BRIEF REPORT | Genetics inMedicine

Estimate of *de novo* mutation frequency in probands with *PTEN* hamartoma tumor syndrome

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Purpose: *PTEN* hamartoma tumor syndrome is an autosomal dominant disorder with increased risks of neoplasias, macrocephaly, and developmental disabilities. While both familial and sporadic cases exist, actual *de novo* mutation frequency remains unknown. We sought to estimate this within our *PTEN*-mutation positive patient series.

Methods: Patients were prospectively accrued if they had known pathogenic germline *PTEN* mutations or phenotypic features suspicious for PHTS. Only families with pathogenic *PTEN* mutations were included. Likelihood for *de novo* mutation was graded from 1 (confirmed inherited) to 5 (confirmed *de novo*) based on family history and mutation status. Fisher's two-tailed exact and unpaired *t*-tests were used to compare between groups.

Results: 187 pathogenic *PTEN*-mutation positive families were eligible for this study. *De novo* (grade 5) status was confirmed in 20 (10.7%) probands, and in 36 (19.3%) was suspected based on family history. Demographics, mutations, and phenotypes were similar for probands graded 1 vs. 5 (all $P > 0.06$). In grade 1 probands, mutations were inherited equally from maternal and paternal lineages ($P = 0.55$).

Conclusions: The frequency of *de novo PTEN* mutation is at minimum 10.7% and at best 47.6%. Absence of PHTS features within a family history should not preclude consideration of this diagnosis for patients with relevant personal history.

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Key Words: Bannayan–Riley–Ruvalcaba syndrome; Cowden syndrome; *de novo* mutation; germline PTEN mutation; inherited cancer syndrome

INTRODUCTION

PTEN hamartoma tumor syndrome (PHTS) is an umbrella term used to describe patients with variable phenotypes, most often Cowden syndrome (CS, OMIM #158350) or Bannayan–Riley–Ruvalcaba syndrome (BRRS, OMIM #153480), and germline mutation of the *PTEN* tumor suppressor gene.[1,2] Patients with PHTS are at increased risk for breast, epithelial thyroid, endometrial, renal, and colorectal cancers,[3,4] making timely diagnosis and identification of at-risk relatives critical for risk management. Both familial and apparently sporadic cases have been reported;[2,5–7] however, the frequency of patients with *de novo* vs. inherited mutations has yet to be established as it has for other autosomal dominant conditions.[8–10] We therefore sought to estimate the relative frequencies of *de novo* and inherited mutations in PHTS patients via review of family history data from our *PTEN*-mutation positive patient series.

MATERIALS AND METHODS

Patients were prospectively recruited after providing informed consent for Cleveland Clinic IRB# 8458-PTEN substudy who presented with the following: relaxed International Cowden Consortium (ICC) criteria (meaning full diagnostic criteria[11] minus one feature); macrocephaly plus autism/developmental delay/mental retardation; penile freckling; or presence of a known germline *PTEN* mutation. Germline *PTEN* mutation analysis was performed per Eng lab protocols as described elsewhere.[3] Only families with probands found to have pathogenic *PTEN* mutations were eligible for this *de novo* mutation study.

Clinical data and family history information were requested and reviewed for all research participants, with special attention paid to documentation of clinical testing in family members. A five-tiered family history grading system was created to denote the degree of confidence regarding *de novo* mutation status in the proband (**Table 1**). A grade of 5 indicated that the mutation was molecularly proven to have occurred *de novo*. In other words, a *PTEN* mutation positive proband with both parents shown not to carry the same mutation received a grade of 5. A grade of 1 indicated that the mutation was molecularly proven to be inherited from a parent or, in the case in which one or both parents were deceased, was shared with a sibling. For cases in which family members had not undergone molecular testing, inheritance was judged as suspected inherited (grade 2) if the proband had a first-degree relative who met the ICC operational criteria for the diagnosis of CS in a family member.[11] A grade of 3 was given when inheritance could not be predicted because of limited family structure and no first-degree relative met

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the ICC operational criteria for the diagnosis of CS in a family member. Family structure was judged as limited if at least one of the following were met: fewer than two women in either the maternal or paternal lineage survived beyond 50 years;[12] one parent is either an only child or no information was recorded about aunts or uncles; or limited family history information was available for either lineage because of adoptive status or lack of contact. A grade of 4 was assigned when the mutation was suspected *de novo*, family structure was sufficient for analysis, and the proband had no first- or second-degree relatives (excluding descendants) meeting the ICC operational criteria for the diagnosis of CS in a family member. Reports of macrocephaly that were not confirmed by documented occipital-frontal circumference measurement were disregarded. Differences between groups were assessed with Fisher's two-tailed exact test and unpaired *t*-test, and $P < 0.05$ was considered significant.

RESULTS

Among the 3,477 individuals accrued to the main 8458-PTEN study, 225 individuals belonging to 187 unrelated families were found to have clearly pathogenic germline *PTEN* mutations. Twenty mutations were confirmed as *de novo* through familial testing (grade 5) by the Eng research laboratory or testing in a CLIA-certified facility, leading to a conservatively calculated *de novo* mutation frequency of 10.7% (20/187) within all eligible families. If analysis is restricted to only those probands with known familial testing results (grade 1 and 5 probands, $n = 42$), a maximum *de novo* mutation frequency of 47.6% (20/42) is obtained. Combining probands with confirmed (grade 5; **Table 1**) and suspected *de novo* mutations (grade 4), a *de novo* mutation frequency of 29.9% (56/187) is estimated.

Table 1 Grading system reflecting degree of confidence of *de novo PTEN* mutation status in a proband

Grade	Number of probands	Description
1	22	Proband mutation proven to be inherited by molecular testing
2	48	No familial molecular testing performed; strong suspicion for inherited mutation based on presence of first-degree relative meeting ICC operational criteria for CS diagnosis in a family member
3	61	No familial molecular testing performed; unable to predict if mutation *de novo* or inherited because of lack of first-degree relatives meeting ICC operational criteria for CS diagnosis in a family member and limited family structure
4	36	No familial molecular testing performed; strong suspicion for *de novo* mutation based on lack of first- or second-degree relatives meeting ICC operational criteria for CS diagnosis in a family member with sufficient family structure for analysis
5	20	Proband mutation proven to be *de novo* by molecular testing

CS, Cowden syndrome; ICC, International Cowden Consortium.

Within the group molecularly proven to have *de novo PTEN* mutations (grade 5), features identified at presentation for testing were varied (**Table 2**). There were no differences in proportion of mutations that would lead to protein truncation vs. missense mutations ($P = 0.51$), gender ($P = 0.55$), or age at diagnosis ($P = 0.12$) between grade 1 vs. grade 5 probands. In grade 1 probands, mutations were inherited equally from the maternal and paternal lineages ($P = 0.55$). Within both groups, males were significantly younger at diagnosis than females ($P = 0.002$ for both). Given that many PHTS features have gender- and age-related penetrance,[7,13,14] grade 1 and grade 5 groups were stratified by gender to examine whether phenotypic differences were noted between patients; no such differences were found for any PHTS phenotype or for presence of any cancer diagnosis ($P > 0.06$ for all phenotypes).

DISCUSSION

This study conservatively reveals at minimum a 10.7% *de novo PTEN* mutation frequency, and demonstrates at best a 47.6% *de novo* mutation frequency. This range may still be an underestimate given the possibility that patients without a striking family history may not be considered for referral to a genetics clinic for evaluation and testing. When PHTS is a part of the differential diagnosis, clinicians should be mindful of *de novo* mutation frequency and not exclude consideration of this syndrome for a patient who lacks relevant diagnoses in their family history.

We had posited that if present, an overrepresention of one mutation type or phenotype among patients with *de novo* vs. inherited mutations would imply that those *de novo* mutations led to an increased phenotypic severity, causing decrease in survival to age of reproduction or reproductive ability. We did not find evidence to support this hypothesis, and in fact found that when stratified by gender, patients with *de novo* mutations had no appreciable demographic, phenotypic, or genotypic differences from those with confirmed inherited mutations. This finding supports the need for all PHTS patients to adhere to screening guidelines, regardless of family history.

Approximately 60–90% of *PTEN* mutations are inherited. In some families in which a mutation was proven as inherited (grade 1), this result was not surprising given the number of other relatives in the family with relevant diagnoses. However, in other families, in particular when the proband was a young child, there was a lack of known relevant diagnoses in the family history; yet one parent, with no preference for maternal or paternal inheritance, was found to share the child's mutation. Given that many characteristics of PHTS have age-related penetrance,[15,16] this was not an unexpected finding. Examining parents for phenotypic features suspicious for PHTS may help caregivers to predict which parent is more likely to test positive, so that parental testing can be performed in a step-wise and cost-saving manner. Macrocephaly is present in over 94% of persons

with PHTS[17] and is easily assessed by head circumference measurement, making this characteristic a potentially helpful and simple predictor of familial mutation status. Finding that most mutations are likely to be inherited is an important point to discuss with patients, and may increase their motivation to share their mutation status with at-risk family members so that predictive testing of relatives may be facilitated, enabling those testing positive to receive appropriate risk management.

We acknowledge the limitations inherent in this study, most notably the lack of medical record documentation for the majority of family members, for whom medical records could not be obtained if they were not study enrollees in accordance with our center's Institutional Review Board policies. We also regret that paternity testing was not possible given that in many situations, familial testing was performed through one of several clinical laboratories. Although we would have preferred to confirm the accuracy of the reported familial diagnoses and relationships, it may not be practical or possible in a clinical setting to do so, making the degree of diagnostic certainty in this study applicable to "real-life" clinical situations.

Our group has previously published a risk calculator, available online at http://www.lerner.ccf.org/gmi/ccscore/, that predicts the probability of having a germline *PTEN* mutation on the basis of personal medical history.[3] Family history is a crucial component of risk assessment and testing criteria for many inherited cancer syndromes.[18–22] We are currently studying family history diagnoses to determine which family history characteristics may be incorporated into a future version of this risk model.

Table 2 Clinical features of probands with *de novo* (grade 5) germline *PTEN* mutations

Family ID	Sex	Age at diagnosis (years)	Mutation	Consequence	Patient history
180	F	26	c.287C>G (Pro96Arg)	Missense	Macrocephaly, goiter, gastrointestinal polyposis, and lipoma
559	F	40	c.389G>A (Arg130Gln)	Missense	Macrocephaly, goiter, breast cancer dx 29 years, and uterine fibroids
780	M	3	c.44ins16	Truncation	Macrocephaly and lipomatosis
3015	F	41	c.734del4	Truncation	Breast papillomas, goiter, hamartomatous polyps, endometrial cancer dx 39 years, and mucocutaneous papillomatosis
3159	F	9	c.1003C>T (Arg335Ter)	Truncation	Macrocephaly, autism, hypotonia, and lymphangioma
3393	M	35	c.376G>C (Ala126Pro)	Missense	Macrocephaly, goiter, hamartomatous polyps, lipomas, and penile freckling
3429	F	19	c.76A>C (Thr26Pro)	Missense	Macrocephaly, Lhermitte–Duclos dx 19 years, and goiter
3597	F	10	c.1003C>T (Arg335Ter)	Truncation	Macrocephaly, developmental delays, arteriovenous hemangioma, acral keratoses, and lipoma
4366	M	4	Whole gene deletion	Haplo-insufficiency	Macrocephaly and autism
4386	M	2	c.737C>T (Pro246Leu)	Missense	Macrocephaly and developmental delay
4503	M	3	c.486C>G (Asp162Glu)	Missense	Macrocephaly, developmental delay, and hypotonia
4551	M	7	c.75G>T (Leu25Phe)	Missense	Macrocephaly, hydrocephalus, autism, hypotonia, cryptorchidism, and overgrowth
5063	F	12	c.511C>T (Gln171Ter)	Truncation	Macrocephaly, arteriovenous hemangiomas, and mucocutaneous papillomas
5130	M	3	c.420_421insA	Truncation	Macrocephaly, autism, and penile freckling
5319	F	46	c.401T>G (Met134Arg)	Missense	Macrocephaly, breast cancer dx 43 years, and gastrointestinal polyposis
5428	M	3	c.388C>T (Arg130Ter)	Truncation	Macrocephaly and developmental delay
5708	M	5	c.209+5G>A	Splice alteration	Macrocephaly, developmental delay, hypotonia, lipoma, and penile freckling
5833	M	1	c.263A>G (Tyr88Cys)	Missense	Macrocephaly, developmental delay, and hypotonia
5909	M	2	c.1003C>T (Arg335Ter)	Truncation	Macrocephaly, developmental delay, and dermal hamartoma
6052	M	2	Duplication of promoter, exon 1	Uncertain	Macrocephaly, developmental delay, and penile freckling

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Zbuk KM, Eng C. Cancer phenomics: RET and PTEN as illustrative models. *Nat Rev Cancer* 2007;7:35–45.
2. Marsh DJ, Coulon V, Lunetta KL, et al. Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germline PTEN mutation. *Hum Mol Genet* 1998;7:507–515.
3. Tan MH, Mester J, Peterson C, et al. A clinical scoring system for selection of patients for PTEN mutation testing is proposed on the basis of a prospective study of 3042 probands. *Am J Hum Genet* 2011;88:42–56.
4. Heald B, Mester J, Rybicki L, Orloff MS, Burke CA, Eng C. Frequent gastrointestinal polyps and colorectal adenocarcinomas in a prospective series of PTEN mutation carriers. *Gastroenterology* 2010;139:1927–1933.
5. Lynch ED, Ostermeyer EA, Lee MK, et al. Inherited mutations in PTEN that are associated with breast cancer, cowden disease, and juvenile polyposis. *Am J Hum Genet* 1997;61:1254–1260.
6. Marsh DJ, Kum JB, Lunetta KL, et al. PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. *Hum Mol Genet* 1999;8:1461–1472.
7. Tan MH, Mester JL, Ngeow J, Rybicki LA, Orloff MS, Eng C. Lifetime cancer risks in individuals with germline PTEN mutations. *Clin Cancer Res* 2012;18:400–407.
8. Shen MH, Harper PS, Upadhyaya M. Molecular genetics of neurofibromatosis type 1 (NF1). *J Med Genet* 1996;33:2–17.
9. Gray JR, Bridges AB, Faed MJ, et al. Ascertainment and severity of Marfan syndrome in a Scottish population. *J Med Genet* 1994;31:51–54.
10. Gonzalez KD, Buzin CH, Noltner KA, et al. High frequency of *de novo* mutations in Li-Fraumeni syndrome. *J Med Genet* 2009;46:689–693.
11. Pilarski R, Eng C. Will the real Cowden syndrome please stand up (again)? Expanding mutational and clinical spectra of the PTEN hamartoma tumour syndrome. *J Med Genet* 2004;41:323–326.
12. Weitzel JN, Lagos VI, Cullinane CA, et al. Limited family structure and BRCA gene mutation status in single cases of breast cancer. *JAMA* 2007;297:2587–2595.
13. Gorlin RJ, Cohen MM Jr, Condon LM, Burke BA. Bannayan-Riley-Ruvalcaba syndrome. *Am J Med Genet* 1992;44:307–314.
14. Lachlan KL, Lucassen AM, Bunyan D, Temple IK. Cowden syndrome and Bannayan Riley Ruvalcaba syndrome represent one condition with variable expression and age-related penetrance: results of a clinical study of PTEN mutation carriers. *J Med Genet* 2007;44:579–585.
15. Nelen MR, Padberg GW, Peeters EA, et al. Localization of the gene for Cowden disease to chromosome 10q22-23. *Nat Genet* 1996;13:114–116.
16. Eng C. Will the real Cowden syndrome please stand up: revised diagnostic criteria. *J Med Genet* 2000;37:828–830.
17. Mester JL, Tilot AK, Rybicki LA, Frazier TW II, Eng C. Analysis of prevalence and degree of macrocephaly in patients with germline PTEN mutations and of brain weight in Pten knock-in murine model. *Eur J Hum Genet* 2011;19:743–748.
18. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. *Gastroenterology* 1999;116:1453–1456.
19. Parmigiani G, Berry D, Aguilar O. Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. *Am J Hum Genet* 1998;62:145–158.
20. Frank TS, Deffenbaugh AM, Reid JE, et al. Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. *J Clin Oncol* 2002;20:1480–1490.
21. Li FP, Fraumeni JF Jr, Mulvihill JJ, et al. A cancer family syndrome in twenty-four kindreds. *Cancer Res* 1988;48:5358–5362.
22. Fitzgerald RC, Hardwick R, Huntsman D, et al.; International Gastric Cancer Linkage Consortium. Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. *J Med Genet* 2010;47:436–444.