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ORIGINAL RESEARCH—CLINICAL

A Bi-Institutional Study of Gastrointestinal and Hepatic Manifestations in Children With *PTEN* Hamartoma Tumor Syndrome

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BACKGROUND AND AIMS: *PTEN* hamartoma tumor syndrome (PHTS) confers a high risk of specific cancers and is the most common genetic cause of autism spectrum disorder (ASD). Gastrointestinal (GI) phenotypes in PHTS are poorly characterized in children. Thus, we aimed to characterize the GI and hepatic manifestations in children with PHTS and to investigate genotype-phenotype associations. **METHODS:** We performed a retrospective chart review of prospectively accrued children with PHTS at 2 tertiary-care centers. Wilcoxon rank-sum, Chi-squared, and Fisher's exact tests and Firth's logistic regression were utilized to explore associations between variables. **RESULTS:** This series included 80 children with disease-causing *PTEN* variants. Common GI manifestations included constipation in 41 (51%), feeding issues in 31 (39%), and polyps in 22 (28%) children. The polyps were of mixed histologic types. Eosinophilic gastrointestinal disorders were observed in 5 (6%) children. Crohn's disease, celiac disease, and protein-losing enteropathy were observed once each. Eosinophilic gastrointestinal disorders were observed exclusively in patients without ASD ($P = .052$). Nonsense *PTEN* variants were enriched in those with polyps ($P = .029$). Missense *PTEN* variants (OR 2.9, $P = .034$) and upper GI polyps (OR 4.4, $P = .018$) were associated with increased odds of constipation. **CONCLUSION:** Constipation and feeding issues are common in children with PHTS. Polyps are more prevalent in children with PHTS than previously described and associated with nonsense *PTEN* variants. Children without ASD represent a distinct patient subset with a predisposition to eosinophilic gastrointestinal disorders and possibly upper GI polyps. Endoscopic evaluation should continue to be performed in symptomatic children with PHTS, with consideration of closer follow-up in those without ASD.

Keywords: *PTEN* Hamartoma Tumor Syndrome; Autism Spectrum Disorder; Gastrointestinal Polyps; Eosinophilic Gastrointestinal Disorders

Introduction

PTEN hamartoma tumor syndrome (PHTS) is a molecular umbrella term for a group of clinically heterogeneous autosomal dominant disorders caused by pathogenic germline *PTEN* variants, including Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, *PTEN*-related Proteus syndrome, and *PTEN*-related Proteus-like syndrome.[1] Patients with PHTS present with diverse phenotypic features that affect multiple organ systems, in addition to a high lifetime risk of various cancers.[2] More recently, germline variants in *PTEN* have been identified as the most common etiology of monogenic autism spectrum disorder (ASD).[1,3]

Gastrointestinal (GI) polyposis is one of the most common manifestations of PHTS in adults, which can occur throughout the entire GI tract, with a predilection for the colon.[4] Over 90% of patients with PHTS who received a colonoscopy had at least one polyp, with many having multiple polyps with mixed histologic types. Individuals with PHTS also have a 9% lifetime risk of colorectal cancer.[2] Polyposis and malignant manifestations are well-documented features of PHTS. However, the majority of clinical studies are in adults. Endoscopic evaluation in children with PHTS is typically symptom-driven, as polyps

Abbreviations used in this paper: **ASD, autism spectrum disorder; BMI, body mass index; CC, Cleveland Clinic; CHOP, Children's Hospital of Philadelphia; DD, developmental delay; EGIDs, eosinophilic gastrointestinal disorders; GI, gastrointestinal; PHTS, *PTEN* hamartoma tumor syndrome.**

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and GI malignancies are thought to be adult-onset.[4] Hence, GI phenotypes, especially in children, are not well characterized.

While most adults with PHTS come to medical attention due to cancer, the majority of children are brought to medical attention because of neurodevelopmental disorders including macrocephaly, developmental delay (DD), and/or ASD.[5–7] Indeed, PTEN, outside its tumor-suppressive function, plays an essential role in embryogenesis and maintenance of normal physiological functions, especially within the nervous system.[8–11] GI distress is highly prevalent in those with ASD. A review of 84 studies estimating prevalence of GI symptoms in patients with ASD reported that nearly half had GI symptoms including, but not limited, to constipation, diarrhea, abdominal pain, and feeding issues.[12] However, it is unclear if children with PHTS-associated ASD have different or more severe GI manifestations than those without ASD. Given that PTEN is highly expressed within the epithelial cells and enteric nerves of the GI tract, we hypothesize that children with PHTS-associated ASD will have distinct GI phenotypes compared to those without ASD.

We thus comprehensively characterized GI and hepatic manifestations of children with PHTS followed at the Cleveland Clinic (CC) and the Children's Hospital of Philadelphia (CHOP). We also investigated if any genotype or phenotype associations exist, particularly in the context of ASD.

Methods

Patient Selection

Pediatric patients (< 18 years of age at the time of diagnosis) with confirmed disease-causing germline *PTEN* variants were identified in our institutional review board–approved REDCap databases. Patients were identified retrospectively from prospectively accrued patients seen at the *PTEN* Multidisciplinary Clinic and Center of Excellence at the CC (n = 87) between 2005 and 2020, and the Comprehensive *PTEN* Hamartoma Tumor Syndrome Clinic and Center of Excellence at CHOP between 2011 and 2021 (n = 60). Only patients who were evaluated by a *PTEN* GI specialist were eligible for our study.

Of the 87 patients at CC, 47 were excluded because a *PTEN* GI specialist did not evaluate them. Of the 60 patients at CHOP, 20 were excluded because they were not evaluated by a *PTEN* GI specialist (n = 18) or had missing *PTEN* variant information (n = 2). The final series of 80 patients (CC, n = 40; CHOP, n = 40) included 75 patients with pathogenic or likely pathogenic germline *PTEN* variants, 4 patients with *PTEN* variants of uncertain significance, and 1 patient with conflicting variant classification that included pathogenic, likely pathogenic, and variants of uncertain significance. These 5 patients were determined to have disease-causing variants due to having clinical features that met the pediatric clinical criteria for PHTS[5] and were included in this study.

Data Collection

Electronic medical records were reviewed up until the age of 18 years. These records started from the earliest available point (typically between infancy to early childhood) or from when patients were first brought to medical attention for suspected PHTS—whichever instance came first. The chart review was conducted by a team of 4 researchers well-versed in the clinical characteristics of PHTS. A standardized data collection form using structured and free-text fields was utilized by both the institutions. One reviewer from each institution audited a random subset of charts to ensure accuracy and consistency. Records from medical genetics, developmental pediatrics or neurology, dermatology, gastroenterology, endocrinology, and/or oncology were reviewed. Demographic data (age of consent, sex, race, and ethnicity), body mass index (BMI), and *PTEN* genotype were collected at time of PHTS diagnosis. PHTS-related clinical features, including macrocephaly, DD, ASD, epilepsy, hypotonia, thyroid nodules, breast lesions for female individuals, oral and skin lesions, vascular malformations, genitourinary malformations, and cancer were reviewed at the time of PHTS diagnosis as well as postdiagnosis. Patients with a diagnosis of ASD all had a formal diagnosis from neuropsychological evaluation and/or a developmental and behavioral pediatrician.

GI manifestations were identified on gastroenterology records, radiographic imaging, laboratory studies, GI motility testing, endoscopic procedures reports, and pathology reports. Indications for endoscopic evaluations as well as the number of polyps visualized on endoscopy were recorded. Constipation, feeding issues, gastroestophageal reflux disease, and hematochezia were diagnosed by clinical assessment of a board-certified pediatric gastroenterologist. We required biopsy-proven results for consideration of the following phenotypes: eosinophilic gastrointestinal disorders (EGIDs), Crohn's disease, celiac disease, glycogenic acanthosis, polyps, and lymphoid aggregates/hyperplasia. Hamartomatous gastric polyps are often histologically indistinguishable from nonspecific hyperplastic gastric polyps. To avoid confusion, gastric polyps that were histologically classified as hamartomatous, inflammatory, juvenile, or hyperplastic were all classified as hamartomatous polyps. All instances of lymphoid aggregates from pathology reports were noted and classified separate from polyps as they are considered a normal finding in young children with unclear clinical significance in the context of PHTS.

Statistical Analysis

Descriptive statistics were calculated for all variables. GI and other PHTS manifestations were compared by stratifying patients by ASD status. The Wilcoxon rank-sum test was used to compare the medians of continuous variables. Chi-squared and Fisher's exact tests were used to compare the proportions of categorical variables. Firth's logistic regression was performed to explore phenotype as well as genotype associations. This method helps reduce bias in the maximum likelihood estimates that can arise with small sample sizes. ASD and the most prevalent GI manifestations, constipation and feeding issues, were selected as outcome variables. Variables with a *P* value less than or equal to .2 on univariate regression were considered for analysis. Explanatory variables were also chosen based on frequency, relevant *PTEN* biology, and clinical relevance. All statistical analyses were performed in R (version 4.1.2). A *P* value less than .05 was considered as the threshold for statistical significance.

Results

Patient Characteristics

In this series, 80 children with disease-causing germline *PTEN* variants were included. The median (interquartile

Table 1. PHTS Patient Characteristics

Characteristics	N	Overall, N = 80[a]	ASD, N = 41[a]	No ASD, N = 39[a]	*P* value[b]
Age at consent	80	9 (4, 12)	7 (4, 12)	9 (4, 13)	.3
Sex	80				.014
Male		48 (60%)	30 (73%)	18 (46%)	
Female		32 (40%)	11 (27%)	21 (54%)	
Ethnicity	79				>.9
Not Hispanic or Latino		72 (91%)	37 (90%)	35 (92%)	
Hispanic or Latino		7 (8.9%)	4 (9.8%)	3 (7.9%)	
Race	80				>.9
White		68 (85%)	36 (88%)	32 (82%)	
Other		6 (7.5%)	3 (7.3%)	3 (7.7%)	
Black		4 (5.0%)	2 (4.9%)	2 (5.1%)	
Asian		1 (1.3%)	0 (0%)	1 (2.6%)	
Unknown		1 (1.3%)	0 (0%)	1 (2.6%)	
BMI-for-age percentile	75	85 (66, 96)	85 (64, 97)	84 (68, 96)	.5

ASD, autism spectrum disorder; BMI, body mass index; PHTS, *PTEN* hamartoma tumor syndrome.
[a]Median (IQR); N (%).
[b]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test.

range [IQR]) age of consent was 9 years (4, 12) with 48 being male (60%) and 32 being female (40%) (Table 1). Majority of our patients were White (68 of 80, 85%) and non-Hispanic/Latino (72 of 79, 91%). The most common indication prompting PHTS evaluation at initial presentation included macrocephaly (67 of 80, 84%), developmental delay (36 of 80, 45%), and/or ASD (14 of 80, 18%) in isolation or combination. GI complaints, including abdominal pain, constipation, diarrhea, or hematochezia, were reported in 9 (11%) patients at the initial visit for PHTS evaluation (Table A1). Patients were stratified into 2 phenotypically ascertained groups that consist of patients with ASD (n = 41) and patients without ASD (n = 39). Male sex was associated with ASD ($P = .014$). From an anthropometric standpoint, the median (IQR) BMI-for-age percentile was 85 (66, 96). There were no differences in BMI when stratified by ASD status.

PTEN Variation Spectrum

The most common type of *PTEN* variant was missense (31 of 80, 39%), followed by nonsense (21 of 80, 26%), frameshift truncating (10 of 80, 12%), splice site (9 of 80, 11%), and large deletions/duplications (7 of 80, 8.8%) (Table 2). There was no evidence of statistically significant differences across variant types when comparing patients with and without ASD ($P = .7$). Variants in the coding region were the most common (65/80, 81%). Noncoding region variants, specifically deep intronic splice site variants, were reported in 10 (12%).

PHTS-Associated GI and Hepatic Manifestations

The median (IQR) number of GI manifestations per patient was 2 (1, 3). The most common GI features reported were constipation (41 of 80, 51%), followed by feeding issues such as food aversion, dysphagia, and aspiration (31 of 80, 39%), polyps (22 of 80, 28%), gastroesophageal reflux disease (15 of 80, 19%), and hematochezia (14 of 80, 18%) (Figure 1A). We observed lymphoid hyperplasia in 11 (14%) and EGIDs in 5 (6%) patients. Lymphoid hyperplasia and polyposis were comorbid in all patients with EGIDs. Nonspecific endoscopy findings based on histology included esophagitis (9 of 80, 11%), gastritis (6 of 80, 8%), and duodenitis (6 of 80, 8%). GI dysmotility, based on motility studies, was observed in 4 (5%) patients. Mesenteric lesions, typically lipomatosis, occurred in 4 (5%) patients. Glycogenic acanthosis and hepatic steatosis, diagnosed by abdominal imaging (ie, liver ultrasound and computed tomograpy abdomen), was observed twice each. Both patients with hepatic steatosis had a BMI-for-age percentile of 99. One patient with hepatic steatosis developed elevated liver function enzymes with a suspected diagnosis with nonalcoholic steatohepatitis. Two patients had intussusception, with 1 patient requiring exploratory laparotomy and resection of 19 cm their distal small intestine, including the ileocecal valve and appendix, due to high polyp burden with a large obstructive distal ileal polyp. Postsurgery pathology showed florid follicular hyperplasia of the ileal polyp as well as the small intestine, terminal ileum, and cecum. Celiac disease, Crohn's disease, and protein losing enteropathy were observed once each.

Endoscopic, Polyp, and Histologic Data

A total of 30 (38%) patients underwent endoscopic surveillance; of which, 28 (93%) had colonoscopies and 27 (90%) had upper endoscopies (esophagogastroduodenoscopy). The 2 most common indications for colonoscopy or esophagogastroduodenoscopy were hematochezia and abdominal pain (Table A2). During initial endoscopic evaluation, more than 1 polyp was visualized in the stomach of 6 (22%), in the small intestine of 9 (33%), and in the colon of 11 (40%) patients (Table 3). When accounting for

Table 2. *PTEN* Variation Spectrum in Patients With PHTS

PTEN variant type	N	Overall, N = 80[a]	ASD, N = 41[a]	No ASD, N = 39[a]	*P* value[b,c]
Variation classification	80				.4
P/LP		75 (94%)	39 (95%)	36 (92%)	
VUS		4 (5.0%)	1 (2.4%)	3 (7.7%)	
Conflicting (P/LP/VUS)		1 (1.3%)	1 (2.4%)	0 (0%)	
Variation site	80				.047
Coding region		65 (81%)	34 (83%)	31 (79%)	.8
Non-coding region		10 (12%)	3 (7.3%)	7 (18%)	.2
Whole gene deletion		4 (5.0%)	4 (9.8%)	0 (0%)	.1
Large deletion (Exon 3–9)		1 (1.3%)	0 (0%)	1 (2.6%)	.5
Variation effect	80				.7
Missense		31 (39%)	19 (46%)	12 (31%)	
Nonsense		21 (26%)	10 (24%)	11 (28%)	
Frameshift truncating		10 (12%)	4 (9.8%)	6 (15%)	
Splice site		9 (11%)	3 (7.3%)	6 (15%)	
Large del/dup		7 (8.8%)	4 (9.8%)	3 (7.7%)	
Other		2 (2.5%)	1 (2.4%)	1 (2.6%)	

ASD, autism spectrum disorder; BMI, body mass index; Del/Dup, deletion/duplication; PHTS, *PTEN* hamartoma tumor syndrome; P/LP, pathogenic/likely pathogenic; VUS, variant of unknown significance.
[a]N (%).
[b]Fisher's exact test of homogeneity.
[c]Post-hoc pairwise Fisher's exact test, significance on overall Fisher's exact testing likely due to small number of observation and should be interpreted with caution.

subsequent endoscopic evaluations, the cumulative number of patients with multiple polyps visualized increased to 11 (41%) in the stomach, 15 (52%) in the small intestine, and 16 (57%) in the colon. Majority of patients that underwent endoscopic evaluation had histologically confirmed polyps (22 of 30, 73%), with 16 (59%) and 15 (54%) patients having upper and lower GI polyps, respectively (Figure 1C).

All histologic variants of polyps were observed, with the majority of individuals having more than 1 polyp histology (16 of 22, 73%) (Figure 2). The most common type of gastric polyps was fundic gland polyps (7 of 14, 50%) followed by hamartomatous/hyperplastic polyps (5 of 14, 36%). Inflammatory polyps were the most commonly observed polyp type in the small intestine (7 of 13, 54%). Two patients, including the one with intussusception due to florid lymphoid hyperplasia requiring surgical resection, had single adenomatous duodenal polyps. Juvenile polyps (8 of 15, 53%) and ganglioneuromas (7 of 15, 47%) were the most common polyp types observed in the colon. Additionally, 1 patient with EGID was diagnosed with 2 inflammatory esophageal polyps characterized by markedly increased intraepithelial eosinophils.

Phenotype and Genotype Associations

Overall, we observed no statistically significant differences between GI phenotypes when stratifying by ASD status (Figure 1B and C). However, consistent with our clinical observations, we noted an increased frequency of constipation in patients with ASD (59% vs 44%, $P = .2$). Of the 30 subjects who underwent endoscopic evaluation, those without ASD showed an increased propensity to have polyps (82% vs 62%, $P = .2$), specifically upper GI polyps (65% vs 38%, $P = .2$). Notably, EGIDs were observed exclusively in patients without ASD (29% vs 0%, $P = .052$). Interestingly, singular observations of celiac disease and Crohn's disease were only observed in patients without ASD.

Furthermore, patients with ASD had higher median levels of aspartate amino transferase on liver function studies compared to patients without ASD (38 U/L vs 29 U/L; $P = .039$) (Table A3). However, caution should be taken when interpreting the association between ASD and aspartate amino transferase due to unclear clinical significance and concerns for confounding variables not analyzed (eg, medication effects).

On Firth's logistic regression analysis, male sex was associated with increased odds of having ASD (odds ratio [OR], 3.46; 95% confidence interval [CI] [1.35, 9.46]; $P = .01$), while EGIDs were associated with decreased odds of having ASD (OR, 0.08; 95% CI [0.00, 0.79]; $P = .028$) (Table 4). Missense *PTEN* variants (OR, 2.86; 95% CI [1.08, 8.00]; $P = .34$) and upper GI polyps (OR, 4.42; 95% CI [1.28, 17.91]; $P = .018$) were associated with increased odds of constipation. Hypotonia was associated with increased odds of feeding issues (OR, 2.69; 95% [1.07, 7.07]; $P = .034$), while missense *PTEN* variants were associated with decreased odds, albeit this was not statistically significant (OR, 0.45; 95% CI [0.16, 1.16]; $P = .098$).

On closer examination, we found that nonsense *PTEN* variants were associated with the presence of polyps (45% vs 0%, $P = .029$) (Table 5). Additionally, the majority of patients with EGIDs (4 of 5 or 80%) had nonsense *PTEN* variants. In contrast, missense mutations were associated

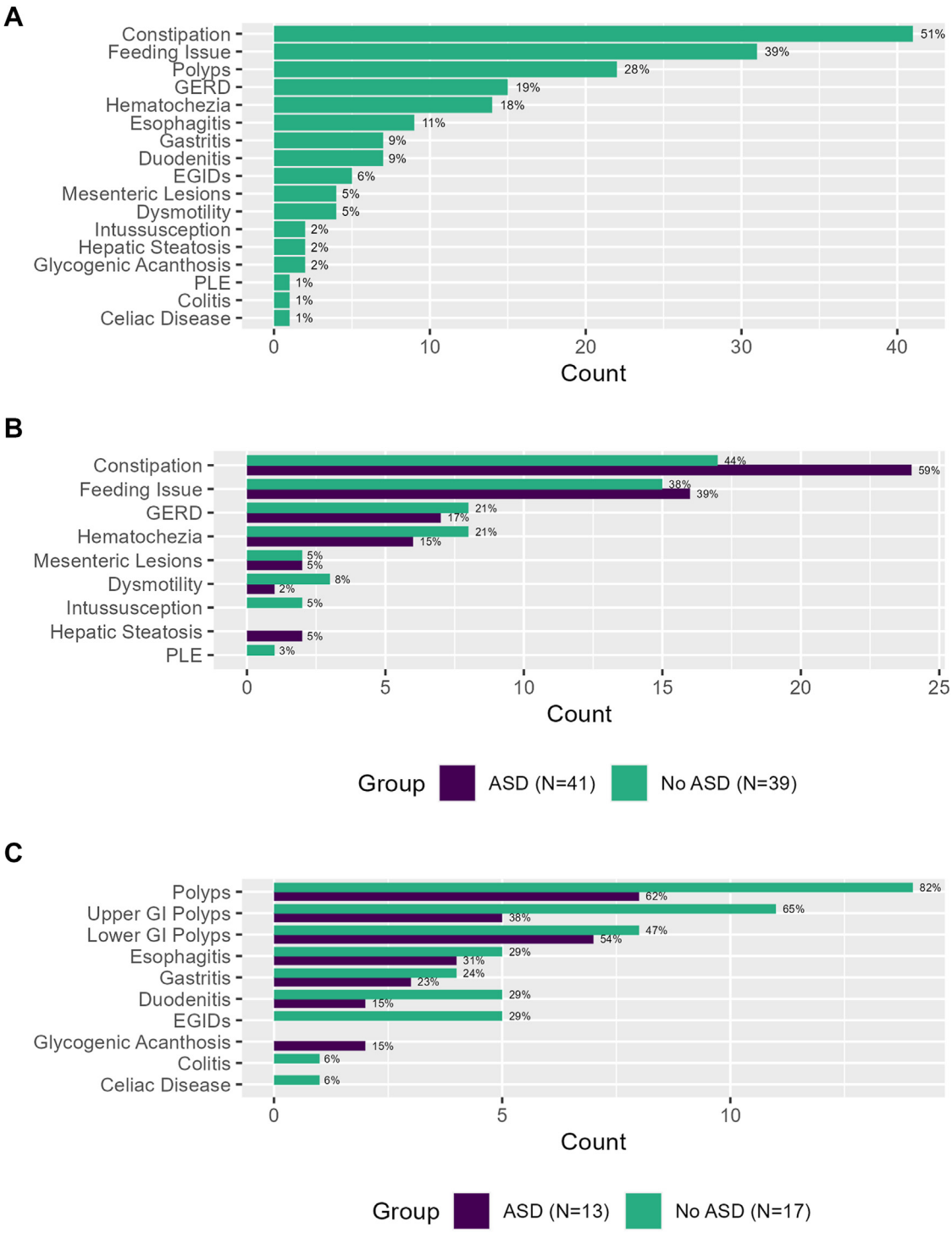

Figure 1. PHTS-associated Gastrointestinal and Hepatic Manifestations. (A) Bar graph illustrating the count and (%) of GI manifestations in the total series (N = 80). (B) Grouped bar graph stratified by ASD status depicting GI manifestations not reliant on endoscopic evaluation (N = 80). (C) Grouped bar graph stratified by ASD status summarizing GI manifestations reliant on endoscopic evaluation (N = 30). Purple (ASD), Green (No ASD). ASD, autism spectrum disorder; GERD, gastroesophageal reflux disease; GI, gastrointestinal; PHTS, *PTEN* hamartoma tumor syndrome; PLE, protein-losing enteropathy.

with the absence of polyps (63% vs 3%, $P = .016$). Large *PTEN* deletions/duplications (27% vs 13%, $P = .63$) were overrepresented in those with polyps, albeit not statistically significant.

Other PHTS-Associated Clinical Features

The most prevalent features in our series were neurodevelopmental disorders, either in isolation or combination, that included macrocephaly (80 of 80, 100%), DD (60 of 80,

Table 3. Number of Polyps Visualized on Endoscopy in Patients With PHTS

	EGD, N = 27[a]		Colonoscopy, N = 28[a]
Polyp number	Stomach	Small intestine	Colon
Polyp number at presentation			
None	18 (67%)	18 (67%)	14 (50%)
One	3 (11%)	0 (0%)	3 (11%)
Less than 10	3 (11%)	6 (22%)	8 (29%)
Greater than 10	3 (11%)	3 (11%)	3 (11%)
Hundreds	0 (0%)	0 (0%)	0 (0%)
Cumulative polyp number			
None	12 (44%)	12 (44%)	11 (39%)
One	4 (15%)	0 (0%)	1 (3.6%)
Less than 10	7 (26%)	6 (22%)	5 (18%)
Greater than 10	4 (15%)	8 (30%)	11 (39%)
Hundreds	0 (0%)	1 (3.7%)	0 (0%)

EGD, esophagogastroduodenoscopy; PHTS, *PTEN* hamartoma tumor syndrome.
[a]N (%), polyps visualized on endoscopy.

75%), ASD (41 of 80, 51%), hypotonia (38 of 80, 48%), and/or epilepsy (12 of 80, 15%) (Table A4). Vascular malformations were overrepresented in patients without ASD compared to those with ASD (38% vs 20%; $P = .061$), although not statistically significant. Six patients developed cancer, with 5 patients having a PHTS-component malignancy: 4 with thyroid cancer and 1 with endometrial cancer. One patient was diagnosed with neuroblastoma at 2 years of age and subsequently developed angiosarcoma at 4 years of age.[13] The median (IQR) age of diagnosis of cancer was 15.5 years (12.5, 17.75).

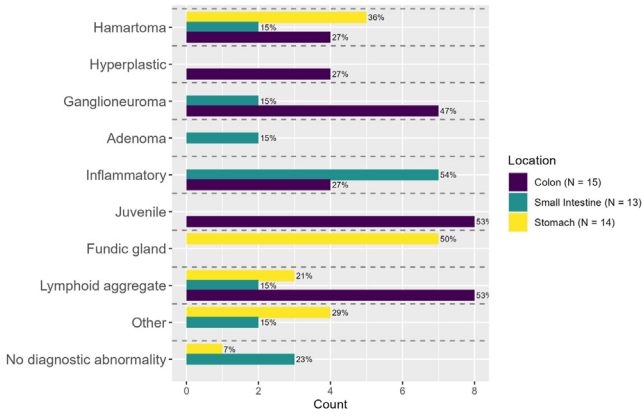

Figure 2. Distribution of Polyp Types by Location in GI Tract in Patients with PHTS. A grouped bar graph that illustrates the count and proportion (%) of different polyp types by location within the gastrointestinal (GI) tract. Colon (purple), Small intestine (green), Stomach (yellow). Gastric polyps classified as hamartoma, inflammatory, juvenile, or hyperplastic were uniformly categorized as hamartomas as they are histologically indistinguishable in the stomach. PHTS, *PTEN* hamartoma tumor syndrome.

Discussion

The majority of PHTS clinical studies are performed in adults. Despite PHTS manifestations displaying variable expression and age-related penetrance, adult phenotypes are often attributed to children.[14] This may lead to suboptimal medical surveillance as well as anticipatory guidance for patients and family members. Thus, we carried out one of the largest surveys of GI and hepatic manifestations to date of a well-phenotyped series of 80 pediatric patients with PHTS. Overall, we observed that approximately half to two-thirds of children with PHTS were referred to our *PTEN* GI specialists due to a variety of GI complaints. This is in contrast to adults who are typically seen for surveillance of polyposis or cancer. We found that children with PHTS display a broad range of GI phenotypes related to aberrant motility as well as immune and metabolic dysregulation but found that polyps are more common in this population than previously believed.

The most common GI phenotype in children with PHTS was unrelated to polyps. Approximately half had constipation, sufficient for referral to our *PTEN* pediatric GI specialists. While it can be argued that constipation is nonspecific, the frequency of constipation, often difficult to manage, argues against coincidence, even in the absence of ASD. Additionally, 5 patients in our series had GI dysmotility. One patient had severe chronic constipation with marked abdominal distention due to severe GI dysmotility secondary to diffuse ganglioneuromatosis, requiring total colectomy with end ileostomy.[15] Murine *Pten* knock-out models result in, among other phenotypes, myelination problems.[9–11] Additionally, high PTEN expression has been noted in enteric nerves in adult humans[15,16]; selective deletion of *Pten* in enteric neuronal cells in murine models resulting in diffuse ganglioneuromatosis leading to an intestinal pseudo-obstruction phenotype.[16,17] It is plausible

Table 4. Firth's Logistic Regression Association Analysis in PHTS

Predictor variables	N	ASD[a]	Constipation[a]	Feeding issues[a]
Sex (male)	48	3.46 (1.35, 9.46)	0.50 (0.17, 1.34)	0.73 (0.29, 1.85)
ASD	41	-	2.49 (0.93, 7.21)	-
Constipation	41	1.85 (0.70, 5.03)	-	-
Hypotonia	38	-	-	2.69 (1.07, 7.07)
Missense mutation	31	1.49 (0.55, 4.06)	2.86 (1.08, 8.00)	0.45 (0.16, 1.16)
Upper GI polyps	16	-	4.42 (1.28, 17.91)	-
Hematochezia	14	-	-	-
EGID	5	0.08 (0.00, 0.79)	-	-

ASD, autism spectrum disorder; EGID, eosinophilic gastrointestinal disorders; GI, gastrointestinal; PHTS, *PTEN* hamartoma tumor syndrome.
[a]OR (95% CI); OR, odds ratio; CI, confidence interval.

that aberrant myelination and proliferation of peripheral nerves, including the enteric nerves, result in GI dysmotility that most commonly manifests as constipation in patients with PHTS.

Over one-third of our patients had feeding issues that ranged from food aversion to dysphagia. Feeding issues have been well-documented in children with ASD.[18] Initially, it was unclear if feeding issues in our patients are related to underlying ASD. However, feeding issues were observed in approximately equal proportion in those with and without ASD. Our clinical observation suggested that feeding issues might be related to hypotonia, which was supported by our findings that hypotonia was associated with increased odds of feeding issues on Firth's logistic regression. However, impaired ASD-related sensory dysfunction may still be a contributor. Decreased sensory functioning, including taste, smell, and sensory under-responsiveness, were recently described in a prospective series of children with PHTS and ASD compared to those with PHTS but no ASD, and to those with ASD without PHTS.[19] Finally, one-fifth of our pediatric patients with PHTS had gastroesophageal reflux disease, which may also contribute to feeding issues. Thus, feeding issues in PHTS are likely multifactorial with those with hypotonia potentially having more serious feeding issues.

Despite the high prevalence of feeding issues, over half of our patients had a BMI greater than the 85th percentile. The latter is consistent with observations that that patients with *PTEN* variants are clinically significantly overweight due to enhanced insulin sensitivity through the PI3K-AKT pathway.[20] Additionally, 2 patients in our series also had hepatic steatosis, with 1 patient developing nonalcoholic steatohepatitis. Liver-specific *Pten* deletion in murine models results in enhanced glycogen and fatty acid synthesis, leading to steatohepatitis and hepatocellular carcinoma.[21,22] There is at least 1 case report of Cowden syndrome complicated by hepatocellular carcinoma due to nonalcoholic steatohepatitis.[23] Taken together, underlying germline *PTEN* variants are associated with a higher BMI potentially due to generalized overgrowth, which may be partly explained by increased insulin sensitivity. However, it is unclear if this overgrowth is associated with increased adiposity and if patients with PHTS are at increased risk of complications that are classically associated with obesity later in life.

From our previous prospective study of PHTS-related GI polyps in primarily adults, polyps were thought to be rare in children with PHTS.[4] In our current series, polyposis was the third most prevalent GI manifestation, observed in 73% of patients who underwent endoscopic surveillance. The majority of these patients had multiple polyps with varying histological types. These findings suggest that polyps in children may be more common than previously described,

Table 5. *PTEN* Variation Spectrum in Patients with PHTS and Polyps

PTEN variant type	N	Overall, N = 30[a]	No polyps, N = 8[a]	Polyps, N = 22[a]	*P* value[b,c]
Variation effect	30				.009
Nonsense		10 (33%)	0 (0%)	10 (45%)	.029
Missense		8 (27%)	5 (63%)	3 (14%)	.016
Large del/dup		7 (23%)	1 (13%)	6 (27%)	.63
Frameshift truncating		3 (10%)	2 (25%)	1 (4.5%)	.16
Splice site		1 (3.3%)	0 (0%)	1 (4.5%)	1.0
Other		1 (3.3%)	0 (0%)	1 (4.5%)	1.0

PHTS, *PTEN* hamartoma tumor syndrome.
[a]N (%).
[b]Fisher's exact test of homogeneity.
[c]Post-hoc pairwise Fisher's exact test.

consistent with other recent retrospective studies in children with PHTS.[6,7] However, no subjects had GI-related malignancies, corroborating our original observation that colon cancer is adult-onset. Thus, supporting the current recommendation of baseline colonoscopy at 35 years in absence of symptoms prior.[24] While not statistically significant, upper GI polyps were overrepresented in those without ASD. Notably, we observed that upper GI polyps were associated with increased odds of having constipation. Additionally, we observe *PTEN* nonsense variants are positively associated with polyps, while missense *PTEN* variants are negatively associated with polyps. While not statistically significant, large *PTEN* deletions/duplications were enriched in those with polyps. Two patients had continuous deletions of *PTEN* and *BMPR1A*, which is associated with juvenile polyposis of infancy.[25] While no absolute genotype-phenotype associations have been identified in PHTS, previous studies show that missense *PTEN* variants are overrepresented in those with ASD.[26] In contrast, a small series of 10 patients with PHTS and GI polyps participating in an active GI program found that all patients had truncating variants.[27] Studies show that variants that are more damaging to PTEN protein structural stability and catalytic function are associated with cancer rather than ASD.[28,29] It is tempting to speculate that patients with PHTS in the absence of ASD represent a subset of patients at increased risk of developing malignancies.

Of relevance, all 5 cases of EGIDs in our series were exclusively observed in patients without ASD. All cases were also associated with lymphoid hyperplasia and intestinal polyposis, with nearly all patients harboring nonsense *PTEN* variants. This corroborates previous findings that demonstrated PHTS is associated with EGIDs that is typically comorbid with polyps.[30] Lymphoid hyperplasia is considered a normal finding in young children but is also observed in children and adults with PHTS. Although its clinical significance is unclear, lymphoid hyperplasia may be pathologic in certain patients with PHTS. Loss of immune tolerance has been shown in both humans and mice with germline *PTEN* variants.[31] Autoimmune phenotypes such as thyroiditis, colitis, celiac disease, hemolytic anemia, and pernicious anemia have been observed in series of patients with PHTS.[32,33] In this series, EGIDs were associated with comorbid atopy (4 of 5, 80%), and autoimmune conditions (3 of 5, 60%). One patient had concurrent celiac disease. This patient also had intussusception due to florid follicular hyperplasia requiring small bowel resection. Two patients—one with comorbid Hashimoto's thyroiditis and type 1 diabetes—had severe anemia from concurrent ulcerative duodenitis/jejunitis that was responsive to steroid and immunosuppression therapy, with 1 case complicated by protein-losing enteropathy. There are at least 2 reports of Bannayan-Riley-Ruvalcaba syndrome associated with celiac disease, with 1 case presenting with concurrent EGIDs and the other case presenting comorbid Hashimoto's thyroiditis and papillary thyroid carcinoma.[34,35] Additionally, in this series, there are twice as many instances of autoimmune thyroiditis in the patients without ASD, as well as a singular case of Crohn's disease. Thus, clinicians should be aware of the high possibility of comorbid autoimmune conditions and polyposis in patients with PHTS and EGIDs.

One strength of our study is the relatively large sample size given the rarity of PHTS. However, several limitations exist in our study. Ascertainment bias should be accounted for as macrocephaly in combination with DD and/or ASD were the most common indications for diagnostic work up for PHTS. Thus, patients with macrocephaly and mild neurodevelopmental abnormalities may be underrepresented. Additionally, due to our accrual method, patients who were not evaluated by our *PTEN* GI specialists were not included in this study. Thus, our findings may overestimate the true prevalence of PHTS-associated GI manifestations. Due to the rarity of PHTS, our sample size inherently limits in-depth analysis of phenotype and genotype associations. Moreover, our sample size constrains precise effect sizes estimates in our multivariable analysis, impacting overall validity and reliability of our results. Finally, due to the exploratory nature of our analysis and our limited sample size, confidence intervals were not adjusted for multiple comparisons. However, our findings are consistent with our clinical observations (ie, increased odds of male and constipation in ASD, increased odds of hypotonia with feeding issues), increasing our confidence in the validity our multivariable analysis results. Larger, independent studies are needed to validate these findings.

Overall, we found that functional constipation and feeding issue are common findings in children with PHTS. Importantly, polyps are more prevalent in children than previously described, and associated with nonsense *PTEN* variants. Our findings suggest children without ASD may represent a distinct patient subset with a predisposition to EGIDs, and possibly upper GI polyps. Missense *PTEN* variants and the presence of upper GI polyps are associated with increased odds of constipation. The current recommendation for surveillance endoscopy for PHTS is to begin at age 35 or earlier if symptomatic. Endoscopic evaluation should continue to be performed in symptomatic children with PHTS, specifically those with concerning symptoms, such as dysphagia or feeding problems, iron deficiency anemia, hematochezia. Closer follow-up should be considered in those without ASD.

Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2023.10.012.

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