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RESEARCH ARTICLE

Exploring the neurological features of individuals with germline PTEN variants: A multicenter study

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Abstract

Objective: *PTEN*, a known tumor suppressor gene, is a mediator of neurodevelopment. Individuals with germline pathogenic variants in the *PTEN* gene, molecularly defined as PTEN hamartoma tumor syndrome (PHTS), experience a variety of neurological and neuropsychiatric challenges during childhood, including autism spectrum disorder (ASD). However, the frequency and nature of seizures and the utilization of allied health services have not been described. **Methods**: Young patients with PHTS and sibling controls were recruited across five centers in the United States and followed every 6–12 months for a mean of 2.1 years. In addition to the history obtained from caregivers, neurodevelopmental evaluations and structured dysmorphology examinations were conducted, and brain MRI findings, received therapies, and epilepsy characteristics were reported. **Results**: One hundred and seven patients with PHTS (median age 8.7 years; range 3–21 years) and 38 controls were enrolled. ASD and epilepsy were frequent among patients with PHTS (51% and 15%, respectively), with generalized epilepsy strongly associated with ASD. Patients with epilepsy often required two antiseizure medications. Neuroimaging revealed prominent perivascular spaces and decreased peritrigonal myelination in individuals with PHTS-ASD. Allied therapy use was frequent and involved physical, occupational, speech, and social skills therapies, with 89% of all patients with PHTS, regardless of ASD diagnosis, utilizing at least one service. **Interpretation**: This prospective, longitudinal study highlights the wide neurological spectrum seen in young individuals with PHTS. ASD is common in PHTS, comorbid with epilepsy, and allied health services are used universally. Our findings inform care discussions with families about neurological outcomes in PHTS.

Introduction

PTEN hamartoma tumor syndrome (PHTS) is a molecular diagnosis characterized by germline pathogenic variants in the *PTEN* (phosphatase and tensin homolog) gene or deletions at the 10q23 locus irrespective of clinical features.[1] Patients with PHTS have macrocephaly and are predisposed to specific malignancies, hamartomas, and overgrowths, with a particularly increased frequency of breast, endometrial, thyroid, skin, and renal cancers.[2,3] Among young individuals with PHTS, a macrocephaly/autism syndrome (OMIM 605309) was identified, and PHTS is recognized as among the most common genetic causes of autism spectrum disorder (ASD).[2,4] Nearly 22% of patients with PHTS have ASD, and 4–17% of individuals with ASD and macrocephaly are suspected to harbor a germline *PTEN* pathogenic variant.[4–7] Despite the recognition of this neurodevelopmental phenotype in patients with PHTS, little is known about the neurological signs and symptoms experienced by these patients and how these change over time and during critical phases of neurodevelopment.

The psychological traits of ASD in patients with PHTS (PHTS-ASD) when compared with individuals with ASD without PHTS, include severe cognitive delays, deficits in attention, increased impulsivity, reduced reaction time, decreased processing speed, and impaired motor coordination, as well as other adaptive behavior and sensory deficits.[8] It is thought that these traits arise from disruption of frontal lobe networks.[5,9,10] Known MRI features of PHTS are from relatively small series and include enlarged perivascular spaces, focal cortical malformations, Chiari I malformation, cerebrovascular malformations, Lhermitte–Duclos disease, and multifocal periventricular white matter abnormalities.[11–13]

PHTS-ASD individuals also experience an increased risk of epilepsy, with an estimated prevalence of 15–20% of patients with PHTS, consistent with higher rates of epilepsy among all individuals with ASD (estimated at 7–10% in a meta-analysis of 48 cross-sectional and cohort studies).[14,15] Epilepsy in individuals with PHTS may be either generalized or focal, and case reports, but not case series, exist of patient with focal epileptogenic lesions who have been considered for epilepsy surgery.[14,16]

The predominant literature on PHTS is focused on cancer, and only recently have reports begun to focus on the neurobehavioral characteristics of these patients. There has been no large-scale, longitudinal characterization of head circumference, seizures, allied health usage, and imaging findings in patients with PHTS, and in this study, we report on these features using a large natural history study (clinicaltrials.gov: NCT02461446). The objective of this study was to report the early life neurologic features, complications, and their treatment, across the participants of this natural history study who had a molecular diagnosis of PHTS, comprising 107 patients and 38 controls. Additional publications have reported the dermatologic, metabolic, and neurobehavioral characteristics of participants in this natural history study.[8,9,17,18]

Materials and Methods

Study participants

Patient information and consent for study inclusion were collected from caregivers or participants themselves if they were able to provide informed consent. The cohort included 145 children, adolescents, and young adults, aged 3–21 years at time of study entry, of whom 38 were controls, and 107 of whom were diagnosed with PHTS, based on a pathogenic variant in *PTEN* or deletion of the *PTEN* locus. Patients were enrolled through the Rare Disease Clinical Research Network Developmental Synaptopathies Consortium (DSC) as part of a longitudinal natural history study of patients with PHTS (clinicaltrials.gov: NCT02461446). Patients were enrolled from five DSC sites (Cleveland Clinic, Boston Children's Hospital, Stanford University, and University of California, Los Angeles). Patients included in this study included the healthy controls, patients with germline pathogenic variants in PTEN, with or without a diagnosis of autism spectrum disorder. Patients without PTEN germline pathogenic variant, and autism spectrum disorder with macrocephaly, also examined in this natural history study, were not included in our analyses. Study visits occurred every 6–12 months. The institutional review board at each institution approved this study protocol, whereby Boston Children's IRB acted as the Reliant site. All the IRBs align with the principles of the Helsinki Declaration, Belmont Report, and Common Rule.

Demographics

Demographic information including age, race, sex, and ethnicity was obtained at the baseline visit and verified at all subsequent study visits.

Seizure evaluation and neuroimaging

Evaluation for suspected seizures or neuroimaging was at the clinician's discretion. Per the study protocol, EEG could be performed if a patient opted in to this testing. MRI was only done if otherwise clinically indicated. MRI findings reported by the treating clinician were reviewed when preparing this study, and notable features were

tabulated. MRI scanners used were those available at each institution, ranging from 1.5 T–3 T in field strength. The diagnosis of seizure and epilepsy were made by the treating clinician. Central imaging review and EEG review were not performed.

Medications

Clinician reported medications, their routes of administration, and doses were reviewed at each study visit. Weight-based dose information was not available.

Inclusion/exclusion criteria

For cohort inclusion, patients had to be 3–21 years of age at the initial visit, have a confirmed pathogenic germline variant in *PTEN* or a germline deletion of the *PTEN* locus, and provide informed consent (by patient, parent, or guardian, as appropriate given patient's age and cognitive ability) for study participation. Scheduled research visits were carried out at baseline, 6 months, 12 months, 18 months, and 24 months after study enrolment.

Data collection

For each patient, the following data were collected: personal and demographic data, *PTEN* genetic profile, dysmorphology findings (excluding dermatological findings), neuroimaging findings, epilepsy history, neurodevelopmental comorbidities (e.g., ASD and Lhermitte–Duclos disease), medications, and types of therapies used. Occipitofrontal circumference was measured by the treating clinician at every visit, and macrocephaly was defined as circumference greater than or equal to the 98th percentile for the matched age and sex. Participants were screened by a clinical psychologist with expertise in ASD to determine whether they met the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition criteria for ASD and underwent confirmatory testing in the form of Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observation Schedule-2 (ADOS). At every visit, in participants with clinician-diagnosed epilepsy, the type of epilepsy, age at seizure onset, and antiseizure medications prescribed were reported. EEG findings were not reported. Allied health visits were reported based on the type of therapy, the duration of visits, and the frequency of visits.

Statistical analyses

Descriptive statistics included frequencies, percentages, median, and interquartile range. Two sample t-tests and Wilcoxon tests were used to compare continuous variables across groups, and two-sided Fisher's exact tests or chi-square analyses were carried out for group comparisons across categorical variables. Group comparisons based on sex, ASD diagnosis, and control status were carried out. All analyses were conducted in Microsoft Excel and R studio version 4.1. In generating the aggregate trajectory for head circumference, individual patient trajectories were co-plotted on a scatter plot, and LOESS (locally estimated scatterplot smoothing) regression was used to interpolate an overall curve. Statistical significance was set at $p < 0.05$. The Bonferroni correction was used to adjust the cutoff for statistical significance when applying multiple hypothesis tests simultaneously.

Results

Demographic characteristics

Between August 2015 and March 2021, 145 patients were enrolled in the study cohort. An average of 2.1 years (range 0.8–3.7 years, IQR 2.0–2.2 years) of natural history data were analyzed per patient and data from baseline, 6-month, 12-month, and 2-year follow-up visits included for all study patients. Baseline demographic characteristics among the study participants are shown in (Table 1). Among the 145 study participants, 107 (74%) were diagnosed with PHTS, of whom 98 (92%) had a confirmed germline pathogenic variant in *PTEN*, and 9 (8%) had a variant of uncertain significance. Thirty-eight patients (26%) were controls, most commonly unaffected siblings of the PHTS index patient. Among the 107 patients with PHTS, 71 were male (66%), and the majority white (82 patients, 77%), and non-Hispanic/Latino (90 patients, 84%).

Fifty-one patients with PHTS (48%) were diagnosed with ASD compared to no controls, also followed for the same period. The median age at baseline evaluation was 8.6 years for patients with PHTS and 12.7 years for sibling controls.

ASD is frequent in young patients with PHTS

ASD was a frequent phenotype observed in this cohort of patients. ASD was diagnosed in 55 patients with PHTS (51%) compared to CDC estimates of baseline autism prevalence at 1 in 36 (2.8%).[19] Among these patients with ASD and PHTS, males were overrepresented, with 42 males (76%) among 55 individuals with PHTS and ASD, compared to 29 males (56%) of 52 individuals in the PHTS non-ASD group ($p = 0.03$, Fisher's exact test). PHTS-ASD males and females were diagnosed at distinct mean ages, though this difference was not statistically significant a (median 3.4 years for males vs. 5 years for

Table 1. Baseline demographics.

	PHTS (N = 107)	Controls (N = 38)
Median age at baseline visit (IQR)	8.6 years (5.3–11.2 years)	12.7 years (8.5–17.3 years)
Sex, *n* (%)		
Male	71 (66%)	17 (45%)
Female	34 (32%)	16 (42%)
Not provided	2 (2%)	5 (13%)
Race, *n* (%)		
White	82 (77%)	12 (32%)
Asian	3 (3%)	5 (13%)
Black	2 (2%)	2 (5%)
Biracial or multiracial	9 (8%)	7 (18%)
Unknown	11 (10%)	12 (32%)
Ethnicity		
Not Hispanic or Latino	90 (84%)	21 (55%)
Hispanic or Latino	13 (12%)	6 (16%)
Unknown	4 (4%)	11 (29%)
ASD, *n* (%)	55 (51%)	–
Macrocephaly, *n* (%)	86 (99%)[a]	–

Demographic variables across patients with PHTS (N = 107), compared with sibling controls (N = 38).
ASD, autism spectrum disorder; IQR, interquartile range; PHTS, PTEN hamartoma tumor syndrome; SD, standard deviation.
[a]Of 87 patients with head circumference reported.

females, $p = 0.44$, Wilcoxon test, Fig. 1A), and we observed greater variability in the age of ASD diagnosis among females with PHTS.

Growth trajectories of head circumference in PHTS

Macrocephaly was present in at least 99% of patients with PHTS (median head circumference 99th percentile, range 55th percentile to >99th percentile). We compiled all measured head (occipitofrontal) circumference values across patients with PHTS, grouped by sex, to generate PHTS-specific growth curves for head circumference (Fig. 1B). As described in the methods, each patient's head circumference trajectory was plotted individually and an interpolated curve was produced to represent the group average. Patients with PHTS, particularly males, had a more rapid relative trajectory of head circumference growth in the early years of life. Patients with PHTS and ASD did not display any statistically significant difference in head circumference when compared with those without ASD. There was no statistically significant difference in head circumference between individuals with PHTS and epilepsy and those without epilepsy. Head circumference values were available after 3 years of age and stabilized after age 6–10 years along a consistent trajectory, with relatively limited data for participants aged 15 years or greater.

Dysmorphological characteristics

While PHTS is not recognized as classically being associated with dysmorphisms, 77 (72%) of the 107 study participants diagnosed with PHTS underwent a thorough dysmorphological examination at a study visit. A total of 27 dysmorphological features were evaluated across patients in the study cohort (complete listing of features in Table S1). Of these 77 patients, 65 (59%) carried a diagnosis of ASD and 81 (73%) were males. Macrocephaly was the most frequently observed characteristic, present in all 75 patients for whom measurements were taken. In addition to macrocephaly, craniofacial dysmorphisms were common across individuals with PHTS, with dolichocephaly (40/77, 52%), long eyelashes (30/77, 39%), high arched palate (26/75 for whom it was evaluated, 35%), and pointed chin (26/77, 34%) being most common. Figure 1C depicts a bar plot of the frequencies of the dysmorphisms in patients with PHTS-ASD and PHTS-non-ASD subtypes. No single dysmorphic feature was associated with increased likelihood of an ASD diagnosis after multiple test correction.

Epilepsy in PHTS is often generalized and associates with ASD

Across patients with PHTS and epilepsy, ASD was a frequent comorbidity. Among the 107 patients with PHTS, 11 patients (10%) were diagnosed with epilepsy, of whom 8 (73%) were diagnosed with comorbid ASD. Among the 11 patients with PHTS and epilepsy, 6 patients (55%) had generalized epilepsy, 3 (27%) had focal epilepsy, and 2 (18%) had both focal and generalized epilepsy. Of the eight total patients with generalized epilepsy, two patients had tonic–clonic seizures, three had absence epilepsy, one had myoclonic seizures, one had epileptic spasms, and another had an unspecified seizure type. Two of these eight patients additionally reported unclassified events, not clearly diagnostic of seizure. No patients with solely clonic, tonic, or atonic seizures were observed. Among the five patients with focal epilepsy, four were with impairment of consciousness and one was without impairment of consciousness. One patient also had reported a history of autonomic seizures.

Among the six patients with PHTS and epilepsy for whom drug treatment information was available, one was on monotherapy, three were on two antiseizure medications, and two were treated with three or more medications. The most frequently used antiseizure medications in this population were lamotrigine, clobazam, and

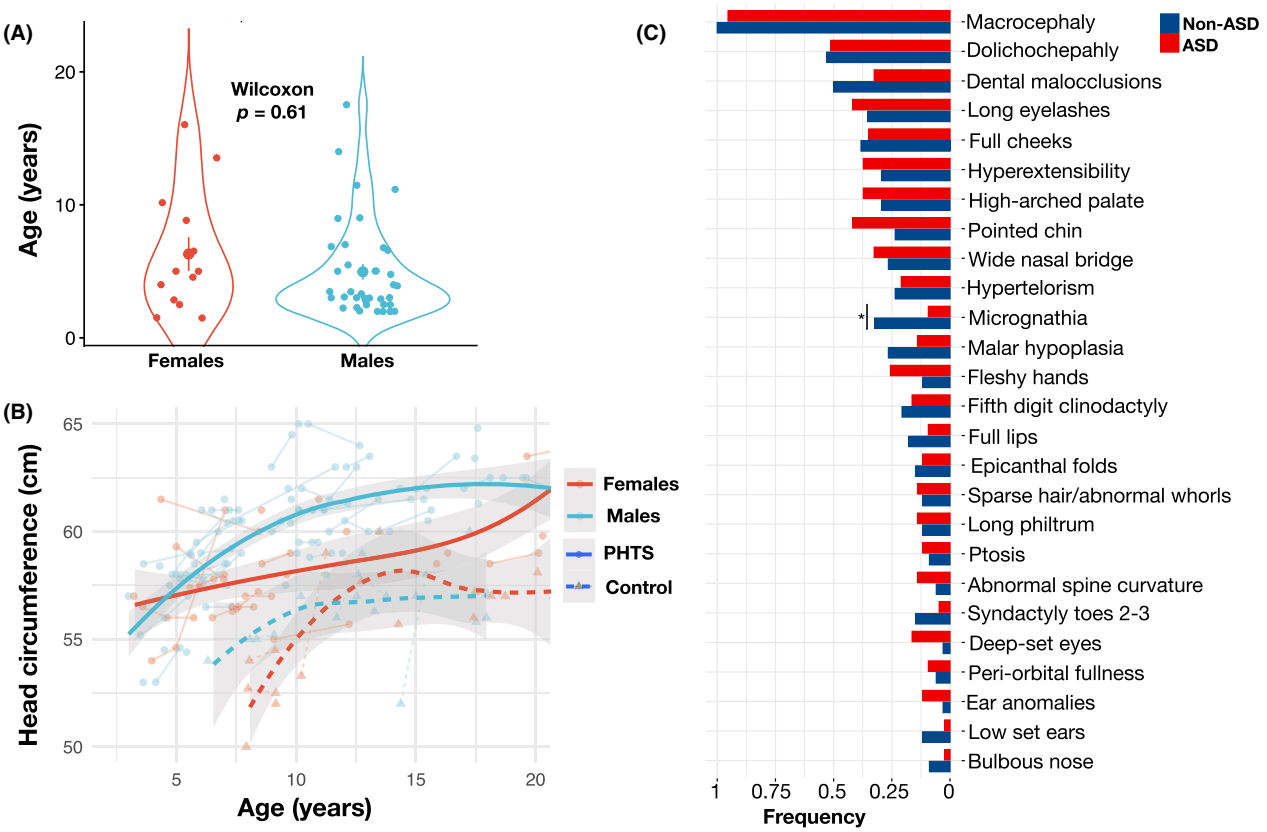

Figure 1. Clinical findings in patients with PHTS. A. Violin plots depicting the age of ASD diagnosis stratified by sex. B. Head circumference growth trajectories in patients with PHTS (solid lines) compared to healthy controls (dashed lines) for males (blue) and females (red). C. Bar plot depicting the frequency of dysmorphic features in PHTS with and without comorbid ASD. PHTS refers to PTEN hamartoma tumor syndrome. ASD refers to autism spectrum disorder.

levetiracetam (used in 3 patients each), followed by topiramate and oxcarbazepine (2 patients each). The three patients who were on combinations of two antiseizure medications used: lamotrigine and levetiracetam, clobazam and topiramate, and clobazam and lamotrigine. Of note, 15 patients with PHTS without an epilepsy diagnosis were prescribed antiseizure medications, and of these, 9 patients also had a diagnosis of ASD. While the indication for medication usage was not available in our dataset, it was potentially for neuropsychiatric indications. Among these patients with PHTS who did not have epilepsy and were prescribed antiseizure medications, topiramate (3 patients) was the most prescribed medication.

MRI findings

Serial MRIs were not done routinely in patients with PHTS as part of this longitudinal study and MRIs were done, in general, at the discretion of the treating provider if there was a clinical indication. Among the 35 patients that did have MRI scans of the brain (19 of whom had ASD, and 10 of 11 patients with epilepsy), we observed a wide spectrum of findings. The most frequent changes were prominent perivascular spaces (6 of 35 patients, 17%), periventricular white matter abnormalities (4 of 35 patients, 11%), and ventricular prominence (6 of 35 patients, 17%). Three patients (9%) of those who underwent MRI were reported to have a Chiari I malformation, two of whom underwent surgical decompression, though the degree to which this was symptomatic or provided benefit was unreported. Two patients had choroid plexus cysts. Three patients with PHTS-ASD were found to have incomplete peritrigonal myelination, but this was not seen in the PHTS-non-ASD patients, and the ages at which these patients underwent MRIs was not available. Lipomas of the spine were seen in two patients, one at the T2 vertebral body, and another without specified location. Presumed infarcts were seen in three patients, but further information to ascertain whether these were true infarcts was not available. Based on available data, no cerebrovascular imaging or other systemic vascular imaging was done for these patients to better understand the nature of

the cerebral ischemia. Two patients with PHTS-ASD were found to have focal cortical dysplasia, and one of these patients also had complete agenesis of the corpus callosum, associated with focal seizures in both cases. Increased corpus callosum thickness was reported in one patient. We did not observe any cases of Lhermitte–Duclos disease or polymicrogyria in our cohort.

Physical, occupational, and speech therapies were widely utilized in patients with PHTS

We next examined the frequency and duration of various types of adjunctive therapy visits in patients with PHTS. 94% of individuals with PHTS-ASD utilized at least one type of adjunctive therapy, as did 83% of individuals with PHTS and without ASD. Language concerns were seen in 45% of PHTS-ASD versus 33% of PHTS-non-ASD ($p = 0.23$, Fisher's exact test); hearing concerns were reported in 11% of PHTS-ASD versus 3% of PHTS-non--ASD ($p = 0.27$, Fisher's exact test); and behavioral concerns were present in 25% of PHTS-ASD versus 8% of PHTS-non-ASD ($p = 0.02$, Fisher's exact test). Patients with PHTS and ASD more often attended speech/language therapies, behavioral health therapies (e.g., applied behavior analysis therapy), and social skills groups than non-ASD patients (74% vs. 38%, 50% vs. 22%, and 33% vs. 10%, respectively, $p < 0.007$ in all cases, Fisher's exact test). Participants with ASD were more likely to use other therapies (e.g., complementary and alternative medicine approaches) with frequency 52% versus 25% ($p = 0.006$, Fisher's exact test). Although the percentage of PHTS patients with concerns for motor development differed among those with and without ASD, at 64% and 35%, respectively ($p = 0.004$, Fisher's exact test), the rates of therapy usage between ASD and non-ASD participants for occupational therapy, physical therapy, and counseling were similar (78% vs. 52%, 65% vs. 46%, and 17% vs. 19%, $p > 0.008$ in all cases, Fisher's exact test). There were no statistically significant differences in therapy visits observed between males and females. Session durations ranged from 15 min to 480 min and were on average similar across patients with and without ASD, and males and females, at median 50–60 mins per session. Therapy sessions were administered at least weekly for most patients.

Discussion

In this study, we report the neurological phenotypes and allied health utilization in a large natural history database of prospectively enrolled patients with PHTS, to better understand the early interaction between PHTS, neurodevelopmental comorbidities, and associated physical and imaging characteristics. Our findings highlight the increased frequency of ASD in this condition, the dysmorphisms seen in PHTS, the MRI features associated with PHTS-ASD and PHTS more generally, as well as the seizure phenotype among patients with PHTS. We also describe differences in the utilization of therapy services between PHTS-ASD and PHTS non-ASD participants. Our study is unique in its prospective, multicenter design, paired with detailed clinical observation in a rare and understudied aspect of PHTS, particularly among young patients.

About 51% of individuals with PHTS were diagnosed with ASD, most of whom were males and diagnosed early in life, between 3 and 4 years of age. PHTS with ASD was frequently comorbid with generalized epilepsy. While the association between PHTS and ASD has been previously observed, our study highlights the overrepresentation of males among individuals with PHTS and ASD, consistent with the ASD population more generally, in which males are overrepresented.[20] We also highlight the MRI features of patients with PHTS and ASD, though the lack of routine brain MRI in our series may challenge the specificity of these findings.[21–23] Patients with PHTS and ASD had white matter abnormalities, such as incomplete peritrigonal myelination on brain MRI, seen in three individuals with ASD. This may be related to ASD pathogenesis, though this finding was limited to a small number of patients and may also be seen in non-ASD individuals with PHTS.

In addition to white matter abnormalities, incomplete myelination, and dilated perivascular spaces, in our series, brain MRIs revealed megalencephaly, increased corpus callosum thickness, and Chiari I malformation, which have been previously observed in patients with PHTS.[24,25] Two patients in our series underwent neurosurgical intervention for Chiari I decompression, though the degree to which they were improved is not known. As such, we recommend cautious interpretation of brain MRI in individuals with PHTS, to avoid surgical intervention without clear benefit. Related to the young age of our cohort, no meningiomas were reported, and because of the lack of vascular imaging, no intracranial vascular malformations were reported. We did not observe Lhermitte–Duclos disease in our cohort of patients, underscoring its relative rarity, even in a pediatric population with PHTS.

Generalized epilepsy was the most frequent subtype in patients with PHTS in our cohort, in contrast to other neurodevelopmental syndromes such as tuberous sclerosis complex, in which the majority of seizures are focal. Patients were generally treated with at least two antiseizure medications. Most commonly, lamotrigine, levetiracetam, and clobazam were used in individuals with epilepsy. Moreover, there were a number of patients prescribed traditionally antiseizure medications for

non-seizure indications, including, contrary to our experience in individuals with ASD and epilepsy, topiramate in three cases. A small subset of patients with PHTS had focal seizures, and brain MRI in two cases revealed focal cortical dysplasia, seen also in prior series.[26–29]

Adjunctive therapies administered by allied health professionals are a cornerstone for success early in life, and lead to improved long-term outcomes. Our series highlights a high utilization of therapy services across individuals with PHTS (89% overall), particularly in those with ASD (94%). We also illustrate the differential utilization of therapy services, with higher use of speech, behavioral, and social therapies in patients with ASD and similar physical and occupational therapy utilization. Adjunctive therapy was multimodal and frequent at young ages, and patients received therapy generally at least weekly, suggesting the need to continue to provide strong support for young patients with PHTS across allied health domains.

The strengths of our study include the long-term follow-up, the detailed clinical reporting, and the unique nature of our patient population. We sought to comprehensively characterize the clinical care of young patients with PHTS. In doing so, we characterized epilepsy subtypes in individuals with PHTS, its treatment, and the association with ASD in young patients with PHTS, as well as the utilization of allied health services. Our study is limited by the lack of a robust control cohort, and there are very likely elements of ascertainment and selection bias present in our results. The children who participated in the study were also majority white and were able to access care at one of the enrolling academic institutions, potentially not representative of the overall cohort of young patients with PHTS. As with any observational study, confounders that were not measured, or reporting errors may have occurred, underscoring the need for replication and validation efforts in larger and more representative populations. Thus, autism spectrum disorders are likely overestimated in our data, as many individuals with PHTS without ASD are undoubtedly underdiagnosed or diagnosed at later ages into adulthood. Lastly, while most cases were *de novo* variants, these data were (*de novo* or not) not collected as part of the parent natural history study. Thus, it remains unknown whether inherited or novel variants contribute differentially to neurologic phenotypes in PHTS. Further we note that this study is limited by sample size and is underpowered for a genotype–phenotype association analysis for seizures, therapy usage, or autism spectrum disorder co-occurrence in individuals with PHTS.

In conclusion, across a prospective cohort of 107 young individuals with PHTS, we identified ASD as a frequent comorbidity in 51% of patients. There is no specific, targeted medical therapy for neurocognitive changes in individuals with PHTS. A recently published pilot Phase II randomized placebo-controlled trial of everolimus in 46 individuals with PHTS by Srivastava *et al.* was conducted, but did not meet its primary endpoint for efficacy in neurocognitive outcomes. However, this trial did show direction towards improvement in secondary outcomes including motor skills, verbal learning, autism symptoms, nonverbal IQ, and adaptive behavior, as well as global improvement.[30] Our results suggest that therapy utilization is frequent in both patients with PHTS-ASD and PHTS non-ASD and is a reasonable candidate for a clinical trial endpoint for future therapeutics in PHTS. Moreover, generalized epilepsy was common in PHTS, and associated with ASD. In most cases, patients with PHTS and generalized epilepsy required two or more antiseizure medications, suggesting that seizure freedom may be an additional relevant clinical trial endpoint in this population. Lastly, our findings underscore the need for detailed neurological examination and routine neurodevelopmental assessment in patients with PHTS, and the need for further mechanism-directed research into the relationship between abnormal myelination and ASD in PHTS.

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Author Contributions

AD, MS, and CE contributed to the conception and design of the study; AD, SB, DL, RB, PK, TWF, SS, SP, GEH, NRF, DMR, AYH, and JAM contributed to the acquisition and analysis of data; AD, SB, DL, RB, and CE contributed to drafting a significant portion of the manuscript and figures. All authors critically reviewed the manuscript and gave final approval.

Conflict of Interest

The authors have no conflict of interest to declare.

Data Availability Statement

The de-identified data from this study are available from the DSC PTEN principal investigator (Dr. Eng) with reasonable request.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1.

