Yonago Acta medica 2009;52:57–72

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.

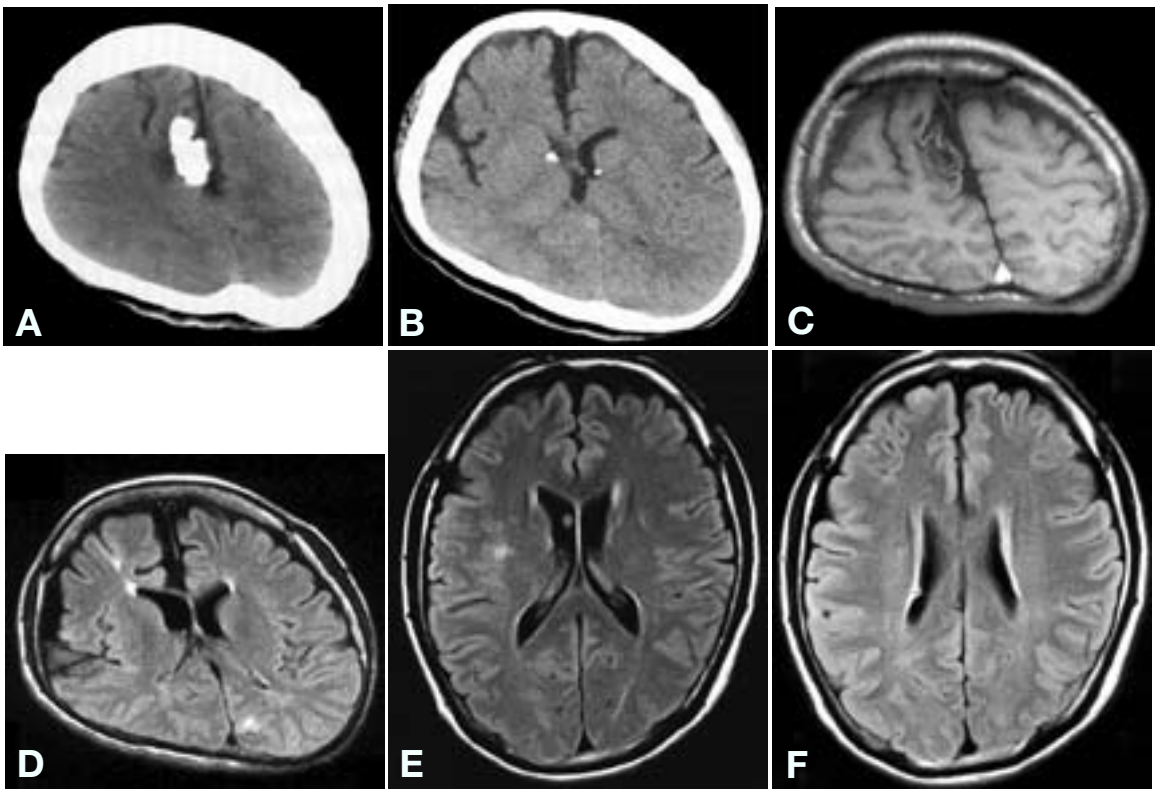


Fig. 1. Neuroimaging of patients with *TSC1* p.R509X mutation. **A** to **D**: Patient 1. **E** and **F**: Patient 2. **A** and **B**: computed tomography (CT). **C** to **F**: magnetic resonance (MR) imaging [**C**: T1 weighted image, **D** to **F**: Fluid-attenuation recovery images].

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.

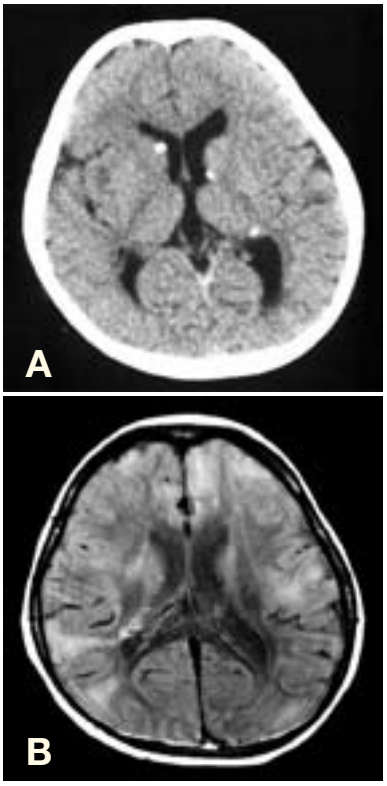


Fig. 2. Neuroimaging of Patient 4 with *TSC2* p.Q1119X mutation.
A: CT image.
B: Fluid-attenuation recovery MR image.

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)

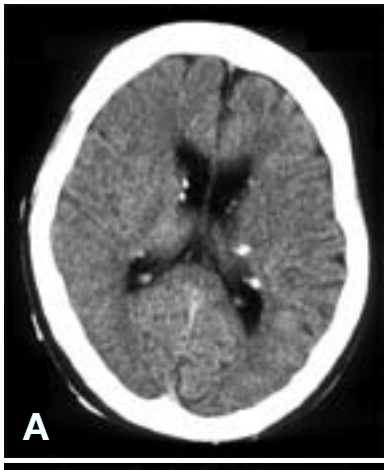
Patient 4: This 8-year-old girl suffered from infantile spasms at age 6 months. Residual seizures are currently controlled by potassium bromide, but she shows severe intellectual disability and autistic behavior including panic and self injury. Numerous cortical tubers and subependymal nodules are noted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

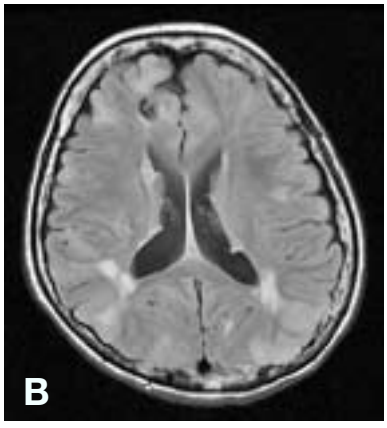
Patient 5: A large renal tumor, histologically identified as cystic dysplasia with renal cancer, was resected when this girl was 3 months old. She suffered from febrile hemiconvulsion at age 13 months, but seizure never recurred thereafter, and electroencephalography remained normal. Intracranial calcifications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

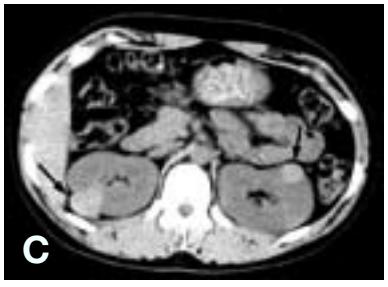
Table 3. Clinical features of patients with *TSC2* c. 4393 C>T (p. R1459X) mutation

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.

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

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-382	F	TSC2	exon 16	1849C>T	R611W			MM	MR		+		CT (+)		+	+	+	+	Renal ab (+)				Eye (–)
Ali (2004)																							
TS-07	S	TSC2	exon 16	1832G>A	R611Q	9		MM	MR (–)	NA	+	NA	NA	NA	+	+	–	+	NA			NA	
TS-23	S	TSC2	exon 16	1831C>T	R611W	2		MM	MR	+	+	+	+	–	+	–	–	+	NA			NA	
Hung (2006)																							
29	S	TSC2	exon 16	1832G>A	R611Q		f	MM	MR		+	+			+	–	–	+	–				
Zhang (1999)																							
22	F	TSC2	exon 16	1850G>A	R611Q	6	f	MM	MR(–)		+		+		+	–							
Beauchamp (1998)																							
F03-01	F	TSC2	exon 16	1832G>A	R611Q	5		MM	MR(–)		+	Brain findings (+)			+	–	–	–	–			–	
Hung (2006)																							
82	S	TSC2	exon 17	1939G>A	D647N		m	MM	MR (–)		+	–			–	–	–	–	–				
Zhang (1999)																							
32	S	TSC2	exon 17	1957G>A	D647N	2	m	MM	NA		+		+		+	NA							
Zhang (1999)																							
21	F	TSC2	exon 20	2324T>G	V769E	44	f	MM	MR (++)		NA		NA		+	+							
2	S	TSC2	exon 20	2324T>G	V769E	32	m	MM	MR (++)		+		+		+	+							
Verhoef (1999)																							
Family 3 sib 1	F	TSC2	intron 20	2374-2 A>C			f	SP	Moderate MR		+		(+)?		+	+		+				+	
Family 3 sib 2	F	TSC2	intron 20	2374-2 A>C			f	SP	Severe MR		+		(+)?			+	+		+			+	
Beauchamp (1998)																							
F08–01	F	TSC2	exon 23	2714G>A	R905Q	10		MM	MR (–)		+	Normal	Normal		+	+	+	+	NA			NA	
Jansen (2006)																							
Family A ($n = 25$)	F	TSC2	exon 23	2714G>A	R905Q	6 – 61		MM	MR (–) 12, LD 10, mild CI 3		15/25	5/15 WML	1	1	23/25				1		1	0/12	0/16
Family B ($n = 3$)	F	TSC2	exon 23	2714G>A	R905Q	NA		MM	MR (–) 1, mild CI 2		2	2			3	1			NA			NA	
Family C ($n = 9$)	F	TSC2	exon 23	2714G>A	R905Q	NA		MM	MR (–) 6, impaired 1, severe CI 1		6	1/1 examined			8	1	2	2				1/1 examined	
Family D ($n = 1$)	F	TSC2	exon 23	2714G>A	R905Q	NA		MM	MR (–)		+	–			+			+	NA		NA	NA	
Family E ($n = 1$)	F	TSC2	exon 23	2714G>A	R905Q	NA		MM	MR (–)		+	abn			+				–		–	–	
P1	S	TSC2	exon 23	2713C>T	R905W	NA		MM	NA		–	+	+		+	+			NA		NA	NA	
P2	S	TSC2	exon 23	2713C>T	R905W	NA		MM	Mild CI		Remitted	+	+		+	+		+	–		–	–	Retinal HM
P3	S	TSC2	exon 23	2713C>T	R905W	NA		MM	Moderate CI		Active	+	+		+				–		–	+	
P4	S	TSC2	exon 23	2713C>T	R905W	NA		MM	Severe CI		Active LGS	+	+		+				–		–	–	
P5	S	TSC2	exon 23	2713C>T	R905W	NA		MM	MR(–)		–	+		+	+	+					+	–	
P6	S	TSC2	exon 23	2713C>T	R905W	NA		MM	Severe CI		+	+	+		+	+			–			NA	
P7	S	TSC2	exon 23	2713C>T	R905W	NA		MM	Severe CI		+	NA	NA	NA					NA		NA	NA	
P8	S	TSC2	exon 23	2713C>T	R905W	NA		MM	Mild CI		Remitted IS	+	+		+	+	+	+	+		–	NA	

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 [iv]

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
Au (1998)																							
TS95-12		TSC2	exon 23	2713C>T	R905W			MM	MR		+	+	+		+	–	–	–	–		–	–	
Yamamoto (2002)																							
8	S	TSC2	exon 23	2713C>T	R905W	21	f	MM	IQ 40		+	+	+		+	+				Renal tumor			
Yamashita (2000)																							
1		TSC2	exon 23	2713C>T	R905T			MM	Moderate MR		IS				+	+							
Yamamoto (2002)																							
7	S	TSC2	exon 23	2713C>G	R905G	3	m	MM	Neurological symptoms (+)		IS	+	+		+							+	
Hung (2006)																							
64	S	TSC2	exon 26	2974C>T	Q992X		m	NM	MR (–)		+	+			+	–	–	–	–				
Beauchamp (1998)																							
S17-01	S	TSC2	exon 26	2974C>T	Q992X	5		NM	Moderate MR		+	Brain findings (+)			+	+	–	–	–			NA	Retinal findings (+)
The present study																							
Patient 4	S	TSC2	exon 28	3355C>T	Q1119X	8	f	NM	DQ 17	+	IS	+	+		+	+			–			+	
Feng (2004), the present study																							
4, Patient 5	S	TSC2	exon 28	3355C>T	Q1119X	23	f	NM	MR (–)	–	Febrile Sz	NA	+		–	–			+	Renal tumor			Liver AML
Humphrey (2004)																							
Twin A	F	TSC2	exon 29	3043delC	truncation 1210?	3	m	FS	DQ 45	+	Partial Sz	Extensive											
Twin B	F	TSC2	exon 29	3043delC	truncation 1210?	3	m	FS	DQ 71	Partial	IS	+											
Wilson (1996)																							
TSC-001-1 father	F	TSC2	exon 29	3616C>T	R1199W		m	MM		Beh/ LD (+)	+	MRI (–)	CT (–)		+	–	–	–					
TSC-001-2 sib	F	TSC2	exon 29	3616C>T	R1199W			MM				MRI (+)	CT (–)		+	+	–						
TSC-001-3 sib	F	TSC2	exon 29	3616C>T	R1199W			MM	MR		+	MRI (–)	CT (–)		+	–	–	+					Eye (–)
Lyczkowski (2007)																							
D-I-1	F	TSC2	exon 33	4422–4423del		NA	m	FS	NA		Controlled	NA	NA	NA	+				NA	NA	NA	NA	
D-II-2	F	TSC2	exon 33	4422–4423del		NA	m	FS	NA		Controlled	NA	NA	NA	+				NA	NA	NA	NA	
D-II-3	F	TSC2	exon 33	4422–4423del		NA	f	FS	NA		Controlled	NA	NA	NA	+				NA	NA	NA	NA	
D-III-1	F	TSC2	exon 33	4422–4423del		9	f	FS	NA		IS	39	+	+	+	+			–			+	
D-III-2	F	TSC2	exon 33	4422–4423del		6	f	FS	IQ 85		IS	+	+	+	+	+			–			+	
D-III-3	F	TSC2	exon 33	4422–4423del		3	m	FS	IQ 101		IS	49	+	+	+				+				

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 [v]

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
Niida (1999)																							
171	S	TSC2	exon 33	4421–4422del	R1474fs 1521X			FS	MR		+	Brain image (+)			+	+	+	+	NA		NA	NA	
311	S	TSC2	exon 33	4422–4423del	R1474fs 1521X			FS	MR (–)		+	Brain image (+)			+	–	–	–	NA		NA	NA	
Niida (1999)																							
187	F	TSC2	exon 33	4375C>T	R1459X			NM	MR		+	Brain image (+)			+	+	–	+	–		–	+	Retinal HM
Zhang (1999), the present study																							
6, Patient 6	F	TSC2	exon 33	4393C>T	R1459X	18	m	NM	IQ 33	+	Controlled	+	+		NA	NA			+				
8, Patient 7	S	TSC2	exon 33	4393C>T	R1459X	19	f	NM	IQ 41	+	Surgery	+	+		+	+			+				
Smalley (1994)																							
n = 17	F	TSC2	exon 34	4508A>C	Q1503P			MM	IQ<70 4/17			(–) in 10	–										Psychiatric disorder 13/17
Wilson (1996)																							
TSC-422-1	F	TSC2	exon 34	4519–4547 dup	L1510fs 1541X			FS	MR		+		CT (+)		+	+	–	+	Renal ab (+)				Eye (–)
TSC-422-2	F	TSC2	exon 34	4519–4547 dup	L1510fs 1541X			FS	NA		–				+	+	+	+	Renal ab (+)				Eye (–)
Jansen (2006)																							
Family F II-3	F	TSC2	exon 35	4642G>A	E1542K			MM	MR (–)		–	+			+	+	+		+				
Family F III-3	F	TSC2	exon 35	4642G>A	E1542K			MM	MR (–)		–	+			+	+	–						
Family F III-5	F	TSC2	exon 35	4642G>A	E1542K			MM	MR (–)		–	+			+	+	–						
Family F IV-3	F	TSC2	exon 35	4642G>A	E1542K			MM	MR (–)		+	+			+	+	–						
Family F IV-4	F	TSC2	exon 35	4642G>A	E1542K			MM	MR (–)		–	+			+	+	–						
Family F IV-7	F	TSC2	exon 35	4642G>A	E1542K			MM	MR (–)		–	+		+	+	+	–						
Family F IV-8	F	TSC2	exon 35	4642G>A	E1542K			MM	MR (–)		–	+			+	+	–						
Family F IV-9	F	TSC2	exon 35	4642G>A	E1542K			MM	MR (–)		–	+			+	+	–						
Family F V-3	F	TSC2	exon 35	4642G>A	E1542K			MM	MR (–)		+	+			–	–	–						
Family F V-4	F	TSC2	exon 35	4642G>A	E1542K			MM	MR (–)		–	+			–	–	–						
Lyczkowski (2007)																							
E-I-1	F	TSC2	exon 35	4662G>A	1554Q	62	m	SP	IQ 106		NA	7	+	+					NA	NA	NA	NA	
E-II-1	F	TSC2	exon 35	4662G>A	1554Q	39	f	SP	IQ 115		NA	12	+						NA	NA	NA	NA	
E-II-2	F	TSC2	exon 35	4662G>A	1554Q	38	m	SP	NA		None		+	+		+			+		+		Hepatic cyst
E-II-4	F	TSC2	exon 35	4662G>A	1554Q	34	f	SP	IQ 105		NA	8	+						NA	NA	NA	NA	
E-III-1	F	TSC2	exon 35	4662G>A	1554Q	5	m	SP	IQ 102		Controlled	14	+	+	+	+		+	–	–	–	+	Retinal pigment
E-III-2	F	TSC2	exon 35	4662G>A	1554Q	3	m	SP	IQ 121		None	18	+	+	+	+			–	–	–	–	

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 [vi]

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
Mayer (2007)																						
IV-3	F	TSC2 exon 36	4684G>A	G1556S	3	m	MM	MR (–)		–		–		+							+	
IV-1	F	TSC2 exon 36	4684G>A	G1556S	12	m	MM	MR (–)?		+	–	–		+				–				
III-3	F	TSC2 exon 36	4684G>A	G1556S		m	MM	MR (–)		–		–		+			+					
III-2	F	TSC2 exon 36	4684G>A	G1556S		f	MM	MR (–)				–		+				+				
Verhoef (1998)																						
Family A	S	TSC2 exon 36	4882 delTT	1628X	14	m	FS	MR (–)		±		+		+	+	+	–	–	–		–	Dental pits
Family B	F	TSC2 exon 36	4882 delTT	1628X	18	f	FS	MR (–)		+		+		+	+	+	+	+	–		+	
Family B mother	F	TSC2 exon 36	4882 delTT	1628X	40	f	FS	MR (–)		+		+		+	+	+	+	+	–		+	
Niida (1999)																						
185	S	TSC2 exon 37	4858C>T	H1620Y			MM	MR		+	Brain image (+)			+	+	–	+	–		–	NA	
Au (1998)																						
TS94–53		TSC2 exon 37	4859A>T	N1620I			MM	MR	Beh/ LD (+)	+	+	NA		+	+	–	–	–		±	–	Eye (–)
Maheshwar (1997)																						
$n = 4$, unrelated	S	TSC2 exon 38	5042C>T	P1675L			MM	MR (–) 1, moderate MR 1, severe MR 2		3								1/1 examined				
Niida (1999)																						
277	S	TSC2 exon 38	5024C>T	P1675L			MM	MR		+	Brain image (+)			+	–	–	–	+		–	+	Retinal HM
Zhang (1999)																						
28	S	TSC2 exon 38	5042C>T	P1675L	3	f	MM	MR		+		+		+	NA							
Feng (2004)																						
7	F	TSC2 exon 38	5042C>T	P1675L	15	f	MM	Severe MR														
Hung (2006)																						
4	S	TSC2 exon 40	5227C>T	R1743W		m	MM	MR (–)		+	+			+	+	–	+	(+)?				
50	S	TSC2 exon 40	5227C>T	R1743W		m	MM	MR		+	+			+	+	–	+	NA				
Choi (2006)																						
12	S	TSC2 exon 40	5227–5244 del	R1743–K1748 del		m	IF			+	Tuber/ SEN (–)			+						+	+	Retinal HM
13	S	TSC2 exon 40	5227–5244 del	R1743–K1748 del		m	IF			+	Tuber/ SEN (+)			+							+	
Martin (2003)																						
Twin M	F	TSC2 exon 40	5256–73 del	1740–1745 del	6	m	IF	Severe MR		IS		+		+	+			+		+		
Twin T	F	TSC2 exon 40	5256–73 del	1740–1745 del	6	m	IF	Severe MR		Partial Sz	+	+		+	+		+	+			+	

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 [vii]

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
Rok (2005)																							
Patient A	S	TSC2	exon 40	5238–5255 del	del 1746 HIKRLR	10	f	IF	MR(+)		Partial Sz	+	+		+	+	–	–	+		–	+	
Patient B	S	TSC2	exon 40	5238–5255 del	del 1746 HIKRLR	9	m	IF	MR(+)		IS	+	+		+	+	–	–	+		–	–	Retinal HM
Patient C	S	TSC2	exon 40	5238–5255 del	del 1746 HIKRLR	9	f	IF	MR(–)		IS	+	+		+	+	–	+	+		–	+	
Patient D	S	TSC2	exon 40	5238–5255 del	del 1746 HIKRLR	1.5	?	IF	MR(–)		IS	+	+		+	–	–	–	–		–	+	
Dabora (2001)																							
n = 9		TSC2	exon 40	5238–5255 del	del 1746 HIKRLR	7 – 28		IF	MR none–severe		All (+)					None–severe			None–extensive				
Niida (1999)																							
113	S	TSC2	exon 40	5328–5255 del	H1746Q/ 1747–52 del			MM/ IF	MR (–)		–	NA			+	+	+	–	+		–	–	
Hung (2006)																							
10	S	TSC2	exon 40	5238–5255 del			f		LD		+	+			+	+	–	–	–				
25	S	TSC2	exon 40	5238–5255 del			m		LD		+	+			+	+	–	+	–				
Beauchamp (1998)																							
S18-01	S	TSC2	exon 40	5328–5255 del	H1746Q/ 1747–52 del	9		MM/ IF	Mild MR		+	Brain findings (+)			+	+	+	+	+			+	
The present study																							
Patient 8	S	TSC2	exon 40	5238–5255 del	del 1746 HIKRLR	3	m	IF	Moderate–severe MR	–	IS	+	+		+	+			–			+	
Patient 9	S	TSC2	exon 40	5238–5255 del	del 1746 HIKRLR	6	f	IF	Severe MR	+	IS, partial Sz	+	+		+	–			–				
Patient 10	S	TSC2	exon 40	5238–5255 del	del 1746 HIKRLR	13	m	IF	Moderate MR	Partial	IS	+	+		+	–			–		+		
Lyczkowski (2007)																							
B-II-1 (twin)	F	TSC2	IVS1, exon 1	1838 bp del		6	f	del	IQ 85		Occasional	65	+		+	+			+		+	+	
B-II-2 (twin)	F	TSC2	IVS1, exon 1	1838 bp del		6	f	del	IQ < 42		IS	60	+			+		+	+		+	+	

–, absent; +, present; ab, abnormality; AF, angiofibroma; AML, angiomyolipoma; Beh, behavioral problems; CI, cognitive impairment; CR, cardiac rhabdomyoma; DQ, developmental quotient; f, female; F, familial; FS, frameshift; HM, hamartoma; HPM, hypopigmented macule; IF, in-frame deletion; IS, infantile spasms; IVS, intervening sequence within an intron; LD, learning disability; LGS, Lennox-Gastaut syndrome; m, male; MM, missense mutation; MR, mental retardation; NA, not available; NM, nonsense mutation; PUF, periungual fibroma; RCC, renal cell carcinoma; S, sporadic; SEGA, subependymal giant cell astrocytoma; SEN, subependymal nodule; s/o, suspect of; ShgP, Shagreen patch; SP, splice mutation; Sz, seizure; TSC, tuberous sclerosis complex; Var, variable; WML, white matter lesion.

Discussion

Previous studies report that either TSC1 or TSC2 mutations are found in 70% to 80% of TSC patients (Hung et al., 2006; Au et al., 2007). The relatively low proportion of positive results in our series may have resulted from a selection bias that doctors in charge of patients with ambiguous clinical phenotype tend to ask the genetic analysis to confirm the diagnosis. However, mutations could be detected even in individuals with partial expression of TSC phenotype, for example, Patient 5 who did not show any cutaneous symptoms.

Some factors have been elucidated that could explain the variability of clinical manifestations in TSC patients, particularly neurological symptoms. These include the mutated gene (TSC1 versus TSC2) (Dabora et al., 2001; Lewis et al., 2004; Sancak et al., 2005; Au et al., 2007), somatic mosaicism, history of infantile spasms (Lewis et al., 2004), and the number and volume of cortical tubers (Jansen et al., 2008). Higher prevalence of severe intellectual disability in TSC patients with TSC2 mutations rather than TSC1 mutations may be related to the fact the tuberin plays a critical role in the phosphorylation of mTOR through its GAP activity and hamartin binds to tuberin and stabilizes the latter (Chong-Kopera et al., 2006). Somatic mosaicism in parents can explain the emergence of more severe phenotypes in their children (Rose et al., 1999). Certain aspects of the data in our series of patients with identical mutations may be related to these mechanisms. However, somatic mosaicism cannot explain the mild phenotype of Patient 2, who was born to an affected TSC mother. In addition, the basis for the differential manifestation of epilepsy and cortical tuber load between siblings, monozygotic twins, and members within a single family, remains unclear. As for the two-hit theory, the second hit as somatic mutations have been detected in angiomyolipoma and giant cell astrocytoma of TSC patients, but not in ungual fibroma, pulmonary lymphangiomyolipomatosis, and cortical tubers (Niida et al., 2001; Mizuguchi et al., 2004). Pathogenic significance of haploinsufficiency in tumor-suppressive genes has been also assumed in neurofibromatosis 1 (Easton et al., 1993; Henske et al., 1996), where marked intrafamilial variation is prevalent similarly to TSC. Apparently there are other factors that modulate the phenotype of individual TSC genotypes. These may include somatic mutation in other factors within the mTOR and other signaling pathways, and genetic background related to the epileptogenesis, or activation of the inflammatory system (Boer et al., 2008). In addition, the significance and pathogenesis of mTOR pathway in the synaptic plasticity (Kelleher et al., 2004), and decreased volume of subcortical gray matter (Ridler et al., 2007) in TSC patients need to be further explored to understand the variability of neurological manifestations.

Most of the TSC1 mutations, and 2/3 of TSC2 mutations, causes truncation of the gene product proteins (Au et al., 2007). The data in the identical mutation list (Table 5) correlate with this overall tendency. As shown in this list, most of the mutations of TSC1/TSC2 genes in patients with mild intellectual disability are missense mutations. Relatively preserved tuberin-hamartin complex function may explain the mild phenotype in certain cases with missense mutations (Jansen et al., 2006). In addition, given that the proportion of missense mutations is relatively high in the GAP domain (Au et al., 2007), which has an essential role in the tuberin function, this type of mutations outside the GAP domain might remain subclinical and regarded as polymorphism. On the other hand, various truncating mutations of TSC2 gene, whose GAP domain either preserved or untranslated, can result in both mild and severe intellectual and behavioral disabilities. This again supports that other factors than the truncated gene product itself play critical roles in the determination of neurological phenotype.

Accumulation of mutation data with detailed clinical information is mandatory for a better understanding of genotype-phenotype correlation and the exploration of background mechanism. However, the mutation database and individual journal articles are often insufficient for collecting

clinical data and draw reliable conclusion. Due to the aforementioned modifying factors of TSC phenotypes, interpretation of mutation data in individual patient is most confusing. We hope that the review data in this article would help the assessment of mutations and provide research interest by doctors and investigators.

Acknowledgments: We thank Dr. Shinjiro Akaboshi (Division of Pediatrics, Tottori Medical Center), Dr. Mari Kaneda (Department of Dermatology, Course of Integrated Medicine, Graduate School of Medicine, Osaka University), Dr.Jun Tohyama (Division of Pediatrics, Nishi-Niigata Chuo National Hospital), Dr. Masako Kubo (Department of Pediatric Surgery, Yamanashi Prefectural Central Hospital), Dr. Ichiro Suzaki (Division of Pediatrics, Tottori Prefectural Kousei Hospital), Drs. Saori Tanabe and Takashi Shiihara (Division of Pediatrics, Nihonkai General Hospital), Dr. Nobuaki Iwasaki (Department of Pediatrics, Ibaraki Prefectural University of Health Science) and Dr. Yoshihiro Maegaki (Division of Child Neurology, Tottori University), for providing the clinical information of each patient.

References

1 Ali M, Girimaji SC, Markandaya M, Shukla AK, Sacchidanand S, Kumar A. Mutation and polymorphism analysis of TSC1 and TSC2 genes in Indian patients with tuberous sclerosis complex. Acta Neurol Scand 2005;111:54–63.

2 Au KS, Rodriguez JA, Finch JL, Volcik KA, Roach ES, Delgado MR, et al. Germ-line mutational analysis of the TSC2 gene in 90 tuberous-sclerosis patients. Am J Hum Genet 1998;62:286–294.

3 Au KT, Williams AT, Roach ES, Batchelor L, Sparagana SP, Delgado MR, et al. Genotype/phenotype correlation in 325 individuals referred for a diagnosis of tuberous sclerosis complex in the United States. Genet Med 2007;9:88–100.

4 Beauchamp RL, Banwell A, McNamara P, Jacobsen M, Higgins E, Northrup H, et al. Exon scanning of the entire TSC2 gene for germline mutations in 40 unrelated patients with tuberous sclerosis. Hum Mutat 1998;12:408–416.

5 Boer K, Jansen F, Nellist M, Redeker S, van den Ouweland AMW, Spliet WGM, et al. Inflammatory processes in cortical tubers and subependymal giant cell tumors of tuberous sclerosis complex. Epilepsy Res 2008;78:7–21.

6 Choi JE, Chae JH, Hwang YS, Kim KJ. Mutation analysis of TSC1 and TSC2 in Korean patients with tuberous sclerosis complex. Brain Dev 2006;28:440–446.

7 Chong-Kopera H, Inoki K, Li Y, Zhu T, Garcia-Gonzalo FR, Rosa JL, et al. TSC1 stabilizes TSC2 by inhibiting the interaction between TSC2 and the HERC1 ubiquitin ligase. J Biol Chem 2006;281:8313–8316.

8 Dabora SL, Jozwiak S, Franz DN, Roberts PS, Nieto A, Chung J, et al. Mutational analysis in a cohort of 224 tuberous sclerosis patients indicates increased severity of TSC2, compared with TSC1, disease in multiple organs. Am J Hum Genet 2001;68:64–80.

9 Easton DF, Ponder MA, Huson SM, Ponder BAJ. An analysis of variation in expression of neurofibromatosis (NF) type 1 (NF1): evidence for modifying genes. Am J Hum Genet 1993;53:305–313.

10 Feng JH, Yamamoto T, Nanba E, Ninomiya H, Oka A, Ohno K. Novel TSC2 mutations and decreased expression of tuberin in cultured tumor cells with an insertion mutation. Hum Mutat 2004;26:245–250.

11 Gomez MR, Kuntz NL, Westmoreland BF. Tuberous sclerosis, early onset of seizures, and mental subnormality: Study of discordant homozygous twins. Neurology 1982;32:604–611.

12 Henske EP, Scheithauer BW, Short MP, Wollmann R, Nahmias J, Hornigold N, et al. Allelic loss is frequent in tuberous sclerosis kidney lesions but rare in brain lesions. Am J Hum Genet 1996;59:400–406.

13 Humphrey A, Higgins JNP, Yates JRW, Bolton PF. Monozygotic twins with tuberous sclerosis discordant for severity of developmental deficits. Neurology 2004;62:795–798.

14 Hung C, Su Y, Chien S, Liou HH, Chen CC, Chen PC, et al. Molecular and clinical analyses of 84 patients with tuberous sclerosis complex. BMC Medical Genetics 2006;7:72.

15 Jansen AC, Sancak O, D'Agostino MD, Badhwar AP, Roberts P, Gobbi G, et al. Unusually mild tuberous sclerosis phenotype is associated with TSC2 R905Q mutation. Ann Neurol 2006;60:528–539.

16 Jansen FE, Vincken KL, Algra A, Anbeek P, Braams O, Nellist M, et al. Cognitive impairment in tuberous sclerosis complex is a multifactorial condition. Neurology 2008;70:916–923.

17 Jobert S, Bragado-Nilsson E, Samolyk D, Pedespan JM, Marchal C, Reichert S, et al. Deletion of 11 amino acids in tuberin associated with severe tuberous sclerosis phenotypes: evidence for a new essential domain in the first third of the protein. Eur J Hum Genet 1997;5:280–287.

18 Kelleher III RJ, Govindarajan A, Tonegawa S. Translational regulatory mechanisms in persistent forms of synaptic plasticity. Neuron 2004;44:59–73.

19 Kwiatkowska J, Jozwiak S, Hall F, Henske EP, Haines JL, McNamara P, et al. Comprehensive mutational analysis of the TSC1 gene: observations on frequency of mutation, associated features, and nonpenetrance. Ann Hum Genet 1998;62:277–285.

20 Lewis JC, Thomas HV, Murphy KC, Sampson JR. Genotype and psychological phenotype in tuberous sclerosis. J Med Genet 2004;41:203–207.

21 Lyczkowski DA, Conant KD, Pulsifer MB, Jarrett DY, Grant PE, Kwiatkowski DJ, et al. Intrafamilial phenotypic variability in tuberous sclerosis complex. J Child Neurol 2007;22:1348–1355.
22 Maheshwar MM, Cheadle JP, Jones AC, Myring J, Fryer AE, Harris PC, et al. The GAP-related domain of tuberin, the product of the TSC2 gene, is a target for missense mutations in tuberous sclerosis. Hum Mol Genet 1997;6:1991–1996.
23 Martin N, Zügge K, Brandt R, Friebel D, Janssen B, Zimmerhackl LB. Discordant clinical manifestations in monozygotic twins with the identical mutation in the TSC2 gene. Clin Genet 2003;63:427–430.
24 Mayer K, Goedbloed M, van Zijl K, Nellist M, Rott H-D. Characterisation of a novel TSC2 missense mutation in the GAP related domain associated with minimal clinical manifestations of tuberous sclerosis. J Med Genet 2004;41:e64.
25 Mizuguchi M, Mori M, Nozaki Y, Momoi MY, Itoh M, Takashima S, et al. Absence of allelic loss in cytomegalic neurons of cortical tuber in the Eker rat model of tuberous sclerosis. Acta Neuropathol 2004;107:47–52.
26 Niida Y, Lawrence-Smith N, Banwell A, Hammer E, Lewis J, Beauchamp RL, et al. Analysis of both TSC1 and TSC2 for germline mutations in 126 unrelated patients with tuberous sclerosis. Hum Mutat 1999;14:412–422.
27 Niida Y, Stemmer-Rachamimov AO, Logrip M, Tapon D, Perez R, Kwiatkowski DJ, et al. Survey of somatic mutations in tuberous sclerosis complex (TSC) hamartomas suggests different genetic mechanisms for pathogenesis of TSC lesions. Am J Hum Genet 2001;69:493–503.
28 Ramesh V. Aspects of tuberous sclerosis complex (TSC) protein function in the brain. Biochem Soc Transac 2003;31:579–583.
29 Ridler K, Suckling J, Higgins NJ, de Vries PJ, Stephenson CME, Bolton PF, et al. Neuroanatomical correlates of memory deficits in tuberous sclerosis complex. Cereb Cortex 2007;17:261–271.
30 Roach ES, Gomez MR, Northrup H. Tuberous sclerosis complex consensus conference: revised clinical diagnostic criteria. J Child Neurol 1998;13:624-628.
31 Rok P, Kasprzyk-Obara J, Dómanska-Pakieła D, Jóźwiak S. Clinical symptoms of tuberous sclerosis complex in patients with an identical TSC2 mutation. Med Sci Monit 2005;11:230–234.
32 Rose VM, Au KS, Pollom G, Roach ES, Prashner HR, Northrup H. Germ-line mosaicism in tuberous sclerosis: how common? Am J Hum Genet 1999;64:986–992.
33 Sancak O, Nellist M, Goedbloed M, Elfferich P, Wouters C, Maat-Kievit A, et al. Mutational analysis of the TSC1 and TSC2 genes in a diagnostic setting: genotype-phenotype correlations and comparison of diagnostic DNA techniques in Tuberous Sclerosis Complex. Eur J Hum Genet 2005;13:731–741.
34 Smalley S, Burger F, Smith M. Phenotypic variation of tuberous sclerosis in a single extended kindred. J Med Genet 1994;31:761–765.
35 Swiech L, Perycz M, Malik A, Jaworski J. Role of mTOR in physiology and pathology of the nervous system. Biochim Biohpys Acta 2008;1784:116–132
36 Verhoef S, Bakker L, Tempelaars AMP, Hesseling-Janssen ALW, Mazurczak T, Jozwiak S, et al. High rate of mosaicism in tuberous sclerosis complex. Am J Hum Genet 1999;64:1632–1637.
37 Verhoef S, Vrtel R, Bakker L, Stolte-Dijkstra I, Nellist M, Begger JH, et al. Recurrent mutation 4882delTT in the GAP-related domain of the tuberous sclerosis TSC2 gene. Hum Mutat 1998;Suppl 1:S85–87.
38 Vrtel R, Verhoef S, Bouman K, Maheshwar MM, Nellist M, van Essen AJ, et al. Identification of a nonsense mutation at the 5' end of the TSC2 gene in a family with a presumptive diagnosis of tuberous sclerosis complex. J Med Genet 1996;33:47–51.
39 Wilson PJ, Ramesh V, Kristiansen A, Bove C, Jozwiak S, Kwiatkowski DJ, et al. Novel mutations detected in the TSC2 gene from both sporadic and familial TSC patients. Hum Mol Genet 1996;5:249–256.
40 Yamamoto T, Pipo JR, Feng JH, Takeda H, Nanba E, Ninomiya H, et al. Novel TSC1 and TSC2 mutations in Japanese patients with tuberous sclerosis. Brain Dev 2002;24:227–230.
41 Yamashita Y, Ono J, Okada S, Wataya-Kaneda M, Yoshikawa K, Nishizawa M, et al. Analysis of all exons of TSC1 and TSC2 genes for germline mutations in Japanese patients with tuberous sclerosis: report of 10 mutations. Am J Med Genet 2000;90:123–126.
42 Zhang H, Nanba E, Yamamoto T, Ninomiya H, Ohno K, Mizuguchi M, et al. Mutation analysis of TSC1 and TSC2 genes in Japanese patients with tuberous sclerosis complex. J Hum Genet 1999;44:391–396.

Received December 22, 2008; accepted January 6, 2009

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