Risk of colorectal cancer for carriers of a germ-line mutation in *POLE* or *POLD1*

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Background: Germ-line mutations in the exonuclease domains of the *POLE* and *POLD1* genes are associated with an increased, but yet unquantified, risk of colorectal cancer (CRC).

Methods: We identified families with *POLE* or *POLD1* variants by searching PubMed for relevant studies prior to October 2016 and by genotyping 669 population-based CRC cases diagnosed in patients under 60 years of age, from the Australasian Colorectal Cancer Family Registry. We estimated the age-specific cumulative risks (penetrance) using a modified segregation analysis.

Results: We observed 67 CRCs (mean age at diagnosis = 50.2 (SD = 13.8) years) among 364 first- and second- degree relatives from 41 *POLE* families, and 6 CRCs (mean age at diagnosis = 39.7 (SD = 6.83) years) among 69 relatives from 9 *POLD1* families. We estimated risks of CRC up to the age of 70 years (95% confidence

interval) for males and females, respectively, to be 40% (26–57%) and 32% (20–47%) for *POLE* mutation carriers and 63% (15–99%) and 52% (11–99%) for *POLD1* mutation carriers.

Conclusion: CRC risks for *POLE* mutation carriers are sufficiently high to warrant consideration of annual colonoscopy screening and implementation of management guidelines comparable to those applied in cases of Lynch syndrome. Refinement of estimates of CRC risk for *POLD1* carriers is needed; however, clinical management recommendations could follow those made for *POLE* carriers.

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INTRODUCTION

Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide. Identifying people at high risk of developing CRC and optimizing their screening can reduce the incidence of, and mortality from, CRC. Up to 35% of CRC cases are estimated to be attributable to genetic factors;¹ however, known hereditary CRC syndromes caused by high-penetrance germ-line mutations account for only 3–5% of all CRCs.² In recent years, gene discovery efforts have identified several novel CRC susceptibility genes, including rare germ-line variants within the polymerase proofreading domains of the *POLE* and *POLD1* genes, which are reported to be associated with CRC and polyposis (referred to as polymerase proofreading-associated polyposis).^{3–5} These novel CRC susceptibility genes are now included on multigene sequencing panels for clinical testing, but the age-specific cumulative risks (penetrance) of CRC for people who carry mutations in these genes have not yet been quantified, which is an

impediment to optimizing personalized clinical management. The aim of this study was to estimate the age-specific cumulative risks of CRC separately for male and female carriers of *POLE* and *POLD1* gene mutations.

MATERIALS AND METHODS

We searched in PubMed for relevant studies published before October 2016 reporting pedigree and cancer data for families with germ-line *POLE* or *POLD1* variants that were either novel or previously observed at population frequency ≤ 0.002 according to the non-Finnish European population in the Exome Aggregation Consortium (ExAC) database,⁶ given recent evidence that shows low-variant allele frequency is an important guide in determining disease-causing variants.⁷ Variants identified from the literature were re-annotated, for consistency, via *in silico* methods using Annovar⁸ with default settings. We applied a criterion for predicting pathogenicity of missense variants in both genes, as recommended

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by the American College of Medical Genetics and Genomics,⁹ namely using (i) multiple commonly used *in silico* tools (SIFT, PolyPhen2, MutationTaster, CADD, GERP, REVEL, and M-CAP (**Supplementary Table S1** online)) and (ii) a high level of consensus between multiple *in silico* tools for prediction of deleterious effect. For this study, we applied the recommended or default thresholds for prediction of deleteriousness for each of the seven *in silico* tools (see **Supplementary Table S1** for thresholds). For each variant, the sum of *in silico* tools that reported the variant to be deleterious was calculated (maximum score of 7). A variant was considered to be likely pathogenic for this study where at least four of seven *in silico* tools predicted the variant has a deleterious effect (**Supplementary Table S1**). Families were excluded from the penetrance analysis if they were (i) families of probands with variants not predicted to be pathogenic, (ii) discovery families in which the *POLE* c.1270C>G p.Leu424Val and *POLD1* c.1433G>A p.Ser478Asn variants were originally described,³ (iii) families of carriers of *de novo* mutations, or (iv) uninformative owing to missing information on sex or age at cancer diagnosis of probands. For the families included in the analysis, information regarding mutation carrier status, sex, cancer- or polyp-affected status, age at cancer or polyp/polypoid diagnosis, last known age or death, and country of study of families was extracted, where possible, from identified studies.

We searched for *POLE* or *POLD1* mutation carrier families by genotyping 669 population-based probands diagnosed with CRC before 60 years of age from the Australasian Colorectal Cancer Family Registry (ACCFR)^{10,11} (**Supplementary Table S2**) for 17 rare germ-line variants within the exonuclease domains of the *POLE* and *POLD1* genes (**Supplementary Table S3** and **Supplementary Materials and Methods**). These 17 variants were selected based on multiple sources, namely:

1. Rare germ-line variants in the exonuclease domains of *POLE* and *POLD1* reported in the ExAC database (≤ 0.002 allele frequency)
2. Variants, identified from our in-house whole-genome and whole-exome sequencing studies of 100 multiple-case CRC-affected families from the clinic-based recruitment arm of the ACCFR that were either rare or novel variants according to the ExAC database (≤ 0.002 allele frequency)
3. Variants reported in the discovery paper by Palles et al.³
4. Variants predicted to be deleterious by at least four of the seven *in silico* tools

Using data from both published studies and the ACCFR, we estimated the hazard ratio (HR) and corresponding 95% confidence interval of CRC for mutation carriers compared with the general population (based on age-, sex-, and country-specific incidences) and the age-specific cumulative risks (penetrance), using a modified segregation analysis that incorporated data for all family members, whether genotyped or not, and whether affected or not. We properly adjusted for ascertainment of families in which each pedigree's data was conditioned on the proband's genotype, cancer status, and

age at diagnosis (for population-based families) or on the proband's genotype, and the cancer statuses and ages at diagnoses of all family members (for clinic-based families) to produce unbiased estimates (see details in **Supplementary Materials and Methods**). All statistical tests were two-sided, and *P* values lower than 0.05 were considered statistically significant.

RESULTS

From the literature, we identified 15 studies reporting 37 rare variants (minor allele frequency ≤ 0.002) within the exonuclease domains of *POLE* and *POLD1*. Of these 37 variants, 32 were predicted to be pathogenic by *in silico* criteria (**Supplementary Table S1**). Of the 89 families with *POLE* (*n* = 70) or *POLD1* (*n* = 19) mutations, 42 were excluded (7 families with variants not predicted to be pathogenic, 3 families from the study by Palles et al.³ that initially reported the association of *POLE* and *POLD1* with CRC, 3 families of *de novo* mutation carriers, 29 families of probands without age or sex information). From the ACCFR, two unrelated carriers of the *POLE* c.861T>A p.Asp287Glu variant and one carrier of the *POLE* c.1336C>T p.Arg446Trp variant were identified (pedigrees shown in **Supplementary Figure S1**).

We included 47 families (38 *POLE* and 9 *POLD1*) from published studies, and 3 families with *POLE* mutations from the ACCFR (**Supplementary Figure S1**), in the penetrance analysis. Of these, 28 (21 *POLE* and 7 *POLD1*) families were ascertained because they had a family history of cancer, and 22 (20 *POLE* and 2 *POLD1*) were ascertained via population-based cancer registries, regardless of family history. We observed 67 CRCs with a mean age at diagnosis of 50.2 (SD = 13.8) years among 364 first- and second-degree relatives (53% female) from 41 families with *POLE* mutations, and 6 CRCs with a mean age at diagnosis of 39.7 (SD = 6.83) years among 69 first- and second-degree relatives (45% female) from 9 families with *POLD1* mutations (**Table 1**).

We estimated cumulative risks of CRC to age 70 years for males and females to be, respectively, 28% (95% CI, 10%–42%) and 21% (95% CI, 7%–33%) for *POLE* mutation carriers and 90% (95% CI, 33%–99%) and 82% (95% CI, 26%–99%) for *POLD1* mutation carriers (**Figure 1**). The CRC HR was estimated to be 12.2 (95% CI, 7.35–20.2) and 87.2 (95% CI, 15.3–495) for *POLE* and *POLD1* mutation carriers, respectively (**Table 2**). The HRs decreased with age, being 38.7 (95% CI, 17.5–85.4) for <50 years compared with 8.21 (95% CI, 4.24–15.9) for ≥ 50 years for *POLE* mutation carriers (*P* = 0.003), and 201 (95% CI, 62.0–651) for <50 years compared with 3.34 (95% CI, 0.22–50.1) for ≥ 50 years for *POLD1* mutation carriers (*P* = 0.007; **Table 2**). There was no evidence for a difference in HRs by sex (all *P* > 0.1).

The *POLE* c.1270C>G, p.Leu424Val mutation has been reported in 19 families. For these specific mutation carriers, the estimated cumulative risks of CRC to age 70 years were 97% (95% CI, 85%–99%) for males and 92% (95% CI,

Table 1 Numbers and mean ages at diagnosis of cancers in the first- and second-degree male and female relatives of probands from *POLE* and *POLD1* mutation families

Site of cancer	Male		Female	
	<i>n</i>	Mean age (SD)	<i>n</i>	Mean age (SD)
<i>POLE</i>		(<i>n</i> = 172)		(<i>n</i> = 192)
Colon/rectum	36	48.5 (14.9)	31	52.1 (12.5)
Cutaneous malignant melanoma	5	46.8 (29.9) ^a	11	54.4 (18.7)
Duodenum	1	45	0	—
Renal	1	86	0	—
Ureter	1	46	0	—
Esophagus	1	85	1	77
Pancreas	1	46	1	78
Brain	1	69	2	68 ^a
Bone	1	UK	0	—
Lung	2	61.0 (5.66)	1	92
Liver	0	—	1	UK
Leukemia	2	74.0 (12.7)	0	—
Astrocytoma	3	50.7 (30.9)	0	—
Ewing's sarcoma	0	—	1	14
Intra-abdominal	0	—	1	UK
Head and neck	1	52	3	74.0 (2.65)
Breast	0	—	4	64.5 (21.4)
Endometrium	NR	NR	3	53.0 (9.85)
Ovary	NR	NR	4	43.8 (6.29)
Prostate	5	74.0 (14.2) ^a	NA	NA
<i>POLD1</i>		(<i>n</i> = 38)		(<i>n</i> = 31)
Colon/Rectum	4	42.3 (7.04)	2	34.5 (2.12)
Stomach	1	72	1	85
Renal	0	—	1	74
Urinary bladder	0	—	1	UK
Hodgkin lymphoma	0	—	1	30
Head and neck	1	56	0	—
Breast	0	—	3	58.3 (10.7)
Endometrium	NR	NR	3	55.0 (2.65)
Ovary	NR	NR	1	31

—, Not applicable; N, total number of affected relatives; NR, not relevant; UK, age at diagnosis unknown.

^aAge at diagnosis was unknown for one person.

75%–99%) for females. The corresponding HR was 131 (95% CI, 71.3–242) when both sexes were combined, and 75.0 (95% CI, 37.9–149) for males and 269 (95% CI, 111–650) for females. Results for penetrance and HR estimates were not materially different between analyses with and those without imputing missing ages.

DISCUSSION

To our knowledge, this is the first report of both relative and cumulative risks of CRC for people who carry germ-line mutations within the exonuclease domains of the *POLE* or *POLD1* genes. Our analysis included all of the reported rare germ-line variants within the exonuclease domains of *POLE* and *POLD1* predicted to be pathogenic by multiple commonly used *in silico* tools. However, it is possible that not all variants

result in the same level of risk as is evidenced by the recurring *POLE* c.1270C>G, p.Leu424Val mutation.

CRC has been the predominant cancer identified in *POLE* and *POLD1* mutation carriers to date (perhaps because of the way the families had been selected for genetic testing). However, a broader extracolonic spectrum of cancers is being revealed as additional carrier families are identified. In addition to CRC, cancers of the endometrium, ovaries, pancreas, brain, and small intestine have been reported for carriers, similar to what has been observed for DNA mismatch repair (MMR) gene mutation carriers (Lynch syndrome).¹² The presence of 10 to <100 adenomas and/or the presence of duodenal polyps could be distinguishing features of *POLE* or *POLD1* mutation carriers from MMR gene mutation carriers.⁵ Interestingly, carriers of the *POLE*

Figure 1 Cumulative risks (unbroken lines) and corresponding 95% confidence intervals (dotted lines) of colorectal cancer. Risks are shown for (a) *POLE* mutation carriers, (b) *POLD1* mutation carriers, and the general population (dashed lines). Blue and red represent males and females, respectively. Information for the general population is based on SEER (Surveillance, Epidemiology and End Results) data (nine registries) for race/ethnicity cancer incidence between 2003–2007. A full color version of this figure is available at the *GENETICS in MEDICINE* journal online.

Table 2 Hazard ratio (95% confidence interval) of colorectal cancer for carriers of a germ-line mutation in *POLE* or *POLD1*

	<i>POLE</i>	<i>POLD1</i>	<i>POLE</i> p.Leu424Val
Overall HR (95%CI)	12.2 (7.35–20.2)	87.2 (15.3–495)	131 (71.3–242)
Sex			
Male	12.3 (5.35–28.1)	723 (102–5140)	75.0 (37.9–149)
Female	12.1 (5.64–16.1)	75.0 (19.2–292)	269 (111–651)
<i>P</i> difference	0.9	0.1	0.03
Age			
< 50 years	38.7 (17.5–85.4)	201 (62.0–651)	238 (95.6–593)
≥ 50 years	8.21 (4.24–15.9)	3.34 (0.22–50.1)	98.0 (44.1–218)
<i>P</i> difference	0.003	0.007	0.2

HR, hazard ratio.

c.1270C > G, p.Leu424Val mutation showed no predilection for site and present with different histological types including mucinous adenocarcinoma, suggesting variability in phenotype even for the same mutation, implicating potential environmental or genetic modifiers.

Additional phenotypic variability was observed with regard to tumor DNA MMR status where the majority of CRCs from *POLE* and *POLD1* carriers were MMR-proficient or microsatellite-stable. However, a small subset of CRCs in *POLE* mutation carriers showed tumor MMR deficiency, without evidence of a germ-line MMR gene mutation.^{13,14} Therefore, germ-line *POLE* exonuclease domain variants may account for a proportion of people with tumor MMR-deficient phenotype not explained by germ-line MMR gene mutations or acquired *MLH1* promoter hypermethylation (suspected Lynch syndrome).¹⁵ Furthermore, somatic *POLE* and *POLD1* mutations have been reported in both colorectal and endometrial cancers,^{16,17} supporting the hypothesis that loss of polymerase proofreading and the resultant hypermutator tumor phenotype can underlie inactivation of the MMR genes through somatic mutations.

This study has several limitations, including the lack of detailed information regarding how cancer histories of family members in published studies were verified. A large proportion of families were excluded from the analysis owing to missing information for age and sex of probands, thereby reducing the precision of our risk estimates. We used *in silico* predictions to assign pathogenicity for variant inclusion in the penetrance analysis; however, it has been shown that *in silico* tools and their algorithms for missense variant effect prediction are only 65–80% accurate when examining known disease-causing missense variants.¹⁸ The *POLE* and *POLD1* variants included in the analysis were predicted to be deleterious by multiple *in silico* variant effect prediction tools (by at least four out of the seven tools used in this study), as recommended by the American College of Medical Genetics and Genomics,⁹ and further selected based on a variant allele frequency filter of ≤ 0.002 , adding confidence that our CRC risk estimates were based on only those variants likely to be pathogenic. We genotyped 17 *POLE* and *POLD1* rare, likely pathogenic variants to identify additional carriers, but we cannot exclude the possibility that other *POLE* and *POLD1* variants besides those genotyped may exist within the ACCFR CRC-affected individuals. Apart from CRC, we were unable to estimate risks of other cancers because there were too few cancer diagnoses. Finally, our estimates might not necessarily be applicable to non-Caucasians.

In summary, the increased CRC risks for all carriers of a *POLE* pathogenic or likely pathogenic exonuclease domain variant, particularly for the recurrent *POLE* c.1270C > G, p.Leu424Val mutation, warrant consideration of annual colonoscopy screening and clinical management guidelines comparable to those currently recommended for people with Lynch syndrome or familial adenomatous polyposis. As yet the risk of metachronous CRC is not known, but is likely to be similarly increased, raising consideration of subtotal

colectomy rather than segmental resection for *POLE* mutation carriers. Functional studies to support variant classification may help to further refine the CRC risk estimates, as will additional carrier families. For *POLD1* mutation carriers, refinement of penetrance estimates for CRC is needed; however, clinical management recommendations could follow those suggested for *POLE* mutation carriers.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/gim>

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AUTHOR CONTRIBUTIONS

DDB - study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; study supervision. JS - acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; technical support. MC - acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; technical support. CR - study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; technical support. KM - analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; technical support. BJP - analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; technical support. MAJ - study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual

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DISCLAIMER

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DISCLOSURE

The authors declare no conflict of interest.

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