The Canadian Journal of Neurological Sciences (2024), **51**, 636–643
doi:10.1017/cjn.2023.332

Canadian Journal of Neurological Sciences
Journal Canadien des Sciences Neurologiques

Original Article

Retrospective Analysis of Canadian Adults with Tuberous Sclerosis Complex

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ABSTRACT: ***Background:*** Our study goal was to characterize the relative frequencies of molecular and phenotypic traits of tuberous sclerosis complex (TSC) in a Canadian adult population. Previous studies have sought to identify TSC-related genotypic and phenotypic trends in pediatric cohorts, but little is known about clinical manifestations and severity when it presents in adults. ***Methods:*** We conducted a retrospective chart review of adult patients seen at the TSC clinic at the University Health Network genetics clinics (Toronto, Ontario) to compare trends in the relative frequency of TSC manifestations with genotype. ***Results:*** Fifty-one patients were eligible for this study. Eight patients had a pathogenic/likely pathogenic variant in the tuberous sclerosis complex 1 (*TSC1)* gene, 18 had a tuberous sclerosis complex 2 (*TSC2)* pathogenic/likely pathogenic variant, 6 patients had multiple variants identified in *TSC1*/*TSC2* or *TSC2*/*PKD1*, 11 had no mutation identified (NMI) and 8 had no genetic testing done. Patients with a pathogenic/likely pathogenic variant in *TSC2* presented with an increased involvement of multiple systems and a higher frequency of TSC-related manifestations relative to the other mutation groups. ***Conclusion:*** Previous studies comparing the wide phenotypic variability with TSC genotype have mainly comprised pediatric cohorts. With a focus on adults, we found trends to be similar across previous literature. An informed multidisciplinary approach should be taken to ensure proper surveillance and management of adults with TSC until a correlation between genotype and phenotype, especially past infancy, is better understood.

RÉSUMÉ : Analyse rétrospective d'adultes canadiens atteints de sclérose tubéreuse de Bourneville. ***Contexte :*** Dans cette étude, notre objectif a été de caractériser les fréquences relatives des traits moléculaires et phénotypiques de la sclérose tubéreuse de Bourneville (STB) au sein d'une population adulte canadienne. Notons que des études antérieures ont cherché à identifier les tendances génotypiques et phénotypiques liées à la STB dans des cohortes pédiatriques. Cela dit, on sait peu de choses sur les manifestations cliniques et la gravité de cette maladie lorsqu'elle se manifeste chez l'adulte. ***Méthodes :*** Nous avons effectué une étude rétrospective des dossiers de patients adultes vus à la clinique de STB qu'on retrouve au sein des University Health Network Genetics Clinics (Toronto, Ontario) afin de comparer entre elles les fréquences relatives de manifestations de la STB en fonction du génotype. ***Résultats :*** Au total, 51 patients étaient admissibles à cette étude. Soulignons que 8 d'entre eux présentaient un variant pathogène/probablement pathogène dans le gène TSC1 tandis que 18 d'entre eux présentaient un variant pathogène/probablement pathogène dans le gène TSC2. Plus encore, 6 patients présentaient plusieurs variants identifiés dans les gènes TSC1, TSC2 ou encore TSC2/PKD1, tandis que 11 patients ne présentaient aucune mutation identifiée (AMI) et 8 n'avaient pas effectué de tests génétiques. Les patients présentant un variant pathogène/probablement pathogène dans le gène TSC2 ont présenté une implication accrue de plusieurs systèmes et une fréquence plus élevée de manifestations liées à la STB par rapport aux autres catégories de mutations. ***Conclusion :*** Les études précédentes comparant la grande variabilité phénotypique au génotype de la STB comprenaient principalement des cohortes pédiatriques. En nous concentrant sur des adultes, nous avons constaté que les tendances étaient similaires à celles de la littérature antérieure. Une approche multidisciplinaire éclairée devrait ainsi être adoptée pour assurer une surveillance et une prise en charge adéquates des adultes atteints de STB, et ce, jusqu'à ce que la corrélation entre le génotype et le phénotype, en particulier après la petite enfance, soit mieux comprise.

Keywords: Tuberous sclerosis complex; mTOR inhibitors; lesions; management; genetics

(Received 19 June 2023; final revisions submitted 28 November 2023; date of acceptance 21 December 2023; First Published online 27 December 2023)

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Cite this article: Pal D, Forster N, Madan M, Whitney R, Farncombe KM, and Kim RH. (2024) Retrospective Analysis of Canadian Adults with Tuberous Sclerosis Complex. *The Canadian Journal of Neurological Sciences* **51**: 636–643, https://doi.org/10.1017/cjn.2023.332

Introduction

Tuberous sclerosis complex (TSC) is a rare, autosomal-dominant disorder characterized by hamartomas in multiple organs.[1] It affects an estimated 1 in 6,000 to 10,000 individuals at birth[2] and is caused by germline pathogenic variants in the tumor suppressor genes tuberous sclerosis complex 1 (*TSC1)* and tuberous sclerosis complex 2 (*TSC2),* which function as part of the mammalian target of rapamycin (mTOR) pathway to regulate cell growth, size and proliferation.[3] *De novo* pathogenic variants are identified in two-thirds of individuals with TSC, while the remainder of variants are inherited.[4]

Individuals with TSC often show multiple organ system involvement, including the brain, skin, kidneys, heart, lungs and eyes.[4] Nearly all patients with TSC present with skin manifestations, including hypomelanotic macules, facial angiofibroma and shagreen patches.[4] Central nervous system involvement is seen in 90% of patients with TSC, including subependymal nodules (SEN), cortical tubers and subependymal giant cell astrocytoma (SEGA).[4] Furthermore, 80% of individuals with TSC will have a renal lesion by 10.5 years of age,[5] including benign angiomyolipoma and renal cysts.[4] Neurological and renal malignancies are the leading causes of death in TSC patients.[4]

A clinical diagnosis of TSC is made in patients showing two major characteristics or one major characteristic and two minor characteristics.[6] In addition, a pathogenic variant in *TSC1* or *TSC2* is sufficient for a diagnosis of TSC.[4] Although both genetic and clinical criteria are used to diagnose TSC, wide phenotypic variability often complicates the diagnosis, and 10%–25% of individuals with TSC do not have a germline pathogenic variant identified by regular genetic testing, referred to as no mutation identified (NMI).[7] Due to wide phenotypic variability, patient care and management vary depending on an individual's clinical features.

Previous studies have reported that patients with *TSC2* pathogenic variants usually present with a more severe phenotype (i.e., when compared to *TSC1* and those with NMI), which consists of a greater number of tubers, earlier age of seizure onset and greater risk for intellectual disability.[8] Eighty percent of those affected by TSC are diagnosed in infancy or early childhood; therefore, some individuals have unrecognized manifestations and do not receive a diagnosis until adulthood.[9] Recent studies involving patients with TSC have a strong focus on children, leaving a gap in knowledge in the clinical manifestations in adults with TSC. Furthermore, multisystem involvement in adults with TSC has yet to be reported in depth in the current literature, which brings the need to highlight how the progression or delayed diagnosis of TSC may affect the adult population. As a result, it is imperative to explore genotype-phenotype correlations to ensure structured management and surveillance are maintained with the variable phenotypic expression seen over time in adult patients with TSC.[10] In this study, we aimed to characterize the phenotypic and molecular characteristics of a Canadian adult TSC population to improve multidisciplinary care and adult patient management.

Materials and Methods

Participant Inclusion

We performed a retrospective chart review of adult TSC patients who were followed at the Fred A. Litwin Center for Genetic Medicine (University Health Network, Toronto, Canada) between January 1, 2001–August 10, 2020. Male and female patients ≥ 18 years of age with a clinical[6] or genetic diagnosis of TSC were reviewed; however, only individuals with a definitive diagnosis of TSC were included in the analysis. Data was collected through electronic patient records and paper charts, with information entered into a REDCap database. Approval was obtained from the Research Ethics Board at the University Health Network.

REDCap Database

The Research Electronic Data Capture (REDCap) database was developed as a secure, web-based platform for research teams to collect, store and disseminate project-specific information.[11] An internally housed REDCap database was constructed to collect patient medical history, family history and information relevant to the adult TSC experience. All patients were offered screening for possible TSC manifestations, per updated clinical surveillance guidelines.[6] Patients were typically offered a follow-up appointment every 12–16 months, where their interval medical history was reviewed and investigations were arranged.

The REDCap database included relevant information related to demographics, family history, genetic testing and TSC manifestations. TSC clinical features, such as neurological, skin, renal, eye, cardiac, respiratory, liver and reproductive systems were captured for analysis. In addition, behavioral information was also recorded, as was patient management information, such as imaging of affected systems and current/previous interventions. Finally, genetic testing results, including variant type, variant nomenclature, inheritance pattern and certainty of diagnosis, were recorded.

Genetic Testing

Of the 51 patients in our cohort, eight did not have genetic testing done (16%). The remaining 43 patients (84%) had genetic testing done by next-generation sequencing, sanger sequencing and multiligation-dependent probe amplification. Genetic testing results were classified according to the American College of Medical Genetics standards and include pathogenic, likely pathogenic and variants of unknown significance.[12] All genetic test results were reviewed by the clinic's Genetic Counsellor and Medical Geneticist. For our study, eligible patients were grouped according to their genetic testing results (see Fig. 1).

Statistical Analysis

The frequency of clinical manifestations was compared between the *TSC1* and *TSC2* groups, as well as between the *TSC1* or *TSC2* and the NMI group. R-studio (version 2022.7.1.554) was used to perform a chi-square test for the categorical data collected in this study.[13] P-values < 0.05 were considered statistically significant.

Results

Our cohort included 51 patients with a confirmed clinical diagnosis of TSC. The age range of the patients was 19 years to 66 years, with a median age of 32 years. Fifty-one percent (26/51) of the cohort were male and 49% (25/51) were female. Sixteen percent (8/51) of individuals had a *TSC1* variant and approximately 35% (18/51) of patients had a *TSC2* variant confirmed by genetic testing. In addition, four individuals had variants identified in both the *TSC1* and *TSC2* genes, and two patients had whole deletions of both the *TSC2* and *PKD1* genes. Twenty-two percent (11/51) of our cohort had NMI through germline genetic testing and an additional eight individuals did not have confirmatory genetic

https://doi.org/10.1017/cjn.2023.332 Published online by Cambridge University Press

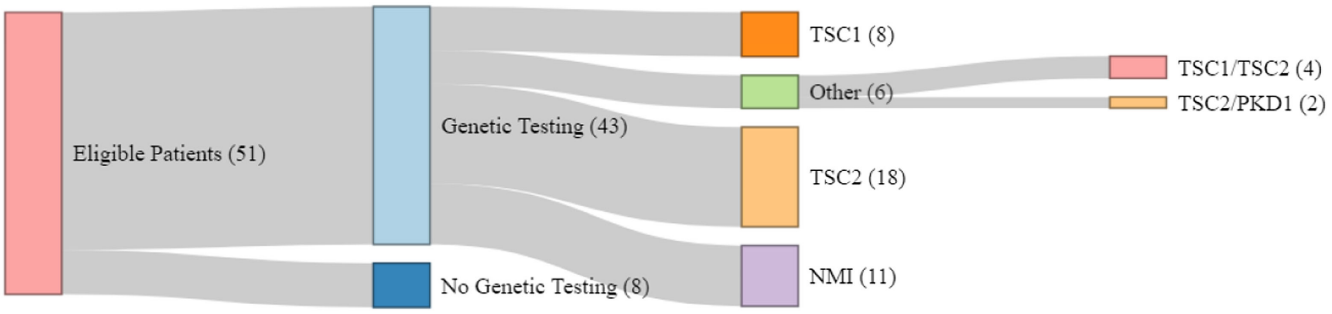

Figure 1: Categorization of 51 eligible patients into groups based on genetic testing results.

Table 1: Variants associated with TSC Canadian adults. *TSC2* was the most frequent variant identified in this cohort. Reference sequences: NM_000368.5 (TSC1); NM_000548.5 (TSC2)

Gene	Study ID	Variant	Codon Change	Classification
TSC1	7	c.2074C>T	p. Arg692*	Pathogenic
	8	c.363 + 5G>A	None	VUS
	11	c.2757_2760del	p. Lys919Asnfs*11	Likely pathogenic
	13	c.2110dup	p. Tyr704Leufs*2	Likely pathogenic
	29	c.2509_2512del	p. Asn837Valfs*11	Pathogenic
	35	c.733C>T	p. Arg245*	Pathogenic
	45	c.1579C>T	p. Gln527*	Pathogenic
TSC2	1	c.2638_2639 + 2del	None	Likely pathogenic
	2	c.1832G>A	p. Arg611Gln	Pathogenic
	3	c.1844T>C	p. Phe615Ser	VUS
	4	c.5227C>T	p. Arg1743Trp	Pathogenic
	9	c.2353C>T	p. Gln785*	Pathogenic
	14	c.2370C>G	p. Tyr790*	Pathogenic
	17	c.246G>A	p. Trp82*	Pathogenic
	18	c.1689delC	p. Val564Serfs*134	Likely pathogenic
	20	c.5288_5289delCCinsT	p. Pro1763Leufs*63	Likely pathogenic
	27	c.3611-2A>G	None	Likely pathogenic
	28	c.4504delC	p. Leu1502Cysfs*74	Pathogenic
	31	c.5009A>C	p. His1670Pro	VUS
	42	c.3611_3614delGGAAinsCTCGCTGCCG	p. Gly1204_Asn1205delinsAlaArgCysArg	VUS
	47	c.3610G>A	p. Gly1204Arg	Pathogenic
	48	c.3212C>G	p. Thr1071Arg	VUS
TSC1, TSC2	6	c.566A>C c.2180C>T	p. His189Pro p. Pro727Leu	VUS VUS
	16	c.2000T>A c.821A>G	p. Leu667* p. Tyr274Cys	Likely pathogenic VUS
	19	c.1425dup c.856A>G	p. Asp478Argfs*2 p. Met286Val	Likely Pathogenic Benign
	36	c.1379del c.5017G>C	p. Pro460Glnfs*4 p. Val1673Leu	Pathogenic Benign
TSC2/PKD1	39	Whole deletion (arr[GRCh37] 16p13.3(2032065_2155562)x1)	–	
	43	Whole gene deletion (breakdown points unknown)	–	

None = Splice site variant; VUS = Variant of unknown significance.

testing; however, they met the clinical criteria of TSC. Table 1 displays genetic testing results of patients included in the study, grouped by gene.

For the purpose of this analysis, we focused on the most prevalent systems affected: neurological, skin, renal, eyes, liver, cardiac and the lungs. Within our cohort, the most prevalent manifestations included: SEN, SEGA, cortical tubers, renal angiomyolipoma's (AMLs) and skin features, such as hypomelanotic macules (Tables 2 and 3). Organ system involvement varied among patients within the cohort. The systems that were observed to be most affected in our cohort were the neurological, dermatological and renal systems. However, only the

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Table 2: P-values yielded from chi-square test comparing frequency of manifestations between the *TSC1* and *TSC2* groups and comparing *TSC1/TSC2* group with the NMI group. *P*-values < 0.05 are considered statistically significant

Clinical Manifestation		Frequency (%)			p-values	
		TSC1 (n = 8)	*TSC2 (n = 18)*	*NMI (n = 11)*	*TSC1* vs *TSC2*	*TSC1/TSC2* vs NMI
Neurological findings	Cortical Tubers	50	67	55	0.66	0.85
	SEN	63.5	72	66	0.67	0.29
	SEGA	0	39	18	0.07	0.05
	Seizures	100	83	36	0.53	0.022
	Epilepsy	25	44	9	0.43	0.15
	TAND	12.5	6	9	0.22	0.68
	ASD	25	28	18	0.87	1.0
Skin Features	Hypomelanotic macules	38	78	83	0.43	
	Shagreen patches	88	33	67	0.003*	
	Facial angiofibroma	88	83	0		
	Fibrous cephalic patches	13	11	33		
Renal Features	AML	50	83	81	0.14	0.19
	Renal cysts	50	44	36	1.0	0.93
Cardiac Features	Cardiac rhabdomyoma	0	50	9	0.01*	0.02*
Liver Features	Liver AML	0	22	9	0.78	
	Liver nodules/tubers	0	6	9	0.49	
	Hamartoma	0	6	0		
Lung features	LAM	25	22	18	1.0	0.74

ASD=autism spectrum disorder; AML=angiomyolipoma; LAM=lymphangiomyomatosis; SEN=subependymal nodules; SEGA=subependymal giant cell astrocytoma; TAND=TSC-associated Neuropsychiatric disorder.
*indicates p-value<0.05; statistically significant,

dermatological and cardiac manifestations had statistically significant differences when compared across the phenotypes (Table 2). Dermatological manifestations were present among all groups, with hypomelanotic macules being the most frequent feature. Other prevalent features include shagreen patches and facial angiofibroma. Cardiac rhabdomyoma was the most prevalent cardiac feature amongst our cohort and was recorded only in the *TSC2* group, "other" variants and the NMI group.

Although there were no statistically significant differences detected between the other systems affected in TSC and genotype, some notable findings were observed. TSC-associated neuropsychiatric features (TAND) varied among the different groups, with behavioral disturbances and cognitive impairment observed most frequently. Of the individuals with a contiguous mutation in *TSC2/PKD1*, interestingly both presented with AMLs and cysts, which led to hypertension. mTOR treatment was given in 35% (18/51) patients; 16 patients were given Everolimus and two patients were given Sirolimus to manage systemic features but with an emphasis on renal manifestation management.

A summary of the observed frequency of clinical manifestations and statistical significance of our analysis based on chi-square tests between *TSC1* and *TSC2* and between the *TSC* group with the NMI group can be found in Table 2. A summary of clinical manifestations and genetic testing results can be seen in Table 3.

Discussion

Our study aimed to examine genotype and phenotype relative frequencies in adults with TSC. Our cohort consisted of 51 adult patients, with an age range of patients 19 to 66 years of age, consisting of 51% (26/51) males and 49% (25/51) females. The most prevalent manifestations in our cohort included: SEN, SEGA, cortical tubers, renal AMLs and skin features, such as hypomelanotic macules. We observed that approximately 16% of individuals had a *TSC1* variant and 35% presented with a *TSC2* variant. This finding is consistent with previous reports from the literature, showing that *TSC2* variants are more common than variants in *TSC1*.[14]

TSC2 variants are associated with a more severe phenotype with age;[15] in our cohort, those with *TSC2* variants presented with SEN, SEGA and cortical tubers more frequently than those with *TSC1* variants. However, due to the lack of statistical significance, our data does not confidently support previous studies showing that SEN and SEGA are more common in patients with a *TSC2* variant.[15] This is likely secondary to small sample size and lack of power to detect such differences. In reviewed literature, however, tubers often did not differ between the two groups.[15] In addition, *TSC2* variants are associated with higher risk of intellectual disability.[16] Our data was consistent with these findings, as individuals with a *TSC2* variant presented with a higher frequency of cognitive impairment and ASD, but again the finding was not statistically significant. In the literature, multiple TAND symptoms are associated with approximately 90% of affected individuals.[17] In addition, it is important to consider that TAND symptoms may arise later in life due to the diversity of psychosocial factors that influence the diagnosis of neuropsychiatric symptoms.[17] Our cohort underrepresents this metric, which reinforces the idea that TAND symptoms may be undiagnosed or untreated when TSC is diagnosed later in life.[17] Studies report that TAND was only screened in 4% of patients at the time of their referral to a TSC

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Table 3: Clinical manifestations present in an adult TSC cohort of 43 patients. Manifestations in the brain, kidney and skin were the most frequently reported. NM_000368.5 (TSC1); NM_000548.5 (TSC2)

			Brain					Kidney			Skin					Heart		Lungs	Liver			Eyes		Dental
Mutation Group	Study ID	Variant	CT	SEN	SEGA	Seizures	CI	AML	RC	PKD	HM	SP	FA	FP	UF	CR	LAM	Nod.	Ham.	AML	Cysts	RH	Pits	GF
TSC1	7	c.2074C>T (p. Arg692*)		✓		✓	✓	✓	✓			✓	✓	✓			✓							
	8	c.363 + 5G>A	✓			✓						✓	✓											
	11	c.2757_2760del (p. Lys919Asnfs*11)	✓	✓		✓		✓			✓	✓	✓		✓		✓						✓	
	13	c.2110dup (p. Tyr704Leufs*2)				✓			✓			✓	✓		✓									
	29	c.2509_2512del (p. Asn837Valfs*11)		✓		✓			✓			✓	✓		✓								✓	✓
	35	c.733C>T (p. Arg245*)				✓		✓	✓		✓	✓	✓											
	41			✓		✓						✓	✓		✓									
	45	c.1579C>T (p. Gln527*)		✓		✓		✓			✓													
TSC2	1	c.2638_2639 + 2delAAGT		✓		✓		✓			✓	✓	✓	✓	✓	✓	✓					✓		✓
	2	c.1832G>A (p. Arg611Gln)	✓	✓							✓											✓		
	3	c.1844T>C (p. Phe615Ser)			✓	✓		✓	✓								✓	✓					✓	
	4	c.5227C>T (p. Arg1743Trp)	✓			✓		✓			✓	✓	✓		✓		✓							
	9	c.2353C>T (p. Gln785*)		✓	✓	✓	✓	✓	✓		✓		✓	✓		✓						✓		
	10		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓							✓		✓
	14	c.2370C>G (p. Tyr790*)	✓	✓		✓	✓	✓				✓	✓		✓									
	17	c.246G>A (p. Trp82*)	✓	✓				✓			✓		✓			✓				✓				
	18	c.1689del (p. Val564Serfs*134)		✓		✓	✓	✓			✓		✓		✓		✓					✓	✓	
	20	c.5288_5289delCCinsT (p. Pro1763Leufs*63)	✓			✓			✓		✓		✓			✓								
	23		✓	✓	✓	✓	✓	✓			✓		✓			✓								
	27	c.3611-2A>G	✓	✓		✓	✓	✓	✓				✓			✓				✓		✓		
	28	c.4504del (p. Leu1502Cysfs*74)			✓	✓	✓	✓								✓								
	31	c.5009A>C (p. His1670Pro)	✓	✓		✓	✓	✓	✓	✓	✓		✓		✓									
	33		✓	✓	✓	✓	✓	✓			✓		✓		✓	✓			✓					
	42	c.3611_3614delGGAAinsCTCGCTGCCG (p.1204fs)	✓	✓		✓		✓			✓	✓	✓							✓				
	47	c.3610G>A (p. Gly1204_Asn1205delinsAlaArgCysArg)	✓	✓	✓	✓	✓	✓	✓		✓		✓			✓				✓		✓		✓
	48	c.3212C>G (p. Thr107Arg)					✓		✓		✓	✓	✓											

Multiple	6	*TSC1*: c.566A>C (p. His189Pro) *TSC2*: c.2180C>T (p. Pro727Leu)		✓			✓	✓		✓	✓	✓	✓		✓								
	16	*TSC1*: c.2000T>A (p. Leu667*) *TSC2*: c.821A>G (p. Tyr274Cys)	✓	✓				✓		✓	✓	✓			✓								
	19	*TSC1:* c.1425dup (p. Asp476Argfs*2) *TSC2:* c.856A>G (p. P.Met286Val)	✓	✓		✓		✓		✓	✓	✓		✓	✓							✓	
	36	*TSC1:* c.1379del (p. Pro460Glnfs*4) *TSC2:* c.5017G>C (p. Val1673Leu)	✓	✓		✓			✓	✓	✓	✓	✓										
	39	TSC2/PKD1 Whole Deletion: arr[GRCh37] 16p13.3(2032065_2155562)x1 -	✓							✓		✓		✓	✓					✓	✓		
	43	TSC2/PKD1 Whole Deletion: breakdown points unknown						✓		✓		✓			✓						✓		
NMI	5	–		✓				✓			✓	✓		✓		✓							
	12	–			✓							✓											
	15	–						✓				✓			✓							✓	
	22	–	✓					✓				✓										✓	
	24	–	✓			✓		✓															
	25	–						✓		✓		✓											
	30	–	✓	✓				✓		✓		✓											
	32	–	✓			✓			✓			✓											
	34	–	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓	✓								
	46	–				✓		✓	✓	✓							✓						
	49	–	✓	✓				✓	✓	✓		✓							✓				

AML=angiomyolipoma; CI=cognitive impairment; CT=cortical tubers; CR=cardiac rhabdomyoma; FA=facial angiofibroma; FP=forehead plaque; GF=gingival fibroma; Ham=[liver] hamartoma; HM=hypomelanotic macules; LAM=lymphangioleiomyomatosis; NMI=no mutation identified; Nod=[liver] nodules; RC=renal cysts; RH=retinal hamartoma; PKD=polycystic kidney disease; SEN=subependymal nodules; SEGA=subependymal giant cell astrocytoma; SP=shagreen patches; UF=ungual/peri-ungual fibroma.

clinic,[18] contributing to its underdiagnosis in adult patients. Our findings support the idea of reevaluation of TAND features on a regular basis.[17]

As we did not have access to pediatric records, we cannot make confident conclusions on trends surrounding early age of onset of TSC symptoms, such as infantile spasms and epilepsy. Our patient health information regarding progression of epilepsy was inconsistent for those included in our analyses, thus we cannot make claims on the details of TSC-related epilepsy in adults. For the purpose of this study, we defined patients with epilepsy as those who presented with a history of multiple (two or more) seizures.[19] Previous literature states that very little is known about the evolution of TSC-related epilepsy in adulthood.[20] However, our data revealed that a history of epilepsy was again more prevalent in patients with a variant in *TSC2*, which is also consistent with findings reported in previous studies.[15] Our cohort presented with a rate of epilepsy of approximately 25% (12/51), compared to previous literature indicating 62%–93% of TSC patients will have epilepsy.[21]Therefore, our data showed adults with TSC experienced epilepsy at lower rates and the burden of epilepsy may decrease over time. Moreover, about 70% of individuals with TSC experience seizure onset in the first year of life – making a *de novo* occurrence of epilepsy unlikely in adulthood, though little is known about the evolution of epilepsy in adulthood.[20] According to a study within a cohort of 231 patients, only four patients (12%) without a history of seizures developed epilepsy in adulthood, demonstrating an overall decreased risk with age.[22] However, some literature reports that epilepsy in adulthood may be underreported, especially in milder cases where clinical features may be more subtle or infrequent and this may have been the case in our cohort too.[21]

Patients with a variant of *TSC2* also present with an increased rate of systemic involvement.[23] Renal involvement, including both AMLs and cysts, was seen more frequently in those with a variant in *TSC2* in our cohort. However, when compared with other genotypes, this finding was not statistically significant. Previous studies have reported similar trends.[24] The *TSC2* and *PKD1* genes lie adjacent to each other on chromosome 16p, thus large deletions can disrupt the function of both genes.[25] Of the two patients who had a whole deletion of *TSC2/PKD1* genes, hepatic involvement was observed only in the *TSC2* group, which is consistent with previous studies.[24] Cardiac involvement between the two groups varied in the literature;[26] our data showed that cardiac rhabdomyoma was observed in patients with *TSC2* and NMI, but not *TSC1* variants. Since we did not have access to pediatric records, trends regarding cardiac rhabdomyoma may be underestimated as they typically regress by adulthood.[27]

Our findings regarding trends in adults with TSC were mostly consistent with those previously reported in the pediatric population, but our sample size was small, and the study was underpowered. Generally, patients in the *TSC2* group showed increased systemic involvement and overall frequency of TSC-related features. Future directions include examining severity in relation to inheritance type (*de novo*, familial, mosaic) to better understand the molecular basis of TSC manifestations.

Some limitations to our study may include being a single-center cohort, therefore, limiting our data to a smaller set of patients. Even within our cohort, eight patients did not have genetic testing and were therefore not part of the overall analysis. Moreover, due to the nature of the clinical findings found within the various affected systems, some statistical analyses grouped more than one type of clinical manifestation. We also conducted multiple comparisons as part of our statistical analysis, which can increase the possibility of a type I error, and adjustments were not made to the P value in the study to account for this.[28] In addition, the difference in *TSC1* and *TSC2* in the literature may be affected by reporting bias in different regions of the world.[29] The more severe phenotype in *TSC2* deficient patients may result in a reporting bias, leading to an overrepresentation of *TSC2* in the literature. Furthermore, we are also unable to look for somatic variants, as data regarding tumor testing were not obtained. Finally, the retrospective nature of our study brings a limited follow-up period and does not allow us to make claims on current experiences of patients involved in this study. Our study relied solely on retrospectively collected clinical data; therefore, it is possible that some findings are underreported. As data regarding age of diagnosis in our patient medical records were incomplete, we were unable to focus our analysis on the patients who were diagnosed with TSC in adulthood and further exploring age of onset for certain findings, such as epilepsy or cardiac rhabdomyoma.

Conclusions

Our study has explored and analyzed the large phenotypic variability associated with TSC genotypes in an adult population. Overall, our findings were consistent with previous literature, but elucidates lower epileptic trends amongst adults; which requires confirmation from other cohort studies. This supports that a multidisciplinary approach should be taken in order to manage TSC-related features in adults and provide appropriate care despite variants identified.

Data availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments. We would like to acknowledge the following individuals from the Hospital for Sick Children (Toronto, Ontario) who developed the pediatric REDCap database: Mathieu Lemaire, Blathnaid McCoy and Maria Zak. Our appreciation to Derek Wong for his assistance in the statistical analysis, and Carolina Sanabria Salas for her assistance in the variant analysis.

Statement of authorship. This study was designed and supervised by RHK and KMF. Data curation and analysis were performed by DP with guidance from RW. Clinical care for the patients was provided by NF, MM and RHK. The first draft of the manuscript was written by DP and KMF, and all authors commented on previous versions of the manuscript. All authors reviewed and approved the final manuscript.

Funding statement. R.H.K. is supported by Anonymous (2), the Bhalwani Family Charitable Foundation, Goldie R. Feldman, Karen Green and George Fischer Genomics and Genetics Fund, Lindy Green Family Foundation, the FDC Foundation, the Shar Foundation, the Devine/Sucharda Charitable Foundation, the Princess Margaret Cancer Foundation, Leslie E. Born and the Hal Jackman Foundation. D.P. received support from the FDC Foundation.

Competing interests. None.

Ethics approval. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Research Ethics Committee at the University Health Network (#20-5813).

Consent to participate. A consent waiver was obtained by the University Health Network Research Ethics Board for this study.

https://doi.org/10.1017/cjn.2023.332 Published online by Cambridge University Press

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https://doi.org/10.1017/cjn.2023.332 Published online by Cambridge University Press