile spasms when he was 5 months old. He gained the ability to utter meaningful words since 2 years of age. He was hyperactive and had difficulties in social relationships during early childhood. However, his social skills improved since he was 5. When he was 13, he was no longer autistic, but showed occasional, compulsive behaviors.

TSC patients with identical mutations in the literature (Table 5)

By adding the 10 patients in this study, we could identify 5 TSC1 mutations in 17 patients, and 33 TSC2 mutations found in 179 patients (Table 5), which were found in 2 or more individuals. Most of these groups with identical mutations, either truncating or non-truncating, included both individuals with normal and disabled intelligence. In contrast, individuals with L61R mutation in TSC1, and K12X, E1542K, 1554Q, G1556S, and 1628X mutations in TSC2, did not show intellectual disabilities, even having multiple cortical tubers. R905Q mutation of TSC2 gene also showed resultant tendency of milder cognitive impairment. Out of the 14 missense mutations in TSC2, 6 were concentrated at exons 34 to 38, which corresponds to the GAP homology domain (Maheshwar et al., 1997).

Table 5. TSC patients with identical mutations in the literature [i]

Reference Patient	Heredity			DNA change	Protein	Age (year)	Sex	Mutation type	Intelligence	Autism	Seizure	Tuber	SEN	SEGA	HPM	AF	PUF	ShgP	AML	RCC	Renal cyst	CR	Others
Lyczkowski (2007)																							
A-I-1 (father)	F	TSC1	exon 4	403T>G	L61R	51	m	MM	IQ 100		Controlled	14	+		+	+	+			+		–	
A-II-1 (sib)	F	TSC1	exon 4	403T>G	L61R	21	f	MM	IQ 101		Occasional	12	+		+	+	+	+		–		–	
A-II-2 (sib)	F	TSC1	exon 4	403T>G	L61R	17	f	MM	IQ 72		Intractable	18	+		+	+	+						
Kwiatkowska (1998)																							
Mother	F	TSC1	exon 15	2105 del AAAG		27	f	FS					+			+	+	+					
Sib 1	F	TSC1	exon 15	2105 del AAAG		7		FS	Severe MR		+		+										
Sib 2	F	TSC1	exon 15	2105 del AAAG		3		FS	MR (–)		–				+								
Hung (2006)																							
61	F	TSC1	exon 15	1525C>T	R509X	3.5	m	NM	LD		+	+			+	+	–	–	–			–	
Zhang (1999), the present study																							
3; Patient 1	S	TSC1	exon 15	1746 C>T	R509X	41	f	NM	Severe MR	–	IS, partial Sz	+	++		–	–		–	+				
26; Patient 2	F	TSC1	exon 15	1746 C>T	R509X	27	m	NM	MR (–)	–	–	+	+		+	–			+				
The present study																							
Patient 3	S	TSC1	exon 15	1746 C>T	R509X	62	m	NM	MR (–)	–	+	+			+	+	+	+	+				Liver cyst s/o, adrenal nodule
Niida (1999)																							
Family 26	F	TSC1	exon 17	2295 C>T	R692X			NM	MR		+	Brain image (+)			+	+	Var	Var	–			–	(retinal HM)
Family 38	F	TSC1	exon 17	2295 C>T	R692X			NM	MR (–)/LD		+	Brain image (+)			–	–	+	–	+			NA	
Lyczkowski (2007)																							
C-I-1	F	TSC1	exon 20	2790insG		88	m	FS	NA		NA	NA	NA	NA		+	+	+		NA	+		
C-II-2	F	TSC1	exon 20	2790insG		55	f	FS	NA		NA	NA	NA	NA	+	+	+			NA	NA	NA	
C-II-3	F	TSC1	exon 20	2790insG		52	m	FS	NA		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
C-III-1	F	TSC1	exon 20	2790insG		31	m	FS	IQ 87		Intractable	+	+	+		+	+		–	–	–	–	
C-III-2	F	TSC1	exon 20	2790insG		30	m	FS	NA		Controlled	+	+	+	+				–	–	–		Retinal HM
C-III-3	F	TSC1	exon 20	2790insG		28	m	FS	Impaired		Intractable	NA	NA	NA	+	+	+	+	–	–	–	–	
C-III-4	F	TSC1	exon 20	2790insG		27	m	FS	IQ 79		Controlled	9	+		+				+			–	
C-III-5	F	TSC1	exon 20	2790insG		23	f	FS	IQ 73		Intractable	45	+		+				–	–	–		Retinal HM
Vrtel (1996)																							
Father	F	TSC2	exon 1	52A>T	K12X (1–49 del?)	30	m	IF?							–								Gingival fibroma
Son	F	TSC2	exon 1	52A>T	K12X (1–49 del?)	1.5	m	IF?	MR (–)						+							+	
Verhoef (1999)																							
Family 4 sib 1	F	TSC2	intron 1	156+1G>A	31X		m	FS	MR (–)		IS	+	+		+	+							
Family 4 sib 2	F	TSC2	intron 1	156+1G>A	31X		m	FS	Mild MR		+	+	+		+	+						+	Gingival fibroma

Table 5, continued to the next page. Footnotes, refer to Table 5 [vii], end of this table.

Table 5. TSC patients with identical mutations in the literature [ii]

Reference Patient	Heredity		DNA change	Protein	Age (year)	Sex	Mutation type	Intelligence	Autism	Seizure	Tuber	SEN	SEGA	HPM	AF	PUF	ShgP	AML	RCC	Renal cyst	CR	Others
Wilson (1996)																						
TSC-028-1	F	TSC2 exon 12	1365G>C	M449I			MM	MR(–)	Beh/ LD (–)	+		CT (+)		+	–	+	–					Eye (+)
TSC-028-2	F	TSC2 exon 12	1365G>C	M449I			MM	MR		+				+	+	–	–					Eye (–)
TSC-028-3	F	TSC2 exon 12	1365G>C	M449I			MM					CT (–)			+	+		Renal ab (+)				
TSC-028-4	F	TSC2 exon 12	1365G>C	M449I			MM	MR		+		CT (+)		+	–	–	–					Eye (–)
Beauchamp (1998)																						
F17-01	F	TSC2 exon 12	1348G>T	E450X	3		NM	Mild MR		+	Brain findings (+)			+	–	–	–	NA			–	
F16-01	F	TSC2 exon 12	1348G>T	E450X	3		NM	MR(–)		+	Brain findings (+)			+	–	–	–	NA			–	
Niida (1999)																						
326	S	TSC2 exon 12	1348G>T	E450X			NM	MR		+	Brain image (+)			+	–	–	–	–			+	Retinal HM
Jobert (1997)																						
B17 II-1 (father)	F	TSC2 exon 14	1462del33 mRNA	482–492 del		m	IF	MR (–)		+	MRI (+)	CT (+)		+	+	+	+	Renal ab (+)				Eye (–)
B17 III-2 (son)	F	TSC2 exon 14	1462del33 mRNA	482–492 del		m	IF	MR		+				+	+	+	+	Renal ab (–)				
B95 I-1 (mother)	F	TSC2 exon 14	1462del33 mRNA	482–492 del		f	IF	MR (–)		+	MRI (+)	CT (+)		–	+	+	–	Renal ab (+)				Eye (+)
B95 II-1 (son)	F	TSC2 exon 14	1462del33 mRNA	482–492 del		m	IF	MR		+	MRI (+)	CT (+)		+	+	+	+	Renal ab (+)				Eye (+)
Au (1998)																						
TS94-96		TSC2 exon 14	1513C>T	R505X			NM	MR	Beh/ LD (+)	+	+	+		+	+	+	+				+	Eye (–)
Wilson (1996)																						
TSC-037-1	F	TSC2 exon 14	1531C>T	R505X			NM	MR (–)		+		CT (+)			+	+						
TSC-037-2	F	TSC2 exon 14	1531C>T	R505X			NM	MR (–)	Beh/ LD (+)	+				+	+	+	–					
Hung (2006)																						
20	S	TSC2 exon 14	1513C>T	R505X		m	NM	MR (–)		+	+			+	+	–	–	(+)?				
57	S	TSC2 exon 14	1513C>T	R505X		f	NM	MR		+	+			+	+	–	+	–				
Niida (1999)																						
53	F	TSC2 exon 16	1832G>A	R611Q			MM	MR (–)		+	Brain image (+)			+	+	–	–	NA			+	
Au (1998)																						
TS94-31		TSC2 exon 16	1832G>A	R611Q			MM	MR	Beh/ LD (+)	+	+	+		+	+	–	–	–		–	+	Eye(+)
TS93-29		TSC2 exon 16	1832G>A	R611Q			MM	MR		+	+	+		+	–	–	–	–		–	–	

Table 5, continued to the next page. Footnotes, refer to Table 5 [vii], end of this table.