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POLE and *POLD1* mutations in 529 kindred with familial colorectal cancer and/or polyposis: review of reported cases and recommendations for genetic testing and surveillance

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Purpose: Germ-line mutations in the exonuclease domains of *POLE* and *POLD1* have been recently associated with polyposis and colorectal cancer (CRC) predisposition. Here, we aimed to gain a better understanding of the phenotypic characteristics of this syndrome to establish specific criteria for *POLE* and *POLD1* mutation screening and to help define the clinical management of mutation carriers.

Methods: The exonuclease domains of *POLE* and *POLD1* were studied in 529 kindred, 441 with familial nonpolyposis CRC and 88 with polyposis, by using pooled DNA amplification and massively parallel sequencing.

Results: Seven novel or rare genetic variants were identified. In addition to the *POLE* p.L424V recurrent mutation in a patient with polyposis, CRC and oligodendroglioma, six novel or rare *POLD1* variants (four of them, p.D316H, p.D316G, p.R409W, and p.L474P, with strong evidence for pathogenicity) were identified in nonpolyposis CRC families. Phenotypic data from these and previously reported *POLE*/*POLD1* carriers point to an associated phenotype characterized by attenuated or oligo-adenomatous colorectal polyposis, CRC, and probably brain tumors. In addition, *POLD1* mutations predispose to endometrial and breast tumors.

Conclusion: Our results widen the phenotypic spectrum of the *POLE*/*POLD1*-associated syndrome and identify novel pathogenic variants. We propose guidelines for genetic testing and surveillance recommendations.

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Key Words: adenomatous polyposis; genetic testing; hereditary nonpolyposis colorectal cancer; polymerase proofreading-associated polyposis

INTRODUCTION

Germ-line mutations in the exonuclease (proofreading) domain of DNA polymerases Pol δ and Pol ε have been associated with a dominantly inherited syndrome that confers increased risk to colorectal cancer (CRC) and polyposis.[1] Two recurrent pathogenic variants, *POLE* p.L424V and *POLD1* p.S478N, have been identified in 21 and 3 families, respectively.[1–4] A novel mutation in *POLD1*, p.L474P, was found in a hereditary nonpolyposis CRC family.[2]

Patients carrying *POLE* and *POLD1* exonuclease domain mutations show variable phenotypes including multiple adenomas and CRC, and endometrial cancer in the case of female *POLD1* mutation carriers.[1–4] A better characterization of the syndrome is currently required to establish specific criteria for *POLE* and *POLD1* exonuclease mutation screening and to help define the clinical management of mutation carriers. To fulfill this aim, here we study the complete exonuclease domains of *POLE* and *POLD1* in 529 independent families characterized by the presence of familial or early-onset mismatch repair (MMR), proficient CRC, and/or *APC*-negative and *MUTYH*-negative polyposes.

MATERIALS AND METHODS

Study sample

A total of 544 CRC cases belonging to 529 families were included in the study: 456 familial CRC cases from 441 uncharacterized MMR-proficient families, including 60 Amsterdam-positive families, and 88 polyposis cases. Most of them, 526 cases (511

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families), were previously genotyped for *POLE* p.L424V and *POLD1* p.S478N (included in series no. 1 in the work by Valle et al.[2]). All of them were referred to the Genetic Counseling Units of the Catalan Institute of Oncology in the Spanish region of Catalonia between 1999 and 2012. Referral was based on family history of CRC or polyps, presence of early-onset CRC, and/or personal history of polyposis. Eighteen additional CRC patients belonging to unrelated Amsterdam I MMR-proficient families were included in the study. These were recruited through the Human Genetics Program of the Spanish National Cancer Research Center (CNIO).

Among the 456 MMR-proficient cases (441 families), 49 (10.7%) fulfilled Amsterdam I, 11 (2.4%) fulfilled Amsterdam II, and 390 (85.5%) fulfilled the Bethesda criteria. No specific information on family history was available for six patients. The mean age at cancer diagnosis was 48.98 (SD: 12.54) for the tested individuals. Nonpolyposis cases were MMR-proficient, i.e., their tumors showed microsatellite stability and expression of the MMR proteins MLH1, MSH2, MSH6, and PMS2.

Clinical features of polyposis cases, which included adenomatous polyposes (17.0%), attenuated adenomatous polyposes (47.7%), and nonadenomatous polyposes (15.9%), were detailed by Valle et al.[2] For these cases, screening of *MUTYH* and *APC* mutations was performed as previously described.[2]

Informed consent was obtained from all subjects and the study received the approval of the Ethics Committee of the Institut d'Investigació Biomèdica de Bellvitge (IDIBELL) (PR073/12).

Germ-line mutation identification in pooled samples

Mutation screening of the exonuclease coding regions of *POLE* and *POLD1* was performed by using a combination of pooled samples, PCR amplification (*POLE* exons 9–14 and *POLD1* exons 6–12), and high-throughput sequencing, as previously described.[5] Amplification of the DNA pools was performed using Phusion High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA) and custom-designed primers covering the exons and intron–exon boundaries (**Supplementary Table S1** online). Next-generation sequencing was performed on a HiSeq-2000 at the Centro Nacional de Análisis Genómico (CNAG, Barcelona, Spain).

Direct automated sequencing

Direct automated (Sanger) sequencing was used to validate the results obtained by massive parallel sequencing, to identify the mutated cases within pools, and to perform the co-segregation studies. Sequencing was performed on an ABI Sequencer 3730 (Applied Biosystems, Life Technologies, Foster City, CA) using a standard protocol. Data were analyzed using Mutation Surveyor (version 3.10) (Softgenetics, State College, PA, USA). The primers used were the same as those used for germ-line

Table 1 Germ-line variants in the exonuclease domain (amino acids R311 to L526) and adjacent regions of *POLD1* identified in 456 familial or early-onset nonpolyposis CRC and 88 polyposis cases

				Protein function prediction				3D structure prediction		
Variants[a]	Population MAF (dbSNP/ESP)	Protein domain/ secondary structure element	Evolutionary conservation (PhyloP)[d]	PPH2 (HumDiv/ HumVar)	SIFT	Mutation taster	Condel	CUPSAT/ I-Mutant/ERIS	Mutator phenotype[f] (*S. cerevisiae*)	Variant classification
c.883G>A; p.(V295M)	n.a./0.0003 (rs199545019)	N-terminal/loop	**1.096**	0.410/ 0.103 (N)	0.09 (N)	N	0.371 (N)	**Destabilize (all)**	n.a.	Uncertain significance
c.946G>C; p.(D316H)	**0/0**[c]	**Exonucl. (catalytic res.)/ β-strand**	**6.977**	**1/1 (PbD)**	**0 (D)**	**D**	**0.690 (D)**	No effect/ **destabilize** (I-Mut, ERIS)[e]	**Yes**[13,14]	**Pathogenic**
c.947A>G; p.(D316G)	**0/0**[c]	**Exonucl. (catalytic res.)/β-strand**	**6.549**	**1/1 (PbD)**	**0 (D)**	**D**	**0.688 (D)**	**Destabilize (all)**	**Yes**[13,14]	**Pathogenic**
c.1225C>T; p.(R409W)	**0/0**[c]	**Exonucl./α-helix**	**1.115**	**1/1 (PbD)**	**0 (D)**	**D**	**0.610 (D)**	**Destabilize (all)**	n.a.	**Probably pathogenic**
c.1421T>C[b]; p.(L474P)	**0/0**[c]	**Exonucl./α-helix**	**5.593**	**1/1 (PbD)**	**0 (D)**	**D**	**0.702 (D)**	**Destabilize (all)**	**Yes**[14]	**Pathogenic**
c.1562G>A; p.(R521Q)	n.a./0.00035 (rs143076166)	**Exonucl./α-helix**	**8.762**	**0.816**/ 0.195 (**PsD**/N)	**0.05 (D)**	**D**	0.435 (N)	No effect/ **destabilize** (I-Mut, ERIS)[e]	n.a.	Uncertain significance

Indicated in bold is the evidence that supports the damaging nature of the variants. In silico algorithm NNSplice did not predict splice site effects for any of the variants. Loss of *POLD1* wild-type allele could not be identified in five of six tumors analyzed (**Supplementary Table S3** online), in agreement with the haploinsufficiency model previously proposed.[1]

D, damaging or deleterious; ESP, NHLBI Exome Sequencing Project; Exonucl., exonuclease domain; MAF, minor allele frequency; N, neutral; n.a., not available; PbD, probably damaging; PPH2, Polyphen 2; PsD, possibly damaging; Res., residue.

[a]NM_002691. [b]Previously reported in familial CRC.[2] [c]Not reported in dbSNP, 1000 Genomes, and NHLBI GO Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), or found in 500 alleles of Spanish origin.[18] [d]PhyloP100way_vertebrate annotations in dbNSFP v.2.8. PhyloP (phylogenetic *P* values) conservation score is based on the alignments of 100 vertebrate genomes. Positive scores indicate constraint (if score >0.95, the variant is conserved). The higher the score, the more conserved is the residue. [e]Protein destabilization also predicted by PoPMuSiC. [f]The homologous residue in *Saccharomyces cerevisiae* (*Pol3* gene) when mutated causes a mutator phenotype.

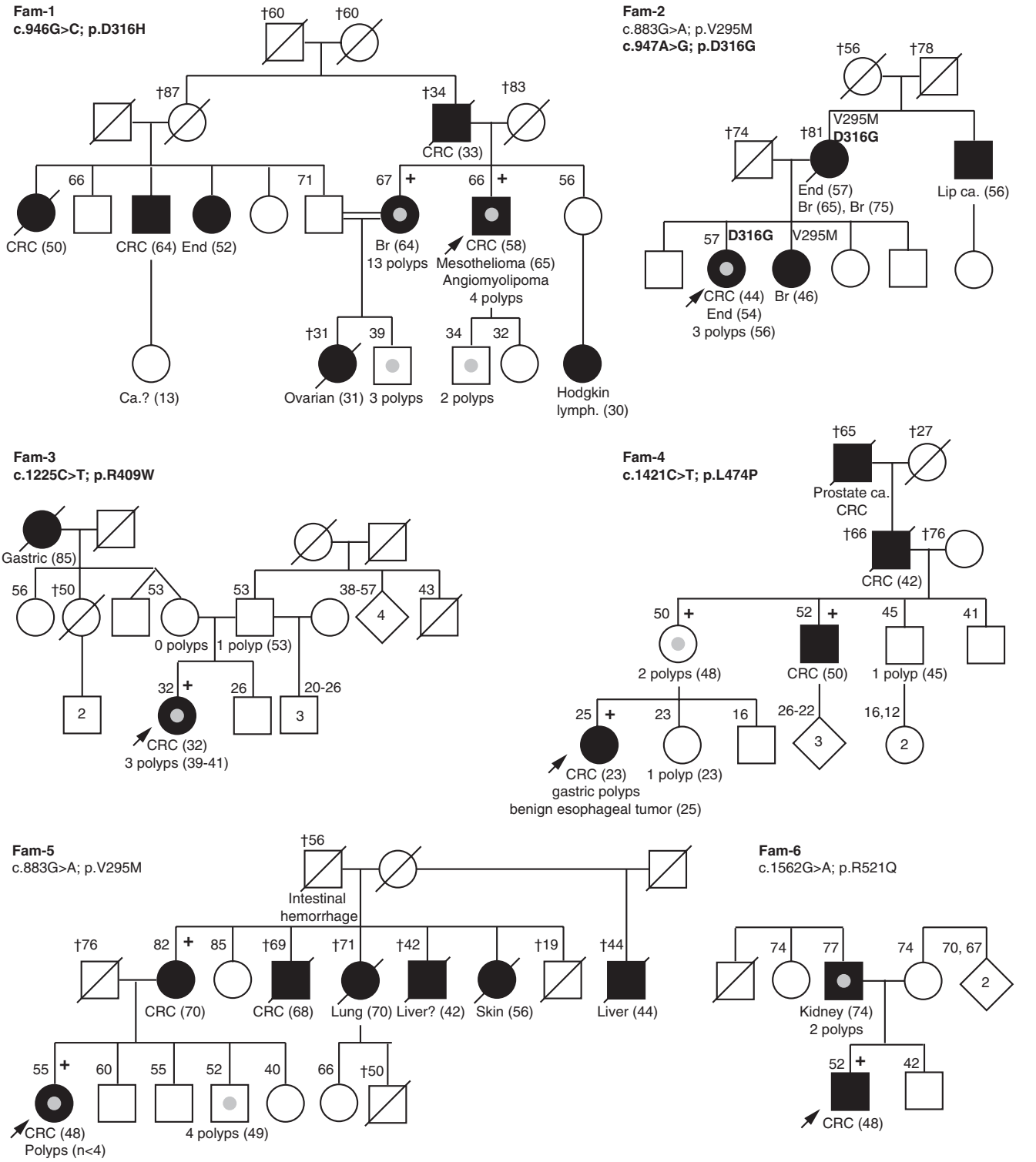

Figure 1 Pedigrees of the families with germ-line *POLD1* variants. Demonstrated pathogenic variants are indicated in bold, and variants of unknown significance are shown in regular case. Filled symbol, cancer; centered gray circle, presence of ≥2 polyps (adenomatous or hyperplastic); +, mutation carrier; –, wild-type. Ages at information gathering or at death (†), when available, are indicated on the top-left corner. Probands are depicted by an arrow. End, endometrial cancer; Br, breast cancer; Lymph, lymphoma.

Table 2 Phenotypic characteristics of the carriers of pathogenic mutations in the exonuclease domain of *POLE* (literature and current study)

POLE mutation	Family (ind) (gender)[a]	Tumors	Colonic polyps	Gastric polyps	MSI status	Publication
p.L424V	SM7 (II.1) (♀)	-(>61 y)	43 AP (34–61 y)	n/a	—	Palles et al.[1]
p.L424V	SM7 (II.2) (♀)	-(>60 y)	Multiple AP (46–60 y)	n/a	—	Palles et al.[1]
p.L424V	SM7 (II.4) (♂)	CRC (43 y)	5 AP (43 y)	n/a	MSS (2 AP)	Palles et al.[1]
p.L424V	SM7 (II.6) (♀)	CRC (40 y)	27 AP (40–54 y)	n/a	MSS (CRC, 17 AP)	Palles et al.[1]
p.L424V	SM7 (II.8) (♀)	-(>51 y)	9 AP, 1 HP (38–51 y)	n/a	MSS (1 AP)	Palles et al.[1]
p.L424V	SM7 (III.6) (♀)	-(>33 y)	9 AP (26–33 y)	n/a	—	Palles et al.[1]
p.L424V	SM7 (III.7) (♀)	-(>30 y)	68 AP (23–30 y)	n/a	—	Palles et al.[1]
p.L424V	A (III.1) (♀)	CRC (48 y)	n/a	n/a	MSS (CRC)	Palles et al.[1]
p.L424V	B (III.1) (♀)	2x CRC (48 y)	3 AP, 1 HP (48–63 y)	n/a	—	Palles et al.[1]
p.L424V	B (IV.2) (♂)	CRC (28 y)	18 AP (29–41 y)	n/a		Palles et al.[1]
p.L424V	C (II.2) (♂)	CRC (50 y)	n/a	n/a	MSS (CRC)	Palles et al.[1]
p.L424V	C (II.3) (♂)	-(>60 y)	6 AP, 5 HP (41–60 y)	n/a	—	Palles et al.[1]
p.L424V	C (III.1) (♂)	CRC (37 y)	Polyps (37–42 y)	n/a	—	Palles et al.[1]
p.L424V	D (IV.2) (♀)	CRC (37 y)	n/a	n/a	—	Palles et al.[1]
p.L424V	E (III.1) (♀)	-(>75 y)	>40 AP (53–75 y)	n/a	—	Palles et al.[1]
p.L424V	F (IV.1) (♂)	-(>74 y)	4 AP (60–74 y)	n/a	MSS (3 AP)	Palles et al.[1]
p.L424V	G (III.1) (♂)	-(>55 y)	2 AP (37–55 y)	n/a		Palles et al.[1]
p.L424V	H (III.2) (♀)	CRC (53 y)	6 AP (52 y)	n/a	MSS (1 AP)	Palles et al.[1]
p.L424V	I (II.3) (♂)	2x CRC (46 y, 62 y), ureter cancer (46 y)	5 AP (46 y)	n/a		Palles et al.[1]
p.L424V	I (II.9) (♂)	CRC (64 y)	10 AP, 2 HP (64–82 y)	n/a	MSS (10 AP)	Palles et al.[1]
p.L424V	I (III.4) (♀)	CRC (40 y)	Multiple polyps (40–56 y)	n/a	MSS (1 AP)	Palles et al.[1]
p.L424V	I (III.9) (♀)	2x CRC (47 y)	2 AP (47–54 y)	n/a	—	Palles et al.[1]
p.L424V	I (III.10) (♂)	-(>51 y)	11 AP (38–51 y)	n/a	—	Palles et al.[1]
p.L424V	J (II.4) (♀)	-(>57 y)	1 AP (57 y)	n/a	—	Palles et al.[1]
p.L424V	K (II.3) (♀)	2x CRC (40 y, 42 y), breast cancer (42 y)	1 AP (40–62 y)	n/a	—	Palles et al.[1]
p.L424V	K (III.3) (♀)	-(>37 y)	49 AP (27–37 y)	n/a	MSS (1 AP)	Palles et al.[1]
p.L424V	K (III.4) (♀)	-(>36 y)	63 AP (23–36 y)	n/a	—	Palles et al.[1]
p.L424V	L (n/a) (♀)	CRC (45 y)	n/a	n/a	—	Palles et al.[1]
p.L424V (de novo)	Fig1A (III.6) (♀)	CRC (28 y), anaplastic oligodendroglioma (30 y)	>31 AP, 1 HP, 1 mixed polyp	n/a	—	Valle et al.[2], current study
p.L424V	Family 1 (PT1) (♀)	CRC (40 y), EC (50 y)	~30 AP	n/a	MSI/ MSH6 loss (CRC), MSS (EC)	Elsayed et al.[3]
p.L424V	Family 1[b] (PT2) (♂)	CRC (30 y), astrocytoma (15 y)	n/a	n/a	MSI (CRC)	Elsayed et al.[3]
p.L424V	Family 2 (PT3)	CRC (34 y)	Multiple APs (34 y)	n/a	MSI/MSH2 and MSH6 loss (CRC), MSS (1 AP)	Elsayed et al.[3]
p.L424V (de novo)	Family 3 (PT4)	CRC (33 y)	Polyps (33 y)	n/a	MSS (CRC)	Elsayed et al.[3]
p.L424V	F156/F381 (F156_III:5) (♂)	CRC (27 y)	51–100 AP (37 y)	Duodenum + jejunum (APs)	—	Spier et al[4c]
p.L424V	F156 /F381 (F156_III:1) (♀)	-(>35 y)	>100 AP, HP (35 y)	Duodenum (APs), gastric fundic gland	—	Spier et al.[4c]
p.L424V	F156 /F381 (F156_III:3) (♀)	CRC (42 y)	Multiple APs (32 y)	Duodenum (APs)	—	Spier et al.[4c]

Table 2 Continued on next page

Table 2 Continued

POLE mutation	Family (ind) (gender)[a]	Tumors	Colonic polyps	Gastric polyps	MSI status	Publication
p.L424V	F156/F381 (F156_III:4) (♂)	5x CRC (30 y (4×), 46 y)	51–100 AP, HP (30 y)	Gastric fundic gland	—	Spier et al.[4c]
p.L424V	F156/F381 (F156_II:6) (♂)	4x CRC (36 y (2×), 56 y, 66 y)	51–100 AP (36 y)	Duodenum (APs)	MSS	Spier et al.[4c]
p.L424V	F156 /F381 (F156_III:1) (♂)	CRC (36 y), duodenal cancer (45 y)	21–50 (34 y)	0	MSS (duodenal cancer)	Spier et al.[4c]
p.L424V	F156 /F381 (F156_III:2) (♀)	-(>37 y)	21–50 AP (37 y)	0	—	Spier et al.[4c]
p.L424V	F156/F381 (F156_III:5) (♀)	-(>32 y)	11–20 AP (32 y)	Duodenum (1 AP), gastric fundic gland	—	Spier et al.[4c]
p.L424V	F354 (II:5) (♂)	3x CRC (27 y, 38 y, 39 y), neuroendocrine colon cancer (41 y), glioblastoma (47 y)	51–100 AP (27 y)	Duodenum (APs)	—	Spier et al.[4c]
p.L424V	F354 (III:5) (♂)	-(>16 y)	11–20 AP, HP (16 y)	0	—	Spier et al.[4c]
p.L424V	F1505 (index)	CRC (38 y)	51–100 AP (38 y)	0	MSS	Spier et al.[4c]
p.L424V	H1427 (III:1) (♂)	2x CRC (46 y, 48 y)	<10 AP	0	MSS	Spier et al.[4]
p.L424V	H1427 (II:2) (♀)	CRC (63 y)	<10 AP	0	—	Spier et al.[4]
p.L424V	H1427 (III:8) (♀)	3x CRC (45 y (2×), 53 y), ovarian cancer (33 y)	21–50 AP, HP	Duodenum (1 AP)	—	Spier et al.[4]

AP, adenomatous polyp; CRC, colorectal cancer; EC, endometrial cancer; HP, hyperplastic polyp; ind, individual; MSI, microsatellite instability; MSS, microsatellite stability; n/a, not available information; y, years; ♀, female; ♂, male.

[a]According to the corresponding publication. The gender is indicated whenever available in the corresponding publication. [b]PT2 was diagnosed with neurofibromatosis (originated in the family branch without *POLE* L424V). [c]Selection criteria for polyposis patients included in the study: ≥20 synchronous or ≥40 metachronous colorectal adenomas.

mutation identification in pooled samples (**Supplementary Table S1** online).

In silico prediction analysis

Protein damage prediction of missense genetic variants was performed by using the in silico algorithms PolyPhen-2, SIFT, Condel, and Mutation Taster.[6–9] dbNSFP (version 2.8) was used to obtain the evolutionary conservation PhyloP scores.[10] Possible alterations of the splice sites were evaluated using NNSplice0.9.[11]

Structural analysis

The human 3D model of POLD1 was obtained from ModBase (http://modbase.compbio.ucsf.edu/) and improved by the RepairPDB and Optimize commands of FoldX (http://foldx.crg.es). This model was calculated using as a template the crystallographic structure of the homologous yeast protein Pol3 (PDB ID: 3iay, chain A), which shares 51% sequence identity with the human POLD1.[12] Based on this model and domain annotations from the yeast protein Pol3, the exonuclease domain of *POLD1* comprises amino acids R311-L526. For the identified variants, protein stability calculations were performed using CUPSAT (http://cupsat.tu-bs.de), I-Mutant 2.0 (http://folding.biofold.org/i-mutant/i-mutant2.0.html), ERIS (http://troll.med.unc.edu/eris/), and PoPMuSiC (http://dezyme.com).

Loss of heterozygosity

Six microsatellite flanking *POLD1* and spanning 2.06 Mb, three centromeric (D19S867, D19S585, D19S904) and three telomeric (D19S246, D19S907, and D19S601), were used to assess loss of heterozygosity (LOH) in DNA extracted from formalin-fixed paraffin-embedded tissue. Also, SNaPshot targeting the corresponding *POLD1* mutation was used to assess LOH and to discriminate wild-type and mutated alleles. LOH was scored if the intensity of any allele was reduced by ≥50% relative to the other allele after taking account of the relative allelic intensities in paired constitutional DNA. Primers and conditions are shown in **Supplementary Table S1** online.

RESULTS AND DISCUSSION

Germ-line mutations in the exonuclease domain of *POLE* and *POLD1* in a series of familial CRC and/or polyposis

In a previous study, we analyzed by targeted genotyping the recurrent mutations *POLE* p.L424V and *POLD1* p.S478N in a Spanish familial CRC series, which included 438 MMR-proficient familial nonpolyposis (423 families) and 88 unrelated polyposis CRC patients, among others (series no. 1).[2] We identified a de novo *POLE* p.L424V in a CRC and polyposis patient with anaplastic oligodendroglioma recently diagnosed at age 30.

Here, we extended the analysis by sequencing the complete exonuclease domains of *POLE* and *POLD1* in the

Table 3 Phenotypic characteristics of the carriers of pathogenic mutations in the exonuclease domain of *POLD1* (literature and current study)

POLD1 mutation	Family (ind) (gender)[a]	Tumors	Colonic polyps	MSI status	Publication
p.S478N	SM6 (IV.2) (♂)	-(>65 y)	10 AP (43–65 y)	MSS (5 AP)	Palles et al.[1]
p.S478N	SM6 (IV.6) (♀)	EC (52 y)	28 AP, 2 HP (52–68 y)	—	Palles et al.[1]
p.S478N	SM6 (IV.8) (♀)	EC (45 y)	17 AP (53–65 y)	—	Palles et al.[1]
p.S478N	SM6 (V.2) (♂)	CRC (28 y)	28 AP (28–35 y)	MSS (tumor, 1 AP)	Palles et al.[1]
p.S478N	SM6 (V.10) (♂)	2 astrocytomas (26 y)	7 AP, 2 HP (33–45 y)	—	Palles et al.[1]
p.S478N	SM6 (V.12) (♂)	-(>39 y)	6 AP, multiple HP (29–39 y)	—	Palles et al.[1]
p.S478N	SM4 (III.2) (♀)	2x CRC (29 y, 51 y)	Multiple AP (48 y)	—	Palles et al.[1]
p.S478N	SM4 (III.6) (♀)	CRC (41 y), EC (50 y)	>19 AP (41–53 y)	—	Palles et al.[1]
p.S478N	SM4 (III.8) (♂)	CRC (32 y)	45 AP, 1 HP (32–52 y)	MSS (CRC)	Palles et al.[1]
p.S478N	SM4 (III.9) (♀)	CRC (33 y), EC (45 y)	40 AP (33–49 y)	—	Palles et al.[1]
p.S478N	n.a.	CRC (28 y)	n/a	—	Palles et al.[1]
p.L474P	Fig. 1B (II.2) (♀)	EC (52 y)	n/a	—	Valle et al.[2]
p.L474P	Fig. 1B (II.6) (♀)	CRC (33 y), EC (56 y)	0	—	Valle et al.[2]
p.L474P	Fig. 1B (III.1) (♀)	CRC (36 y), GIST (36 y)	0	—	Valle et al.[2]
p.D316H	Fam-1 (III.7) (♀)	Breast cancer (64 y)	11 AP, 2 HP	—	Current study
p.D316H	Fam-1 (III.8) (♂)	CRC (58 y), angiomyolipoma (65 y), mesothelioma (65 y)	3 AP, 1 HP	MSS (CRC)	Current study
p.V295M; D316G	Fam-2 (II.2) (♀)	EC (57 y), 2x breast cancer (65 y, 75 y)	n/a	MSS (EC)	Current study
p.D316G	Fam-2 (III.2) (♀)	CRC (44 y), EC (54 y)	2 AP, 1 HP	MSS (CRC)	Current study
p.R409W	Fam-3 (III.3) (♀)	CRC (32 y)	3 HP (39–41 y)	MSS (CRC)	Current study
p.L474P	Fam-4 (III.1) (♀)	-(50 y)	2 polyps	—	Current study
p.L474P	Fam-4 (III.2) (♂)	CRC (50 y)	0	—	Current study
p.L474P	Fam-4 (IV.1) (♀)	CRC (23 y), benign esophageal tumor (25 y)	0 (gastric HPs)	MSS and loss of MSH6 (CRC)	Current study

AP, adenomatous polyp; CRC, colorectal cancer; EC, endometrial cancer; HP, hyperplastic polyp; ind, individual; MSS, microsatellite stability; n/a, not available information y, years; ♀, female; ♂, male.

[a]According to the corresponding publication. The gender is indicated whenever available in the corresponding publication.

aforementioned patients, adding 18 additional MMR-proficient Amsterdam I–positive families, thus making a total of 456 MMR-proficient familial nonpolyposis CRC cases (441 families) and 88 polyposes. It corresponds to the largest series of hereditary nonpolyposis CRC where the complete exonuclease domains of *POLE* and *POLD1* have been studied.

Although no additional mutations were identified in *POLE*, six novel or rare nonsynonymous variants were detected in *POLD1*, five of them in previously genotyped patients. In silico predictions, evolutionary conservation, absence in control populations, functional effects on yeast,[13,14] and/or affectation of an exonuclease catalytic residue, strongly support the pathogenic effect of four of the six identified variants: *POLD1* p.D316H, p.D316G, p.R409W, and p.L474P (**Table 1**). Localization within the exonuclease domain of the amino acids affected by these mutations is shown in **Supplementary Figure S1** online. CRC was diagnosed in 62.5% (5/8) of mutation carriers, and both endometrial and breast cancers were diagnosed in 33.3% (2/6) of female carriers; one was diagnosed with two primary breast tumors (see complete tumor spectrum of mutated families in **Figure 1** and **Supplementary Table S2** online). Only one *POLD1* mutation carrier (1/8; 12.5%) was diagnosed with more than five colonic adenomas (cumulative resection of 11 adenomas until age 67), suggesting that *POLD1* mutations do not show strong association with polyposis and agreeing with the findings observed in mice.[15]

Not so evident are the functional effects of *POLD1* p.V295M and p.R521Q (minor allele frequencies ≤0.035%), which are classified as variants of unknown significance (**Table 1**). However, p.V295M, outside but close to the exonuclease domain (**Supplementary Figure S1** online), occurs in two independent affected families (**Figure 1**). In one of them, p.V295M is found in trans with p.D316G (catalytic residue) (individual Fam-2, II.2). The patient was diagnosed with endometrial cancer and two metachronous breast tumors. To date, no biallelic mutation in *POLE* or *POLD1* has been reported. Considering the likely pathogenic variants identified in this study, *POLD1* exonuclease mutations account for 1% (4/411) of MMR-proficient familial/early-onset nonpolyposis CRC cases, including 1.7% (1/60) of Amsterdam-positive cases (familial CRC type X).

Table 4 Common phenotypic features observed in carriers of pathogenic mutations in the exonuclease domains of *POLE* and *POLD1* reported to date[1–4] and in the current study, and clinical suspicion or recommended guidelines for genetic testing

Observed features	Guidelines for genetic testing
POLE exonuclease mutation carriers (*n* = 47; 20 families)	*POLE*
Colonic adenomas: Mean: 19.3 (range: 1–68; *n* = 23 carriers)[a]	Attenuated adenomatous polyposis (20–100 adenomas)
≥2 adenomas: 81.8% (27/33) carriers	Amsterdam I (only CRC)
≥5 adenomas: 73.9% (17/23) carriers	CRC and oligopolyposis (5–20 adenomas), both diagnosed before age 50
CRC: 63.8% (30/47) carriers. Mean age at first diagnosis: 40.7	CRC or oligopolyposis (5–20 adenomas) and first-degree relative with CRC diagnosed before age 50
Duodenal adenomas: 50% (7/14) carriers[b]	CRC or oligopolyposis (5–20 adenomas) and two or more first- or second-degree relatives with CRC, regardless of age
Brain tumor: 6.4% (3/47) carriers. Mean age at diagnosis: 30.6	—
POLD1 exonuclease mutation carriers (*n* = 22; 8 families)	*POLD1*
Colonic adenomas: Mean: 12.3 (range: 0–45; *n* = 16 carriers)	Attenuated adenomatous polyposis (20–100 adenomas)
≥2 adenomas: 63.6% (14/22) carriers	Amsterdam II (only CRC and EC)
≥5 adenomas: 55.5% (10/18) carriers	CRC before age 50 or EC before age 60, and oligopolyposis (5–20 adenomas) diagnosed before age 50
CRC: 59.1% (13/22) carriers. Mean age at first diagnosis: 35.9	CRC or EC or oligopolyposis (5–20 adenomas) and first-degree relative with CRC before age 50 or EC before age 60
EC: 57.1% (8/14) female carriers. Mean age at diagnosis: 51.4	CRC or EC or oligopolyposis (5–20 adenomas) and two or more first- or second-degree relatives with CRC or EC, regardless of age
Breast cancer: 14.3% (2/14) female carriers	—
Brain tumor: 4.5% (1/22; two synchronic at age 26)	Pending additional evidence, the presence of brain and/or breast tumors in the context of the characteristic features (CRC, polyposis, and/or endometrial cancer) may be an indicator of the polymerase proofreading-associated syndrome

Tumor MMR deficiency should not be an exclusion criterion based on its identification in two colorectal tumors developed by *POLE* mutation carriers.[3] A preliminary screening of the recurrent mutations *POLE* p.L424V, *POLD1* p.S478N, and *POLD1* p.L474P may be considered. When using gene panels (next-generation sequencing), the proposed guidelines may be used for prioritization purposes in the data analysis.

CRC, colorectal cancer; EC, endometrial cancer; MMR deficiency, mismatch repair deficiency (it includes microsatellite instability and/or loss of expression the MMR proteins).

[a]Data from Spier et al.[4] is not included because the number of adenomas is given in ranges (e.g., 21–50 or 21–100). [b]The presence of upper gastrointestinal findings has not been systematically evaluated in *POLD1* exonuclease mutation carriers yet.

Review of the phenotypic data from the reported carriers of *POLE* and *POLD1* exonuclease pathogenic mutations

Phenotypic data from the 69 carriers (29 families) of *POLE/POLD1* exonuclease pathogenic mutations reported to date (refs. 1–4 and in the present study) are recapitulated in **Table 2** (*POLE*) and **Table 3** (*POLD1*) and are summarized according to the mutated gene in **Table 4**. Altogether, available information point to an associated phenotype characterized by attenuated or oligo-adenomatous colorectal polyposis (>80% of *POLE* and >60% of *POLD1* mutation carriers were diagnosed with ≥2 adenomas; on average, 16 adenomas), CRC (60–64% of carriers), and probably brain tumors (5.8%). Gastroduodenal (mostly duodenal) adenomas were detected in 57.1% of carriers who underwent gastroduodenoscopies (only 14 *POLE* exonuclease mutation carriers evaluated).[4] Moreover, the *POLD1* phenotypic spectrum includes endometrial (57.1% of female carriers) and breast tumors (14.3% of female carriers). All 21 *POLE/POLD1* mutation carriers without cancer underwent resection of colorectal adenomas, indicating complete or very high expressivity of the associated phenotype (associated carcinomas and/or adenomas), but precluding any conclusion about CRC penetrance.

Ascertainment bias due to the inclusion of CRC and/or polyposis families in the studies may have led to overrepresentation or under-representation of the *POLE/POLD1*–associated tumors. In particular, the high prevalence of endometrial cancer in *POLD1* families might be biased by the inclusion of families fulfilling the Amsterdam or Bethesda criteria. However, the fact that endometrial cancer is extremely rare among female *POLE* mutation carriers (1/26 compared to 8/14 female *POLD1* carriers) supports its relevance in the *POLD1*-associated phenotype.

Recommendations for genetic testing and clinical surveillance

Clinical suspicion of the polymerase proofreading-associated syndrome may arise when the clinical characteristics depicted in **Table 4** (left column) are fulfilled. Due to the current need of genetic testing guidelines for *POLE/POLD1*, we attempt to define the first preliminary recommendations for their use in the routine practice. Based on the overlapping phenotypes of this entity with Lynch syndrome and attenuated adenomatous polyposis (*APC/MUTYH*),[16] we took as a model the criteria for hereditary nonpolyposis CRC (revised Bethesda), adapted them to the attenuated or oligo-polyposis scenario, and took into consideration additional specific *POLE/POLD1* characteristics. The resulting recommendations are shown in **Table 4** (right column). Although preliminary, they will help guide in routine genetic testing and counseling until larger series of mutation carriers are described and standardized guidelines defined.

The implementation in routine genetic testing of targeted next-generation sequencing using multi-gene panels will help alleviate the issue of overlapping phenotypes among familial CRC and polyposis syndromes. In this context, the proposed guidelines may be used for prioritization purposes in the analysis of data.

More extensive phenotypic data from mutation carriers are needed to establish standardized surveillance recommendations. In the meantime, based on the clinical features of *POLE* and *POLD1* mutation carriers (**Table 4**) and the guidelines recommended for Lynch syndrome and attenuated adenomatous polyposis,[17] whose features largely overlap with those observed in the polymerase proofreading-associated syndrome, we recommend colonoscopies every 1–2 years and gastroduodenoscopies every 3 years, starting at age 20–25 (reevaluate periodicity according to the findings), adding endometrial cancer screening beginning at age 40 for *POLD1* female carriers. The predisposition to breast tumors may eventually influence the age to start and the frequency of mammogram screenings. For both *POLE* and *POLD1* mutation carriers, the possibly increased susceptibility to brain tumors may be taken into consideration.

SUPPLEMENTARY MATERIAL

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

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DISCLOSURE

The authors declare no conflict of interest.

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