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ORIGINAL ARTICLE

POLE mutations in families predisposed to cutaneous melanoma

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Abstract Germline mutations in the exonuclease domain of POLE have been shown to predispose to colorectal cancers and adenomas. POLE is an enzyme involved in DNA repair and chromosomal DNA replication. In order to assess whether such mutations might also predispose to cutaneous melanoma, we interrogated whole-genome and exome data from probands of 34 melanoma families lacking pathogenic mutations in known high penetrance melanoma susceptibility genes: *CDKN2A*, *CDK4*, *BAP1*, *TERT*, *POT1*, *ACD* and *TERF2IP*. We found a novel germline mutation, POLE p.(Trp347Cys), in a 7-case cutaneous melanoma family. Functional assays in *S. pombe* showed that this mutation led to an increased DNA mutation rate comparable to that seen with a Pol ε mutant with no exonuclease activity. We then performed targeted sequencing of *POLE* in 1243 cutaneous melanoma cases and found that a further ten probands had novel or rare variants in the exonuclease domain of POLE. Although this frequency is not significantly higher than that in unselected Caucasian controls, we observed multiple cancer types in the melanoma families, suggesting that some germline *POLE* mutations may predispose to a broad spectrum of cancers, including melanoma. In addition, we found the first mutation outside the exonuclease domain, p.(Gln520Arg), in a family with an extensive history of colorectal cancer.

Keywords Cutaneous melanoma · POLE · Germline mutation

Introduction

Familial melanoma represents approximately 5–10 % of all cutaneous malignant melanoma (CMM) cases reported [1–7]. Susceptibility in approximately half of families is attributable to known high penetrance genes. As yet, the cause of increased risk of CMM development in the remaining families is largely unknown but could be due to a combination of low or medium penetrance mutations (often detected through genome-wide association studies) or due to rare as yet undiscovered high penetrance mutations. To address the latter possibility, we have used next-generation sequencing to study high-density CMM families to uncover novel high-penetrance melanoma predisposition genes. This has led to the identification of rare mutations in *POT1*, *ACD* and *TERF2IP* [8, 9], but the cause of predisposition in the bulk of CMM families is as yet undetermined. In some families there are plausible candidate

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mutations, which require functional assessment to establish their involvement in CMM predisposition. In one such family we identified a novel (i.e. unreported in publically available databases) mutation in the exonuclease domain of the catalytic subunit of DNA directed polymerase ε (POLE). POLE is an enzyme involved in DNA repair and chromosomal DNA replication. Given that such mutations are associated with colon cancer susceptibility [10], another cancer type with a high environmental mutation burden, we felt that *POLE* was also a good candidate CMM predisposition gene. The aim of this study was to determine the role of *POLE* mutations in CMM predisposition.

Materials and methods

Ethics

Ethics approval was obtained from the QIMR Berghofer Human Research Ethics Committee and the Committee of Biomedical Research Ethics of the Capital Region of Denmark. Written consent was obtained from each participant in this study.

Samples

Australian samples were ascertained as part of the Q-MEGA project [11] which is a population-based study from Queensland investigating the link between genetics and environment in melanoma development. Q-MEGA consists of four study samples: the Queensland Study of Childhood Melanoma (N = 101), the study of Melanoma in Adolescents (N = 298), the Study of Men over 50 (N = 178) and the Queensland Familial Melanoma Project (QFMP; N = 1897) [12]. The Childhood study included children diagnosed with CMM under the age of 15 between 1987 and 1994 while the adolescent study consisted of cases diagnosed between the age of 15 and 19 in the same period [13–15]. In the study of men over 50, individuals were diagnosed with CMM between July 1993 and June 1994 and were at least 50 years old at diagnosis [16]. In the QFMP study, individuals that presented to the Queensland Cancer Registry with histologically confirmed CMM between the years 1982 and 1990 were approached. Detailed information on personal and family cancer history was ascertained and where an individual had relatives with CMM, these additional family members were also asked to participate. Danish cutaneous melanoma cases were ascertained through the Danish Project of Hereditary Malignant Melanoma. For each participant, genomic DNA was extracted from whole blood using standard salting out methods [17]. In some instances DNA was extracted from transformed lymphoblastoid cell lines.

Next-generation sequencing

Whole-genome sequencing for ten Q-MEGA cases and exome sequencing for a further 77 Q-MEGA cases was performed using the Hiseq 2000 platform (Illumina) combined with SureSelect Human All Exon V4 + UTRs enrichment kits (Agilent). 100 bp paired-end reads were generated with samples having a mean coverage of 96X. Using the BWA alignment algorithm, the sequence output was mapped to the UCSC human genome reference build 19 [18]. SNPs and indels were detected using bcftools and SAMtools mpileup with disabled BAQ computation [19]. Variants were filtered for stringency using quality score (>70) and alternate read counts (>2 and >20 % of all reads at a given position). Samples selected for this study were wild type for previously identified high-risk CMM genes: *CDKN2A*, *CDK4*, *TERT*, *POT1*, *ACD*, and *TERF2IP* [2, 7–9, 20–22].

Targeted sequencing of *POLE* on randomly selected probands, from Q-MEGA and the Danish Project of Hereditary Malignant Melanoma, was performed using Ion 318v2 chips on an Ion Torrent Personal Genome Machine (Life Technologies) with AmpliSeq library kits and custom designed primer pools. The exonic regions had 99.4 % coverage; with amplicon lengths of 150–250 bp. The missing region encompasses 44 nucleotides of exon 1 which does not form part of the crucial exonuclease domain. Samples had minimum sequence coverage of 30X across all regions. Variants were filtered for stringency and were required to have minimum of four reads for the reference and four reads for the alternative alleles. As with the exome/genome data, variants were filtered using quality score (>70) and alternate read counts (>20 % of all reads at a given position). Variants from next-generation sequencing data sets that passed the filtering criteria were validated by Sanger sequencing. See Supplementary Table 1 for primers.

Fission yeast strain construction and mutation rate assays

Strains used in this study are listed in Supplementary Table 2. Standard genetic methods were used for strain construction [23]. The *pol2-F348C* mutant strain encoding a variant equivalent to human POLE p.(Trp347Cys) was constructed by amplifying *pol2* segments with primer pairs 1083 + 1156 and 1084 + 1155 (Supplementary Table 3). The products were purified, annealed and amplified using primers 1083 and 1084. The product was digested with BglII and AscI and inserted into BamHI, AscI-cleaved pFA6a-*kanMX6* [24]. The *pol2-F348W* strain (encoding a protein equivalent to wild-type human POLE) was

constructed in the same way using initial primer combinations of 1083 + 1159 and 1084 + 1157. As a control, a *pol2*$^{+}$ (wild-type) strain was also constructed using the same procedure, using primers 1083 and 1084 to amplify the wild-type sequence, in case the sequence changes in the vicinity of the *pol2*$^{+}$ gene affected *pol2*$^{+}$ expression. To construct the exonuclease null *pol2-D276A*, *E278A*, primer pairs 1083 + 1136 and 1084 + 1135 were initially used. Plasmids were integrated into the *pol2* locus after linearization with BamHI and selecting for G418 resistance. Constructs were verified by Sanger sequencing. Mutation rates were determined by fluctuation analysis as previously described [25]. For canavanine resistance assays, PMA plates were used, containing 70 μg/ml canavanine (Sigma). For fluoro-orotic acid (FOA) resistance, cells were plated onto EMM medium containing 1 g/l FOA (Formedium). Eleven cultures were used for each experiment, and plates were scored after 8 (Ade) and 12 days (canavanine resistance), respectively. Since background growth on the primary FOA-containing plates made it difficult to score FOA-resistant colonies, the primary plates were replica plated onto fresh FOA media after 3 days and scored after an additional 3 days. Mutation rates were calculated using the MSS-Maximum Likelihood Estimator [26]. Two independently generated strains were used to generate biological replicates and mutation rates were calculated from two independent fluctuation assays.

Results

Whole-genome or exome sequencing was performed on a total of 34 CMM families (87 cases) to identify novel, high-risk CMM predisposition genes. A novel *POLE* mutation (i.e. not reported in any publically available database) was found in all three cases sequenced in one family. The mutation at position chr12:133252386 (c.1041G > T; NM_006231; build hg19) results in a missense substitution, p.(Trp347Cys), occurring within the exonuclease domain of the large subunit of POLE. One case presented with uveal melanoma (UMM) at age 21, the second case presented with CMM at age 14 and the third case presented with multiple primary melanoma first diagnosed at age 70 (Fig. 1). This mutation has not been reported in dbSNP, ESP6500 NHLBI or the 1000 Genomes Project. Overall, this family had a total of seven confirmed cases of CMM and a case with UMM (Fig. 1). Co-segregation analysis using Sanger sequencing found that 5 of the 6 melanoma cases that were available for testing carried the p.(Trp347Cys) mutation. This included two individuals with multiple primary melanomas, and a case that developed three different primary cancers: melanoma, renal cell carcinoma and prostate cancer (Supplementary Table 4). The individual that was wild type for the mutation developed CMM at the latest age (94 years) of all melanoma cases in the family.

While tryptophan 347 is highly conserved in Pol ε across vertebrates (Supplementary Figure 1) it is not perfectly conserved in invertebrates (*Drosophila* or *C. elegans*), members of the eukaryote fungi kingdom (*S. cerevisiae* or *S. pombe*), or the eukaryote plant kingdom (*A. thaliana*). However the position homologous to Trp347 is conserved as an aromatic amino acid, not only in Pol ε but also in other B family polymerases, such as Pol δ and distantly related bacterophage RB69/T4 polymerases (Supplementary Figure 2). In *S. cerevisiae* Pol ε, this residue does not fall into a conserved region within the exonuclease domain [27]. Examining its location using PyMOL shows that it is not adjacent to the exonuclease active site, so it is difficult to assess its effect on Pol ε function (Supplementary Figure 3) [28, 29]. To assess whether this mutation was functionally important, an equivalent mutation was constructed in *S. pombe* and mutation rate assays were carried out. In fission yeast Pol ε (Pol2) the position equivalent to human Trp347 is Phe348, so in addition to mutating this to cysteine, a strain expressing F348W was also constructed. Using three different mutation rate assays, strains expressing Pol ε Phe348Cys were shown to have a mutator phenotype. For instance the mutation rate of *ura4/ura5* genes (giving FOA-resistance) is nearly 40 times the mutation rate of a wild-type strain (Fig. 2). In contrast, the conservative change to the human wild type amino acid p.(Phe348Trp) only resulted in a slight increase in the mutation rate. The mutation rates are comparable to those seen within a strain expressing Pol ε D276AE278A, where mutations in the exonuclease active site are predicted to inactivate the proof-reading ability of the enzyme [30]. Thus these results suggest that the human p.(Trp347Cys) mutation will cause an increased mutation rate, comparable to a polymerase mutant defective in proof-reading.

Given the co-segregation pattern in this family together with the mutation causing functional impairment in yeast, we consider it a likely cause of melanoma susceptibility in this family. To determine whether *POLE* mutations are responsible for other melanoma families we conducted targeted sequencing analysis in a large series of CMM cases. Overall, 1243 probands from the Q-MEGA (N = 1093) and Danish (N = 150) samples were sequenced at sufficient depth to give adequate coverage of the *POLE* gene. This led to the identification of an additional ten probands with novel (N = 5) or rare (N = 5) mutations occurring in the exonuclease domain (amino acids 223–517) of POLE (Table 1) The predicted effect of these variants on POLE function was modelled using PyMOL (Supplementary Figure 4).

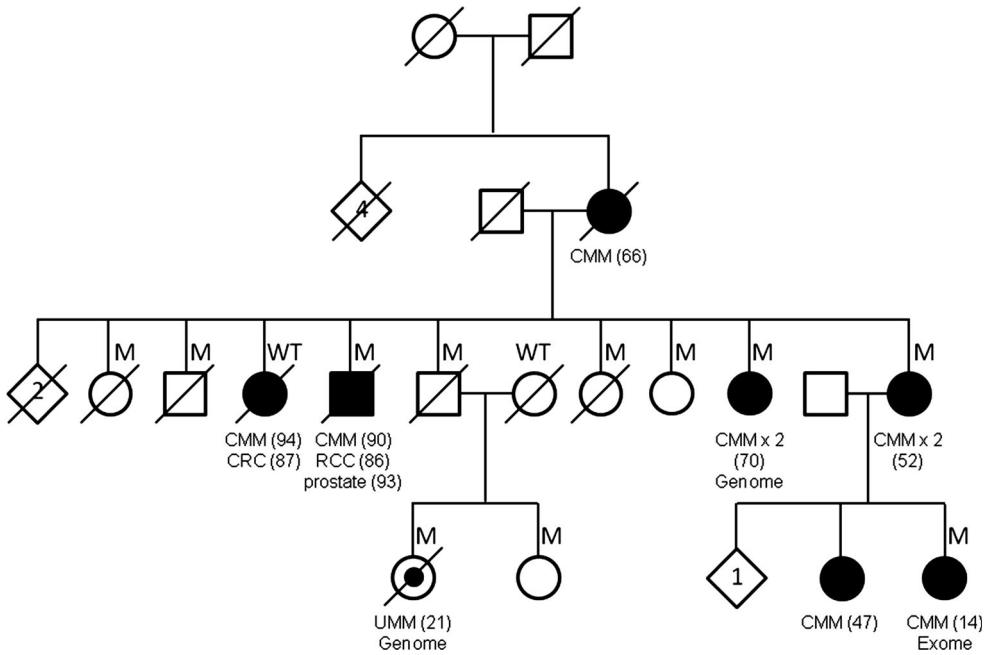

Fig. 1 Segregation analysis of *POLE* p.(Trp347Cys) in family 40972. *Black symbols* represent individuals with cutaneous malignant melanoma (CMM). The *black dot* indicates a case with uveal malignant melanoma (UMM). *M* indicates *POLE* p.(Trp347Cys) mutation carrier while *WT* indicates the individual is wild type. The age at diagnosis of cutaneous or other cancer is indicated in the *brackets*. In instances where a person has developed multiple primary melanomas, the *number* is indicated and the age of first *CMM* diagnosis is indicated. *Circles* are females and *squares* are males. A *line through a symbol* indicates they are deceased. Unaffected and/or ungenotyped cases are represented by a *diamond with the number* of individuals indicated in the *centre of the symbol*. *CRC* colorectal cancer, *RCC* renal cell carcinoma

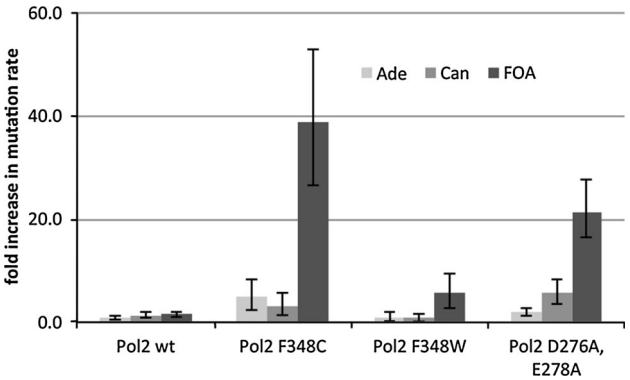

Fig. 2 Mutation rates of *S. pombe* strains expressing mutant Pol ε equivalent to human POLE p.(Trp347Cys). Shown are fold changes of mutation rates for Ade^{+} reversion of the *ade6-485* allele (Ade), and the rates of spontaneous mutation to canavanine resistance (Can) and 5-fluoroorotic acid (FOA) resistance relative to the wild type strain (WT: *S. pombe* strain 2840). Numbers are calculated from two experiments with two independently generated strains per 10^8 cell divisions. Human Trp347 is partially conserved in *S. pombe* (Phe348). The phenylalanine was therefore mutated to both the human wild-type tryptophan residue (Phe348Trp) and a mutant cysteine residue (Phe348Cys). Both *pol2* (pol ε) variants were integrated into strain 2840. The wild-type *pol2* gene was integrated into the 2840 strain in the same way (Pol2 WT), to determine if plasmid integration in the vicinity of the *pol2* locus affected the mutation rate. *Error bars* indicate the 95 % confidence intervals

Of the novel mutations observed, POLE p.(Arg259Cys) occurred in a sporadic case that developed CMM at age 34 and had family history of breast and bladder cancer. The second mutation, POLE p.(Gln352Pro) occurred in an individual who developed CMM at age 54. They also had a sibling with CMM (DNA unavailable for testing) and two other siblings who developed either astrocytoma (age 70) or leukaemia (age 65). They also had a parent who developed squamous cell carcinoma (SCC) of the oesophagus at age 85 (Supplementary Figure 5A). The third mutation, p.(Lys425Arg), was detected in a sporadic CMM case (age 55) without a family history of cancer. The fourth mutation, p.(Val460Met) occurred in a CMM case (age 29) who had an extensive family history of cancer. Her father, who also carried the mutation, had colorectal cancer (CRC) at age 74, prostate cancer at age 70 and acute myeloid leukaemia at age 83 (Supplementary Figure 5A). Additionally, there were family members with prostate cancer, oesophageal cancer and CRC (unavailable for testing). The fifth mutation occurred in a 3-case CMM family (Supplementary Figure 5A). The two cases available for genotyping both carried the p.(Ile515Met) mutation and developed CMM at ages 36 and 41. Additionally, a mutation occurring three amino acids after the boundary

Table 1 Heterozygous mutations found in the exonuclease domain of POLE

Family ID	Position	NT change	AA change	SNP ID	ESP6500 MAF EA	CADD PHRED score	SIFT	PP2	GERP++	Total CMM cases	Cases with mutation/cases genotyped	Ages of CMM onset	Other cancers in family (age of onset)
D05	133253975	C775T	R259C	–	0	25.4	T	B	5.64	1	1/1	34	Bladder, breast
062657	133253197	C844T	P282S	rs138207610	0.000349	29.6	D	P	5.06	1	1/1	55	–
41398	133253180	T861A	D287E	rs139075637	0.001281	23.8	D	P	1.59	1	1/1	57	Pharynx SCC (77)
40374	133253180	T861A	D287E	rs139075637	0.001281	23.8	D	P	1.59	6	2/6	22, 30, 32, 34, 47, 73	Mouth SCC (73), breast (78), NHL (73), Ewing's sarcoma (14)
41355	133253180	T861A	D287E	rs139075637	0.001281	23.8	D	P	1.59	3	1/2	40, 40, 61	–
40972	133252386	G1041T	W347C	–	0	28	D	B	5.31	7	5/6	14, 52, 66, 70, 90, 94	UMM (21), CRC (87), RCC (86), prostate (93)
41358	133252372	A1055C	Q352P	–	0	24.4	T	P	5.31	2	1/1	54, 55	Astrocytoma (70), leukaemia (65), oesophagus SCC (85)
012859	133250246	A1274G	K425R	–	0	25	D	P	5.27	1	1/1	55	–
002507	133250183	G1337A	R446Q	rs151273553	0.000582	26.5	T	P	5.27	1	1/1	38	Lung (65)
001355	133249845	G1378A	V460M	–	0	29	D	P	5.02	1	1/1	29	AML (83), CRC (74), CRC (80), oesophagus (77), prostate (70), prostate (74), +2 unconfirmed CRC
41188	133249354	C1545G	I515M	–	0	24.8	D	P	3.85	3	2/2	36, 41, 65	–
F89	133249340	A1559G	Q520R[a]	–	0	25.4	T	P	5.13	2	1/2	20, 47	CRC (45), CRC (49), CRC (54) salpinx (72), +2 CRC cases

Position is quoted according to refSeq NM_006231 hg19

CADD Combined Annotation Dependant Depletion. A score >20 indicates that the substitution is amongst the 1 % most deleterious in the human genome. *GERP* Genomic Evolutionary Rate Profiling score. A score >5 denotes a region of high evolutionary constraint. *B* benign, *D* damaging, *P* probably damaging, *T* tolerated, *CMM* cutaneous malignant melanoma, *CRC* colorectal cancer, *AML* acute myeloid leukaemia, *NHL* non-Hodgkin lymphoma, *RCC* renal cell carcinoma, *SCC* squamous cell carcinoma

[a] Variant occurs outside of the exonuclease domain

of the exonuclease domain, p.(Gln520Arg), was identified in a Danish proband who had an extensive history of CRC with three confirmed cases and a further two unconfirmed reports as well as a case of salpinx cancer. Her mother, who also carried the mutation, was diagnosed with CRC at age 45. While no further DNA samples were available for testing, there was a strong family history of CRC on the mother's side.

In addition to the novel mutations, we identified rare population variants that occurred in five probands (Table 1, Supplementary Figure 5B). POLE p.(Phe282Ser) (rs138207610; minor allele frequency (MAF) = 0.000349 in NHLBI ESP6500 European American population) occurred in an individual that developed ten primary CMM tumours with the age of onset being 55 years as well as a Merkel cell carcinoma at age 76. POLE p.(Asp287Glu) (rs139075637; MAF = 0.001281 in NHLBI ESP6500 European American population) was identified in three CMM probands. In one family it did not segregate with disease, occurring in 2 out of 6 cases. One individual developed CMM at 73 and non-Hodgkin lymphoma at 40. The second individual had eight primary CMMs as well as breast cancer and SCC of the mouth. POLE p.(Asp287Glu) was also seen in a three case family where there was incomplete segregation and the carrier developed CMM at 61. The final instance of p.(Asp287Glu) occurred in sporadic CMM case with age of onset of 57 years. Lastly, p.(Arg446Gln) (rs151273553; MAF = 0.000582 in NHLBI ESP6500 European American population) occurred in a sporadic CMM case (age 38).

By comparison to the CMM probands above, there were 20 variants reported in the exonuclease domain of the European American population in the NHLBI ESP6500 database, only 16 of which were rare (MAF < 0.001). Overall, 29 out of 4300 control individuals had rare variants in this region of the gene, equating to a MAF of 0.007 (Supplementary Table 5). In the melanoma probands (N = 1243), we observed ten novel or rare (MAF < 0.001) variants in the exonuclease domain of POLE (MAF = 0.008), the frequency not being statistically different to that of controls.

Discussion

Here we identified a POLE p.(Trp347Cys) mutation in a family predisposed to CMM as well as a range of other cancers, which included renal cell carcinoma, prostate cancer and UMM. The CMMs in this family appeared on both sun-exposed and non-sun-exposed body sites. The mutation was carried by six unaffected family members screened. It is possible that these carriers may develop CMM or other cancers at older ages after additional exposure to environmental mutagens. The particular variant in family 40972 (Fig. 1) has not been seen in 60,706 individuals contained within the Exome Aggregation Consortium database (exAC). This database includes approximately 10,000 cancer cases. The mutation lies in the exonuclease domain of the polymerase and modelling this mutation in yeast shows a clear mutator phenotype. Mutations causing proof-reading defects in Pol δ and ε show partial dominance in mice and yeast [30, 31] and our hypothesis is consistent with the notion that individuals heterozygous for POLE p.(Trp347Cys) have cancer predisposition via an increased probability of mutations in genes relevant to CMM development. Further work will be required to determine whether the mutation inactivates exonuclease activity by distorting the active site, or affects some other process such as partitioning the nascent terminus to the exonuclease domain. It is also possible that tumour development is dependent on loss of the wild type POLE allele, and further studies comparing somatic and tumour genotypes will be necessary to establish whether loss of heterozygosity (LOH) is relevant. Unfortunately no archival tumor material was available from carriers in family 40972 for LOH assessment. However, it is not clear from CRC if POLE acts as a classical two-hit tumor suppressor gene, since LOH is only seen in the minority of cases [10].

To further assess the role of POLE as a potential rare melanoma predisposition gene, we also sequenced the POLE exonuclease domain in a large population-based sample of melanoma cases and compared this to the variant frequency in unselected public domain controls. Statistically there was no significant difference in the frequency of exonuclease variants between cases and controls. However, the possibility remains that specific mutations may still be associated with melanoma susceptibility, although to date, CMM has not been reported in any of the CRC families with *POLE* exonuclease mutations.

In the analysis of the population-based samples of CMM cases we noted that several *POLE* mutation carriers had developed three distinct primary cancers. In one case this included CRC, for which germline *POLE* mutations have been shown to cause predisposition [10]. While still speculative at this stage we contend that mutations occurring in the exonuclease domain of POLE might be responsible for susceptibility to a broader range of cancers than just CRC, and our data support the notion that CMM is one of these cancer types. In a study by Rohlin et al. [32] a *POLE* mutation was found to predispose to a multi-tumour phenotype in a family in which two mutation carriers had each developed three different cancer types including, CRC, ovarian, endometrial and pancreatic cancers. Overall, this data suggests that *POLE* may be a general tumour suppressor and the development of cutaneous melanoma may

be part of the disease spectrum. Further larger studies will be needed to determine the proportion of cancer risk that is attributed to such mutations.

In addition to the exonuclease variants discussed above, we also identified a novel mutation three amino acids after the boundary of the exonuclease domain, p.(Gln520Arg), in a Danish family (Fam 89, Supplementary Figure 5A) that presented with several cases of CRC. This implies that the region adjacent to the exonuclease domain plays a role in CRC susceptibility, thus suggesting a more comprehensive analysis of mutations throughout the entire POLE protein in relation to functionality of the enzyme and cancer predisposition is warranted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Gruis NA, van der Velden PA, Sandkuijl LA et al (1995) Homozygotes for CDKN2 (p16) germline mutation in Dutch familial melanoma kindreds. Nat Genet 10(3):351–353. doi:10.1038/ng0795-351
2. Hussussian CJ, Struewing JP, Goldstein AM et al (1994) Germline p16 mutations in familial melanoma. Nat Genet 8(1):15–21. doi:10.1038/ng0994-15
3. MacGeoch C, Bishop JA, Bataille V et al (1994) Genetic heterogeneity in familial malignant melanoma. Hum Mol Genet 3(12):2195–2200
4. Soufir N, Avril MF, Chompret A et al (1998) Prevalence of p16 and CDK4 germline mutations in 48 melanoma-prone families in France. The French Familial Melanoma Study Group. Hum Mol Genet 7(2):209–216
5. Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA. http://evs.gs.washington.edu/EVS/. Accessed 27 Feb 2014, Cited 14 June 2013
6. Walker GJ, Hussussian CJ, Flores JF et al (1995) Mutations of the CDKN2/p16INK4 gene in Australian melanoma kindreds. Hum Mol Genet 4(10):1845–1852
7. Zuo L, Weger J, Yang Q et al (1996) Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. Nat Genet 12(1):97–99. doi:10.1038/ng0196-97
8. Robles-Espinoza CD, Harland M, Ramsay AJ et al (2014) POT1 loss-of-function variants predispose to familial melanoma. Nat Genet. doi:10.1038/ng.2947
9. Aoude LG, Pritchard AL, Robles-Espinoza CD et al (2015) Nonsense mutations in the shelterin complex genes ACD and TERF2IP in familial melanoma. J Natl Cancer Inst. doi:10.1093/jnci/dju408
10. Palles C, Cazier JB, Howarth KM et al (2013) Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. Nat Genet 45(2):136–144. doi:10.1038/ng.2503
11. Baxter AJ, Hughes MC, Kvaskoff M et al (2008) The Queensland Study of Melanoma: environmental and genetic associations (Q-MEGA); study design, baseline characteristics, and repeatability of phenotype and sun exposure measures. Twin Res Hum Genet 11(2):183–196. doi:10.1375/twin.11.2.183
12. Aitken JF, Green AC, MacLennan R, Youl P, Martin NG (1996) The Queensland Familial Melanoma Project: study design and characteristics of participants. Melanoma Res 6(2):155–165
13. Whiteman DC, Valery P, McWhirter W, Green AC (1997) Risk factors for childhood melanoma in Queensland, Australia. Int J Cancer 70(1):26–31
14. Whiteman D, Valery P, McWhirter W, Green A (1995) Incidence of cutaneous childhood melanoma in Queensland, Australia. Int J Cancer 63(6):765–768
15. Youl P, Aitken J, Hayward N et al (2002) Melanoma in adolescents: a case-control study of risk factors in Queensland, Australia. Int J Cancer 98(1):92–98
16. Whiteman DC, Parsons PG, Green AC (1998) p53 expression and risk factors for cutaneous melanoma: a case-control study. Int J Cancer 77(6):843–848
17. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16(3):1215
18. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25(14):1754–1760. doi:10.1093/bioinformatics/btp324
19. Li H, Handsaker B, Wysoker A et al (2009) The sequence alignment/map format and SAMtools. Bioinformatics 25(16):2078–2079. doi:10.1093/bioinformatics/btp352
20. Horn S, Figl A, Rachakonda PS et al (2013) TERT promoter mutations in familial and sporadic melanoma. Science 339(6122):959–961. doi:10.1126/science.1230062
21. Kamb A, Shattuck-Eidens D, Eeles R et al (1994) Analysis of the p16 gene (CDKN2) as a candidate for the chromosome 9p melanoma susceptibility locus. Nat Genet 8(1):23–26. doi:10.1038/ng0994-22
22. Bahuau M, Vidaud D, Jenkins RB et al (1998) Germ-line deletion involving the INK4 locus in familial proneness to melanoma and nervous system tumors. Cancer Res 58(11):2298–2303
23. Moreno S, Klar A, Nurse P (1991) Molecular genetic analysis of the fission yeast *Schizosaccharomyces pombe*. Methods Enzymol 194:795–823
24. Bahler J, Wu JQ, Longtine MS et al (1998) Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. Yeast 14(10):943–951
25. Marti TM, Mansour AA, Lehmann E, Fleck O (2003) Different frameshift mutation spectra in non-repetitive DNA of MutSalpha- and MutLalpha-deficient fission yeast cells. DNA Repair (Amst) 2(5):571–580
26. Hall BM, Ma CX, Liang P, Singh KK (2009) Fluctuation analysis CalculatOR: a web tool for the determination of mutation rate using Luria–Delbruck fluctuation analysis. Bioinformatics 25(12):1564–1565. doi:10.1093/bioinformatics/btp253
27. Shevelev IV, Hubscher U (2002) The 3′5′ exonucleases. Nat Rev Mol Cell Biol 3(5):364–376. doi:10.1038/nrm804
28. Jain R, Rajashankar KR, Buku A et al (2014) Crystal structure of yeast DNA polymerase epsilon catalytic domain. PLoS ONE 9(4):e94835. doi:10.1371/journal.pone.0094835

29. Hogg M, Osterman P, Bylund GO et al (2014) Structural basis for processive DNA synthesis by yeast DNA polymerase varepsilon. Nat Struct Mol Biol 21(1):49–55. doi:10.1038/nsmb.2712
30. Morrison A, Sugino A (1994) The 3′ → 5′ exonucleases of both DNA polymerases δ and ε participate in correcting errors of DNA replication in Saccharomyces cerevisiae. Mol Gen Genet 242(3):289–296
31. Albertson TM, Ogawa M, Bugni JM et al (2009) DNA polymerase epsilon and delta proofreading suppress discrete mutator and cancer phenotypes in mice. Proc Natl Acad Sci USA 106(40):17101–17104. doi:10.1073/pnas.0907147106
32. Rohlin A, Zagoras T, Nilsson S et al (2014) A mutation in POLE predisposing to a multi-tumour phenotype. Int J Oncol 45(1):77–81. doi:10.3892/ijo.2014.2410