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Evaluation of Tuberous Sclerosis Complex Patients

Tüberoskleroz kompleksi tanılı hastaların değerlendirilmesi

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ABSTRACT

Aim: Tuberous sclerosis complex (TSC) is a multisystem genetic, autosomal-dominant disorder predisposing to multiple organ manifestations. The aim of this study is to determine TSC the frequency of findings including diagnostic and non-diagnostic criteria.

Patients and Metod: Thirty-five patients diagnosed with tuberous sclerosis complex were examined retrospectively. The diagnosis of the patients were evaluated according to the diagnostic criteria of TSC that were updated in 2012. As non-diagnostic criteria, we reviewed epilepsy, drug-resistant epilepsy, electroencephalography (EEG) types (focal, diffuse-multifocal and hypsarrhythmia) and TAND (TSC-associated neuropsychiatric disorders) (intellectual disability and/or autism and learning disability).

Results: Twenty-one cases (60%) presented with seizures, 9 cases (26%) with hypopigmented patches and 5 cases (14%) with cardiac rhabdomyomas. The most common finding with brain magnetic resonance imaging (MRI) was cortical tubers (85%). EEG examinations revealed diffuse and multifocal epileptic disorder in 5 (24%), focal epileptic disorder in 8 (38%), and hypsarrhythmia in 8 (38%) patients. 38% of the patients with epilepsy were diagnosed with refractory epilepsy. Severe intellectual disability and / or autism were detected in 11 (32%) patients. The number of patients with renal angiomyolipoma (p:0.001) were significantly higher in drug resistant epilepsy patients and also TSC-associated neuropsychiatric disorders (TAND) (p:0.001) rate was significantly higher in epilepsy patients.

Conclusion: The disease should be followed with a multidisciplinary approach. Although not included in the diagnostic criteria, it should be kept in mind that epilepsy, intellectual disability and neuropsychiatric disorders frequently accompany.

Keywords:Tuberoussclerosis Complex, Epilepsy, Intellectual Disability

ÖZ

Amaç: Tüberoskleroz kompleksi (TSK) vücutta birçok organın tutulumu ile karakterize, otozomal dominant kalıtım gösteren genetik bir rahatsızlıktır. Bu çalışmada TSK tanı kriterlerinin ve tanı kriterleri dışındaki bulguların sıklığını belirlemek amaçlanmıştır.

Hastalar ve Yöntemler: TSK tanılı 35 hastanın verileri geriye dönük olarak incelendi. Hastaların tanısı, 2012 yılında güncellenen TSK'nin tanı kriterlerine göre değerlendirildi. Tanısal olmayan kriterler olarak; epilepsi, ilaca dirençli epilepsi, elektroensefalografi (EEG) tiplerini (fokal, diffüz-multifokal ve hiperaritmi) ve TAND'ı (TSC ile ilişkili nöropsikiyatrik bozukluklar) (zihinsel yetersizlik ve / veya otizm ve öğrenme yetersizliği) inceledik.

Bulgular: Yirmi bir hasta (%60) nöbet geçirme, 9 hasta (%26) hipopigmente lekelenmeler ve 5 hasta (%14) kardiyak rabdomiyomlar nedeniyle başvurmuştu. Beyin magnetik rezonans görüntüleme (MRG) ile en sık saptanan bulgu kortikal tüberlerdi (%85). Nöbet geçiren hastaların EEG incelemelerinde 5'inde (%24) yaygın ve multifokal epileptik bozukluk, 8'inde (%38) fokal epileptik bozukluk ve 8'inde (%38) hipsaritmi paterni saptandı. Epilepsi tanısı ile izlenen olguların %38'i dirençli epilepsi tanısına sahipti. Ağır derecede entelektüel yetersizlik ve/veya otizm 11 (%32) hastada saptandı. Dirençli epilepsi grubunda böbrek anjiomiyolipomaları olan hasta sayısı anlamlı olarak fazlaydı (p:0.001) ve aynı zamanda tüberoskleroz ile ilişkili nöropsikiyatrik bozuklukların oranı anlamlı olarak epilepsi grubunda yüksekti (p:0.001).

Sonuç: Hastalığın multidisipliner bir yaklaşım ile takip edilmesi gerekmektedir. Tanı kriterlerinde yer almasa da epilepsi, entelektüel yetersizlik ve nöropsikiyatrik bozuklukların sık eşlik ettiği akılda tutulmalıdır.

Anahtar Sözcükler:Tüberoskleroz Kompleksi, Epilepsi, Entelektüel Yetersizlik

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INTRODUCTION

Tuberosclerosis complex (TSC) is a single gene disorder with an autosomal dominant inheritance pattern and a frequency of 1/6000 – 1/10000 live births [1,2]. It is caused by a mutation in the TSC1 and TSC2 genes which impairs the function of hamartin and tuberin complex, and clinical findings appear because of the inhibitory effect of rapamycin on the mammalian target (mTOR) signaling pathway [2,3]. Involvement of different organs are observed in different age groups. It is known that brain and heart findings can be seen from the prenatal period, skin and kidney findings are seen during childhood and pulmonary findings frequently present in adulthood [4,5].

Central nervous system (CNS) involvement occurs in more than 90% of patients. This involvement is observed as cortical-subcortical tubers, subependymal nodules, giant cell astrocytoma (SEGA) and white matter radial migration lines. Epilepsy, mental retardation, autism and additional neuropsychiatric disorders occur in these patients as a result of CNS involvement [6]. All types of epileptic seizures can be observed in TSC and these seizures are generally resistant to antiepileptic treatment [7].

The diagnostic criteria of the disease were updated in 2012 by the International Tuber Sclerosis Complex Consensus Group [4,5]. In this study, we retrospectively evaluated data of the patients with TSC disease and analysis the frequency of signs and symptoms that were both included and non-included in diagnostic criteria.

PATIENTS and METHODS

A total of 35 patients with TSC were recruited at the Pediatric Neurology Department from January 2013 to December 2017. Clinical, laboratory and imaging findings of the patients were evaluated retrospectively. The study included patients aged 1-17 years who were followed up for a diagnosis of TSC for at least 1 year. The diagnosis of the patients were evaluated according to the diagnostic criteria of TSC that were updated in 2012 by the International Tuber Sclerosis Complex Consensus Group [4,5]. As non-diagnostic criteria, we reviewed epilepsy, drug-resistant epilepsy, electroencephalography (EEG) types (focal, diffuse-multifocal and hypsarrhythmia) and TAND (TSC-associated neuropsychiatric disorders) (intellectual disability and/or autism and learning disability).

This study was approved by the ethics review committee of the Akdeniz University Clinical Research with 06.26.2019 date number of 607 decision in accordance with the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects.

Statistical Analysis: All analyses were performed on SPSS v17.00 (SPSS Inc., Chicago, IL, USA). Descriptive data of the patients were analyzed. In respect to normality of distribution, data given as mean ± standard deviation or median (minimum - maximum) for continuous variables, and frequency (percentage) for categorical variables. Categorical comparisons between groups were performed by the Chi-Square and p values lower than 0.05 were considered as statistically significant.

RESULTS

We included 35 patients (51.4% male) into our study, median age was 1.9 years (range from 2 months-14 years). Twenty-one patients (60%) presented with seizures, 9 patients (26%) with hypopigmented spots and 5 patients (14%) presented with cardiac rhabdomyomas (2 of these had been diagnosed in the intrauterine period). Ten (28%) patients had a family history of TSC. Hypomelanotic macules on the skin were present in all patients except for one patient. Cortical tubers (n = 30, 85%) and subependymal nodules (n = 29, 83%) were the most common findings on brain magnetic resonance imaging (MRI) (Figure 1 and 2).

In addition to CNS and skin findings, 8 patients (23%) had rhabdomyoma in the heart, 13 patients (37%) had angiomyolipoma in the kidney and 1 patient (3%) had eye involvement (Table 1). Electroencephalography (EEG) examinations of the patients with seizures revealed diffuse and multifocal epileptic disorder in 5 (24%), focal epileptic disorder in 8 (38%), and hypsarrhythmia pattern in 8 (38%) patients. Seven (33%) patients were receiving treatment with a single antiepileptic drug, 6 (29%) patients were using two, and 8 (38%)

patients were using multiple (>2) antiepileptic drugs. Twenty-two (62.8%) patients had various degrees of intellectual disability and/or autism and learning disability (Table 2). Categorical comparisons between diagnostic and non-diagnostic criteria groups were given in Table 3. The number of patients with renal angiomyolipoma (p:0.001) was significantly higher in drug-resistant epilepsy patients than non-drug resistant epilepsy patients (50% vs 9.5%) and also TSC-associated neuropsychiatric disorders (TAND) (p:0.001) rate was significantly higher in epilepsy patients than non epileptic patients (85.3% vs 28.5%).

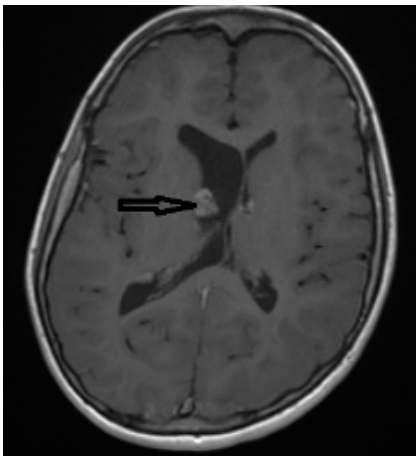

Figure 1. Axial FLAIR MRI shows subependymal nodule along the lateral wall of the lateral ventricle (black arrow).

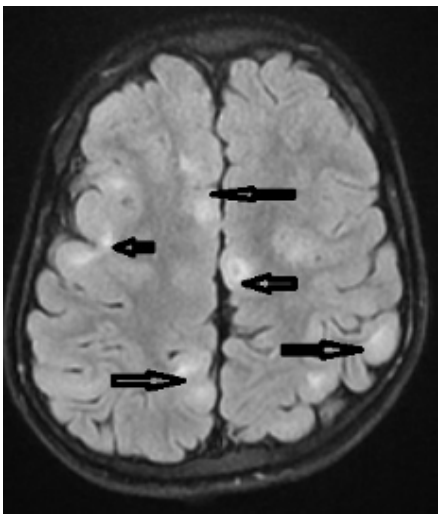

Figure 2. Axial FLAIR MRI shows multiple cortical tubers (black arrow).

DISCUSSION

In this study, we found that hypomelanotic macule was the most commonly seen diagnostic criteria. Also among the symptoms which were not included in diagnostic criteria, the most commonly seen one was learning and intellectual disability. Tuberosclerosis complex is a disease with different symptoms and signs depending on the age of onset and the severity of the disease [8]. In this study, the most common examination finding was hypomelanotic macula (97%). In the literature, hypomelanotic macular incidence in the childhood was reported as 61–97.2% [9,10]. In our study, other common skin findings were facial angiofibroma (34%) and shagreen patch (6%).

Table 1. Evaluation of 35 TSC patients in terms of diagnostic criteria

Criteria	Number of patients (%)
Hypomelanotic macules	34 (%97)
Shagreen patch	2 (%6)
Multiple retinal hamartomas	1 (%3)
Facial angiofibromas	12 (%34)
Cortical tuber	30 (%85)
Subependymal nodules	29 (%83)
Subependymal giant cell astrocytoma (SEGA)	4 (%11)
Cardiac rhabdomyoma	8 (%23)
Renal angiomyolipoma	13 (%37)
Nonrenal hamartomas	7 (%20)
Multiple renal cysts	9 (%25)

Table 2. Evaluation non-diagnostic clinical features of 35 TSC patients

Criteria	Number of patients (%)
Epilepsy	21 (%60)
Learning disability	6 (%22)
Mild type intellectual disability	5 (%14)
Severe type intellectual disability and/or autism	11 (%32)

In this study, 60% of patients had epilepsy diagnosis. This rate ranges between 72-85% in the literature, and was lower in our study [11]. Different types of seizures may occur in patients with TSC. In this study, infantile spasm was present in 38% of patients, and this rate was reported to range from 32.7% to 40% in the literature [12]. In the current study, focal seizures were seen in 38% of the patients, while multifocal seizures were seen in 24%. Similarly, the literature reports that early onset seizures are frequently focal seizures and infantile spasms [13]. Epileptic seizures are also common in TSC, but are not included in the major diagnostic criteria of the disease. There is no clear consensus on the course of epileptic seizures, but they are known to be resistant to treatment

Table 3. Comparison of diagnostic and non-diagnostic criteria in TSC patients

	Epilepsy			EEG types				Drug-resistant epilepsy			TAND*		
	Yes (%)	No (%)	p	H. (%)	F. (%)	M.F(%)	p	Yes (%)	No (%)	p	Yes (%)	No (%)	p
Male	57.9	42.1	0.78	27.2	45.6	27.2	0.56	15.8	84.2	0.28	63	37	0.96
Female	62.5	37.5	0.78	50	30	20	0.56	32.1	68.8	028	62.5	37.5	0.96
Hypomelanotic macules	61.8	38.2	0.21	38	38	24	0.82	23.5	76.5	0.58	61.8	38.2	0.43
Facial angiofibromas	58.3	41.7	0.88	42.8	28.6	28.6	0.81	25	75	0.82	50	50	0.25
Shagreen patch	100	0	0.74	38	38	24	0.94	0	100	0.42	50	50	0.69
Multiple retinal hamartomas	0	100	0.21	NA	NA	NA	NA	0	100	0.58	0	100	0.18
Cortical tuber	56.6	43.4	0.32	41.1	35	23.9	0.81	20	80	0.32	63.3	36.7	0.88
Subependymalnodules	65.5	34.5	0.14	42.1	31.5	26.4	0.16	27.5	72.5	0.14	62	38	0.83
SEGA**	50	50	0.66	50	0	50	0.45	25	75	0.91	50	50	0.57
Cardiac rhabdomyoma	62.5	37.5	0.86	60	20	20	0.48	25	75	0.86	50	50	0.39
Renal angiomyolipoma	58.3	41.7	0.88	57.1	28.5	14.4	0.035	50	50	0.001	66.6	33.4	0.73
Multiple renal cysts	77.7	22.3	0.20	43	28.5	28.5	0.81	33.3	66.7	0.38	66.6	33.4	0.78
Nonrenal hamartomas	42.8	57.2	0.30	0	33.4	66.6	0.13	14.2	85.8	0.54	57.1	42.9	0.72

F: Focal, H: Hypsarrhythmia, M.F:Multifocal , NA:Not Available, TAND* (TSC-associated neuropsychiatric disorders), **Subependymal giant cell astrocytoma (SEGA)

[6,13]. In our study, resistant epilepsy was found in 38% of the patients. Intellectual, behavioral and psychosocial disorders reportedly occur in 44-65% of the patients with TSC throughout their lives [14]. Autism spectrum disorders, attention deficit and hyperactivity disorder, various degrees of intellectual disabilities, learning disabilities in different fields (mathematics, reading and writing) or memory and executive dysfunctions may be seen in patients with TSC [5,14]. Our results showed that 46% of the patients had mild to severe intellectual disability and / or autism. In 22% of the patients, learning disabilities were found in different areas despite normal mental development. In 2012, the Tuberosclerosis Neuropsychiatry Panel of the Consensus Group of the International Tuberous Sclerosis Complex Group highlighted this issue and this group of symptoms and conditions were named as 'TSC associated neuropsychiatric disorders' (TAND) [4,5]. We found that neuropsychiatric disease rate was higher in epilepsy group. In another study, they also found this correlation [15]. There has been increasing concern regarding the cumulative neurobiological burden associated with the risk of progressive cognitive impairment and epilepsy. Timing and treatment modalities of epilepsy become important for reducing of TAND [16].

Central nervous system lesions seen in TSC include cortical tubers, white matter heterotopia, subependymal nodules and subependymal giant cell astrocytoma [17]. It has been reported that lesions are often found in the frontal and temporal regions, are seen in 80-95% of patients, and the size and number of lesions are often unrelated to the clinical situation [11-12]. Tubers occur in the intrauterine period and the number of tubers do not change in the postnatal period. Tubers do not develop into neoplasms and become calcified over time [18]. The frequency of subependymal giant cell astrocytoma has been reported to be between 10-20% [11,12]. In our study, cortical tubers were found in 85% of the patients and subependymal nodules in 83%, while subependymal giant cell astrocytoma was detected in 11% of the patients.

The disease may also cause cardiac involvement characterized by cardiac rhabdomyomas. They are usually multiple and have good prognosis [19]. Cardiac rhabdomyomas develop in the intrauterine period during which many patients are diagnosed and referred to pediatric neurology departments in the newborn period with a preliminary diagnosis of TSC [4,5]. Approximately 96% of patients with cardiac rhabdomyoma are diagnosed with TSC [12]. In our study, a total of 5 patients (14%) were diagnosed with cardiac rhabdomyoma, of which 2 were intrauterine.

In our study, renal angiomyolipoma was detected in 37% of the patients. The incidence of renal

lesions in TSC increases with age. The most common lesion is angiomyolipoma and it is frequently detected in multiple and bilateral forms. In our series, the number of renal angiomyolipoma patients were significantly higher in drug-resistant epilepsy patients. This result may be related with TSC2 mutation that causes more severe phenotype of TSC [20,21]. In the literature TSC2 mutations more related with severe phenotype and also renal angiomyolipomas [22]. However our patients were not evaluated for genetic mutations. And also TSC2 gene is in close proximity with the autosomal dominant polycystic kidney disease (PCKD) gene, so PCKD may also be seen in these patients [23]. Renin-dependent hypertension may occur in patients with renal lesions. It is recommended that patients with TSC should undergo abdominal MRI examinations both at diagnosis and after diagnosis at intervals of 1 to 3 years. In addition, arterial blood pressure measurement is required in the follow-up of patients. [4,5].

This study has some limitations. The most important limitation of this study is its retrospective nature. Secondly; the limited number of cases evaluated in the study reduces the reliability of statistical analysis between the groups. In addition, for some of patients our follow up period was short so the patients without epilepsy may develop epilepsy in future. Finally, the diagnoses of the patients in the study were based on clinical and radiological findings, none of them had genetic analysis. However, despite these limitations, we think that it will contribute to the literature because of the evaluation of comorbid conditions not included in the diagnostic criteria of tuberous sclerosis.

CONCLUSION

The results of our study are consistent with the literature. TSC is a multisystemic disease and should be followed with a multidisciplinary approach. Individual differences of the patients should be considered. Although epilepsy, intellectual disability and neuropsychiatric disorders are not included in the TSC diagnostic criteria, they often accompany the disease. It is possible that physical and neuropsychiatric characteristics of the disease may change or emerge over time, therefore regular follow-up and controls are very important.

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Ethic: This study was approved by the ethics review committee of the Akdeniz University Clinical Research with 06.26.2019 date number of 607 decision in accordance with the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects.

Conflict of interest: There is no conflict of interest to declare.

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REFERENCES

1. Curatolo P, Bombardieri R, Jozwiak S. Tuberous sclerosis. Lancet. 2008;372(9639):657–68. PMID:18722871
2. Hallett L, Foster T, Liu Z, Blieden M, Valentim J. Burden of disease and unmet needs in tuberous sclerosis complex with neurological manifestations: systematic review. Curr Med Res Opin. 2011;27(8): 1571-83. PMID:21692602
3. Schwartz RA, Fernández G, Kotulska K et al. Tuberous sclerosis complex: advances in diagnosis, genetics, and management. J Am Acad Dermatol. 2007; 57(2):189-202. PMID:17637444
4. Northrup H, Krueger D. International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. Pediatr Neurol 2013; 49(4):243–54. PMID:24053982
5. Krueger DA, Northrup H. International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex surveillance and management: recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. Pediatr Neurol. 2013; 49(4):255–265. PMID:24053983
6. Curatolo P, Moavero R, de Vries PJ. Neurological and neuropsychiatric aspects of tuberous sclerosis complex. Lancet Neurol. 2015;14(7):733-45. PMID:26067126
7. Holmes GL, Stafstrom CE; Tuberous Sclerosis Study Group. Tuberous sclerosis complex and epilepsy: recent developments and future challenges. Epilepsia. 2007;48(4):617-30. PMID:17386056
8. Smalley SL, Burger F, Smith M. Phenotypic variation of tuberous sclerosis in a single extended kindred. J Med Genet. 1994;31(10):761-5. PMID:7837252
9. Saltık S, Karatoprak EY, Taşel B. Characteristics and the clinical course of epilepsy in patients with tuberous sclerosis complex. Türk Pediatri Arş. 2013;48(2):123-30. doi:10.4274/tpa:116
10. Józwiak S, Schwartz RA, Janniger CK,Michałowicz R, Chmielik J. Skin lesions in children with tuberous sclerosis complex: their prevalence, natural course, and diagnostic significance. Int J Dermatol. 1998;37(12):911-7. PMID:9888331
11. Au KS, 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(2):88-100. PMID:17304050
12. Mettin RR, Merkenschlager A, Bernhard MK, Elix H, Hirsch W, Kiess W et al. Wide spectrum of clinical manifestations in children with tuberous sclerosis complex–Follow-up of 20 children. Brain Dev. 2014;36(4):306-14. PMID:23751858
13. Chu-Shore CJ, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. Epilepsia. 2010; 51(7):1236–41. PMID:20041940
14. Joinson C, O'Callaghan FJ, Osborne JP, Martyn C, Harris T, Bolton PF. Learning disability and epilepsy in an epidemiological sample of individuals with tuberous sclerosis complex. Psychol Med. 2003;33(2):335–44. PMID:12622312
15. Wang YY, Pang LY, Ma SF, Zhang MN, Liu LY, Zou LP. Epilepsy may be the major risk factor of mental retardation in children with tuberous sclerosis: A retrospective cohort study. Epilepsy Behav. 2017;77:13-8. PMID:29073472
16. Jozwiak S, Kotulska K, Domanska-Pakiela D, Lojszczyk B, Syczewska M, Chmielewski D, et al. Antiepileptic treatment before the onset of seizures reduces epilepsy severity and risk of mental retardation in infants with tuberous sclerosis complex. Eur J Paediatr Neurol Sep 2011;15(5):424–31. PMID: Epilepsy Behav. 2017;77:13-8.

17. Mizuguchi M, Takashima S. Neuropathology of tuberous sclerosis. Brain Dev. 2001; 23(7): 508-15. PMID:11701246

18. Goodman M, Lamm SH, Engel A Shepherd CW, Houser OW, Gomez MR. Cortical tuber count: a biomarker indicating neurologic severity of tuberous sclerosis complex. J Child Neurol. 1997;12(2):85-90. PMID:9075016

19. Jóźwiak S, Domańska-Pakieła D, Kwiatkowski DJ, Kotulska K. Multiple cardiac rhabdomyomas as a sole symptom of tuberous sclerosis complex: case report with molecular confirmation. J Child Neurol. 2005;20(12):988-9. PMID:16417848

20. Camposano SE, Greenberg E, Kwiatkowski DJ, Thiele EA. Distinct clinical characteristics of tuberous sclerosis complex patients with no mutation identified. Ann Human Genet. 2009;73(2):141-6. PMID:19133941

21. Peron A, Vignoli A, La Briola F, Morenghi E, Tansini L, Alfano RM et al. Deep phenotyping of patients with Tuberous Sclerosis Complex and no mutation identified in TSC1 and TSC2. Eur J Med Genet. 2018;61(7):403-10. PMID: 29432982

22. Lin S, Zeng JB, Zhao GX, Yang ZZ, Huang HP, Lin MT et al. Tuberous Sclerosis Complex in Chinese patients: Phenotypic analysis and mutational screening of TSC1/TSC2 genes. Seizure. 2019;71:322-7. PMID:31525612

23. 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(1):64-80. PMID:11112665