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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

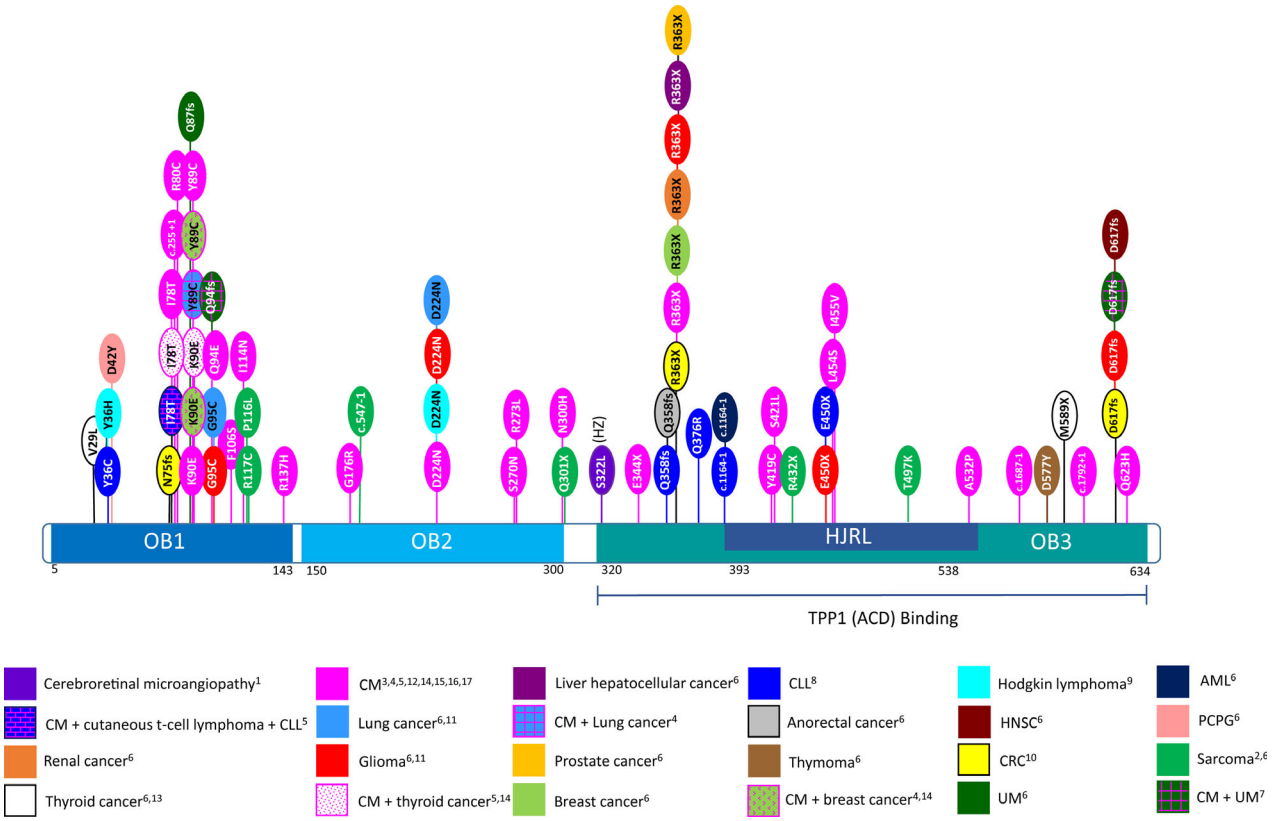

Fig 1. Germline variants in *POT1* predispose to various tumour types. Germline variants in *POT1* that predispose to various types of cancers have been identified across the entire protein domain. The POT1 protein structure is comprised of three oligosaccharide/oligonucleotide (OB) folds and a Holliday 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

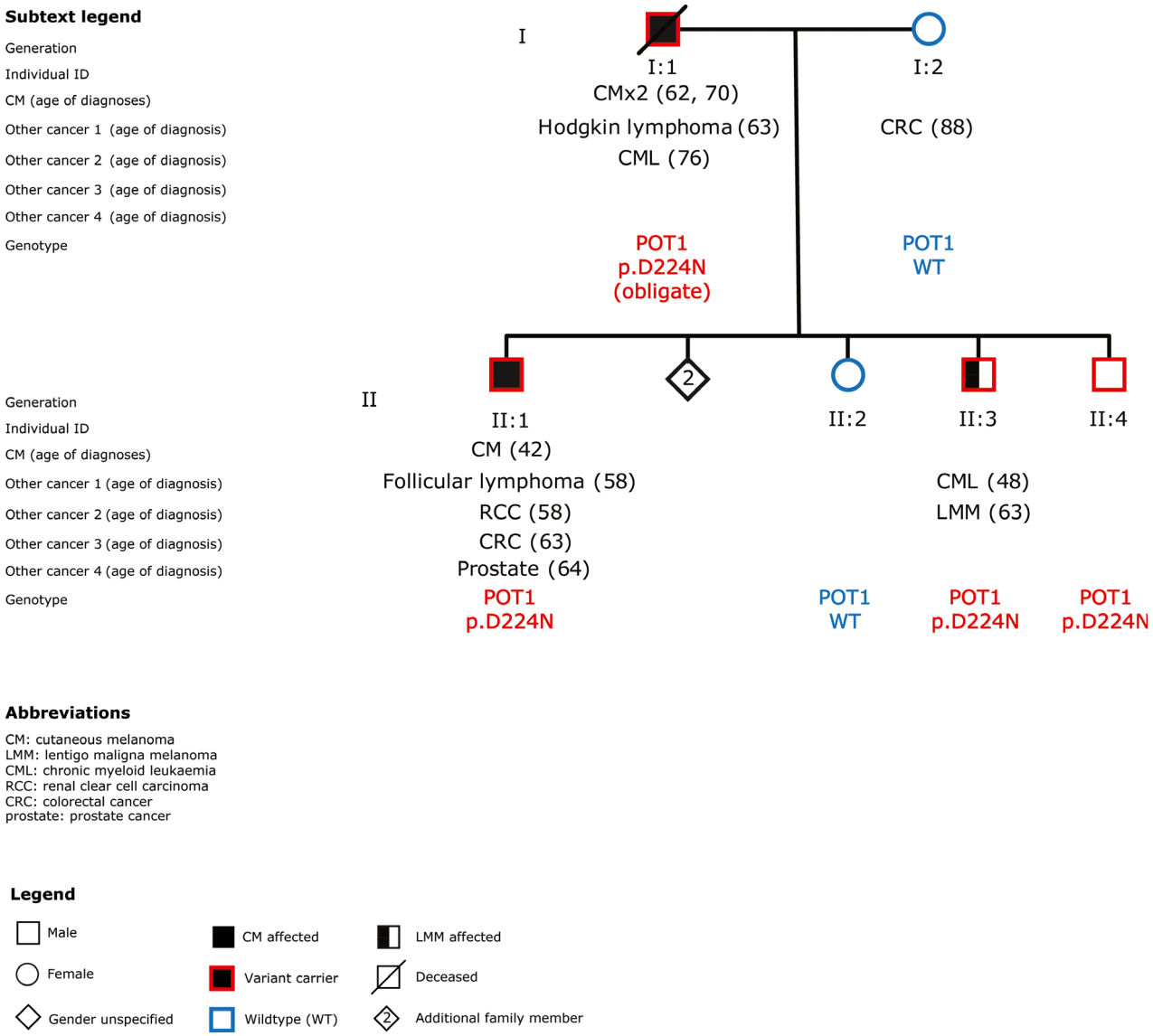

Fig 2. Co-segregation of rare *POT1* missense variant p.D224N is present in a family with melanoma and haematological malignancies. *POT1* missense variant p.D224N is present in individuals with both melanoma and at least one type of haematological malignancy. Proband II:1 was diagnosed with follicular 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.

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

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Conflict of interest

The authors have no competing interests.

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Supporting Information

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

Data S1. Supplementary material.

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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