Loss-of-function variants in POT1 predispose to uveal melanoma

Nathan, Vaishnavi; Palmer, Jane M; Johansson, Peter A; Hamilton, Hayley R; Warrier, Sunil K; Glasson, William; McGrath, Lindsay A; Kahl, Vivian F S; Vasireddy, Raja S; Pickett, Hilda A; Brooks, Kelly M; Pritchard, Antonia L; Hayward, Nicholas K

Published in:
Journal of Medical Genetics

Publication date:
2020

Loss-of-function variants in *POT1* predispose to uveal melanoma

Vaishnavi Nathan[1,2], Jane M. Palmer[1], Peter A. Johansson[1], Hayley R. Hamilton[1], Sunil K. Warrior[3], William Glasson[3], Lindsay A. McGrath[3], Vivian F.S. Kahl[4], Raja S. Vasireddy[5], Hilda A. Pickett[4], Kelly M. Brooks[1], Antonia L. Pritchard[1,6], Nicholas K. Hayward[1*]

*Corresponding author: Nicholas K. Hayward, 300 Herston Rd, Herston, 4006, QLD, Australia
Nicholas.hayward@qimrberghofer.edu.au

[1]Department of Genetics and Computational Biology, QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia
[2]Faculty of Medicine, University of Queensland, Brisbane, QLD, Australia
[3]Queensland Ocular Oncology Service, The Terrace Eye Centre, Brisbane, QLD, Australia
[4]Children's Medical Research Institute, University of Sydney, Westmead, NSW, Australia
[5]Children's Hospital at Westmead, Sydney Children's Hospitals Network, Westmead, NSW, Australia
[6]Division of Biomedical Sciences, University of the Highlands and Islands, Inverness, Scotland, United Kingdom

Summary

Pathogenic germline variants in protection of telomeres 1 (*POT1*) result in a tumour predisposition syndrome (POT1-TPDS), which includes cutaneous melanoma (CM), glioma, chronic lymphocytic leukaemia (CLL), thyroid cancer and sarcoma [1]. Through whole-genome sequencing (WGS) of 20 Australian individuals affected with both CM and uveal melanoma (UM), our study identified two truncating variants in *POT1*. Functional analyses assessing telomere length indicated longer telomeres in variant carriers, compared to healthy age-matched controls, similar to observations in CM patients with loss-of-function *POT1* variants [2].

Word Count (excl. title page, references, figures/tables and required statements): 1217

Body

The risk for development of cancers such as CM and UM is influenced by genetics. In UM, this has mainly been attributed to loss-of-function variants in *BAP1* [3]. Predisposition in CM, is largely due to multiple low-penetrance susceptibility alleles; however, 5-12% of cases report 1st or 2nd degree relatives with CM and in a proportion of these families disease segregates with high-penetrance single gene variants in *CDKN2A*, *CDK4* and the telomere maintenance genes *POT1*, *ACD*, *TERF2IP* and *TERT* (reviewed in [4]). Pathogenic germline variants in *POT1* result in an increase in telomere length and susceptibility to many cancer types including CM, glioma, CLL, thyroid cancer, colorectal cancer, angiosarcoma [1] and osteosarcoma [5], now termed the POT1-TPDS. There is also recent evidence to suggest histological differences in melanomas of *POT1* variant carriers vs non-carriers, highlighting the importance of telomere dysfunction on tumour biology [6]. POT1 binds to telomeric single-stranded DNA (ssDNA) overhangs, preventing telomerase accessibility. Highly conserved oligonucleotide/oligosaccharide-binding (OB) folds in POT1 are essential for specific binding to ssDNA and loss of function of these OB domains leads to increased telomere elongation due to an inability to inhibit telomerase. Certain germline missense variants occurring in these OB domains, and other protein truncating variants, have been reported to disrupt POT1 function, leading to significantly longer telomeres [2]. Here, we extend the POT1-TPDS spectrum to include UM.

All patients (n=20) were recruited in Queensland, Australia and were diagnosed with both CM and UM. Detailed information on personal and family histories of cancer were obtained. Participants were consented for this study under ethics approval granted by the QIMR Berghofer Medical Research Institute Human Research Ethics Committee (HREC; reference

number: HREC/14/QPAH/495). Saliva (kits from DNA Genotek, Canada) or blood samples were used to extract genomic DNA using standard protocols. For the telomere length studies, peripheral blood was collected from healthy individuals through the Department of Hematology Telomere Length Testing Facility, Sydney Children's Hospitals Network. Informed consent was obtained, and the studies were approved by the Sydney Children's Hospitals Network HREC (reference number: HREC/11/SCHN/184). WGS was performed by Macrogen (South Korea) and bioinformatic analyses were performed as previously described [7]. Patients were negative for pathogenic variants in all other known high-penetrance CM and UM susceptibility genes (*CDKN2A*, *CDK4*, *TERT*, *ACD*, *TERF2IP*, *BAP1* and *MBD4*).

Deleterious germline variants in *POT1* (NM_015450) were identified in two patients (Table 1) and were Sanger sequence verified (Supplementary Figure 1). Loss-of-function *POT1* variants in 2/20 patients corresponds to a 2.5% allele frequency, 74 times higher than in the general population (one-sided binomial test, P = 8.7e-5) [8]. Individual MEL1 is heterozygous for variant p.Q94Rfs*12, where an AG deletion causes a frameshift and premature termination at p.106. This deletion occurs in the first OB domain, has been classified by ClinVar (VCV000545902.2) as 'likely pathogenic', has not been reported in the literature and is not present in gnomAD. Individual MEL1 was diagnosed with UM at the age of 57 years, and developed two primary CMs, one at 65 years and the other at 68 years. The second individual, MEL2, is heterozygous for variant p.D617Efs*8, and was diagnosed with UM (age 70) after a CM at age 66. The TA deletion causes a premature termination at p.625, deleting highly conserved C-terminus residues, predicted to affect ACD binding. This variant, although classified of 'uncertain significance' in ClinVar (VCV000209095.2), has been linked to risk of glioma [9] and colorectal cancer [10] components of the POT1-TPDS, and was recently identified in a rare subtype of non-small cell lung cancer, pulmonary sarcomatoid carcinoma

[PSC; [11]]. Although germline assessment was not performed in that study, it is possible that the PSC patient was a germline carrier of the p.D617fs variant. Of note, MEL2 was also diagnosed with a parotid mucinous cystadenoma, another rare epithelial malignancy. No other DNA samples from either family were available to assess *POT1* genotype. All reported cancers in relatives of MEL1 and MEL2 are from family recollections and not all can be confirmed through formal means at the regional cancer registry. While MEL1 reported no known POT1-TPDS cancer types in their family, there is history of other cancers: unconfirmed liver (53; father), unconfirmed ovarian (50) and confirmed pancreatic (80; mother), and unconfirmed genital mucosal lentiginous melanoma (55; sibling). No cancers could be confirmed for relatives of MEL2 who reported: CM (age unknown; paternal uncle), pancreatic cancer (78; father), and Hodgkin's lymphoma (39) and breast cancer (50) in one daughter. Notably, pathogenic POT1 variants have previously been linked to Hodgkin's lymphoma [12].

Table 1: Rare heterozygous frameshift deletions in *POT1* identified in two individuals with both UM and CM. Details of both the frameshift variants in *POT1* (NM_015450) with regard to chromosome position (hg19), nucleotide and amino acid change, and population allele frequency.

Sample ID	Chromosome position	Nucleotide change	Amino acid change	Population frequency (gnomAD)	rsID
MEL1	chr7:124503667	c.281_282del	p.Q94Rfs*12	nil	rs1397398300
MEL2	chr7:124464068	c.1458_1459del	p.D617Efs*8	2.4×10^{-5}	rs758673417

To formally assign pathogenicity to these variants, telomere-lengths of *POT1* variant carriers were assessed by quantitative PCR (qPCR), and calculated as described previously [13]. Healthy individuals (n=240) across a range of age groups (0-88 years of age) were collected through the Sydney Children's Hospitals Network Telomere Length Facility and used as population controls for generating standard curves. Telomere lengths were measured in biological duplicates (blood and saliva samples) for the *POT1* carriers and compared with the control population. MEL1 and MEL2 had longer telomeres than the 95^{th} percentile of their age-matched population (Figure 1). No significant difference was seen between telomere length and sample type (Supplementary Figure 2). These results are consistent with previous observations in pathogenic *POT1* variant carriers [2 9] and we conclude that rs1397398300 and rs758673417 should be classified as pathogenic.

Functional classification of variants of unknown significance in known high penetrance susceptibility genes is essential to determine pathogenicity; this in turn influences clinical practice, notably management of carriers. The POT1-TPDS has similarities to the *BAP1* tumour predisposition syndrome, where carriers of pathogenic *BAP1* variants are predisposed to UM, CM, mesothelioma and renal cell carcinoma [14]. Both syndromes involve increased risk of a spectrum of cancers with differing degrees of penetrance and presentation within families,

thus influencing clinical implications when considering screening, diagnosis and treatment. *POT1* variant carriers have also been associated with developing significantly more Spitzoid melanomas compared to non-carriers [6], similar to features seen in tumours of *BAP1* variant carriers [15]. We saw no such association in our small cohort as MEL1 had lentigo maligna melanomas and MEL2 had an *in situ* melanoma of superficial spreading type. Besides the two UM patients reported here, a pan-cancer study of predisposition genes identified a truncating germline *POT1* variant p.Q87Sfs*4 in a UM patient (diagnosed with UM at 51 years) [16], further supporting that *POT1* loss-of-function variants predisposes to UM. Loss-of-heterozygosity (LOH) of *POT1* was not assessed in MEL1 or MEL2 due to insufficient tumour material. Absence of LOH was reported in the UM p.Q87Sfs*4 variant carrier [16], which accords with observations in other tumour types predisposed by pathogenic *POT1* variants [1 16]. This indicates that *POT1* is not a two-hit tumour suppressor gene, and loss of function of one copy is sufficient for predisposition.

This study demonstrates the complexity of tumour predisposition syndromes and the need for taking thorough and on-going family histories, as well as regular screening for tumour development. Given the broadening spectrum of cancers associated with the POT1-TPDS, screening of families with a range of other tumour types is warranted in order to better characterize the tumours predisposed by germline variants in *POT1*. Defining the POT1-TPDS in detail will allow clinicians and health professionals to recognise high-risk individuals and families, in turn granting patients the ability to make more informed decisions regarding their disease management.

Acknowledgements

We are grateful to the patients and their families who participated in this study. All authors reviewed the manuscript and provided feedback. The study and NKH is funded by the National Health and Medical Research Council (NHMRC; 1093017, 1117663), ALP is funded by Highland Island Enterprise (HMS 9353763), KMB is funded by Cure Cancer Australia. VN is supported by an Australian Government Research Training Program (RTP) Scholarship.

Author contributions

VN designed the research study, prepared samples, conducted experiments, analysed data and prepared the manuscript. JMP recruited patients, consented patients, collected samples, acquired data and prepared the manuscript. PAJ designed the research study, analysed data and prepared the manuscript. HRH acquired data and prepared the manuscript. SKW, WG and LAM recruited patients and prepared the manuscript. VFSK, RSV and HAP conducted experiments, analysed data and prepared the manuscript. KMB prepared samples, supervised the project and prepared the manuscript. ALP designed the research study, supervised the project and prepared the manuscript. NKH provided funding for the study, designed the research study, supervised the project and prepared the manuscript.

***Competing interest statement*:** The authors declare no conflicting or competing interests.

***Data availability statement*:** Details of identified variants are available in Table 1 and Supplementary Figure 1.

Figure legends

Figure 1: *POT1* loss of function variant carriers have longer telomeres than their age-matched population. Telomere lengths of blood samples from MEL1 and MEL2 were measured by qPCR and compared against healthy individuals (n=240). Each measurement was done in technical triplicates. Circles indicate the mean and error bars the standard error of the mean for *POT1* variant carriers Curves represent the 99^{th}, 95^{th}, 50^{th}, 5^{th} and 1^{st} percentiles of telomere lengths obtained from the age-standardised controls.

Supplementary Figure1: Sanger sequence chromatograms of two distinct heterozygous frameshift deletions in *POT1.* Two base-pair deletions were Sanger sequence verified in both MEL1 (p.Q94Rfs) and MEL2 (p.D617Efs).

Supplementary Figure 2: Comparative analysis of saliva and blood samples from *POT1* variant carriers. Telomere lengths of saliva and blood samples from MEL1 and MEL2 measured by qPCR were compared as biological replicates of *POT1* variant carriers. Each measurement represents technical triplicates. Error bars indicate the standard error of the mean. Statistical analyses showed non-significance (denoted n.s.) performed through a two-tailed, unpaired t-test.

References

1. Calvete O, Garcia-Pavia P, Dominguez F, et al. The wide spectrum of POT1 gene variants correlates with multiple cancer types. Eur J Hum Genet 2017;**25**(11):1278-81 doi: 10.1038/ejhg.2017.134[published Online First: Epub Date]|.
2. Robles-Espinoza CD, Harland M, Ramsay AJ, et al. POT1 loss-of-function variants predispose to familial melanoma. Nature genetics 2014;**46**(5):478-81 doi: 10.1038/ng.2947[published Online First: Epub Date]|.
3. Abdel-Rahman MH, Pilarski R, Cebulla CM, et al. Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. Journal of medical genetics 2011;**48**(12):856-9 doi: 10.1136/jmedgenet-2011-100156[published Online First: Epub Date]|.
4. Read J, Wadt KA, Hayward NK. Melanoma genetics. Journal of medical genetics 2016;**53**(1):1-14 doi: 10.1136/jmedgenet-2015-103150[published Online First: Epub Date]|.
5. Mirabello L, Zhu B, Koster R, et al. Frequency of Pathogenic Germline Variants in Cancer-Susceptibility Genes in Patients With Osteosarcoma. JAMA Oncol 2020 doi: 10.1001/jamaoncol.2020.0197[published Online First: Epub Date]|.
6. Sargen MR, Calista D, Elder DE, et al. Histologic features of melanoma associated with germline mutations of CDKN2A, CDK4, and POT1 in melanoma-prone families from the United States, Italy, and Spain. J Am Acad Dermatol 2020 doi: 10.1016/j.jaad.2020.03.100[published Online First: Epub Date]|.
7. Pritchard AL, Johansson PA, Nathan V, et al. Germline mutations in candidate predisposition genes in individuals with cutaneous melanoma and at least two independent additional primary cancers. PloS one 2018;**13**(4):e0194098 doi: 10.1371/journal.pone.0194098[published Online First: Epub Date]|.
8. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 2020;**581**(7809):434-43 doi: 10.1038/s41586-020-2308-7[published Online First: Epub Date]|.
9. Bainbridge MN, Armstrong GN, Gramatges MM, et al. Germline mutations in shelterin complex genes are associated with familial glioma. Journal of the National Cancer Institute 2015;**107**(1):384 doi: 10.1093/jnci/dju384[published Online First: Epub Date]|.
10. Chubb D, Broderick P, Dobbins SE, et al. Rare disruptive mutations and their contribution to the heritable risk of colorectal cancer. Nature communications 2016;**7**:11883 doi: 10.1038/ncomms11883[published Online First: Epub Date]|.
11. Shen E, Xiu J, Bentley R, et al. Frequent Mutations of POT1 Distinguish Pulmonary Sarcomatoid Carcinoma From Other Lung Cancer Histologies. Clin Lung Cancer 2020 doi: 10.1016/j.cllc.2020.04.002[published Online First: Epub Date]|.
12. McMaster ML, Sun C, Landi MT, et al. Germline mutations in Protection of Telomeres 1 in two families with Hodgkin lymphoma. British journal of haematology 2018;**181**(3):372-77 doi: 10.1111/bjh.15203[published Online First: Epub Date]|.
13. Pinese M, Lacaze P, Rath EM, et al. The Medical Genome Reference Bank contains whole genome and phenotype data of 2570 healthy elderly. Nature communications 2020;**11**(1):435 doi: 10.1038/s41467-019-14079-0[published Online First: Epub Date]|.
14. Walpole S, Pritchard AL, Cebulla CM, et al. Comprehensive Study of the Clinical Phenotype of Germline BAP1 Variant-Carrying Families Worldwide. Journal of the National Cancer Institute 2018 doi: 10.1093/jnci/djy171[published Online First: Epub Date]|.
15. Wiesner T, Obenauf AC, Murali R, et al. Germline mutations in BAP1 predispose to melanocytic tumors. Nature genetics 2011;**43**(10):1018-21 doi: 10.1038/ng.910[published Online First: Epub Date]|.
16. Huang KL, Mashl RJ, Wu Y, et al. Pathogenic Germline Variants in 10,389 Adult Cancers. Cell 2018;**173**(2):355-70 e14 doi: 10.1016/j.cell.2018.03.039[published Online First: Epub Date]|.