

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

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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), colorectal cancer, 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 with healthy age-matched controls, similar to observations in CM patients with loss-of-function *POT1* variants.²

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 first-degree or second-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 ref 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, angiosarcoma¹ and osteosarcoma,⁵ now termed the POT1-TPDS. There is also recent evidence to suggest histological differences in melanomas of *POT1* variant carriers versus 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. 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 (online 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 years) after a CM at age 66 years. 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 glioma⁹ and colorectal cancer¹⁰ components of the POT1-TPDS and was recently identified in a rare subtype of non-small cell lung cancer, pulmonary sarcomatoid carcinoma (PSC¹¹). Although germline assessment was not performed in that study, it is possible that the PSC patient was

Table 1 Rare heterozygous frameshift deletions in *POT1* identified in two individuals with both UM and CM

Sample ID	Chromosome position	Nucleotide change	Amino acid change	Population frequency (gnomAD)	rsID
MEL1	chr7:124 503 667	c.281_282del	p.Q94Rfs*12	nil	rs1397398300
MEL2	chr7:124 464 068	c.1458_1459del	p.D617Efs*8	2.4×10 ⁻⁵	rs758673417

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. CM, cutaneous melanoma; *POT1*, protection of telomeres 1; UM, uveal melanoma.

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

To formally assign pathogenicity to these variants, telomere lengths of *POT1* variant carriers were assessed by quantitative PCR 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 there was no significant difference seen between sample types (online supplementary figure 2). MEL1

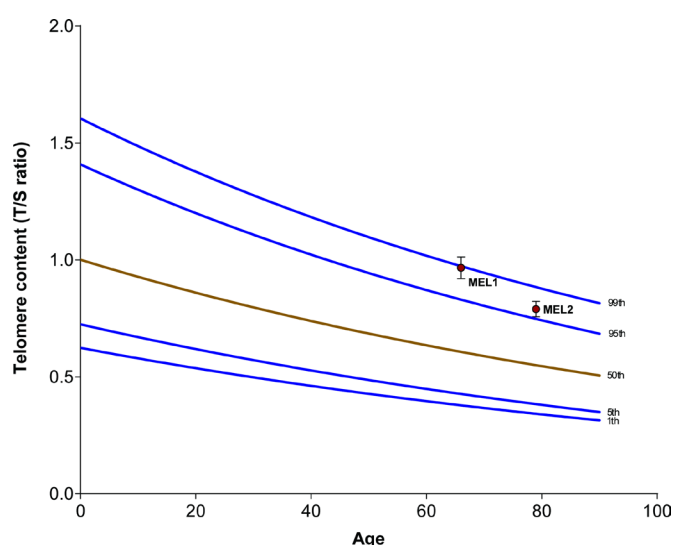


Figure 1 *POT1* loss-of-function variant carriers have longer telomeres than their age-matched population. Telomere lengths of blood samples from MEL1 and MEL2 were measured by quantitative PCR and compared against healthy individuals (n=240). Each measurement was done in technical triplicates. Circles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

and MEL2 had longer telomeres than the 95th percentile of their age-matched population (figure 1), which is consistent with previous observations in pathogenic *POT1* variant carriers.^{2,9} 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 with 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 characterise 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.

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Contributors 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.

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