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Estimate of *de novo* mutation frequency in probands with *PTEN* Hamartoma Tumor syndrome

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Abstract

Purpose—*PTEN* Hamartoma Tumor Syndrome (PHTS) 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 2-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 versus 5 (all $p>0.06$). In grade 1 probands, mutations were inherited equally from maternal and paternal lineages ($p=0.55$).

Conclusion—The frequency of *de novo PTEN* mutation is minimally 10.7% and maximally 47.6%. Absence of PHTS features within a family history should not preclude consideration of this diagnosis for patients with relevant personal history.

Keywords

Germline *PTEN* mutation; Cowden syndrome; Bannayan-Riley-Ruvalcaba syndrome; inherited cancer syndrome; *de novo* mutation

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Conflict of Interest Notification The authors declare no conflicts of interest.

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,[3-5] making timely diagnosis and identification of at-risk relatives critical for risk management. Both familial and apparently sporadic cases have been reported;[2,6,7] however, the frequency of patients with *de novo* versus 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 5-tiered family history grading system was created to denote 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 where one or both parents were deceased, was shared with a sibling. For cases where family members had not undergone molecular testing, inheritance was judged as suspected inherited (grade of 2) when 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 due to limited family structure and no first-degree relative met 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: Less 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 due to adoptive status or lack of contact. A grade of 4 was assigned when the mutation was suspected *de novo* when 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 OFC measurement were disregarded. Differences between groups were assessed with Fisher's 2-tailed exact test and unpaired *t*-test, with $p<0.05$ 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.

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 difference in proportion of mutations that would lead to protein truncation versus missense mutations (p=0.51), gender (p=0.55), or age at diagnosis (p=0.12) between grade 1 versus grade 5 probands. In grade 1 probands, mutations were inherited equally from the maternal and paternal lineages (p=0.55). Within both groups, males had significantly younger ages at diagnosis than females (p=0.002 for both). Given that many PHTS features have gender- and age-related penetrance, 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 a 10.7% *de novo PTEN* mutation frequency, and demonstrates the potential of 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 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 over-represention of one mutation type or phenotype among patients with *de novo* versus inherited mutations would imply 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 where 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,[13,14] this was not an unexpected finding. Examining parents for phenotypic features suspicious for PHTS may help caregivers 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[15] 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 IRB 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/, which predicts the probability of having a germline *PTEN* mutation based on personal medical history.[3] Family history is a crucial component of risk assessment and testing criteria for many inherited cancer syndromes.[16-20] We are currently studying family history diagnoses to determine which family history characteristics may be incorporated into a future version of this risk model.

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REFERENCES

1. Zbuk KM, Eng C. Cancer phenomics: RET and PTEN as illustrative models. Nat Rev Cancer. Jan; 2007 7(1):35–45. [PubMed: 17167516]
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. Mar; 1998 7(3):507–515. [PubMed: 9467011]
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. Jan 7; 2011 88(1):42–56. [PubMed: 21194675]
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. Dec; 2010 139(6):1927–1933. [PubMed: 20600018]
5. Tan MHMJ, Ngeow J, Rybicki L, Orloff M, Eng C. Lifetime Cancer Risks in Individuals with Germline PTEN Mutations. Clin Cancer Res. In press.
6. 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. Dec; 1997 61(6):1254–1260. [PubMed: 9399897]
7. 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. Aug; 1999 8(8):1461–1472. [PubMed: 10400993]
8. Shen MH, Harper PS, Upadhyaya M. Molecular genetics of neurofibromatosis type 1 (NF1). J Med Genet. Jan; 1996 33(1):2–17. [PubMed: 8825042]

9. Gray JR, Bridges AB, Faed MJ, et al. Ascertainment and severity of Marfan syndrome in a Scottish population. J Med Genet. Jan; 1994 31(1):51–54. [PubMed: 8151638]
10. Gonzalez KD, Buzin CH, Noltner KA, et al. High frequency of de novo mutations in Li-Fraumeni syndrome. J Med Genet. Oct; 2009 46(10):689–693. [PubMed: 19556618]
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. May; 2004 41(5): 323–326. [PubMed: 15121767]
12. Weitzel JN, Lagos VI, Cullinane CA, et al. Limited family structure and BRCA gene mutation status in single cases of breast cancer. JAMA. Jun 20; 2007 297(23):2587–2595. [PubMed: 17579227]
13. Nelen MR, Padberg GW, Peeters EA, et al. Localization of the gene for Cowden disease to chromosome 10q22-23. Nat Genet. May; 1996 13(1):114–116. [PubMed: 8673088]
14. Eng C. Will the real Cowden syndrome please stand up: revised diagnostic criteria. J Med Genet. Nov; 2000 37(11):828–830. [PubMed: 11073535]
15. Mester JL, Tilot AK, Rybicki LA, Frazier TW 2nd, 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. Feb 23.2011
16. 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. Jun; 1999 116(6):1453–1456. [PubMed: 10348829]
17. Parmigiani G, Berry D, Aguilar O. Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. Am J Hum Genet. Jan; 1998 62(1):145–158. [PubMed: 9443863]
18. 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. Mar 15; 2002 20(6):1480–1490. [PubMed: 11896095]
19. Li FP, Fraumeni JF Jr. Mulvihill JJ, et al. A cancer family syndrome in twenty-four kindreds. Cancer Res. Sep 15; 1988 48(18):5358–5362. [PubMed: 3409256]
20. Fitzgerald RC, Hardwick R, Huntsman D, et al. Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. J Med Genet. Jul; 2010 47(7):436–444. [PubMed: 20591882]

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 due to 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.

Table 2

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

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