A rare missense variant in protection of telomeres 1 (POT1) predisposes to a range of haematological malignancies

Nathan, Vaishnavi; Johansson, Peter A.; Palmer, Jane M.; Hamilton, Hayley R.; Howlie, Madeleine; Brooks, Kelly M.; Hayward, Nicholas K.; Pritchard, Antonia L.

Published in:
British Journal of Haematology

Publication date:
2021

Publisher rights:
Free access

The Document Version you have downloaded here is:
Publisher's PDF, also known as Version of record

The final published version is available direct from the publisher website at:
10.1111/bjh.17218

8. Jager U, Barcellini W, Broome CM, Gertz MA, Hill A, Hill QA, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: Recommendations from the First International Consensus Meeting. *Blood Rev*. 2020;**41**:100648.
9. Garrison LP Jr, Neumann PJ, Erickson P, Marshall D, Mullins CD. Using real-world data for coverage and payment decisions: the ISPOR Real-World Data Task Force report. *Value Health*. 2007;**10**(5):326–35.
10. Hill A, Hill QA. Autoimmune hemolytic anemia. *Hematology Am Soc Hematol Educ Program*. 2018;**2018**(1):382–9.
11. Yndigegn T, Hofmann R, Jernberg T, Gale CP. Registry-based randomised clinical trial: efficient evaluation of generic pharmacotherapies in the contemporary era. *Heart*. 2018;**104**(19):1562–7.
12. Hoque DME, Kumari V, Hoque M, Ruseckaite R, Romero L, Evans SM. Impact of clinical registries on quality of patient care and clinical outcomes: a systematic review. *PLoS One*. 2017;**12**(9):e0183667.

A rare missense variant in protection of telomeres 1 (*POT1*) predisposes to a range of haematological malignancies

Protection of telomeres 1 (POT1) is a component of the shelterin complex of six subunits encoded by adrenocortical dysplasia protein homologue (*ACD*; also known as TPP1), *POT1*, telomeric repeat binding factor 1 (*TERF1*), telomeric repeat binding factor 2 (*TERF2*), TERF1-interacting nuclear factor 2 (*TINF2*) and TERF2 interacting protein (*TERF2IP*), which binds to single-stranded telomeric DNA to regulate telomere elongation and integrity.[1] The telomerase and shelterin complexes play specific roles in telomere maintenance and prevention of activation of DNA damage response pathways at telomeres, by protecting single-stranded DNA (ssDNA) overhangs.[1] Conserved oligonucleotide/oligosaccharide-binding (OB) domains in *POT1* recognise specific ssDNA motifs with high affinity and are required for *POT1* function.[1] Acquired and inherited variants in *POT1* that alter these OB folds are associated with longer telomeres due to disruption of shelterin function.[2] Rare germline pathogenic variants in *POT1* predispose to chronic leucocyte leukaemia (CLL), glioma, angiosarcoma, osteosarcoma, thyroid cancer, colorectal cancer and cutaneous melanoma (CM), collectively termed the POT1-tumour predisposition syndrome (POT1-TPDS; Fig 1).

Here we describe an Australian family (Family 1, Fig 2) with a germline heterozygous *POT1* missense variant previously associated with susceptibility to CM[2] and Hodgkin lymphoma (HL).[3] Variant carriers in this family presented with tumour subtypes not yet associated with the POT1-TPDS: non-Hodgkin lymphoma (NHL) and chronic myeloid leukaemia (CML).

This family was recruited from the Queensland Familial Melanoma Project (QFMP), and patients consented under ethics approval granted by the QIMR Berghofer Medical Research Institute Human Research Ethics Committee. Proband II:1 was the only individual chosen for whole-genome sequencing (WGS) due to their extensive personal cancer history (MCC_AUS24[4]) to assess the burden of cancer risk alleles among individuals with multiple primary cancers. The WGS was performed by Macrogen (Korea) on the Illumina Hiseq 2000 platform, and data were aligned to reference genome hg19. Single nucleotide variants (SNVs) were detected using 'bcftools' and 'SAMtools',[5] insertion and deletions were detected with 'pindel'[6] and all variants were annotated using ANNOtate VARiation (ANNOVAR).[7] Personal and family cancer history were ascertained by questionnaire, and consented individuals were further followed-up through the Queensland Cancer Registry and clinical/pathology reports. Since our initial report on the proband,[4] extensive follow-up information and co-segregation data were obtained from additional family members. Proband II:1 was negative for known high-penetrance CM susceptibility genes cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and cyclin-dependent kinase 4 (*CDK4*), and only rare variants (population allele frequency <0·0005) were selected for further analysis to identify high-penetrance variants of strong effect.

A known pathogenic variant in *POT1*,[2,3] p.D224N (transcript NM_015450), was found in proband II:1. Sanger sequence verification and co-segregation analyses were carried out for all family members with available germline DNA samples (Fig 2). Individual II:1 was diagnosed with CM (aged 42 years) followed by follicular lymphoma and renal clear cell carcinoma (aged 58 years), colorectal cancer (aged 63 years) and prostate cancer (aged 64 years). As no DNA was available for father I:1, diagnosed with CM (aged 62 and 70 years), HL (aged 63 years) and CML (aged 76 years), obligate carrier status was obtained by genotyping unaffected and wild-type mother I:2. Siblings of II:1, individuals II:3 and II:4, were carriers, while unaffected sibling II:2 was wild-type. Individual II:3 was diagnosed with CML at the age of 48 years, and a lentigo maligna melanoma (LMM) at age 63 years (Fig 2).

The p.D224N variant is at a highly conserved residue in the OB2 domain of *POT1*.[2] Germline variants in these conserved OB domains predispose to various cancers (Fig 1). *In vitro* experimental assays have shown that p.D224N disrupts *POT1* binding to ssDNA telomere oligonucleotides, which leads to longer, fragile telomeres.[3]

Several cancer types have been associated with the POT1-TPDS and here we propose the addition of other haematological malignancies, namely NHL, follicular lymphoma and CML. Genetic predisposition to haematological malignancies

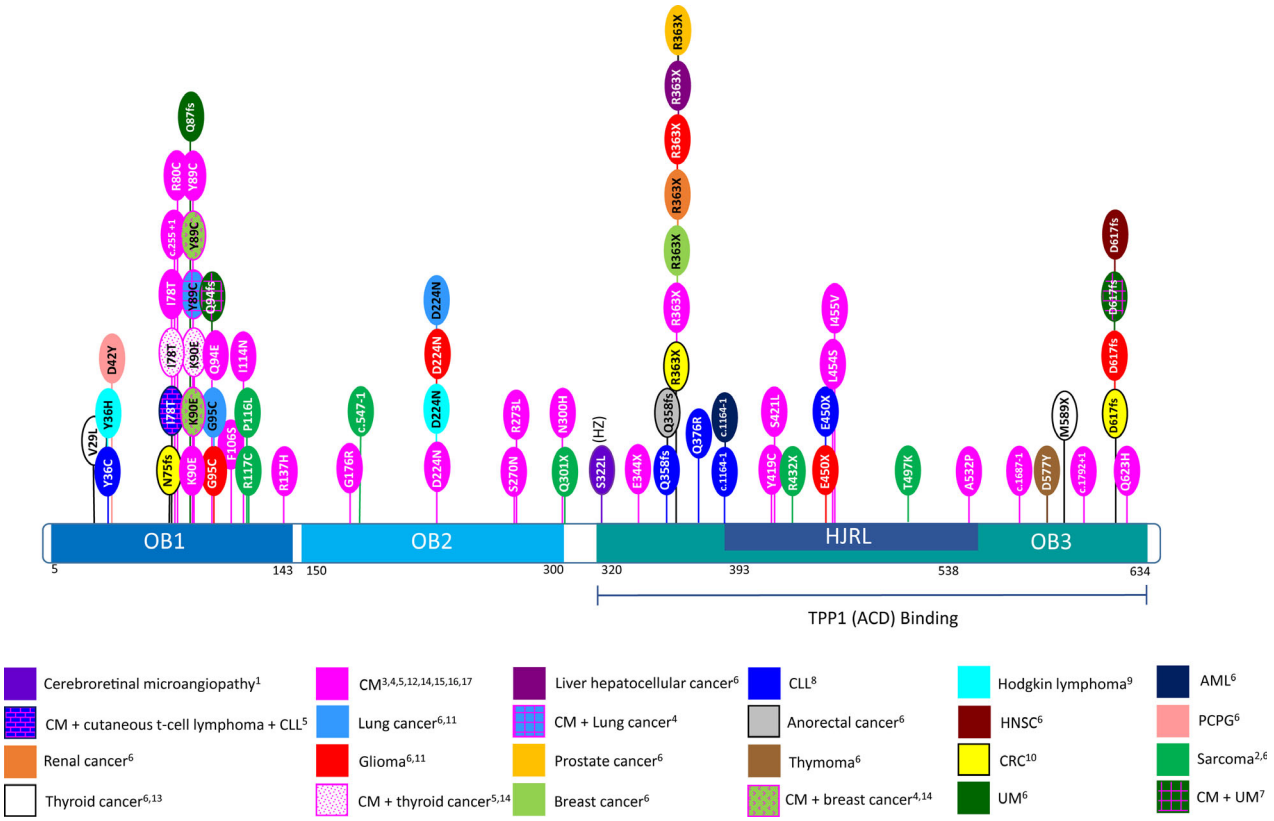

Fig 1. Germline variants in *POT1* predispose to various tumour types. Germline variants in *POT1* that predispose to various types of cancers have been identified across the entire protein domain. The POT1 protein structure is comprised of three oligosaccharide/oligonucleotide (OB) folds and a Holliday junction resolvase-like (HJRL) domain that binds to TPP1 (encoded by *ACD*) embedded within the third OB fold. This lollipop plot shows the position of these variants across the POT1 protein domains, the broad spectrum of cancers associated with the POT1-TPDS, and the overlap of certain variants between cancer types. All rare *POT1* variants (variant allele frequency <0·0005) observed to date in various types of malignancy are shown in this figure, regardless of whether there is sufficient burden of evidence to indicate pathogenicity. All variants are heterozygous except for homozygous (HZ) p.S322L, and depict amino acid changes, except for splice variants c.255+1, c.547−1, c.1164−1 and c.1687−1. Each colour represents a different cancer type, and patterned boxes depict variants in carriers with more than one primary cancer. CM, cutaneous melanoma; UM, uveal melanoma; CRC, colorectal cancer; AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; PCPG, phaeochromocytoma and paraganglioma; HNSC, head and neck squamous cell carcinoma. Variant information sourced from references listed in Data S1. [Colour figure can be viewed at wileyonlinelibrary.com]

is rare, but strong familial association for NHL and follicular lymphoma have been described[8]; however, a familial component to CML is less evident.[9] Additionally, cutaneous T-cell lymphoma, CLL and CM have been observed in two individuals carrying a pathogenic *POT1* variant and a pathogenic splice variant seen in an individual with CLL has also been reported in an individual with acute myeloid leukaemia (AML) (Fig 1). Further support for a role of *POT1* in these haematological malignancies comes from somatic changes. Replicative immortality is a key somatic hallmark of cancer, with telomere dysfunction being the main mechanism by which this is achieved. In leukaemia and lymphoma, one way this is accomplished is through disruption of *POT1*, with loss of function variants/deep (i.e. homozygous) deletion of the locus being present in 6·5% of CLL, 4·7% of T-cell lymphoma and 2% of AML [The Cancer Genome Atlas (TCGA) cBioPortal]. In CLL, *POT1* mutation is associated with the presence of a non-mutated immunoglobulin heavy chain (IgHV) and therefore poorer prognosis.[10] The function of *POT1* in CLL has further been associated with the p53/ataxia-telangiectasia mutated (ATM) axis, whereby the genes encoding these proteins are mutated mutually exclusively (TCGA cBioPortal). This exclusivity indicates converging roles of these gene aberrations in CLL. Data from fibroblasts show that POT1 inactivation results in telomere elongation and aberrant homologous recombination via ATM and p53-dependent replicative senescence.[11] Loss-of-heterozygosity is not typically seen in *POT1* carriers with CLL[10,12] or in other tumour types; mutant *POT1* is therefore considered to act in a dominant-negative manner.[13] Unfortunately, no tumour material was available from the carriers of the *POT1* p.D224N mutation in Family 1, so investigation of the genomic changes in the haematological malignancies present was not possible.

In light of these data, we propose that the high incidence of cancers in this family, particularly the increased

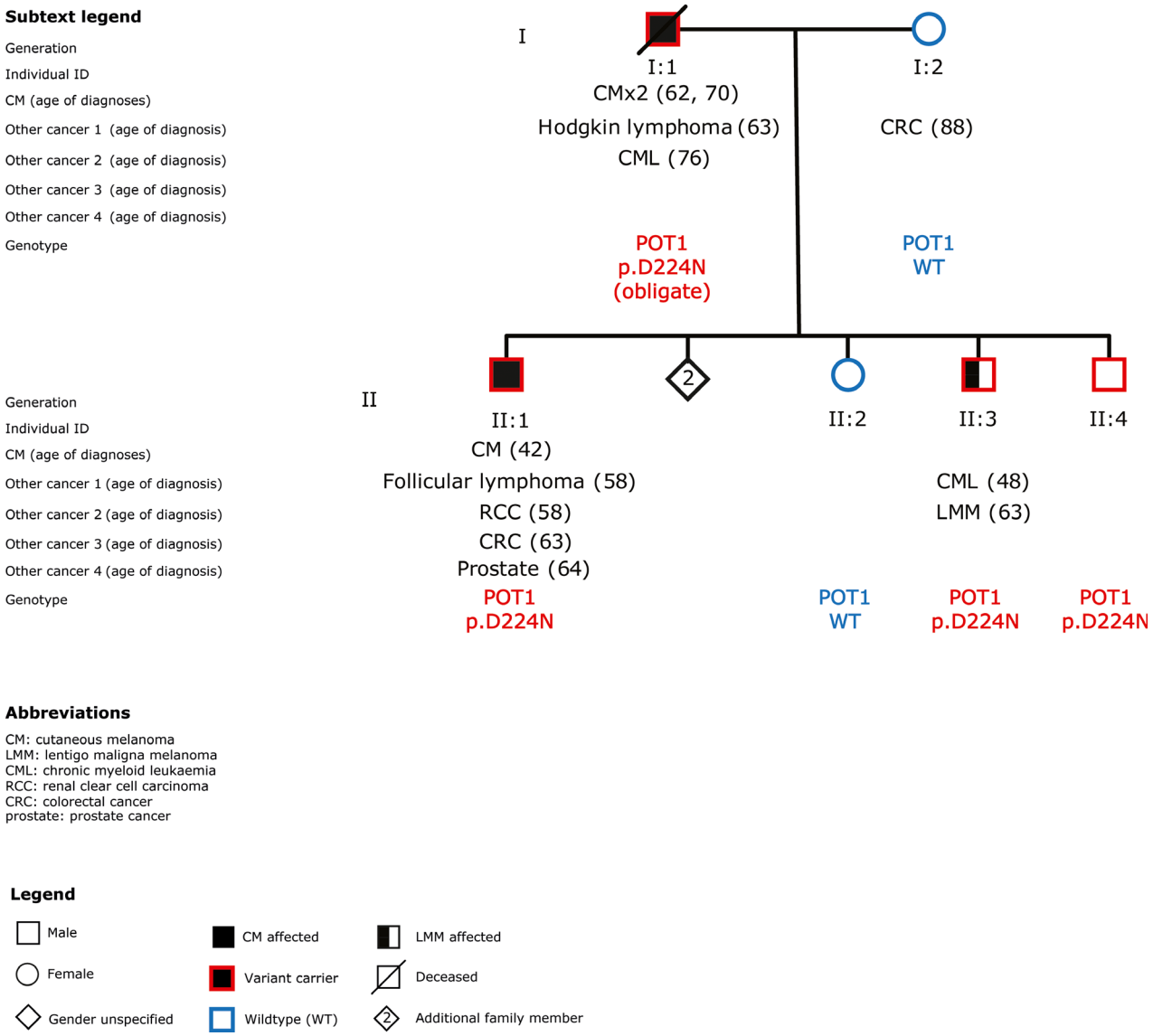

Fig 2. Co-segregation of rare *POT1* missense variant p.D224N is present in a family with melanoma and haematological malignancies. *POT1* missense variant p.D224N is present in individuals with both melanoma and at least one type of haematological malignancy. Proband II:1 was diagnosed with follicular lymphoma, renal cell carcinoma (RCC), colorectal cancer (CRC), prostate cancer and cutaneous melanoma (CM). Obligate carrier I:1 was diagnosed with two CMs, Hodgkin lymphoma and chronic myeloid leukaemia (CML). Brother II:3 was diagnosed with a lentigo maligna melanoma (LMM) and CML. [Colour figure can be viewed at wileyonlinelibrary.com]

susceptibility of various haematological malignancies, is attributed to the inheritance of the pathogenic *POT1* p.D224N variant. This family strengthens the link between predisposition to haematological malignancies and germline *POT1* mutation. The discovery of overlapping cancers between different tumour predisposition syndromes is also of clinical note, particularly when taking family histories and deciding on genes to include in screening. For example, three TP53-negative Li–Fraumeni-like families with cardiac angiosarcoma[14] and two individuals with uveal melanoma[15] who were negative for BRCA1 associated protein-1 (BAP1)-TPDS mutations have been identified with *POT1* germline mutations. Additional knowledge regarding age-of-onset and the expanding tumour spectrum in *POT1*-mutation carriers is required for improved risk management of these patients.

Acknowledgements

We are grateful to the patients and their families who participated in this study.

All authors wrote and reviewed the manuscript; Vaishnavi Nathan performed the laboratory work; Peter A. Johansson performed the bioinformatics; Jane M. Palmer, Hayley R. Hamilton and Madeleine Howlie recruited participants, collected samples and performed participant follow-up; Vaishnavi

Nathan, Kelly M. Brooks, Antonia L. Pritchard and Nicholas K. Hayward designed the research study and analysed the data.

The study and Nicholas K. Hayward is funded by the National Health and Medical Research Council (NHMRC; 1093017, 1117663), Antonia L. Pritchard is funded by Highlands and Islands Enterprise (HMS 9353763), Kelly M. Brooks is funded by Cure Cancer Australia, Vaishnavi Nathan is supported by an Australian Government Research Training Programme (RTP) Scholarship.

The participants were recruited under ethics approval granted by the QIMR Berghofer Medical Research Institute Human Research Ethics Committee (HREC reference number: HREC/14/QPAH/495).

Conflict of interest

The authors have no competing interests.

Vaishnavi Nathan[1,2]
Peter A. Johansson[1]
Jane M. Palmer[1]
Hayley R. Hamilton[1]
Madeleine Howlie[1]
Kelly M. Brooks[1]
Nicholas K. Hayward[1]
Antonia L. Pritchard[1,3]

[1]QIMR Berghofer Medical Research Institute, Herston [2]University of Queensland, Herston, Queensland, Australia and [3]University of Highlands and Islands, Inverness, Scotland.
E-mail: vaishnavi.nathan@qimrberghofer.edu.au

First published online 20 November 2020
doi: 10.1111/bjh.17218

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Supplementary material.

References

1. de Lange T. Shelterin-mediated telomere protection. *Annu Rev Genet*. 2018;**52**:223–47.
2. Shi J, Yang XR, Ballew B, Rotunno M, Calista D, Fargnoli MC, *et al*. Rare missense variants in POT1 predispose to familial cutaneous malignant melanoma. *Nat Genet*. 2014;**46**:482–6.
3. McMaster ML, Sun C, Landi MT, Savage SA, Rotunno M, Yang XR, *et al*. Germline mutations in Protection of Telomeres 1 in two families with Hodgkin lymphoma. *Br J Haematol*. 2018;**181**:372–7.
4. Pritchard AL, Johansson PA, Nathan V, Howlie M, Symmons J, Palmer JM, *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**:e0194098.
5. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, *et al*. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;**25**:2078–9.
6. Ye K, Schulz MH, Long Q, Apweiler R, Ning Z. Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. *Bioinformatics*. 2009;**25**:2865–71.
7. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;**38**:e164.
8. Altieri A, Bermejo JL, Hemminki K. Familial aggregation of lymphoplasmacytic lymphoma with non-Hodgkin lymphoma and other neoplasms. *Leukemia*. 2005;**19**:2342–3.
9. Gunnarsson N, Hoglund M, Stenke L, Sandin F, Bjorkholm M, Dreimane A, *et al*. No increased prevalence of malignancies among first-degree relatives of 800 patients with chronic myeloid leukemia: a population-based study in Sweden. *Leukemia*. 2017;**31**:1825–7.
10. Ramsay AJ, Quesada V, Foronda M, Conde L, Martinez-Trillos A, Villamor N, *et al*. POT1 mutations cause telomere dysfunction in chronic lymphocytic leukemia. *Nat Genet*. 2013;**45**:526–30.
11. Wu CC, Shete S, Amos CI, Strong LC. Joint effects of germ-line p53 mutation and sex on cancer risk in Li-Fraumeni syndrome. *Cancer Res*. 2006;**66**:8287–92.
12. Speedy HE, Kinnersley B, Chubb D, Broderick P, Law PJ, Litchfield K, *et al*. Germ line mutations in shelterin complex genes are associated with familial chronic lymphocytic leukemia. *Blood*. 2016;**128**:2319–26.
13. Kendellen MF, Barrientos KS, Counter CM. POT1 association with TRF2 regulates telomere length. *Mol Cell Biol*. 2009;**29**:5611–9.
14. Calvete O, Martinez P, Garcia-Pavia P, Benitez-Buelga C, Paumard-Hernandez B, Fernandez V, *et al*. A mutation in the POT1 gene is responsible for cardiac angiosarcoma in TP53-negative Li-Fraumeni-like families. *Nat Commun*. 2015;**6**:8383.
15. Nathan V, Palmer JM, Johansson PA, Hamilton HR, Warrier SK, Glasson W, *et al*. Loss-of-function variants in *POT1* predispose to uveal melanoma. *J Med Genet*. 2020 [Online ahead of print]. jmedgenet-2020-107098

Treatment of refractory acute myeloid leukaemia during pregnancy with venetoclax, high-dose cytarabine and mitoxantrone

Treatment of acute myeloid leukaemia (AML) during pregnancy remains a challenge. Most frequently, a regimen consisting of cytarabine and daunorubicin is employed (7 + 3) which has a reasonable safety profile during pregnancy.[1] In the present report, we describe a pregnant patient refractory to standard induction chemotherapy.

A 34-year-old gravida 2, para 1 patient was admitted with AML, in the 21st week of pregnancy. Initial bone marrow