COMMENT

Mosaicism in *PTEN*—new case and comment on the literature

Paula Rofes[1,2], Álex Teulé[1], Lídia Feliubadaló[1,2], Mònica Salinas[1], Raquel Cuesta[1], Sílvia Iglesias[1], Olga Campos[1], Sara González[1,2], Gabriel Capellá[1,2], Joan Brunet[1,2], Jesús del Valle[1,2,3] and Conxi Lázaro[1,2,3]✉

European Journal of Human Genetics (2022) 30:641–644; https://doi.org/10.1038/s41431-022-01052-7

PTEN hamartoma tumor syndrome (PHTS) encompasses a group of related genetic disorders linked to inherited pathogenic variants (PVs) in *PTEN* gene, which includes Cowden syndrome (CS), Bannayan–Riley–Ruvalcaba syndrome (BRRS), Lhermitte–Duclos Disease (LDD) in adults, autism disorders associated with macrocephaly, *PTEN*-related Proteus syndrome (PS) and Proteus-like syndrome [1, 2]. Accumulated evidence suggests that these entities represent variable expression and age-related penetrance of one single condition [2]. CS is a rare autosomal dominant condition with an incidence of ~1:200,000 individuals [2], characterized by a varied spectrum of clinical manifestations, even among patients carrying the same variant. Some of the most common phenotypic features include the development of hamartomatous polyps in the gastrointestinal tract, reported in 35–85% of CS patients, macrocephaly and mucocutaneous lesions as trichilemmomas. Besides, CS patients present increased risks for breast, endometrial and thyroid tumors [1, 2]. The operational diagnosis of CS is based upon the phenotypic manifestations [1] or the identification of a *PTEN* PV, which may be inherited or arise de novo in up to 44% of patients [2]. Interestingly, *PTEN* mosaicism has been reported in 3 patients with a clinical diagnosis of CS, as well as in 3 additional patients with PHTS-associated features z (Table 1).

In June 2018, a 53-years-old white European male without personal history of cancer, nor recurrent infection diseases or immunological alterations was referred to our hospital to undergo genetic testing. He was initially diagnosed with Peutz–Jeghers syndrome (PJS) by gastroenterologists from a community hospital at age 19 due to the presence of gastrointestinal polyposis. Physical examination revealed macrocephaly (61 cm head circumference), multinodular thyroid goiter with endothoracic component, palmoplantar pits and macular pigmentation on the glans penis. No macular pigmentation of oral mucosa or lips was observed. The neuropsychological evaluation did not evidence developmental delays. A thorough examination of the gastrointestinal tract was performed. The esophagogastroduodenoscopy revealed more than 100 sessile polyps in major and minor curvatures of the body and the fundus of the stomach, all of them between 3 and 5 mm and no degenerating appearance. More than 50 polyps were revealed in the second part of the duodenum, two of them semipedicular of a size of 10 and 12 mm respectively. Polyps were observed to continue also in the third part of the duodenum. Some of them were biopsied and were classified as hyperplastic polyps after histological examination. Two hamartomatous polyps were resected from the colon and the duodenum, and an arteriovenous angioma was observed in the sigmoid colon. The last colonoscopy showed 7 polyps in the colon that were resected, 6 of which were hyperplastic polyps and one a lipoma. An endoscopic capsule showed multiple polyps of 3–5 mm along the duodenum and jejunum, 3 duodenal polyps up to 12 mm in diameter, and some other polyps in the distal sections of the intestine. An abdominal ultrasound showed mild hepatic steatosis and small gallbladder polyps, and an abdominal MRI revealed myelolipomas in both adrenal glands.

A four-generation pedigree was elicited at the Genetic Counseling Unit (Fig. 1A). The father of the proband was diagnosed with a double neoplasm of the lung and the stomach (unconfirmed), being deceased at age 70. His daughter is affected of Williams syndrome, a rare genetic disorder with an incidence of 1:10,000 caused by a deletion at 7q11.23, region that includes the elastin gene [3]. It is clinically characterized by cardiovascular and endocrine abnormalities, connective tissue alterations, intellectual disability, a specific cognitive and behavioral profile and growth retardation [3]. A karyotype was performed in the proband, but it didn't show any relevant finding.

Based upon the consensus clinical diagnostic criteria [1], the patient received an operational individual diagnosis of CS in agreement with the following three major criteria: macrocephaly (≥97th percentile: 60 cm for males), macular pigmentation of the glans penis and acral keratosis (≥3 palmoplantar keratotic pits). He additionally met two minor criteria: thyroid structural lesions and vascular anomalies. The presence of gastrointestinal hamartomas could not be considered a major criterion in this patient as we only had histological confirmation for 2 of them (being 3 the minimum required). Accordingly, he was referred for *PTEN* germline genetic testing. Considering the previous diagnosis of PJS, *STK11* was also examined, in addition to opportunistic testing of *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6*.

PTEN c.331T>C, p.(Trp111Arg) variant was identified in 719 out of a total of 3196 sequencing reads, representing a variant allele frequency (VAF) of 22.5% in peripheral blood DNA (Fig. 1B). The presence of the variant was orthogonally validated by conventional Sanger sequencing (Fig. 1C). While this substitution was previously reported in an individual diagnosed with PS [4], it is absent in a large-scale general population reference sequencing datasets [5]. Functional assays have demonstrated that this variant significantly compromises the phosphatase activity of *PTEN* [6, 7]. Accordingly, it is classified as likely pathogenic in ClinVar database

[1]Hereditary Cancer Program, Program in Molecular Mechanisms and Experimental Therapy in Oncology (Oncobell), IDIBELL, Catalan Institute of Oncology, l'Hospitalet del Llobregat, Spain. [2]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain. [3]These authors jointly supervised this work: Jesús del Valle, Conxi Lázaro. ✉email: clazaro@iconcologia.net

Received: 29 September 2021 Revised: 13 January 2022 Accepted: 18 January 2022
Published online: 1 February 2022

Table 1. Summary of previously published *PTEN* mosaicisms and their clinical and genetic features.

Reference	PMID	Patient phenotype	Clinical manifestations (age at diagnosis)	Criteria for genetic testing	Family history (age at diagnosis)	*PTEN* variant (frequency)
Goldenberg et al. (2019)	31796102	ASD of asperger type	Retroauricular glioneural hamartoma, brain and spinal cord lesions, slight hypotonia, moderate coordination disorder, mild social communication disorder and mild learning disability	Glioneuronal hamartoma exhibiting heterogeneous PTEN immunoreactivity	No	*PTEN* c.970dup; p.(Asp324Glyfs*3) (3.5–30%)
Golas et al. (2018)	31062505	BRRS	Macrocephaly, multiple colorectal polyps, craniofacial dysmorphy, multiple nevi, oral papilloma and delayed motor and speech development	Clinical signs of BRRS	No	*PTEN* 10q23.1q23.3 deletion (6 Mb), involving *PTEN, KLLN* and *BMPR1A*, among other genes
Golas et al. (2018)	31062505	BRRS	Macrocephaly, colorectal polyps, hyperpigmented skin and glans penis macules, multinodular thyroid goiter, Arnold–Chiari malformation, hydrocephalus, muscular hypotonia, intellectual disability and delayed speech development	Clinical signs of BRRS	Paternal grandmother: BC (74); paternal grandfather: PC (62); maternal grandmother: TC (38); maternal grandmother's sister: goiter.	*PTEN* 10q23.2q23.3 deletion (4.4 Mb), involving *PTEN*, *KLLN* and *BMPR1A*, among other genes
Salo-Mullen et al. (2014)	24609522	CS	Breast DCIS (40), macrocephaly, hamartomatous and ganglioneuromatous intestinal polyps, lingual and labial papillomatosis, acral keratoses, uterine fibroids, fibrocystic breasts, mucosal fibroma, visceral and cutaneous hemangiomas and pancreatic neuroendocrine tumor	Clinical signs of CS: 2 major and 5 minor features	Nephew: macrocephaly and speech delay; Paternal aunt: BC (50).	*PTEN* partial deletion, comprising at least exons 6–9 (47 ± 10% of cells)
Pritchard et al. (2013)	23619277	CS	Macrocephaly, parietal stroke, LDD, mucosal papillomas, acral keratoses, hamartomatous polyps, ganglioneuroma, multiple lipomas and thyroid goiter	Clinical signs of CS: 4 major and 2 minor features	No	*PTEN* c.767_768del; p.(Glu256Valfs*41) (5–50%[a])
Gammon et al. (2013)	23240978	CS	Macrocephaly and Hashimoto's thyroiditis	Patient did not meet CS consensus criteria	Daughter: CS (20); Daughter: thyroid goiter; Mother: EC (60); Father: melanoma (70)	*PTEN* c.966_967delinsG; p.(Asn323Metfs*21) (<10%)

ASD autism spectrum disorder, *BRRS* Bannayan–Riley–Ruvalcaba syndrome, *CS* Cowden syndrome, *LDD* Lhermitte–Duclos disease, *DCIS* ductal carcinoma in situ, *BC* breast cancer, *EC* endometrial cancer, *PC* prostate cancer, *TC* thyroid cancer.
[a]Variable frequency across different tissue types tested.

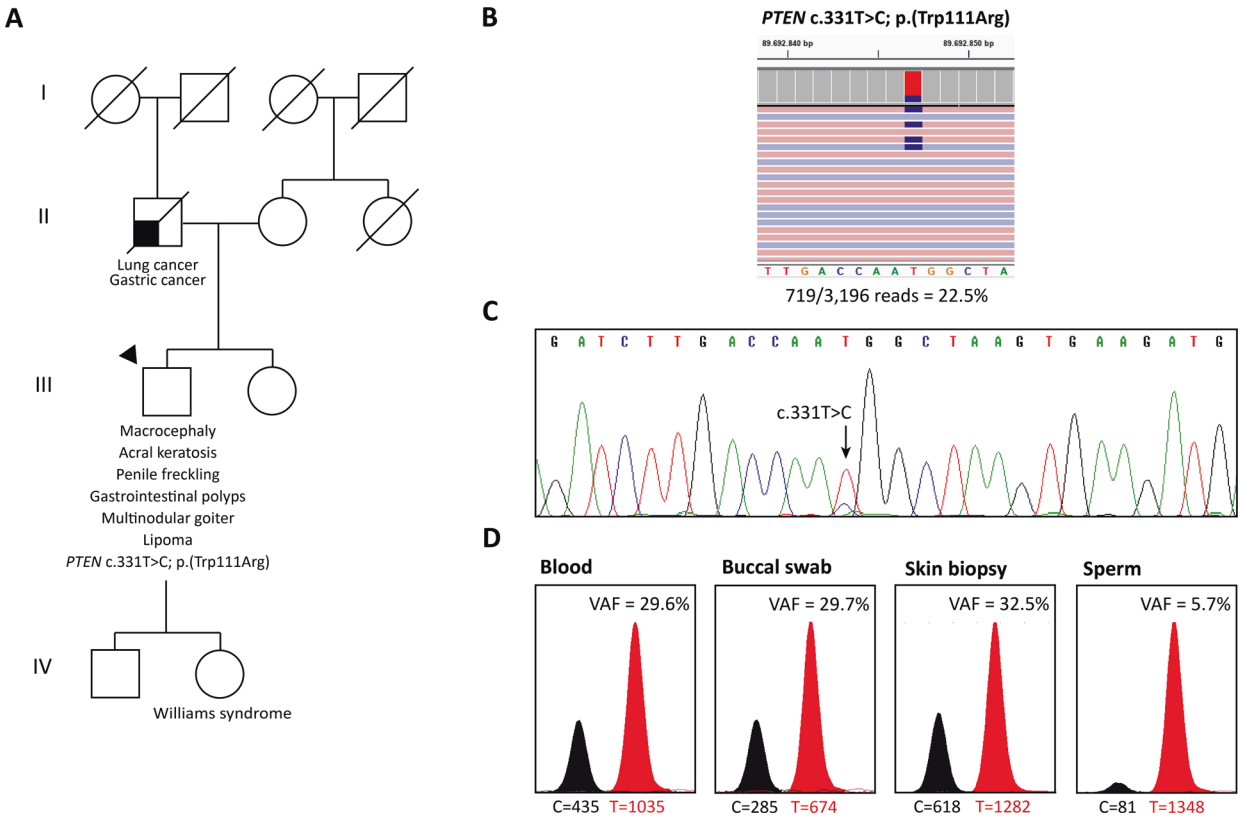

Fig. 1 Cowden syndrome patient and mutational analysis of *PTEN* c.331T>C pathogenic variant. A Pedigree of the Cowden syndrome (CS) patient presented here harboring the mosaic *PTEN* c.331 T > C; p.(Trp111Arg) variant. The black arrowhead is used to indicate the proband. A quarter of an individual symbol shadowed represents an individual diagnosed with cancer. Cancer type is displayed below the individual symbol. **B** Close-up of next-generation sequencing (NGS) data from peripheral blood lymphocyte DNA, visualized in the Integrative Genomics Viewer (IGV). **C** Validation of NGS results by Sanger sequencing. **D** Tissues tested and variant allele frequencies (VAFs) detected by single-nucleotide primer extension (SNuPE) assay.

by ClinGen *PTEN* Variant Curation Expert Panel following *PTEN*-specific ACMG recommendations [8].

Representative samples derived from the three embryonic layers were collected in order to evaluate the extension of mosaicism to other tissues by single-nucleotide primer extension (SNuPE). Since peripheral blood cells originate from the mesoderm, a buccal swab and a skin biopsy were acquired for ectoderm representation, and a urine sample was collected to obtain uroepithelial cells detached from the bladder for endoderm representation. VAFs in ectodermal derivatives ranged between 29 and 34%, suggesting that the variant was only present in a proportion of the cells of these tissues (Fig. 1D). Unfortunately, the presence of the variant in uroepithelial cells could not be evaluated due to a failure in the DNA extraction process. Furthermore, we also aimed at investigating the extension of mosaicism to the germline. In DNA extracted from sperm cells the variant was detected at a frequency of 5.7% (Fig. 1D). Despite confirming germline affectation, both the son and the daughter of the proband refused to undergo carrier testing. Therefore, the transmission of the variant to the offspring could not be assessed. A detailed explanation of the methodology is provided in the Supplementary Methods file. Following the same experimental approach, blood DNA was also tested. The VAF was estimated in 29.6% by SNuPE assay, slightly different to the one obtained by NGS.

The multisystem affectation observed in the patient reported here points out to an early post-zygotic mutational event [9], supported by the patient's phenotype, as he presented macrocephaly, gastrointestinal and vascular alterations and mucocutaneous lesions. At embryonic level, this alteration was possibly acquired before the differentiation of the 3 germ layers (day 8 of the development), considering that mesodermal and ectodermal derivatives appeared affected (blood and epidermal cells, respectively). Unfortunately, endodermal affectation could not be assessed.

One of the most relevant findings of the case presented here is that we were able to confirm the presence of *PTEN* c.331T>C variant in the proband's reproductive cells, although at a significantly low VAF (5.7%). The affectation of the germline has a direct impact in the management of the family, considering that the offspring could be non-mosaic carriers of the same variant. However, we were not able to test the transmission of the variant, as both descendants refused to undergo carrier testing. Since they also refused clinical examination, we could not either evaluate the presence of clinical signs of CS in any of them.

Mosaicism is usually linked to mild phenotypic alterations or later onsets. Interestingly, the proband of this family fulfilled CS clinical diagnostic criteria (3 major and 2 minor criteria) to undergo *PTEN* germline testing, being his phenotype comparable to that of a heterozygous carrier. To our knowledge, only 3 previous cases of *PTEN* mosaicism in CS patients have been reported. Two individuals presented a full penetrance with severe clinical manifestations, despite the low frequency of the variants they harbored. Contrarily, the remaining case had been primarily overlooked, as he did not manifest any CS feature (Table 1). Therefore, it is plausible that nowadays patients harboring mosaic *PTEN* variants remain undetected as they do not meet *PTEN* clinical testing criteria [1]. Besides, 3 additional individuals diagnosed with PHTS-related disorders carrying mosaic *PTEN* variants have also been identified, 2 diagnosed with BRRS and 1 with Asperger (Table 1). This series of patients highlights that *PTEN* mosaicism

does not necessarily correlate with milder phenotypes, and that VAFs cannot predict the severity of the clinical features of carriers.

To date, *PTEN* is included in most hereditary cancer multigene panels, as it is the only clinically actionable gene in the PHTS spectrum. Considering that *PTEN* variant carriers may present increased cancer risks [1, 2], adults should undergo surveillance following the current recommendations, regardless the frequency of the variants detected. Besides, germline *PTEN* variants have been reported in individuals without clinical features of PHTS, indicating that this syndrome likely remains underdiagnosed [2]. In this context, mosaicism could be playing a role, as more cases are being identified than initially suspected. The use of high-throughput sequencing methods, as well as testing affected tissues rather than blood, may help increase sensitivity and effectiveness of conventional genetic testing to overcome this limitation.

DATA AVAILABILITY

All the data generated in this study can be found within the published article and its Supplementary files.

REFERENCES

1. Pilarski R, Burt R, Kohlman W, Pho L, Shannon KM, Swisher E. Cowden syndrome and the PTEN hamartoma tumor syndrome: systematic review and revised diagnostic criteria. J Natl Cancer Inst J Natl Cancer Inst. 2013;105:1607–16.
2. Yehia L, Keel E, Eng C. The Clinical Spectrum of PTEN Mutations. Annu Rev Med Annu Rev Inc. 2020;71:03–16.
3. Twite MD, Stenquist S, Ing RJ. Williams syndrome. Paediatr Anaesth. 2019;29:483–90. https://doi.org/10.1111/pan.13620.
4. Zhou XP, Hampel H, Thiele H, Gorlin RJ, Hennekam RCM, Parisi M, et al. Association of germline mutation in the PTEN tumour suppressor gene and Proteus and Proteus-like syndromes. Lancet 2001;358:210–1.
5. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020;581:434–43. https://doi.org/10.1038/s41586-020-2308-7.
6. Matreyek KA, Starita LM, Stephany JJ, Martin B, Chiasson MA, Gray VE, et al. Multiplex assessment of protein variant abundance by massively parallel sequencing. Nat Genet. 2018;50:874–82.
7. Mighell TL, Evans-Dutson S, O'Roak BJ. A Saturation Mutagenesis Approach to Understanding PTEN Lipid Phosphatase Activity and Genotype-Phenotype Relationships. Am J Hum Genet. 2018;102:943–55.
8. Mester JL, Ghosh R, Pesaran T, Huether R, Karam R, Hruska KS, et al. Gene-specific criteria for PTEN variant curation: Recommendations from the ClinGen PTEN Expert Panel. Hum Mutat. 2018;39:1581–92.
9. Gilbert SF. Early Mammalian Development. 2000. Accessed 9 Dec 2021. https://www.ncbi.nlm.nih.gov/books/NBK10052/.

ACKNOWLEDGEMENTS

We thank CERCA Program/Generalitat de Catalunya for their institutional support. We also wish to thank all the members of the ICO Hereditary Cancer Program.

AUTHOR CONTRIBUTIONS

PR, JV, and CL conceived, designed, and planned the study; PR, LF, RC, OC, SG, GC, JV, and CL contributed to the acquisition, analysis, and/or interpretation of the molecular data; AT, MS, SI, and JB provided samples and clinical data; PR, AT, and CL drafted the manuscript. All authors critically reviewed the manuscript for intellectual content and approved the final version.

FUNDING

Supported by the Carlos III National Health Institute and Ministerio de Ciencia e Innovación, funded by FEDER funds—a way to build Europe—[PI19/00553; PI16/00563; PID2019-111254RB-I00] and CIBERONC [CB16/12/00234]; the Government of Catalonia [Pla estratègic de recerca i innovació en salut (PERIS_MedPerCan and URDCat projects), 2017SGR1282 and 2017SGR496].

COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

Informed written consent for both diagnostic and research purposes was obtained from the patient presented here. The study protocol was approved by the ethics committee of Bellvitge Biomedical Research Institute (IDIBELL; PR278/19).

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41431-022-01052-7.

Correspondence and requests for materials should be addressed to Conxi Lázaro.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.