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The Phenotypic Spectrum of Tuberous Sclerosis Complex: A Canadian Cohort

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Abstract

Objective: We aimed to further elucidate the phenotypic spectrum of Tuberous Sclerosis Complex (TSC) depending on genotype. **Methods:** A retrospective review of patients seen in the TSC clinic at the Hospital for Sick Children was conducted and the frequency of TSC manifestations was compared based on genotype. **Results:** Nineteen-patients had TSC1 mutations, 36 had TSC2 mutations and 11 had no mutation identified (NMI). Patients with TSC2 mutations had a higher frequency of early-onset epilepsy and more frequent systemic manifestations. The NMI group had milder neurologic and systemic manifestations. Our data did not demonstrate that intellectual disability and infantile spasms were more common in TSC2 mutations. **Conclusions:** This is the first Canadian pediatric cohort exploring the genotype-phenotype relationship in TSC. We report that some manifestations are more frequent and severe in TSC2 mutations and that NMI may have a milder phenotype. Disease surveillance and counseling should continue regardless of genotype until this is better elucidated.

Keywords

tuberous sclerosis complex (TSC), genotype, phenotype, no mutation identified (NMI), surveillance

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Tuberous Sclerosis Complex (TSC) is a chronic disease that affects multiple organ systems and has a wide spectrum of clinical manifestations. TSC is most commonly caused by a mutation in TSC1 or TSC2, which encode the proteins hamartin and tuberin, respectively.[1] No mutation is identified in 10-15% of patients with a clinical diagnosis of TSC (hereafter referred to as NMI).[2] Approximately 60-70% of TSC mutations are *de novo* while the remainder are due to autosomal dominant inheritance.[1] For patients with NMI, it is postulated that their manifestations could be the result of somatic mosaicism. It is also possible that there may be an unknown third candidate gene implicated, or that the condition is secondary to an intronic mutation.[2] Overall, it has been observed that patients with TSC2 gene mutations tend to have a more severe manifestation of the disease as compared to those who have a TSC1 mutation. Individuals with NMI have been postulated to have a milder phenotype; although phenotypic variability can occur.[3]

TSC has an estimated population frequency of 1 in 6,000—1 in 10,000 live births and has a population prevalence of approximately 1 in 20,000.[4,5] TSC most commonly manifests as

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benign tumors (hamartomas) that affect the brain, skin, kidney, heart, lung, and bone.[6] The multi-systemic involvement observed in patients with TSC, requires involvement of multiple specialists in medicine and surgery. Tumors that affect the central nervous system are the commonest cause of morbidity and mortality, while renal disease is the second cause of premature mortality. In those who have central nervous system manifestations, 90% develop epilepsy,[7,8] 50% other neuropsychiatric disorders, and 16% autism spectrum disorder.[9,10] TSC is diagnosed clinically and established diagnostic criteria and surveillance monitoring recommendations exist for TSC.[4,11] Given the spectrum of disease observed in individuals with TSC, our goal was to better understand the phenotypic presentation of TSC depending on genotype (TSC1 versus TSC2 versus NMI) in our Canadian cohort.

Methods

We conducted a retrospective review of the medical records of all patients with a clinical diagnosis of TSC, who were followed in the Comprehensive TSC Clinic at the Hospital of Sick Children between January 2016-December 2017. Patients between the ages of 0-18 years with a clinical diagnosis of TSC according to the updated TSC diagnostic criteria (4) with clinical, imaging and genetic testing available were considered for inclusion. Patients without genetic testing were excluded from the analysis. Approval was obtained from the Research Ethics Board at the Hospital for Sick Children, Toronto, Ontario, Canada.

The medical records were reviewed for: demographics, dermatological manifestations, neurological manifestations, ophthalmological manifestations, and systemic involvement. Neuroimaging reports were reviewed at the time of diagnosis of TSC as well as throughout clinic visits to determine the presence of neuroimaging manifestations related to TSC. Neuroimaging findings were extracted from the MRI reports, which were read by neuroradiologists. In most cases, ultrasound of the kidney was performed to evaluate for the presence of renal manifestations associated with TSC, although in some cases when available MRI was utilized. Liver hamartomas were diagnosed by abdominal ultrasound.

For each patient, we reviewed the molecular genetic results which included a mutation in either the TSC1 or TSC2 gene as well as NMI with our genetic counsellor. Mutations were identified by Sanger sequencing with multiplex ligation-dependent probe amplification (MLPA) in the majority of patients, while 2 patients had next generation sequencing (NGS) with MLPA. Only pathogenic and likely pathogenic variants were included. Benign variants and variants of unknown significance (VUS) were excluded. The NMI group included those who had no mutation identified after genetic testing of the TSC1 and TSC2 gene was completed. Data compilation was performed using the Redcap Database.

The frequency of TSC clinical manifestations was compared between TSC1 mutations, TSC2 mutations and NMI. Statistical analysis was performed using IBM SPSS software version 21. Continuous data was represented as mean with standard deviation or median with interquartile range. Qualitative data was represented as proportions or percentages. Comparisons were done using "t-test" for continuous variables and chi-square/Fisher's exact tests for categorical variables. P values <0.05 were considered statistically significant.

Results

Our cohort included a total of 90 patients with a clinical diagnosis of TSC. Twenty percent (18/90) of patients had no genetic testing result available and were therefore excluded from the analysis. Of the 72 patients who had genetic testing available, 6 patients were excluded because they had a VUS. Sixty-six patients with genetic testing remained and were included in the analysis. Of the 66 patients, 31 were females (47%). A TSC1 mutation was detected in 29% ($n = 19$) and a TSC2 mutation in 54% ($n = 36$). Eleven patients (17%) in the cohort had NMI.

The mean current age of patients in our cohort was 9 years (range: 0.4-18 y); while the mean age at diagnosis of TSC was 2.4 years (range 0-17.2 y). Patients with TSC2 mutations were younger at the time of diagnosis and a presumed antenatal diagnosis was seen in both TSC1 and TSC2 mutations, but not in NMI. The mean age at diagnosis for patients with TSC1 mutations was 2.7 years (range 0-11.1 years); in patients with TSC2 mutations, the mean age at diagnosis was 1.2 years (range 0-13.5 years) and in the NMI group the mean age at diagnosis was 5.6 years (range 9 days-17.2 years), ($p = 0.0009$). A summary of the neurologic and systemic manifestations according to mutation type are described below and shown in Table 1.

Neurological Manifestations

Epilepsy

A history of epilepsy was found in 86% (31/36) of TSC2 mutations, 74% (14/19) of TSC1 mutations and 64% (7/11) of the NMI group, ($p = 0.22$). When comparing the age of epilepsy onset, those with TSC2 mutations were younger at epilepsy onset (mean age of onset 1.4 years, range 2 months-9.9 years) when compared to TSC1 mutations (mean age of onset = 4.4 years, range 8 month-10.4 years) and NMI (mean age of onset = 2.8 years, range 2 months-12 years), ($p = 0.0041$) . In addition, patients with TSC2 mutations had an earlier age of onset of epilepsy when compared to TSC1 mutations ($p = 0.0002$). The frequency of refractory epilepsy was similar across the groups, 58% (18/31) in those with TSC2 mutations versus 57% (8/14) in TSC1 mutations and 43% (3/7) in the NMI group ($p = 0.28$). There was no statistical difference in the frequency of medically refractory epilepsy between TSC2 and TSC1 mutations ($p = 0.30$). Infantile spasms were more common in TSC2 mutations, 39% (14/36), when compared to TSC1 mutations, 16% (3/19), and the NMI group,18% (2/11), although this did not reach statistical significance ($p = 0.16$). Moreover, when comparing the frequency of infantile spasms between the TSC2 and TSC1 groups, this approached but did

Table 1. Summary of Clinical Manifestations of TSC According to Mutation Type.

	TSC2, No = 36	TSC1, No = 19	NMI No = 11	TSC1 vs TSC2 Vs NMI, p value	TSC1 Vs TSC2, p value
Age (mean) in years	9.7	8	8.5	0.54	0.15
Age at diagnosis (mean) in years	1.2	2.7	5.6	0.0009	0.03
Antenatal diagnosis, n (%)	12 (32)	4 (21)	None	0.08	0.26
Gender	16M/20F	12M/7F	7M/4F	0.31	0.19
Epilepsy, n (%)	31 (86)	14 (74)	7 (64)	0.22	0.22
Epilepsy onset (mean) in years	1.4	4.4	2.8	0.0041	0.0002
Refractory epilepsy, n (%)	18/31 (58)	8/14 (57)	3/7 (43)	0.28	0.30
Infantile spasms, n (%)	14 (39)	3 (16)	2 (18)	0.16	0.07
Infantile spasms onset (mean) in months	5	10	15.5	NA: Very small number of values in NMI and TSC1	
ID, n (%)	16/30 (53)	5/16 (31)	1/11 (9)	0.04	0.41
Neurobehavioral manifestations, n (%)	10 (28)	5 (26)	3 (27)	0.95	1.00
Tuber, n (%)	35 (97)	18 (95)	9 (82)	0.28	0.58
SEN, n (%)	35 (97)	19 (100)	7 (64)	0.002	0.66
SEGA, n (%)	24 (67)	12 (63)	None	<0.001	0.51
AML, n (%)	26 (72)	7 (37)	4 (36)	0.01	0.01
Multiple Renal cyst, n (%)	11 (31)	None	1 (9)	0.01	0.005
Skin manifestation, n (%)	34/35 (97)	16/18 (89)	10/10 (100)	0.62	0.36
Cardiac rhabdomyoma, n (%)	25 (69)	11 (58)	3 (27)	0.04	0.29
Retinal hamartoma, n (%)	20/34 (59)	2/16 (13)	None	0.001	0.002
Liver hamartoma, n (%)	10 (28)	None	None	0.006	0.009

M: male; F: female; ID: intellectual disability; SEN: subependymal nodule; SEGA: subependymal giant cell astrocytoma; AMLs: angiomyolipoma.

not reach statistical significance ($p = 0.07$). The age of onset of spasms was younger in those with TSC2 mutations (mean age = 5 months, range 2-9 months) when compared to TSC1 mutations (mean age = 10 months, range 4-14 months) and NMI (mean age = 15.5 months, range 7-24 months).

Cognition and Behavior

Although individuals with TSC2 mutations had a higher frequency of intellectual disability (ID), when TSC1 mutations and TSC2 mutations were compared, this did not reach statistical significance ($p = 0.41$). Information on the presence of ID in TSC2 mutations was known in 83% (30/36) and ID was found in 53% (16/30). Information on the presence of ID was known in 84% (16/19) of TSC1 mutations and ID was found in 31% (5/16). Only 9% (1/11) had ID in the NMI group. Neurobehavioral manifestations such as mood disorders, anxiety and aggression were observed similarly across all groups: TSC2 mutations 28% (10/36) versus 26% (5/19) in TSC1 mutations versus 27% (3/11) in the NMI group ($p = 0.95$). There was no statistical difference in the frequency of neurobehavioral manifestations between TSC2 mutations and TSC1 mutations ($p = 1.0$)

Neuroimaging Findings

The presence of tubers on brain magnetic resonance imaging (MRI) was seen at similar frequencies across the groups. Tubers were found in 97% (35/36) of TSC2 mutations versus 95% (18/19) of TSC1 mutations and 82% (9/11) of the NMI group ($p = 0.28$). Subependymal nodules (SENs) were found in 97% (35/36) of TSC2 mutations versus 100% (19/19) of TSC1 mutations and in 64% (7/11) of the NMI group ($p = 0.002$). Subependymal giant cell astrocytoma (SEGA) were found in 67% (24/36) of TSC2 mutations and 63% (12/19) of TSC1 mutations, but not in the NMI group ($p = 0.001$). There was no statistical difference in the presence of tubers, SEN and SEGA between TSC1 mutations and TSC2 mutations (Table 1).

Dermatological Manifestations

Most patients had one or more TSC skin manifestation, regardless of the mutation type. There was no statistical difference found between the 3 groups ($p = 0.62$) with regard to the presence of skin manifestations. Skin manifestations were found in 97% (34/35); (the skin finding was unknown in one patient) of TSC2 mutations and 89% (16/18); (the skin finding was unknown in one patient) of TSC1 mutations. In the NMI group, the status of skin manifestation was known in 10/11 patients and all had skin findings. When TSC2 and TSC1 groups were compared, there was no statistical difference with regard to the frequency of skin manifestations ($p = 0.36$).

Ophthalmological Manifestations

Retinal hamartomas were more common in TSC2 mutations, 59% (20/34) (2 patients with TSC2 mutations had unknown eye findings); when compared to TSC1 mutations, 13% (2/16) (3 patients with TSC1 mutation had unknown eye findings); and none in the NMI group ($p = 0.001$).

Systemic Manifestations

Renal Manifestations

Renal angiomyolipomas (AMLs) and multiple renal cysts were found more frequently with TSC2 mutations. In TSC2 mutations, AMLs were found in 72% (26/36) versus 37% (7/19) in TSC1 mutations and 36% (4/11) in the NMI group, ($p = 0.01$). When the frequency of AMLs was compared between the TSC2 and TSC1 mutations, AMLs were more common in TSC2 mutations and this reached statistical significance ($p = 0.01$). AMLs were almost invariably found bilaterally in the TSC2 mutation group 96% (25/26), and in the NMI group 100% (4/4). In contrast, 43% of those with TSC1 mutations had bilateral lesions (3/7) and 57% had a lesion in a single kidney (4/7). Large AML lesions, defined by a diameter of more than 3 cm, were documented in 23% (6/26) of patients with TSC2 mutations, but none of the other patients. Multiple renal cysts were also significantly more common in the TSC2 group, affecting 31% (11/36), while none were documented in the TSC1 group and only one patient in the NMI group had renal cysts, 9% (1/11), ($p = 0.01$). Two patients with multiple renal cysts had contiguous TSC2 and PKD1 mutations.

Cardiac Manifestations

Cardiac rhabdomyomas were diagnosed in 69% (25/36) of TSC2 mutations versus 58% (11/19) of TSC1 mutations and 27% (3/11) of the NMI group, ($p = 0.04$). Although, when the frequency of cardiac rhabdomyomas was compared between the TSC2 and TSC1 mutations, this did not reach statistical significance ($p = 0.29$).

Liver Manifestations

Liver hamartomas were observed only in those with TSC2 mutations, 28% (10/36) and were not found in patients with TSC1 mutations or NMI, ($p = 0.006$).

Discussion

Our study is the first Canadian cohort examining the genotype-phenotype relationship in TSC and consisted of 66 patients, of whom the majority had either a TSC1 (29%) or TSC2 mutation (54%). Similar to what is previously reported and what is known about the genetics of TSC, 17% of the patients had NMI.[2]

Our results highlight several important findings with regard to the neurological manifestations of TSC. Individuals with TSC2 mutations had an earlier age of onset of epilepsy when compared to TSC1 mutations and NMI. The presence of epilepsy was slightly more common in those with TSC2 mutations in our cohort, but this did not reach statistical significance when compared to the NMI and TSC1 group. Previous studies have found that patients with TSC2 mutations have a higher frequency of epilepsy when compared to TSC1 mutations, but this finding was not replicated in our cohort.[3,12-14] Our data did not demonstrate that individuals with TSC2 mutations may be more at risk for the development of infantile spasms in contrast to previous studies.[8,12] However, the presence of refractory epilepsy was found similarly across all groups in our cohort, which is in keeping with previous reports.[15]

TSC2 mutations have been reported to be associated with a higher risk of ID and more severe ID.[3,13,16,17] However, we did not demonstrate this finding. It has been observed that different variants in the TSC2 gene have been found to be associated with milder phenotypes without ID.[18,19] It is possible that certain TSC2 variants in our cohort may also be associated without ID or with milder cases of ID. However, we did not demonstrate this finding possibly due to our small cohort size and a lack of recurrent variants in our cohort. It is also possible that some cases of mild ID could have gone undetected in our cohort as formal neuropsychological testing was not always available. The cognitive profile in patients with TSC may also not be related to the genotype, but instead related to earlier onset of seizures, polytherapy, multiple seizure types and seizure frequency.[20] We did not demonstrate a difference in the neurobehavioral features of TSC between the groups in our cohort, although this could also depend on the severity of epilepsy rather than on the specific genetic mutation.[21]

The presence of cortical/subcortical tubers has been variably reported in the literature, with some citing tubers to be more frequent in those with TSC2 mutations (13-14) while others have reported no difference between TSC1 and TSC2 mutations, similar to our cohort.[12] The presence of SEGA was similar in TSC1 and TSC2 mutations, and it was found in no individuals with NMI.[15] Our findings contrast what has been reported in the literature with respect to the frequency of SENs and SEGAs. Overall, the presence of SENs and SEGAs have been more frequently reported in those with TSC2 mutations.[12,13] Although we found similar frequencies in the presence of SEGAs between TSC1 and TSC2 mutations in our cohort.

The systemic manifestations in our cohort tended to be more severe in the TSC2 group. AMLs and renal cysts were more frequently seen in individuals with TSC2 mutations and were more likely to be bilateral and large. This is an important clinical parameter since large AMLs have a much higher risk of bleeding and usually require intervention. A higher frequency of AMLs has been previously reported in patients with NMI when compared to TSC1, but we did not replicate this finding.[16]Although, a higher grade of AML involvement has been observed when comparing patients with NMI to TSC1, no difference in severity was observed between NMI and TSC2.[13,15,22] Our results contrast these findings and suggest that the renal phenotype may be more severe in those with TSC2 mutations (more frequent, bilateral and larger AMLs-i.e. more likely to bleed), when compared to TSC1 mutations and NMI. Hepatic involvement in the form of hamartomas was found only in the TSC2 group in our cohort, which is similar to previous reports, although some larger cohorts have documented their presence in all groups.[13,23,24] It has been previously observed that cardiac rhabdomyomas are more common in

TSC2 mutations,[13,15] although our data did not demonstrate this finding.

Overall, we did not replicate some findings previously reported in the literature with respect to the genotype-phenotype relationship in TSC. It is possible that somatic mosaicism could be responsible for the differences in phenotype severity observed across our cohort. Unfortunately, we were unable to fully test for mosaicism. Fifty-eight percent of TSC patients with NMI were observed in one study to have mosaic TSC1/TS2 pathogenic variants.[2] The spectrum of mosaicism in TSC has been described to be variable, with milder phenotypes with isolated cutaneous findings to more severe phenotypes being described.[25,26] Our study has further limitations including that it was small single center retrospective study and we were unable to draw conclusions about specific variant types within the genes. Further, our study represents a subset of patients from a single country and therefore may not be generalizable to all patients with TSC. Finally, there is also the possibility of ascertainment bias, given that an unexpectedly large portion of the patients in our cohort had SEGAs (55%) compared to the expected frequency in TSC (20%). Future directions include performing extended genetic testing on our NMI cohort and pooling of more patients with TSC to better understand the genotype-phenotype relationship depending on the specific mutation type.

Conclusions

In summary, we have highlighted the diverse clinical spectrum of TSC in a population of children in Canada according to the underlying genotype. It is important to emphasize that overlap in disease severity can occur across the mutations and genotype-phenotype correlations are not exact. Disease surveillance and counseling should be similar across patients with TSC regardless of genotype. In the future, larger pooling of patients may allow us to better understand the complex genotype-phenotype relationship of TSC, which may further aid in disease counselling/prognostication.

Author Contributions

Daad Alsowat and Robyn Whitney contributed equally and designated as co-first authors.

Declaration of Conflicting Interests

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