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No correlations between genotype and neurodevelopmental or cancer phenotypes in a cohort of PTEN Hamartoma Tumour Syndrome (PHTS) patients

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Abstract

PTEN hamartoma tumour syndrome (PHTS) is a clinically heterogeneous disorder characterised by benign and malignant proliferative lesions, vascular malformations, and neurodevelopmental disorders (NDD). We analysed clinical and molecular features of 167 individuals from 117 unrelated Italian families with PHTS to explore genotype–phenotype correlations, with special focus on neurodevelopmental disorders and cancer incidence. Patients presenting with neurodevelopmental disorders (42.4%), if compared with those without that phenotype (57.6%), had more frequently de novo PTEN variants ($p<0.001$) and were diagnosed at an earlier age (11.9 vs 37.8 years, $p<0.001$), whereas no differences emerged by type of variants and their predicted impact on the PTEN protein. A cancer diagnosis was reported more frequently in females than males (49.2% vs. 14.3%, OR=5.8, 95% CI 2.6-13.0, $p<0.001$), with cumulative incidence for any cancer, by age 60 years, of 71% (95% CI 57–84%) overall, 90% (76–98%) in females and 42% (22–69%) in males. Breast and endometrial cancer were the most frequent cancer types occurring in females (33.3% and 13.3%, respectively), while thyroid cancer showed the highest cumulative incidence in both sexes (34% by 60 years; 95% CI 18–59%), as well as the earliest age at diagnosis among the typical PHTS tumours (25.0 years on average). These findings do not support the hypothesis that neurodevelopmental versus cancer-predominant phenotypes of PHTS are distinct entities associated with different genotypes, which implies that children diagnosed with PHTS because of NDD may be at high cancer risk and should be managed accordingly.

INTRODUCTION

The *PTEN* (phosphatase and tensin homolog) gene encodes a tumour suppressor protein with lipid and protein phosphatase activity, acting primarily as a negative regulator of the PI3K/AKT/mTOR signalling pathway. Through dephosphorylation of phosphatidylinositol-(3,4,5)-trisphosphate, PTEN limits AKT activation and downstream signalling involved in cellular proliferation, metabolism and survival, thereby maintaining cellular homeostasis across multiple tissues[1]. In addition to its canonical role in PI3K signalling, PTEN contributes to genomic stability, DNA damage response and neuronal development, playing a pleiotropic role in both oncogenesis and neurodevelopment[2].

Germline *PTEN* pathogenic variants cause *PTEN* hamartoma tumour syndrome (PHTS), a rare autosomal dominant condition characterised by multisystem overgrowth, hamartomatous lesions and cancer predisposition. Typical clinical manifestations include macrocephaly, mucocutaneous lesions, vascular anomalies and benign hamartomas affecting multiple organs, and a substantially increased lifetime risks of breast, thyroid, endometrial and renal cancers[3-7]. Cancer penetrance is highly variable, even among individuals carrying identical *PTEN* variants, indicating marked interindividual heterogeneity[4, 5].

A significant proportion of individuals with PHTS present with neurodevelopmental disorders (NDD), including developmental delay, intellectual disability, autism spectrum disorder, behavioural abnormalities and epilepsy, frequently associated with extreme macrocephaly[1, 8]. Neurodevelopmental and cancer features may coexist within the same individual or family, highlighting the broad phenotypic

spectrum of PHTS and suggesting the involvement of modifier mechanisms beyond the primary *PTEN* variant[3].

Recent studies have begun to elucidate biological and molecular factors that may contribute to divergent phenotypic outcomes in PHTS. Functional characterisation of *PTEN* variants has demonstrated allele-specific effects on protein stability, subcellular localisation, and pathway activation, with some variants preferentially impairing neuronal development while others more strongly disrupt tumour suppressor functions[9]. Beyond variant-specific effects, differences in mitochondrial DNA copy number and mitochondrial genomic variation have been associated with ASD-predominant versus cancer-predominant phenotypes, implicating altered metabolic capacity and oxidative stress responses as potential modifiers of disease expression[10]. Indeed, metabolomic profiling has revealed distinct metabolic signatures associated with neurodevelopmental versus cancer phenotypes in individuals with germline *PTEN* pathogenic variants, involving pathways related to energy metabolism, lipid biosynthesis and redox homeostasis[11]. Furthermore, quantitative analyses of DNA damage repair dynamics suggest differential engagement of genomic maintenance pathways in individuals with ASD compared with those who develop malignancies, supporting a role for DNA repair efficiency in long-term phenotypic risk[12]. Broader genomic analyses have also identified variation in additional functionally relevant genes involved in neurodevelopment, cell cycle regulation and cancer susceptibility that may modify phenotypic expression in PHTS[13].

Despite these advances, in daily practice such a clinical heterogeneity poses relevant challenges for personalised management strategies. For instance, current guidelines emphasize the need for cancer surveillance in all patients with germline *PTEN* pathogenic variants but provide limited guidance on neurodevelopmental assessment and support, even as the neuropsychiatric burden becomes increasingly recognized[14, 15].

In order to identify any factors helping to predict clinical presentation and evolution, we explored clinical and molecular features, as well as their correlations, in a cohort of individuals diagnosed with PHTS,

MATERIALS AND METHODS

Study design and population

This is a retrospective multicentric study involving individuals with PHTS referred for diagnosis or follow-up to the Medical Genetics Units of the academic hospitals of the University of Bologna, the University “Aldo Moro” of Bari and the Catholic University of Sacred Heart of Rome, all with extensive experience in diagnosing and managing PHTS patients.

All the patients had confirmed germline *PTEN* pathogenic/likely pathogenic variants (according to ClinVar and/or ClinGen criteria[16]), or a clinical diagnosis of PHTS[3] associated with a germline *PTEN* variant of uncertain significance.

A fraction of patients analysed in this work were also included in previous reports and international multicentric series[5, 7, 17-19].

Data collection

A database was set up to collect clinical and molecular data for the study and was shared among the participating centres. Data were extracted from medical records and laboratory reports already available at the participating centres and anonymised, including age of patients (at diagnosis, at the onset of various clinical manifestations and at the last follow-up), whether they were index cases or family members, the presence or absence of typical clinical manifestations associated with PHTS, and the *PTEN* variants detected. For patients diagnosed with cancer, tumour details (e.g. histology) were also collected.

PTEN variants (missense, non-sense, frameshift and in frame deletions) were mapped on "eased" integrative model of human PTEN[20] (PDB ID: 9A91) using ChimeraX v1.9[21].

Statistical Analyses

Categorical variables were summarized as counts and proportions and compared between groups using Fisher's exact test or chi-square test, as appropriate given the expected cell counts. Continuous variables were summarized using medians and compared between groups using the Mann–Whitney U test due to non-normal distribution. Clinical and molecular features were compared between individuals with and without NDD. A sensitivity analysis was conducted on index cases only. Missing data were handled by pairwise exclusion, such that individuals with unavailable information for a given variable were excluded from the corresponding analysis but retained in other comparisons.

Cumulative cancer incidence was estimated using non-parametric time-to-event analyses with age (in years) as the underlying time scale. Censoring occurred at the age at last follow-up for unaffected individuals and, for thyroid, breast and endometrial cancer, at the age of organ-specific prophylactic surgery when applicable. For tumour-specific analyses, the event time corresponded to the reported age at diagnosis. For "any cancer", age was censored at first tumour diagnosis across all tumour types. Breast and endometrial cancer analyses were restricted to female patients. Cumulative incidence was estimated using the Kaplan–Meier method, and 95% confidence intervals were calculated using Greenwood's formula with log–log transformation of the survival function. Sex-stratified analyses were performed for all tumour types, with breast and endometrial cancer analyses restricted to female patients only. Age-specific cumulative incidence estimates were provided for predefined cut-offs of 30, 40, 50, and 60 years. All statistical tests were two-sided, and the significance level was set to $p<0.05$.

Analyses were performed in Python (version 3.11) using pandas, NumPy, and Matplotlib and R statistical software (version 4.x; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Features of the Patients

Main features of the patients included in the cohort are summarized in Table 1. The cohort included 167 individuals from 117 unrelated families: 92 males and 75 females. The mean age at PHTS diagnosis was 21.2 years (range: 0-77 years), while the mean age at last follow-up was 23.0 years (range: 0-78 years). Seventy-five patients (44.9%) were diagnosed with PHTS under 18 years of age, while the remaining 92 (55.1%) were diagnosed in their adulthood. At the last follow-up, 69 patients (41.3%) were still under 18 years of age of, while the other 98 (58.7%) were adults. Information on the presence or absence of each clinical manifestations investigated was available for at least 100 patients, except for the presence of colorectal polyps, which was available for 91 patients.

One hundred and seventeen (70.1%) of the individuals were the index cases of their families, while 50 (29.9%) were relatives tested positive for the variant previously identified in the index case. Age-adjusted frequencies of clinical manifestations were comparable in index cases and in relatives, suggesting that the two groups were homogeneous and could be analysed together (Supplementary Table 1).

***PTEN* variants**

A total of 87 distinct variants were identified among the 117 index cases, and are detailed in Supplementary Tables 2 and 3. Sixty-five of these 87 variants (74.7%) were reported in ClinVar, while the other 22 (25.3%) were not reported in ClinVar and were classified using the ClinGen criteria[16]. Forty-nine variants (56.3%) were predicted truncating (nonsense, frameshift, deletion or splicing variants), while the remaining 38 (43.7%) were missense or in-frame variants. Variants spanned along the entire sequence of the gene and affected several protein domains (Figure 1).

While 71 variants (81.6%) were present in single families, 16 (18.4%) were detected in multiple families, with the most represented being the c.687C>T;p.Arg233* (found in 11 individuals from eight families).

Comparison between patients with and without Neurodevelopmental Disorder

As shown in Table 2, patients with NDD were significantly more likely to have *de novo* variants than those without NDD ($p<0.001$), while no significant differences emerged between the two groups with respect to variant type, predicted protein effect, or involved protein domain (for missense variants).

Patients with NDD were diagnosed with PHTS at a significantly younger age than those not presenting with that phenotype (11.9 vs 37.8 years -$p<0.001$). Macrocephaly was present in all patients with NDD and in 56/59 (94.9%) of those without NDD ($p=1.000$).

Among the 151 individuals with information on the presence or absence of NDD, clinical manifestations such as benign thyroid disease and cancer were significantly more frequent in patients without NDD, if compared with those with NDD. However, after adjusting the OR for age, no significant difference was observed, demonstrating that the apparent association was caused by age differences in the two groups. (Table 3).

Clinical presentation of carriers of the recurrent c.687C>T variant

Among the 11 carriers of the recurrent c.687C>T variant, five of whom were adults and six were under 18 years of age, all presented with macrocephaly. Of those for whom data on the specific manifestation were available, 4/5 (80.0%) presented with typical mucocutaneous manifestations, 5/9 (55.6%) had benign thyroid disease, 6/7 (85.7%) had gastrointestinal polyps, 8/10 (80.0%) had NDD, and 5/8 (62.5%) developed malignant tumours. Of the latter, one patient developed two tumours in childhood (medulloblastoma at 1 year and thyroid cancer at 10 years), one developed breast cancer at 49 years, one developed breast cancer at 36 years, one developed both breast and thyroid cancer at 26 years, and another developed medulloblastoma at 1 year. Overall, four have both cancer and NDD, four have NDD but not cancer and one has cancer but not NDD.

Tumour characteristics

The types of tumours occurred in the cohort are summarised in Table 4. Among neoplasms belonging to the typical PHTS spectrum, breast cancer was reported in 25 out of 75 female patients (33.3%), endometrial cancer in10 out of 75 female patients (13.3%), thyroid cancer in 16 out of 167 patients (9.6%), renal cancer in 6 out of 167 patients (3.6%) and melanoma and colorectal cancer in 4 out of 67 patients each (2.4%). Seven other tumour types occurred in nine patients. Four patients developed tumours during childhood: two developed medulloblastoma at one year of age, one of those also thyroid cancer at age 10 years, other, two developed thyroid cancers at 14 and 16 years.

The frequency of any tumour was significantly higher in females than males (49.2% vs. 14.3%, OR=5.8, 95% CI 2.6-13.0, p<0.001).

Cumulative cancer incidence analyses (Figures 2 and 3 and Supplementary Table 4) revealed a progressive, age-dependent increase in cancer risk in the cohort. By age 60 years, cumulative incidence of any cancer was 71% (95% CI 57-84%) considering all patients, but was significantly higher in females than in males: 42% (95% CI 22-69%) for male patients and 90% (95% CI 76-98%) for female patients. Thyroid cancer showed the highest cumulative incidence in both sexes (34% by age 60 years; 95% CI 18-59%), with risk starting in paediatric age and markedly increasing during young adulthood. In female patients, breast cancer showed the highest cumulative incidence (88% by age 60 years; 95% CI 71-98%). Moreover, females showed a higher cumulative incidence of thyroid cancer than males (log-rank test, p=0.045), while no differences between males and females were found for colorectal cancer (p=0.118), melanoma, (p=0.315) and other tumors (p=0.929).

DISCUSSION

In this study, we describe clinical and molecular features of a large cohort of Italian PHTS patients. In line with the existing knowledge[4, 22], our data confirm that PHTS is characterised by marked clinical heterogeneity, encompassing neurodevelopmental, mucocutaneous and cancer manifestations. The wide range of ages at which the syndrome and its specific manifestations were diagnosed (from 0 to 77

years) supports the concept of PHTS as a lifelong condition with age-dependent penetrance, but no single clinical feature was universally present, reinforcing the notion of PHTS as a spectrum disorder rather than a homogeneous clinical entity[23].

A key objective of this study was to investigate the phenotypic and genetic differences between PHTS patients with and without NDD. The frequency of different clinical manifestations in our cohort suggested a lower prevalence of malignancies and other benign manifestations in patients presenting with NDD. *PTEN* is known to play a central role in neurodevelopment by regulating neuronal growth, migration, and synaptic plasticity, and germline *PTEN* variants are relatively common as the genetic cause of autism spectrum disorder associated with macrocephaly[8, 22, 24]. Experimental models have demonstrated that altered *PTEN* dosage during critical developmental periods results in abnormal neuronal size and circuitry[25]. Therefore, this observation supports the hypothesis that neurodevelopmental disorders in PHTS are the result of early developmental perturbations of *PTEN*-related signalling pathways, which may be partially independent from mechanisms driving tumour predisposition later in life.

Despite an apparent lower frequency of cancer in individuals with NDD in this cohort, age-adjusted analyses demonstrated that these differences were largely attributable to earlier diagnosis and younger age at follow-up in the NDD group. In fact, by adjusting the analyses for age, no significant differences in tumour occurrence or other major clinical manifestations were observed between patients with and without NDD. Furthermore, in some families in our cohort, the index case was diagnosed with NDD while their older relatives had developed cancer and not NDD. These findings are consistent with large cohort studies on *PTEN* germline variant carriers[6], which highlighted strong age-dependent penetrance of cancer risk and substantial interindividual variability even among carriers of identical variants. Together, these data argue against the existence of clearly separable “neurodevelopmental-predominant” and “cancer-predominant” forms of PHTS and instead support a model of pleiotropic *PTEN* dysfunction modulated by developmental timing, tissue-specific vulnerability, and additional genetic or environmental modifiers. From a clinical perspective, these results underscore the importance of age-aware interpretation of phenotypic data in PHTS and caution against using the absence of cancer in young individuals with NDD as evidence of reduced long-term cancer risk.

The tumour spectrum and age-specific cumulative cancer incidence observed in our cohort are consistent with previously published risk estimates for PHTS; breast, endometrial and thyroid cancers are the most frequent malignancies, and females have a markedly higher cumulative cancer risk than males[3-7].

Thyroid cancer also emerged as one of the most frequent and earliest malignancies in our cohort, with cases occurring in paediatric age. Another paediatric tumour in the cohort was medulloblastoma, reported in two cases, both diagnosed at the age of one year. These findings are consistent with several reports in the literature describing paediatric cases of thyroid and central nervous system tumours in PHTS patients[26-29] and reinforce the suggestion to start cancer surveillance at an early age, as

recommended by most international guidelines, although with different age indications. Nevertheless, surveillance recommendations for children are variable in current guidelines[14, 30-32].

Of note, the occurrence of medulloblastoma in two individuals in our series and in other PHTS patients reported in the literature raises the hypothesis that this type of tumour may fall within the syndrome's cancer spectrum. Further studies are required to confirm this association and to identify reliable markers of early tumour risk in children that could inform future management recommendations.

The cumulative incidence patterns for breast and endometrial cancer in women were also broadly aligned with published data, showing a pronounced increase from early adulthood onwards, with multiple cases of endometrial cancer occurred in premenopausal women[5, 33] often resulting in hysterectomy at a young age. This observation supports the importance of early reproductive and preconception counselling in women with PHTS, for discussion of fertility preservation options, including oocyte or embryo cryopreservation. This approach aligns with emerging paradigms in the care of young women at high cancer risk[34-37].

Limitations of this work include the retrospective nature of data collection and variable completeness of clinical information. Although systematic data harmonisation was applied to mitigate these issues, prospective longitudinal studies would be essential to validate and generalise our findings.

In conclusion, our study reinforces the concept of PHTS as a clinically heterogeneous spectrum disorder characterised by variable neurodevelopmental involvement and age-dependent and sex-specific tumour risk. Our findings do not support the hypothesis that distinct neurodevelopmental- versus cancer-predominant phenotypes of PHTS are distinct entities associated with different genotypes, which implies that children diagnosed with PHTS because of NDD may be at high cancer risk as adults and should be managed accordingly. Future work should integrate deep phenotyping with functional genomics and experimental models to clarify the mechanisms underlying phenotypic variability and to refine personalised risk assessment in PHTS.

Declarations

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AUTHOR CONTRIBUTION STATEMENT

Conceptualization: GI, NR, MG, ELC, DT; methodology: GI, GE, LG, ELC, DT; investigation and data curation: all authors; writing—original draft preparation: GI; writing—review and editing: GI, NR, MG, ELC, DT; All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

Individual patient data cannot be shared due to privacy or ethical restrictions. Requests for aggregate study data can be submitted to the corresponding author.

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ETHICAL APPROVAL

The study was conducted in accordance with the Declaration of Helsinki. All individuals had provided written informed consent to the use of their data for research purposes. The study was approved by the Ethical Board of “Area Vasta Emilia Centro” of Emilia-Romagna region (CB-AVEC), Italy (667/2023/Sper/AOUBo)

COMPETING INTERESTS

No authors have any conflict of interest to declare related with the work presented here.

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Tables

Tables are available in the Supplementary Files section.

Figures

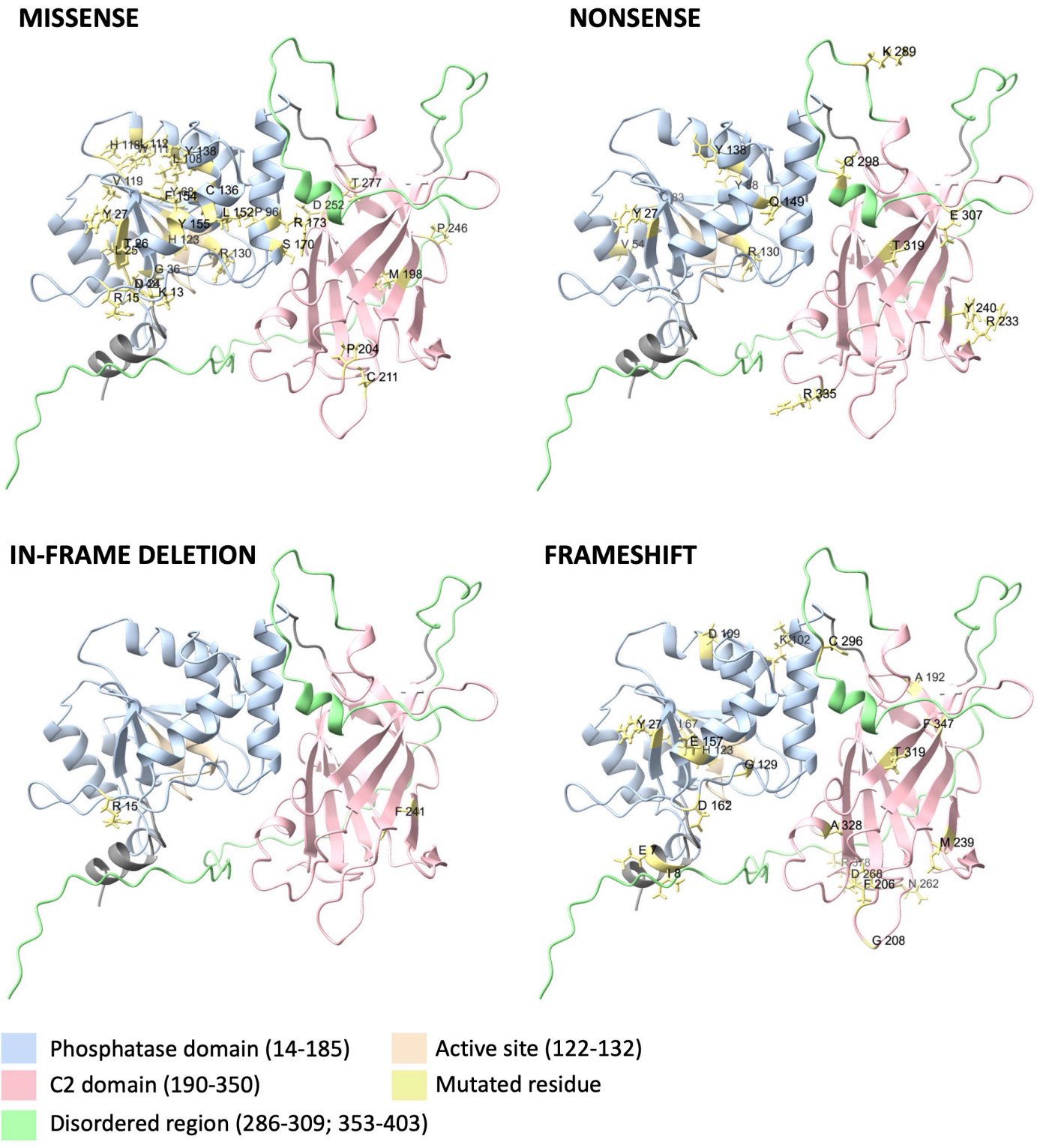


Figure 1

Mapping of *PTEN* variants on 3D protein integrative model (PDB ID 9A91). Protein domains were retrieved from InterPro (P60484) and represented with different colours: phosphatase domain (PD, 14-185) in light steel blue, active site (122-132) in blanched almond, C2 domain (C2 190-350) in pink and disordered regions (286-309 and 353-403) in pale green. Amino acids affected by mutations are indicated in sticks and coloured in khaki. All variants except splicing variants and out-of-frame deletions are represented.

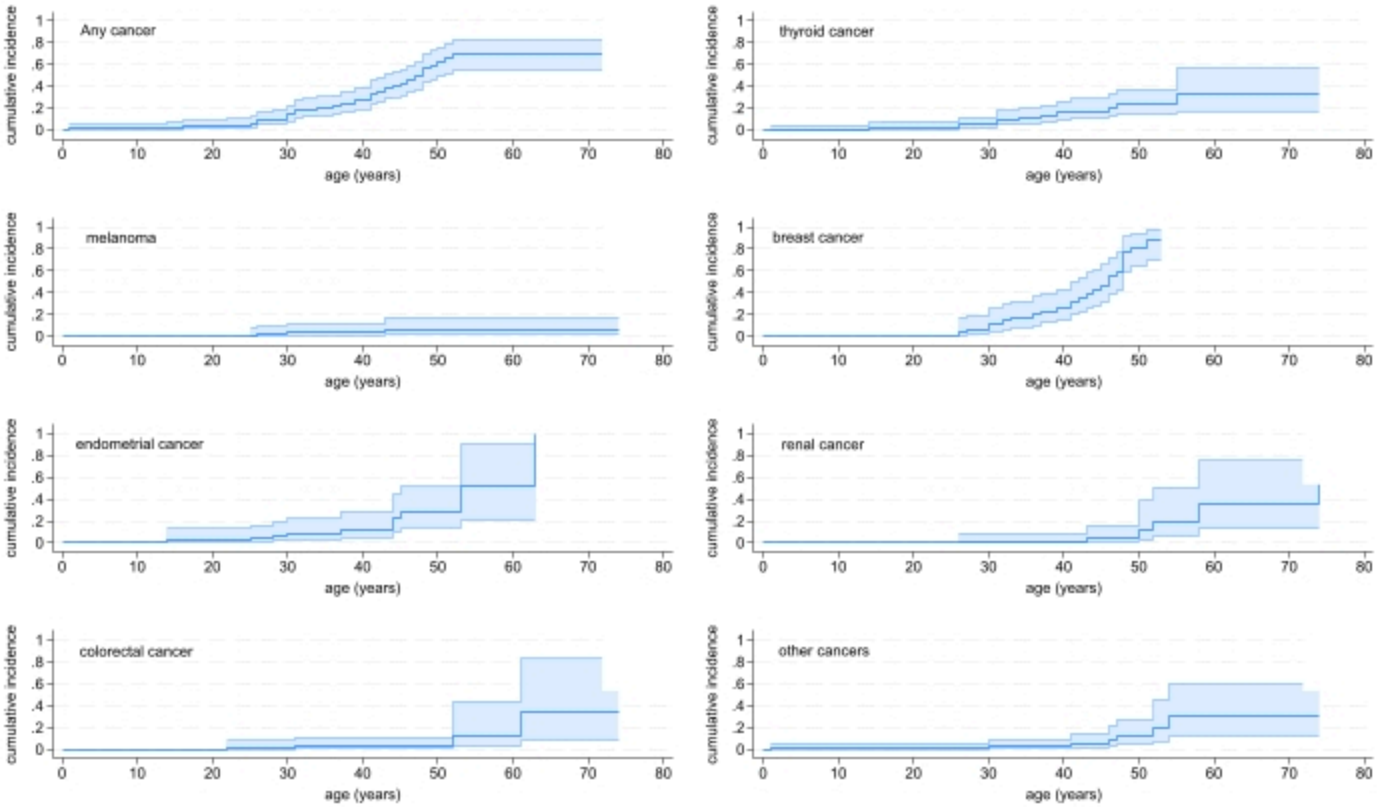


Figure 2

Cumulative incidence of main cancers in the overall PHTS cohort.

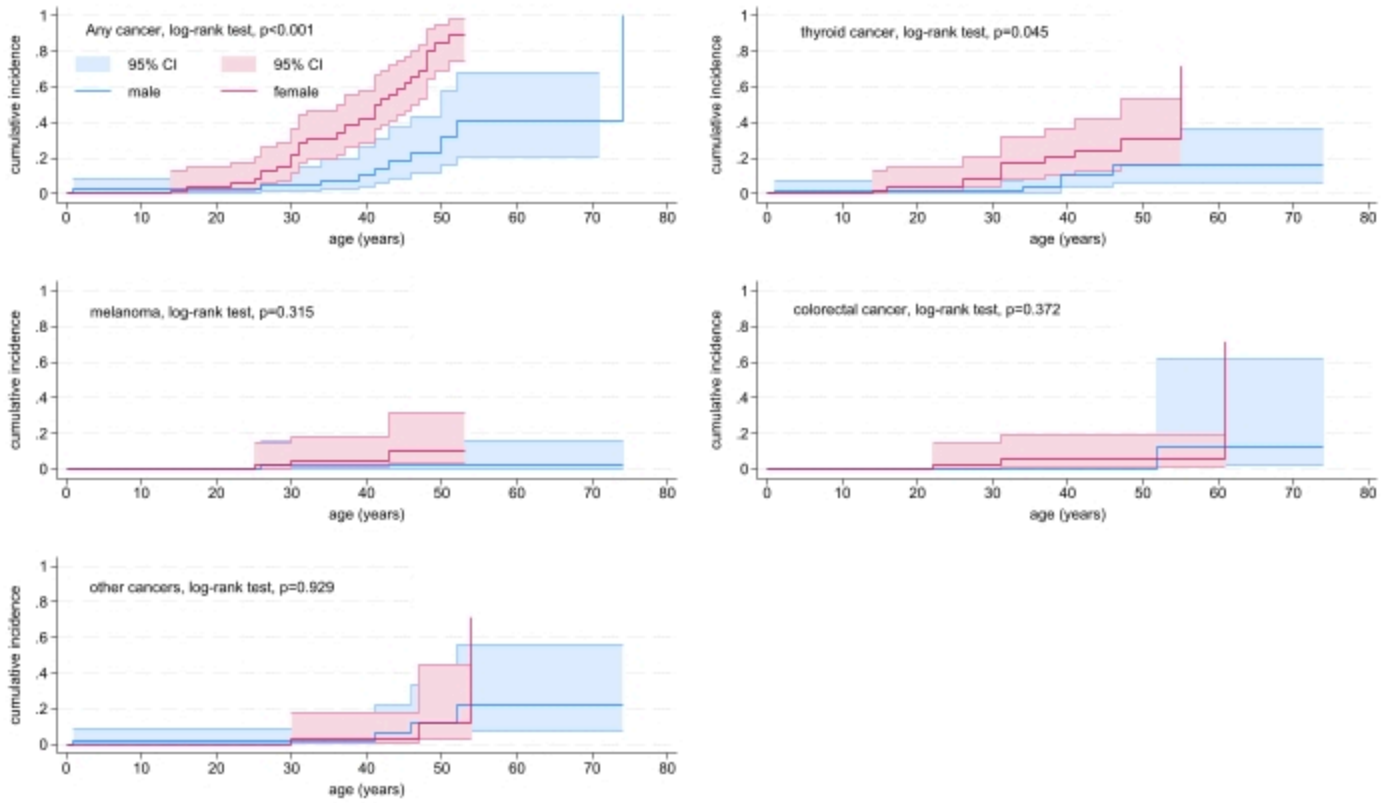


Figure 3

Cumulative incidence of main cancers by sex.

Supplementary Files

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- Table1.docx
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