Genes & Diseases (2024) 11, 524–527

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RAPID COMMUNICATION

# Lack of evidence for germline *WWP1* pathogenic variants in gastrointestinal polyposis and other phenotypes suggestive of PTEN-hamartoma-tumor syndrome

Germline activating variants in *WWP1*, which encodes an E3 ubiquitin ligase that antagonizes PTEN tumor suppressive function, have been proposed as an alternative mechanism of PTEN inactivation in PTEN-hamartoma-tumor syndrome (PHTS)-like patients with wildtype *PTEN*.[1] More specifically, heterozygous, potentially activating *WWP1* variants were first identified by Lee et al in patients affected with gastrointestinal oligopolyposis, including adenomatous, hyperplastic/serrated, and hamartomatous polyps, and occasionally with colorectal cancer (Table 1). Subsequently, based on the PHTS phenotypic features, *WWP1* mutational screening was performed in patients with thyroid nodules,[2] or normocephalic autism spectrum disorder (ASD),[3] where germline *WWP1* variants were also identified (Table S1).

Based on the main findings by Lee et al,[1] we aimed to validate their results and elucidate the role of *WWP1* in the predisposition to polyposis. We sequenced the complete coding region of *WWP1* in 177 adenomatous polyposis (AP) patients without germline pathogenic variants in known polyposis genes. According to previous studies, three variants were recurrently identified in patients with PHTS-like,[1] thyroid nodules,[2] ASD,[3] and AP phenotypes: p.Lys740Asn, p.Asn745Ser, and p.Val726Ile. These variants, together with p.Arg685= , identified in an AP patient, were genotyped in 95 *RNF43*-wildtype, serrated/hyperplastic polyposis (SP) (Methodological details in supplementary material; IDIBELL Ethics Committee approval: PR073/12).

Three *WWP1* germline variants with potential interest [c.2055T>C (p.Arg685=; rs139392205), c.2176G>A (p.Val726Ile; rs144129917), and c.2220G>C (p.Lys740Asn; rs144060832)] were identified in AP patients; the latter two were also identified in patients with PHTS-like, thyroid nodules and/or ASD (Table 1; Table S1). The three variants, each detected in 0.56% (1/177) of AP patients, are also present in Spanish (non-cancer) population individuals with a prevalence of 0.1% (2/1954), 0.3% (6/1907), and 0.7% (14/1954), respectively (source: http://csvs.babelomics.org/), as well as in gnomAD (non-cancer) individuals (Table 1). No SP patients harbored any of the four genotyped variants.

Considering all reported patients with a gastrointestinal tumor phenotype and a germline *WWP1* variant ($n$ = 9; Table 1), the observed phenotypes include gastrointestinal polyposis, diagnosed in the adult age (29–67), with different expressivities regarding number (range: 7 to 114 polyps) and type of polyps, which preferentially include hyperplastic, adenomatous and hamartomatous subtypes. Four (44%) heterozygotes were diagnosed with colorectal cancer (ages at diagnosis: 29–62). These observations are likely influenced by ascertainment bias. No other tumor types were recurrently identified in heterozygotes.

Based on the nature and *in silico* pathogenicity predictions of the variants identified in polyposis patients, only p.Tyr556* (1 patient), p.Lys740Asn (2 patients), and p.Arg685= (1 patient), were considered damaging or predicted damaging, or with a predicted effect on splicing. Nevertheless, gain-of-function mutations are difficult to predict. The significance of loss-of-function mutations, such as p.Tyr556*, remains to be elucidated.

Several of the *a priori* functionally relevant variants, including p.Lys740Asn, p.Asn745Ser, and p.Arg685=, have relatively high population allele frequencies (MAF: 0.04%–0.16% in gnomAD v.2.1.1 non-cancer subset; 0.05%–0.38% in Spanish non-cancer population), considering the rarity of PHTS-suggestive phenotypes (Cowden syndrome has an estimated prevalence of 1:250,000 in European population; supplementary references). These frequencies also exceed the

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2023.03.011

**Table 1** Characteristics of germline *WWP1* variants identified in PHTS-like[1] and gastrointestinal polyposis phenotypes. Variants with population MAF <0.2% and with a SpliceAI score >0.2 in the case of synonymous and intronic variants ( ± 10 nt from exon-intron boundaries) were included unless not reported in the corresponding publication. The variants listed were reported by Lee et al[1] or identified in this study.

| *WWP1* variant; NM_007013.4); protein domain | *Pathogenicity predictions | Population MAF % (gnomAD non-cancer) | Patient's phenotype and family history (age at diagnosis) [individual ID; study] |
|---|---|---|---|
| c.178T>G; p.Ser60Ala; C2 | REVEL (0.133) B; SIFT (0.48) B; Polyphen (0.411) B; CADD (21.7) | 0.004% | Ind_1 [CCF03506; Lee et al, 2020]<br>Macrocephaly, GI polyps (12 hyperplastic, 3 lipomatous) (61)<br>*Family history*: n/a |
| c.1006A>C; p.Met336Leu; disordered region | [a]REVEL (0.042) B; SIFT (1) B; PolyPhen (0) B; CADD (1.079) B | 0.006% | Ind_2 [CCF04145; Lee et al, 2020]<br>GI polyps (18 adenomatous, 2 hamartomatous, 4 hyperplastic, 1 lipomatous, 1 serrated adenoma, 2 other) (48)<br>*Family history*: n/a |
| c.1668T>A; p.Tyr556* | - | 0 | Ind_3 [CCF04959; Lee et al, 2020]<br>GI polyps (3 adenomatous, 5 hyperplastic) (59), colon cancer (62), ovarian cancer (48)<br>*Family history*: n/a |
| c.1709 A>G; p.Asn570Ser; WW4-HECT linker | REVEL (0.161) B; SIFT (0.74) B; Polyphen (0.052) B; CADD (19.88) B | 0.004% | Ind_4 [CCF01687; Lee et al, 2020]<br>Follicular thyroid cancer (62), invasive breast cancer (63), fibrocystic breast cancer, uterine fibroids, GI polyps (8 hyperplastic)<br>*Family history*: n/a |
| **c.2055T>C; p.Arg685=; HECT N-Lobe | Donor loss, Splice AI (0.31); Donor gain, Splice AI (0.12) | 0.039% | Ind_5 [this study]<br>114 colonic polyps (18 adenomatous, 52 hyperplastic), CRC (48)<br>*Family history*: father: CRC (75), multiple polyps; mother: BC (52), KC (77); brother: >10 multiple polyps. All of unknown carrier status. |
| **c.2176G>A; p.Val726Ile; HECT N-Lobe | REVEL (0.181) B; SIFT (0.09) B; Polyphen (0.879) PD; CADD (24.3) | 0.010% | Ind_6 [this study]<br>25 colonic polyps (5 hyperplastic), CRC (53)<br>*Family history*: sister: BC (51); Pat grandmother: BC (56); Mat grandfather: PC (63); Mat aunt: CRC (70); Mat cousin: CRC (54), LC (56). All of unknown carrier status. |
| **c.2220G>C; p.Lys740Asn; HECT N-Lobe | REVEL (0.486); SIFT (0) D; PolyPhen (0.966) PD; CADD (21.5) | 0.048% | Ind_7 [CCF02632_01_001; Lee et al, 2020]<br>GI polyposis, desmoid tumor, basal-cell cancer<br>*Family history*: brother (carrier): GI polyposis; father (carrier): GI polyposis and skin lesions; sister (unk. carrier status): CRC (33); sister (unk. carrier status): CRC (43); great-aunt (unk. carrier status): CRC (49)<br>Ind_8 [this study]<br>>80 adenomatous polyps (29), CRC (29), CRC (30)<br>*Family history*: mother (unk. carrier status): CRC (55); paternal uncle (unk. carrier status): CRC (75) |
| **c.2234A>G; p.Asn745Ser; HECT N-Lobe | REVEL (0.136) B; SIFT (0.5) B; PolyPhen (0.374) B; CADD (19.3) | 0.158% | Ind_9 [CCF03258; Lee et al, 2020]<br>GI polyps (5 hamartomatous, 2 other) (67), bladder cancer (70)<br>*Family history:* n/a |

Abbreviations: B, predicted benign variant; BC, breast cancer; CRC, colorectal cancer; D, predicted deleterious variant; GI, gastrointestinal; Ind, individual ID; KC, kidney cancer; LC, lung cancer; n/a, not available information; PC, prostate cancer; PD, possibly/probably damaging; unk., unknown.

* Pathogenicity prediction scores were obtained from the Ensembl Variant Effect Predictor (VEP). For SIFT and PolyPhen, the scores and significance were included. For REVEL (scores 0 to 1), the closer the score is to 1, the stronger the prediction of deleteriousness. Variants with REVEL scores <0.4 were considered predicted benign, and ≥0.7, deleterious. The range in-between has not been categorized. Phred-style CADD raw scores are displayed, and CADD scores below 20 were considered predicted benign, and CADD scores above 30, deleterious. The range in-between was not categorized. In the case of SpliceAI, we used the recommended cutoff values: 0.2, high recall; 0.5, recommended; and 0.8, high precision (supplementary references).

** The variants genotyped in SP patients. The reference of individual ID (Ind) in the corresponding publication is indicated between brackets.

prevalence of APC-associated polyposis, the most common hereditary polyposis syndrome (supplementary references).

Based on the findings by Lee et al and the presence of thyroid nodules and ASD in PHTS, two research teams studied *WWP1* in patients affected with either phenotype.[2,3] Germline heterozygous variants were identified in both groups of patients (Table S1), some of which — p.Val726Ile, p.Lys740Asn, and p. Asn745Ser — had also been identified in patients with gastrointestinal manifestations. No cancer or family history of cancer or polyposis was reported in any of the heterozygous individuals with either thyroid nodules or ASD. In the case of ASD patients, where segregation analyses were performed in the proband's parents, no disease phenotypes (of any type) were reported in heterozygotes.

The original association of *WWP1* mutations with PHTS-like or gastrointestinal tumor phenotypes was supported by the fact that E3 ubiquitin ligase WWP1 negatively regulates PTEN function.[1] WWP1 mediates PTEN K27-linked polyubiquitination, suppressing PTEN dimerization, membrane recruitment, and function.[4] Unfortunately, Lee et al did not analyze WWP1 and/or PTEN expression in the patients' tumors,[1] and neither did we, due to the unavailability of tumor material. The authors suggested that the identified *WWP1* variants relieved the intra- or inter-molecular WWP1 autoinhibition conferred by the 2,3-linker (a ~30-amino-acid $\alpha$-helical peptide linker positioned between the WW2 and WW3 domains), which would cause WWP1 overactivation.

In 2021, Jiang et al showed, with a robust experimental design, that p.Lys740Asn and p.Asn745Ser do not enhance WWP1 ubiquitin ligase activity, having no effect on WWP1 autoubiquitination or PTEN ubiquitination. In multiple experimental contexts, the variants showed similar results to wild-type WWP1. Moreover, the authors evidenced that on the X-ray crystal structure of WWP1 both Lys740 and Asn745 are rather far from the 2,3-linker-catalytic domain interaction surface, as do most WWP1 variants identified in patients (Fig. S1), making it difficult to rationalize that the variants would destabilize the interaction or stimulate catalysis. Moreover, Jiang et al observed no PTEN suppression caused by the WWP1 variants when the HCT116 colon cancer cell line was transfected with p.Lys740Asn, p.Asn745Ser, or wild-type WWP1, discarding any other possible (unknown) mechanisms of WWP1-driven PTEN inactivation for these two variants.[5] Although the structural study suggests no effect for the other WWP1 variants identified, the available evidence does not unequivocally prove a lack of effect on PTEN through a different (yet unknown) mechanism.

The high heterogeneity and inconsistency of associated phenotypes, absence of disease phenotypes in multiple heterozygotes, relatively high frequency of the most promising variants in the general population, and lack of effect on enhanced PTEN ubiquitination demonstrated by recent comprehensive functional studies, question the causal role of the identified germline *WWP1* variants in PHTS-like and gastrointestinal polyposis predisposition as an alternative to *PTEN* mutations. Nevertheless, because hyperactivation of WWP1 causes the abnormally increased ubiquitination of oncoproteins and tumor suppressors such as PTEN, it is possible that actual *WWP1* activating variants that cause PTEN inactivation — expected to be absent or extremely rare in population controls — cause an increased risk to cancer. If/when those cases are identified, specific preventive measures and cancer treatment options based on the inhibition of WWP1 (supplementary references) might be considered. Until then, the evidence available does not prove a causal association of the germline *WWP1* variants reported to date with the predisposition to PHTS-like or polyposis phenotypes.

## Author contributions

LV and HH conceived the study and interpreted the obtained results. LV supervised the project, analyzed the obtained results, and wrote the manuscript with the assistance of NG-A. NG-A, TF, IQ, MT, and GA performed the experiments, analyzed data, and reviewed the literature. TP performed the computational analyses. JB and GC provided samples and clinical information. All authors critically reviewed the manuscript and approved the submitted and published versions.

## Conflict of interests

HH is on the scientific advisory boards for Genome Medical, Invitae, Promega, and Natera. She is a consultant for GI OnDemand and 23andMe. She has stock/stock options in Genome Medical and GI OnDemand. The other authors declare no conflict of interests.

## Funding

This study was funded by the Spanish Ministry of Science and Innovation (Agencia Estatal de Investigación), co-funded by FEDER funds -a way to build Europe- [PID2020-112595RB-I00 (LV)], Instituto de Salud Carlos III (CIBERONC CB16/12/00234), Government of Catalonia (AGAUR 2021SGR01112, CERCA Program for institutional support), Marie Skłodowska-Curie Individual Fellowship (No. 897064 (NG-A)), and Scientific Foundation "Asociación Española Contra el Cáncer" [AECC Investigador contract (MT)].

## Data availability

Data supporting the reported results may be found in the article and supplementary material.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.03.011.

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17 November 2022
Available online 7 April 2023