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REVIEW

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Contribution to colonic polyposis of recently proposed predisposing genes and assessment of the prevalence of *NTHL1*- and *MSH3*-associated polyposes

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Abstract

Technological advances have allowed the identification of new adenomatous and serrated polyposis genes, and of several candidate genes that require additional supporting evidence of causality. Through an exhaustive literature review and mutational screening of 177 unrelated polyposis patients, we assessed the involvement of *MCM9*, *FOCAD*, *POLQ*, and *RNF43* in the predisposition to (nonserrated) colonic polyposis, as well as the prevalence of *NTHL1* and *MSH3* mutations among genetically unexplained polyposis patients. Our results, together with previously reported data and mutation frequency in controls, indicate that: *MCM9* and *POLQ* mutations are not associated with polyposis; germline *RNF43* mutations, with a prevalence of 1.5–2.5% among serrated polyposis patients, do not cause nonserrated polyposis; *MSH3* biallelic mutations are highly infrequent among European polyposis patients, and the prevalence of *NTHL1* biallelic mutations among unexplained polyposes is ~2%. Although nonsignificant, *FOCAD* predicted deleterious variants are overrepresented in polyposis patients compared to controls, warranting larger studies to provide definite evidence in favor or against their causal association with polyposis predisposition.

KEYWORDS
adenomatous polyposis, FOCAD, genetic predisposition, hereditary cancer, MCM9, MSH3, NTHL1, POLQ, RNF43

1 | INTRODUCTION

Familial adenomatous polyposis is a hereditary colorectal cancer (CRC) syndrome characterized by dozens to hundreds of colorectal adenomas that may potentially develop into CRC. This syndrome is caused by heterozygous germline mutations in *APC*, a tumor suppressor gene involved in the canonical Wnt signaling pathway. Germline mutations affecting the proofreading activity of polymerases epsilon (*POLE*) and delta (*POLD1*) also predispose to adenomatous polyposis, causing the autosomal dominant syndrome called polymerase proofreading-associated polyposis. Autosomal recessive forms of polyposis are caused by biallelic inactivation of the base excision repair genes *MUTYH* and *NTHL1* and of the DNA mismatch repair genes *MSH3*, *PMS2*, *MSH6*, *MSH2*, *MLH1*, and *MLH3*. Other genes, such as *GREM1* and *RNF43* have been associated with mixed and serrated polyposis respectively, and different forms of hamartomatous polyposes are caused by germline mutations in *SMAD4* and *BMPR1A* (Juvenile polyposis), *STK11* (Peutz Jeghers), or

PTEN (PTEN-hamartoma-tumor syndromes) (reviewed by Olkinuora et al., 2018; Valle, 2014, 2017).

In the last years, thanks to the accessibility to genome-wide sequencing and copy number technologies, several candidate polyposis genes have been proposed. However, the evidence gathered supporting their implication in the predisposition to polyposis remains inconclusive, such being the case for *MCM9*, *FOCAD*, and *POLQ* (Goldberg et al., 2015; Raskin et al., 2017; Weren, Venkatachalam, et al., 2015). Although known to be rare, some of the newly described polyposis genes, such as *MSH3* (Adam et al., 2016), lack of reported data on their actual frequency among polyposis cases without mutations in the classically associated polyposis genes (i.e., *APC* and *MUTYH*), or as *NTHL1*, this information is scarce in the literature (Grolleman et al., 2019). On the contrary, five of the 13 reported carriers of *RNF43* mutations, in addition to the serrated/hyperplastic polyps, had developed several adenomas, suggesting a potential implication of *RNF43* in the predisposition to adenomatous polyposis and/or polyposis of multiple polyp types (reviewed by Quintana et al., 2018).

By reviewing the previously reported mutated cases, supported by results obtained from the mutational screening of 177 additional unexplained polyposis patients, here we aim at providing a more definitive answer about (a) the actual contribution of germline mutations in *MCM9*, *FOCAD*, *POLQ*, and *RNF43* to adenomatous polyposis and polyposis of multiple polyp types, and (b) the prevalence of *MSH3* and *NTHL1* biallelic mutations among polyposis patients.

Description of the patients and methods used for gene mutational screening, in silico predictions and statistical analyses, is shown in the Supporting Information Material.

2 | *MCM9* BIALLELIC OR MONOALLELIC (PREDICTED) PATHOGENIC VARIANTS DO NOT CAUSE COLONIC POLYPOSIS

MCM9 encodes a DNA helicase that, together with MCM8, plays an essential role in homologous recombination (HR) DNA repair and maintenance of DNA replication forks (Lutzmann et al., 2012). The helicase activity of MCM9 may also be necessary to repair DNA mismatches by the MMR pathway (Traver et al., 2015). Homozygous mutations in *MCM9* have been associated with predisposition to premature ovarian insufficiency (POI) and hereditary mixed polyposis (Desai et al., 2016; Goldberg et al., 2015; Wood-Trageser et al., 2014). Specifically, by using a combination of whole-genome homozygosity mapping and both exome and targeted gene sequencing, c.672_673delGGinsC (p.E225Kfs*4) was identified in homozygosity in two sisters from a consanguineous Ashkenazi family, exhibiting POI, early-onset mismatch repair (MMR)-proficient metastatic CRC and multiple types of colorectal polyps (Goldberg et al., 2015). In this family, five heterozygous carriers were identified, but clinical history was only available for four of them, one of whom had developed >10 colonic lesions (>10 hyperplastic and adenomatous polyps between ages 53 and 65). Wood-Trageser et al. identified two putatively pathogenic *MCM9* variants in homozygosity in two unrelated POI-affected consanguineous Turkish families of Kurdish ethnicity with no reports of colonic lesions. To further analyze *MCM9* implication in POI, the authors investigated the prevalence of *MCM9* variants in a cohort of 109 women affected by idiopathic POI, a condition characterized by amenorrhea, infertility, hypoestrogenism, and elevated follicle-stimulating hormone serum levels. A nonsense mutation and a predicted damaging variant were found in heterozygosity in two unrelated patients (Wood-Trageser et al., 2014). In a recent study from the same group, *MCM8* and *MCM9* were screened in a cohort of 173 women diagnosed with POI (either with primary or secondary amenorrhea), finding a homozygous *MCM9* nonsense variant in a patient born of a consanguineous marriage, and seven heterozygous variants: one nonsense, one splice site, one missense predicted damaging, and four missense predicted neutral according to REVEL prediction tool (metapredictor; cutoff score: 0.5; Ghosh, Oak, & Plon, 2017; Ioannidis et al., 2016), in nine patients. One of the patients carried two heterozygous variants (one splice-site and one predicted neutral) but their *cis*/*trans* phase was not determined. Another *MCM9* heterozygous carrier (c.911A>G; p.N304S) also harbored a predicted neutral (REVEL) variant in *MCM8* (c.1561G>A; p.D521N) (Desai et al., 2016). That same year, a homozygous nonsense mutation (c.1483G>T; p.E495*) was identified in two POI-affected sisters from a consanguineous Middle Eastern family of Arab origin (Fauchereau et al., 2016). A detailed description of the reported *MCM9* mutation carriers is shown in Table 1. Despite the potential association of *MCM9* mutations with polyposis described by Goldberg et al. (2015), the screening of the gene in polyposis patients has never been reported.

In the herein studied cohort (177 unrelated patients with nonfiliated polyposis), no homozygous or compound heterozygous *MCM9* mutation carriers were identified. Only one variant, c.911A>G (p.N304S; population minor allele frequency, $MAF_{GnomAD} = 0.35\%$), predicted benign, was identified in heterozygosity in an individual with 21–50 polyps and with relatives affected with colorectal polyposis, CRC, and breast and cervix tumors (Table S1). This variant had been previously reported in heterozygosity in two patients with POI, but its role in the disease causation was unknown (Desai et al., 2016). The relatively high frequency of this variant in population-based databases ($MAF_{GnomAD} = 0.35\%$), the lack of predicted impact on protein functionality, and the lack of colonic findings in the two carriers with POI suggest no causal association of c.911A>G with polyposis.

Taking into account the previously reported data and our study (Table 1), only two out of 25 heterozygous carriers of *MCM9* variants were diagnosed with more than five polyps, one of them, carrier of the putatively benign c.911A>G variant, thus supporting lack of association with polyposis predisposition.

If *MCM9* were a polyposis predisposing gene in heterozygosity, one would expect a higher frequency of *MCM9* mutations in polyposis cohorts (such as ours) than in cancer-free population. To evaluate the presence of germline *MCM9* variants in European population, we analyzed the publicly available data from 4,300 Caucasians (European American) analyzed for ESP6500SI-V2 (not

TABLE 1 Clinical and molecular characteristics of the carriers of *MCM9* (NM_017696) variants reported to date

Germline variant,[a] dbSNP (GnomAD MAF %)	REVEL prediction[b]	Allelic state	Phenotype [age at diagnosis]	Frequency in controls[c] (%)	Variant classification (ACMG/AMP guidelines)[d]	References
c.672_673delGGinsC (p.E225Kfs*4) (n.a.)	n.a.	Homozygous	69 polyps (mixed, HP, SA, and Ad.) [34], mCRC [34]; 3–5 polyps [35–37], and POI[e]	0/4,300 (0.000%)	Likely pathogenic	Goldberg et al. (2015)
		Homozygous	<20 polyps (HP, Ad., and 1 with intramucosal carcinoma), mCRC [37], and POI[e]			
		Heterozygous	Clinical data not available[e]			
		Heterozygous	Normal[e]			
		Heterozygous	>10 polyps (HP and Ad.) [53–65][e]			
		Heterozygous	1 Ad. [83][e]			
		Heterozygous	2 Ad. [39][e]			
c.1732+2T>Crs587777871 (0.0139%)	n.a.	Homozygous	POI	0/4,300 (0.000%)	Likely pathogenic	Wood-Trageser et al. (2014)
		Homozygous	POI			
		Heterozygous	Normal			
		Heterozygous	Normal			
		Heterozygous	Normal			
		Heterozygous	Normal (male)			
c.394C>T (p.R132*) rs587777872 (0.0081%)	n.a.	Homozygous	POI	0/4,300 (0.000%)	Pathogenic	
		Heterozygous	Normal			
		Heterozygous	Normal			
		Heterozygous	Normal			
		Heterozygous	Normal (male)			
c.686T>G (p.V229G) rs200078427 (0.0978%)	D (0.727)	Heterozygous	POI	1/4,300 (0.023%)	Uncertain significance	
c.1533C>A (p.Y511*) rs587777873 (n.a)	n.a.	Heterozygous	POI	0/4,300 (0.000%)	Likely pathogenic	
c.1651C>T (p.Q551*) rs1165131021 (n.a.)	n.a.	Homozygous	POI (primary amenhorrea)	0/4,300 (0.000%)	Pathogenic	Desai et al. (2016)
c.905−1G>T and c.1784C>G (p.T595R)[f]	n.a.	Compound heterozygous	POI	1/4,300 (0.023%)	Uncertain significance	
rs149099524 (0.0008%) and n.a.	N (0.217)			0/4,300 (0.000%)	Uncertain significance	
c.686T>G (p.V229G) rs200078427 (0.0978%)	D (0.727)	Heterozygous	POI (secondary amenorrhea) [31]	1/4,300 (0.023%)	Uncertain significance	
c.911A>G (p.Asn304Ser) rs78231991 (0.3542%)	N (0.299)	Heterozygous	POI (primary amenorrhea). Carrier of a predicted-neutral (REVEL) variant in heterozygosis in *MCM8* (c.1561G>A; p.D521N)	35/4,300 (0.814%)	Likely benign	
	N (0.299)	Heterozygous	POI (secondary amenorrhea) [24]			

(Continues)

TABLE 1 (Continued)

Germline variant,[a] dbSNP (GnomAD MAF %)	REVEL prediction[b]	Allelic state	Phenotype [age at diagnosis]	Frequency in controls[c] (%)	Variant classification (ACMG/AMP guidelines)[d]	References
c.1974G>T (p.Q658His) rs78791427 (0.8024%)	N (0.092)	Heterozygous	POI (secondary amenorrhea) [24]	0/4,300 (0.000%)	Likely benign	
c.2011G>T (p.E670*) (n.a.)	n.a.	Heterozygous	Secondary amenorrhea (never diagnosed with POI)	0/4,300 (0.000%)	Uncertain significance	
		Heterozygous	POI (secondary amenorrhea) [23]			
c.2422G>A (p.V808Ile) (n.a.)	N (0.072)	Heterozygous	POI (primary amenorrhea)	0/4,300 (0.000%)	Likely benign	
c.1483G>T (p.E495*) rs1060505058 (n.a.)	n.a.	Homozygous	POI (primary amenorrhea secondary to hypogonadism, complicated by severe osteoporosis) [15]	0/4,300 (0.000%)	Pathogenic	Fauchereau et al. (2016)
		Homozygous	POI (primary amenorrhea)			
		Heterozygous	Normal			
		Heterozygous	Normal			
		Heterozygous	Normal (male)			
c.911A>G (p.N304S) rs78231991 (0.3542%)	N (0.299)	Heterozygous	21–50 Ad. [50]	35/4,300 (0.814%)	Likely benign	Current study

Note: Alternative row shading depicts individual families.

Abbreviations: ACMG/AMP, American College of Medical Genetics and Genomics and Association for Molecular Pathology; Ad., adenoma; CRC, colorectal cancer; D, damaging; ESP, NHLBI GO Exome Sequencing Project; HP, hyperplastic polyp; MAF, minor allele frequency; mCRC, metastatic CRC; N, neutral; n.a., not available information; POI, premature ovarian insufficiency; SA, serrated adenoma.

[a]Only those variants predicted pathogenic by the authors from the original papers have been included.

[b]REVEL: Score ranges from 0 to 1. The larger the score the more likely the mutation may cause functional change; >0.5, predicted-damaging.

[c]Frequency in controls was calculated from ESP6500SI-V2 considering stop-gain, frameshift, splice-site or predicted pathogenic (REVEL) missense variants. ESP6500SI-V2 public data was released by Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (http://evs.gs.washington.edu/EVS/).

[d]ACMG/AMP guidelines (Richards et al., 2015) for variant classification were applied using InterVar software (Li and Wang, 2017). A detailed description of the evidence used for variant classification is shown in Table S2.

[e]Carrier of a predicted-neutral (REVEL) variant in homozygosis in *MUTYH* (c.972G>C; p.Q324H).

[f]Undetermined whether the two variants are located in *cis* or *trans*.

related to cancer), and considered all individuals carrying *MCM9* stop-gain, frameshift, splice-site, and predicted pathogenic (REVEL) missense changes, as well as variants affecting canonical splice-sites, with MAF_{GnomAD} <1%. Under these premises, seven of the 4,300 control individuals (0.16%) were carriers of *MCM9* (predicted) deleterious variants, whereas no (predicted) deleterious variants were identified among the 177 polyposis patients herein analyzed, thus suggesting lack of association of heterozygous *MCM9* mutations with polyposis predisposition. On the contrary, a total of nine homozygous or compound heterozygous *MCM9* mutation carriers (5 families)—all of them with POI—have been reported, two of whom (22%) were diagnosed with polyposis (20–75 polyps of different histologies between ages 30–40). These two patients belong to the same family, where another relative had been diagnosed with >10 polyps but did not carry the *MCM9* mutation in homozygosity. In conclusion, being this the only *MCM9* biallelic mutation carrier family where POI coincides with colonic polyposis (out of 5), together with the fact that one polyposis-affected member of that family is not homozygous for the *MCM9* variant, suggest that most likely other genetic factors are causing the increased polyposis risk in the family.

3 | *FOCAD* (PREDICTED) PATHOGENIC VARIANTS ARE MORE FREQUENT IN POLYPOSIS PATIENTS THAN IN CONTROLS

FOCAD is a tumor suppressor gene that encodes focadhesin, a component of the focal adhesion complex whose disruption, due to somatic homozygous deletions, has been observed in 50% of gliomas and breast tumors (Brockschmidt et al., 2012; Natrajan et al., 2012). Venkatachalam et al. (2011) identified a deletion involving 158 kb in the cytoband 9p21.3 and affecting both *FOCAD* and *hsc-miR491*, in one out of 41 individuals with early-onset or familial CRC. CNV (copy number variation) analysis by targeted high-throughput sequencing in 221 unrelated German patients with unexplained but histologically confirmed colorectal adenomatous polyposis revealed a heterozygous germline deletion affecting exons 20–30 in a patient with 100–500 adenomas (Horpaopan et al., 2015). Two additional (somatic) mutations in *FOCAD* were found in an adenoma of this patient (Table 2). Further analyses in the same cohort revealed several changes in heterozygosity: a stop-gain mutation (c.715C>T; p.Q239*) in a CRC patient affected with attenuated polyposis; and a variant predicted to create a new acceptor site (c.3079–8T>A) in an individual with 11–20 colorectal polyps and CRC (Horpaopan et al., 2015). In a cohort of 1,232 early onset or familial CRC, which included 248 Dutch and German individuals (some of them included in Venkatachalam et al., 2011) and 984 English patients, two different deletions in heterozygosity in the *FOCAD locus* disturbing the open-reading frame of the gene, were identified in two a priori unrelated individuals with personal or family history of CRC and polyposis (Weren, Venkatachalam, et al., 2015). No somatic second hits were found in any of the two *FOCAD* deletion carriers' tumors. Additionally, Weren, Venkatachalam, et al. (2015) performed a mutational screening of *FOCAD* in 117 individuals of the 248-individuals initial cohort and in 33 polyposis cases. Three missense variants were identified in heterozygosity in three polyposes and five early-onset CRC patients, and nine missense variants were found in 10 early-onset CRC patients (Table 2).

In our cohort, no homozygous or compound heterozygous carriers of a novel or rare genetic variants or large deletions in *FOCAD* were identified. Likewise, no heterozygous carriers of pathogenic or predicted pathogenic variants or of copy number alterations were detected. Four heterozygous predicted-neutral (REVEL) missense variants in four unrelated polyposis patients were found (Tables 2 and S1).

Taking into account the previously reported data on CNVs in polyposis cohorts and the current study, only one patient suffering from attenuated adenomatous polyposis carried a large deletion in the *FOCAD* gene (1/398; 0.25%; Horpaopan et al., 2015 and current study). When considering the 2,504 unrelated individuals from 26 populations included in the 1000 Genomes Project, four individuals carried deletions in the *FOCAD* gene affecting >1 Kb of coding exons sequence (6/2,504; 0.24%; Gibbs et al., 2015); a similar proportion than that observed among polyposis patients, therefore suggesting a lack of association with polyposis (Table 2).

On the contrary, while two carriers of nonsense and splice-site variants were identified among 431 polyposis patients subjected to *FOCAD* mutational screening (0.46%; Horpaopan et al., 2015; Weren, Venkatachalam, et al., 2015), 12 of the 4,300 ESP6500SI-V2 controls (0.28%) carried stop-gain, frameshift, splice-site, or predicted pathogenic (REVEL) missense variants ($p = .3691$; Fisher's Exact test; Table 2). Although nonsignificant, the higher frequency of mutations among polyposis patients suggests a potential association with polyposis predisposition. *FOCAD* mutational screening in additional polyposis cases is warranted to definitively confirm or discard this association.

4 | *POLQ* (PREDICTED) PATHOGENIC VARIANTS ARE NOT ASSOCIATED WITH POLYPOSIS

POLQ encodes the DNA polymerase theta (Pol θ) that is required to repair double-strand breaks (DSBs) by microhomology search and removal of nonhomologous tails (θ-mediated end joining, TMEJ). Although it does not compete with NHEJ or HR, TMEJ has an essential role in rescuing DSBs when resection is misregulated or NHEJ is compromised, thus helping sustain cell viability and genome stability (Wyatt et al., 2016). *POLQ* expression is upregulated in human cancers such as colorectal and breast tumors, being also related to poor prognosis in the later (Lemée et al., 2010; Pillaire et al., 2010). Heterozygous missense germline variants in the *POLQ* gene have been identified in both CRC and polyposis patients (Ciavarella et al., 2018; Raskin et al., 2017; Table 3).

By using pooled DNA targeted NGS of *POLQ* in 1,046 familial CRC patients, Raskin et al. (2017) identified two predicted pathogenic heterozygous variants. The variant c.7259A>G (p.Y2420C), located in the polymerase domain, was found in a 50-year-old woman with CRC.

TABLE 2 Clinical and molecular characteristics of the carriers of *FOCAD* (NM_017794) variants reported to date

Germline mutations,[a] dbSNP (GnomAd MAF %)	REVEL prediction[b]	Phenotypes	Tumor/Adenoma molecular features	Frequency of SNVs/INDELs or CNV (%)		Variant classification (ACMG/AMP guidelines)[e]	References
				Polyposes	Controls[c,d]		
c.715C>T (p.Q239*) (n.a.)	n.a.	Polyposis (attenuated; 51–100 Ad.; 52) and CRC. Carrier of a *CTNNB1* whole-gene duplication.	n.a.	1/431 (0.232%)	0/4,300 (0.000%)	Uncertain significance	Horpaopan et al. (2015)
Deletion exons 20–30 (n.a.)	n.a.	Polyposis (attenuated; 100–500 Ad.; 60)	Second hit mutations in Ad.: c.1951C>T (p.L651F) and c.5332+1G>A	1/398 (0.251%)	n.p.	n.a.	
c.3079–8T>A (n.a.)	n.a.	Polyposis (attenuated; 11–20 Ad.; 72) and CRC	n.a.	1/431 (0.232%)	0/4,300 (0.000%)	Uncertain significance	
Deletion exons 4–23 (n.a.)	n.a.	>20 polyps and CRC (33)	n.a.	1/398 (0.251%)	n.p.	n.a.	Venkatachalam et al. (2011)
Deletion exons 2–14 n.a.	n.a.	1 Ad. and CRC (62)	No second hits in FOCAD found	1/398 (0.251%)	n.p.	n.a.	Weren, Venkatachalam, et al. (2015)
Deletion exons 7–20 n.a.	n.a.	Constitutive polyp development (64)	No second hits in FOCAD found	1/398 (0.251%)	n.p.	n.a.	
c.2405C>T (p.A802V) rs79849792 (0.3189%)	N (0.037)	Polyposis	n.a.	1/431 (0.232%)	47/4,300 (1.093%)	Uncertain significance	
		Early-onset CRC	n.a.	Nonpolyposis cohort			
c.4999G>A (p.D1667N) rs117405838 (1.595%)	N (0.065)	Polyposis	n.a.	1/431 (0.232%)	140/4,300 (3.256%)	Uncertain significance	
		Early-onset CRC	n.a.	Nonpolyposis cohort			
		Early-onset CRC	n.a.	Nonpolyposis cohort			
		Early-onset CRC	n.a.	Nonpolyposis cohort			
c.5047G>A (p.A1683T) rs150147497 (0.847%)	N (0.043)	Polyposis	n.a.	1/431 (0.232%)	109/4,300 (2.535%)	Uncertain significance	
		Early-onset CRC	n.a.	Nonpolyposis cohort			
c.2104A>G (p.K702E) rs151286548 (0.1985%)	N (0.092)	Early-onset CRC	n.a.	Nonpolyposis cohort	39/4,300 (0.907%)	Uncertain significance	
c.2276A>T (p.Y759F) rs200978429 (0.0041%)	N (0.189)	Early-onset CRC	n.a.	Nonpolyposis cohort	0/4,300 (0.000%)	Uncertain significance	
c.2600A>G (p.Q867R) rs117863779 (0.4858%)	N (0.239)	Early-onset CRC	n.a.	Nonpolyposis cohort	2/4,300 (0.047%)	Uncertain significance	
c.3253G>A (p.V1085M) rs143564901 (0.0559%)	N (0.369)	Early-onset CRC	n.a.	Nonpolyposis cohort	3/4,300 (0.069%)	Uncertain significance	

(Continues)

TABLE 2 (Continued)

Germline mutations,[a] dbSNP (GnomAd MAF %)	REVEL prediction[b]	Phenotypes	Tumor/Adenoma molecular features	Frequency of SNVs/INDELs or CNV (%)		Variant classification (ACMG/AMP guidelines)[e]	References
				Polyposes	Controls[c,d]		
c.3335A>C (p.E1112A) rs147046649 (0.9802%)	N (0.080)	Early-onset CRC	n.a.	Nonpolyposis cohort	16/4,300 (0.372%)	Uncertain significance	
		Early-onset CRC	n.a.	Nonpolyposis cohort			
c.3937A>G (p.T1313A) (n.a.)	N (0.042)	Early-onset CRC	n.a.	Nonpolyposis cohort	0/4,300 (0.000%)	Uncertain significance	
c.4495C>T (p.P1499S) rs117591845 (0.3602%)	N (0.064)	Early-onset CRC	n.a.	Nonpolyposis cohort	62/4,300 (1.442%)	Uncertain significance	
c.4979C>T (p.S1660F) rs1211734303 (0.0000%)	N (0.085)	Early-onset CRC	n.a.	Nonpolyposis cohort	0/4,300 (0.000%)	Uncertain significance	
c.5362G>C (p.E1788Q) rs745594672 (0.0032%)	N (0.242)	Early-onset CRC	n.a.	Nonpolyposis cohort	0/4,300 (0.000%)	Uncertain significance	
c.401C>T (p.P134L) rs143814736 (0.0149%)	N (0.208)	18–30 ad. and HP (69) *MUTYH* heterozygote	n.a.	1/431 (0.232%)	3/4,300 (0.069%)	Uncertain significance	Current study
c.1393G>A (p.G465R) rs112541381 (0.0122%)	N (0.363)	21–50 polyps and CRC × 3 (71)	n.a.	1/431 (0.232%)	1/4,300 (0.093%)	Uncertain significance	
c.2861C>T (p.P954L) rs200166806 (0.0332%)	N (0.395)	13 ad. (tubular), 1 polyp and CRC (67)	n.a.	1/431 (0.232%)	0/4,300 (0.000%)	Uncertain significance / Likely pathogenic	
c.3041A>G (p.Y1014C) rs137931934 (0.1649%)	N (0.217)	51–100 ad. and CRC × 2 (rectum and colon, 61)	n.a.	1/431 (0.232%)	8/4,300 (0.186%)	Uncertain significance	
			Total PREDICTED-DAMAGING SNV/INDEL frequency	2/431 (0.464%)	12/4,300 (0.279%)	Uncertain significance	All references except Venkatachalam et al. (2011)
			Fisher's test (*p* value)	0.3691			
			Total CNV frequency	1/398 (0.251%)	6/2,504 (0.239%)	No association with polyposis	Horpaopan et al. (2015) and the current study
			Fisher's test (*p* value)	1.000			

Note: Alternative row shading depicts individual families.

Abbreviations: Ad., adenoma; CNV, copy number variant; CRC, colorectal cancer; D, damaging; INDELs, insertion and deletions of few nucleotides; HP, hyperplastic polyp; MAF, minor allele frequency; N, neutral; n.a., not available information; n.p., not present; SNV, single-nucleotide variant.

[a]Only those variants predicted pathogenic by the authors from the original papers have been included.

[b]REVEL: Score ranges from 0 to 1. The larger the score the more likely the mutation may cause functional change; >0.5, predicted-damaging.

[c]Frequency of SNV/INDELs in controls was calculated from ESP6500SI-V2 considering stop-gain, frameshift, splice-site, or predicted pathogenic (REVEL) missense variants. ESP6500SI-V2 public data was released by Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA.

[d]Frequency of CNV in *FOCAD* was obtained from 2,504 unrelated individuals from 26 populations included in the 1000 Genomes Project (Gibbs et al., 2015).

[e]ACMG/AMP guidelines for variant classification (InterVar software; Li and Wang, 2017). A detailed description of the evidence used for variant classification is shown in Table S2.

TABLE 3 Clinical and molecular characteristics of the carriers of *POLQ* (NM_199420) rare/novel variants reported to date (all of them are heterozygous)

Germline mutations,[a] dbSNP (GnomAd MAF%)	REVEL prediction[b]	Phenotype [age at diagnosis]	Tumor/Adenoma molecular features	Frequency in polyposis (%)	Frequency in controls[c] (%)	Variant classification (ACMG/AMP guidelines)[d]	References
c.6743A>G (p.Asn2248Ser) rs376729696 (0.0126%)	D (0.674)	>50 Ad. Carrier of: *APC*: c.3468_3470delAGA (p.E1157del); *EXO1*: c.2212−1G>G; *OGG1*: c.923G>A (p.G308E)	No LOH in Ad.	1/185 (0.540%)	0/4,300 (0.000%)	Uncertain significance	Ciavarella et al. (2018)
c.7259A>G (p.Y2420C) rs150364457 (0.0318%)	D (0.936)	Colon [50]	MSS and IHC-MMR positive	Nonpolyposis cohort	4/4,300 (0.093%)	Uncertain significance	Raskin et al. (2017)
c.872C>T (p.P291L) rs1003257917 (0.0203%)	D (0.523)	Colon [24]	MSI-H and IHC-MSH2/MSH6 negative	Nonpolyposis cohort	0/4,300 (0.000%)	Uncertain significance	
		CRC [55] and polyps	MSS and IHC-MMR positive	Nonpolyposis cohort			
c.6892C>T (p.Q2298*) rs749996073 (0.0004%)	n.a.	CRC and 10 polyps [34]	n.a.	Nonpolyposis cohort	0/4,300 (0.000%)	Uncertain significance	Rosenthal et al. (2018)
c.2021dup (p.K675Efs*16) rs768008424 (0.0029%)	n.a.	20 polyps [44]	n.a.	Nonpolyposis cohort	1/4,300 (0.023%)	Uncertain significance	
c.4684G>T (p.D1562Y) rs3218643 (0.1253%)	N (0.168)	10–20 Ad. [42]	n.a.	1/185 (0.540%)	1/4,300 (0.023%)	Uncertain significance	Current study
c.7537C>T (p.Q2513*) rs148626322 (0.0187%)	Number of aa lost: 78/2590	21–50 Ad. and PC [73]	n.a.	1/185 (0.540%)	1/4,300 (0.023%)	Uncertain significance	
			Total PREDICTED-DAMAGING variant frequency[e]	2/185 (1.081%)	401/4,300 (9.326%)	No association with polyposis	Ciavarella et al. (2018) and current study
			Fisher's test (*p* value)	<0.0001			

Note: Alternative row shading depicts individual families.

Abbreviations: aa, amino acids; Ad., adenoma; CRC, colorectal cancer; D, damaging; ESP, NHLBI GO Exome-Sequencing Project; IHC-MMR, immunohistochemistry of MMR proteins; LOH, loss of heterozygosity; MAF, minor allele frequency; MSI, microsatellite instability (L, low; H, high); MSS, microsatellite stability; N, neutral; n.a., not available information; PC, prostate cancer.

[a]Only those variants predicted pathogenic by the authors from the original papers have been included.

[b]REVEL: Score ranges from 0 to 1. The larger the score the more likely the mutation may cause functional change; >0.5, predicted-damaging.

[c]Frequency in controls was calculated from ESP6500SI-V2 considering stop-gain, frameshift, splice-site or predicted pathogenic (REVEL) missense variants. ESP6500SI-V2 public data was released by Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (http://evs.gs.washington.edu/EVS/).

[d]ACMG/AMP guidelines for variant classification (InterVar software; Li and Wang, 2017). Detailed description of the evidence used for variant classification is shown in Table S2.

[e]Variant c.4262_4268delTACTATT (p.Ile1421ArgfsTer8) has been excluded due to its high frequency in controls, even in homozygosis, and in CRC cases.

The variant c.872C>T (p.P291L), located in the C-terminal helicase domain, was found in two individuals with CRC, one of them also affected with colorectal polyps. On the contrary, Ciavarella et al. (2018) performed whole-exome sequencing in blood DNA of eight sporadic cases with unexplained adenomatous polyposis, selected by the presence of >20 adenomatous polyps by age 35 or >50 adenomas by age 55, and no causative germline mutations in *APC* and/or *MUTYH*. One of these patients, who also carried an APC in-frame deletion of uncertain significance, was found to harbor a predicted damaging (REVEL) heterozygous variant in *POLQ* (c.6743A>G; p.N2248S), together with two rare variants in *OGG1* and *EXO1* (Ciavarella et al., 2018). No somatic second hit analyses were performed in any of the four identified *POLQ* variant carriers. Rosenthal et al. (2018) recently described two likely pathogenic *POLQ* variants in heterozygosity in two unrelated individuals from a cohort of 92 patients with a diagnosis of CRC before age 65 and/or ≥10 adenomatous polyps. Specifically, c.6892C>T (p.Q2298*; MAF_{GnomAD} = 0.00041%) was found in a patient diagnosed with CRC and 10 polyps at age 42, and c.2021dup (p.K675Efs*16; MAF_{GnomAD} = 0.0029%), in 44-year-old individual with 20 polyps.

In our study, two novel/rare variants were identified in two unrelated individuals: c.4684G>T (p.D1562Y; MAF_{GnomAD} = 0.12%), predicted neutral, and c.7537C>T (p.Q2513*; MAF_{GnomAD} = 0.019%), predicted to cause the elimination of 78 amino acids (out of 2,590), included in the DNA-directed DNA polymerase, family A, palm domain (Tables 3 and S1).

We assessed the presence of germline *POLQ* mutations (stop-gain, frameshift, splice-site, and predicted pathogenic (REVEL) missense changes, as well as variants affecting canonical splice-sites, with MAF, <1%) in cancer-free European population (ESP6500SI-V2) and compared it to the mutated cases identified in the polyposis patients, reported to date. No enrichment of *POLQ* (predicted) deleterious variants is observed in the polyposis cases compared to controls (450/4,300 control individuals (10.47%) vs. 2/185 polyposis patients (1.08%; Ciavarella et al., 2018 and current study). The statistically significant higher frequency in controls ($p < .0001$; Fisher's Exact test) clearly indicates a lack of association with polyposis. This observation remains when limiting the analysis to disruptive (stop-gain and frameshift) variants (1/185 control individuals [0.54%] vs. 90/4,300 [2.09%]; $p = .1841$; Fisher's Exact test).

5 | *RNF43* MUTATIONS DO NOT CAUSE NONSERRATED POLYPOSIS

RNF43 is a ubiquitin ligase that functions as a tumor suppressor by exerting a predominant negative feedback mechanism in the Wnt/β-catenin signaling pathway. According to recent evidence, germline mutations in *RNF43* cause serrated polyposis (reviewed by Quintana et al., 2018). The identification of adenomas in five of the 13 *RNF43* mutation carriers reported in the literature, led us to suspect a potential role of germline *RNF43* mutations in the predisposition to adenomatous polyposis or polyposis of multiple polyp types (Table 4). No novel or rare (population MAF, <1%) variants were identified among the 177 (nonserrated) polyposis patients herein studied, suggesting that *RNF43* mutations do not, or very rarely, cause adenomatous polyposis or polyposis of multiple polyp types (serrated/hyperplastic polyposis excluded).

Focusing on serrated polyposis, the frequency of (likely) pathogenic variants (classified according to the ACMG/AMP guidelines) taking into account the two largest unselected serrated polyposis cohorts is 1.76% (3/170; Buchanan et al., 2017; Quintana et al., 2018), and up to 2.63% (5/190) if all European cohorts are considered (Buchanan et al., 2017; Gala et al., 2014; Quintana et al., 2018). These frequencies are higher (Fisher's test; $p \leq .0002$) than the frequency in the cancer-free European population (1/4,300; 0.03%), confirming the association of germline *RNF43* mutations with serrated polyposis (Table 4).

6 | THE PREVALENCE OF BIALLELIC *NTHL1* MUTATIONS AMONG UNEXPLAINED POLYPOSIS CASES IS ~2%

NTHL1 (Nth-Like DNA Glycosylase 1) encodes a glycosylase involved in the DNA base excision repair (BER) pathway. Biallelic inactivation of this gene has been associated with the predisposition to attenuated adenomatous polyposis, CRC and other tumor types, being the presence of multiple primary tumors characteristic of the associated syndrome (Belhadj et al., 2017; Broderick et al., 2017; Grolleman et al., 2019; Groves, Gleeson, & Spigelman, 2019; Rivera, Castellsagué, Bah, van Kempen, & Foulkes, 2015; Weren, Ligtenberg, et al., 2015). To date, a total of 33 *NTHL1* biallelic carriers belonging to 21 unrelated families have been reported (Table S3). The majority of them are carriers of c.268C>T (p.Q90*), either in homozygosity (21/33 carriers) or in combination (compound heterozygosity) with c.235dup (p.A79Gfs*2), c.390C>A (p.Y130*), c.709+1G>A, c.733dup (p.I245Nfs*28), c.806G>A (p.W269*), or c.859C>T (p.Q287*) (6/33 carriers). One family carried a homozygous c.545G>A (p.W182*) (4/33 carriers), and another one, c.806GA (p.W269*) and c.859C>T (p.Q287*) (1/33 carriers). Of the 33 biallelic carriers, 19 (57.6%) had been diagnosed with CRC and 16 (48.5%) exhibited multiple primary tumors either in the colorectum or extracolonic. All biallelic carriers that underwent colonoscopy screening ($n = 28$) were diagnosed with adenomatous polyps (range: 1–200) and five of them (18%) were additionally diagnosed with more than four hyperplastic polyps (range: 4 to >30) (Belhadj et al., 2017; Broderick et al., 2017; Fostira et al., 2018; Grolleman et al., 2019; Rivera et al., 2015; Weren, Ligtenberg, et al., 2015; Table S3).

In our cohort, *NTHL1* was studied in 215 polyposis patients (Belhadj et al., 2017 and current study), finding a total of two unrelated c.268C>T (p.Q90*) homozygous carriers and a heterozygous carrier of c.527T>C (p.I176T; MAF_{GnomAD}= 0.22%; Table S1). One of the biallelic carriers had been diagnosed with CRC and 24

TABLE 4 Clinical and molecular characteristics of heterozygous *RNF43* (NM_017763) mutation carriers reported to date

Germline mutations,[a] dbSNP (GnomAd MAF %)	REVEL prediction[b]	Phenotypes	Tumor/Adenoma molecular features	Frequency in Serrated Polyposis (%)		Frequency in controls[c] (%)	Variant classification (ACMG/AMP guidelines)[d]	References
				All European cohorts	Largest cohorts			
c.337C>T (p.R113*) (n.a.)	n.a.	>30 SSA (51)	n.a.	2/190 (1.053%)		0/4,300 (0.000%)	Pathogenic	Gala et al. (2014)
		7 SSA (52); CLL (42; three siblings with colonic polyps)	n.a.					
c.394C>T (p.R132*) rs786205215 (n.a.)	n.a.	Multiple polyps and CRC (23)	MSS, IHC–MMR+, *KRAS*[e] WT	Single-family study		0/4,300 (0.000%)	Pathogenic[g]	Taupin et al. (2015)
		>80 SP (27)	n.a.					
c.953−1G>A (p.E318fs) (n.a.)	n.a.	>100 polyps (SSA, HP, Ad.; 65)	LOH: yes 100%[f]	Non-European		0/4,300 (0.000%)	Pathogenic	Yan et al. (2017)
		>20 polyps (SSA, HP; 64)	16 SP: *BRAF/KRAS* mutation[e]: 62.5% *BRAF* and 18.75% *KRAS*					
		Rectal cancer and 1 Ad. (49), 1 HP (descending colon; 53)	5 Ad.: 0% *BRAF*					
		2 SSA and 1 Ad. (37)	CIMP: 13 SP: 61.5% CIMP+					
		1 SSA and 2 HP (35)	3 Ad. and 1 CRC: 100% CIMP−					
		Normal colonoscopy						
c.640C>G (p.L214V) rs200626293 (0.0123%)	N (0.137)	>100 SP (18)	n.a.	1/190 (0.526%)	1/170 (0.588%)	3/4,300 (0.070%)	Likely pathogenic[g]	Buchanan et al. (2017)
c.443C>G (p.A148G) rs142178517 (0.0407%)	N (0.170)	34 SP (57)	n.a.	1/190 (0.526%)	1/170 (0.588%)	1/4,300 (0.023%)	Uncertain significance/Likely pathogenic[g]	

(Continues)

TABLE 4 (Continued)

Germline mutations,[a] dbSNP (GnomAd MAF %)	REVEL prediction[b]	Phenotypes	Tumor/Adenoma molecular features	Frequency in Serrated Polyposis (%)		Frequency in controls[c] (%)	Variant classification (ACMG/AMP guidelines)[d]	References
				All European cohorts	Largest cohorts			
c.394C>T (p.R132*) rs786205215 (n.a)	n.a.	>40 polyps (serrated) and CRC (55)	LOH: c.2309−1G>A; *BRAF/KRAS* WTCIMP[h]: 1 CRC positive	1/190 (0.526%)	1/170 (0.588%)	0/4,300 (0.000%)	Pathogenic[g]	Quintana et al. (2018)
Total PREDICTED-DAMAGING variants			All European serrated polyposis cohortsFisher's test (*p* value)	5/190 (2.631%)		1/4,300 (0.023%) <0.0001	Association with serrated polyposis	Gala et al. (2014), Buchanan et al. (2017), and Quintana et al. (2018)
			Largest serrated polyposis cohortsFisher's test (*p* value)		3/170 (1.765%)	1/4,300 (0.023%) 0.0002		Buchanan et al. (2017) and Quintana et al. (2018)
			Non-serrated polyposisFisher's test (*p* value)	0/177 (0.000%)		1/4,300 (0.023%) 1.000	No association with nonserrated polyposis	Current study

Note: Alternative row shading depicts individual families.

Abbreviations: Ad., adenoma; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; CLL, chronic lymphocytic leukaemia; D, damaging; ESP, NHLBI GO Exome Sequencing Project; IHC-MMR, immunohistochemistry of MMR genes; HP, hyperplastic polyp; LOH, loss of heterozygosity; MSS, microsatellite stability; MAF, minor allele frequency; N, neutral; n.a., not available information; SP, serrated polyps; SSA, sessile serrated adenoma; WT, wild-type.

[a]Only those variants predicted pathogenic by the authors from the original papers have been included.

[b]REVEL: Score ranges from 0 to 1. The larger the score the more likely the mutation may cause functional change; >0.5, predicted-damaging.

[c]Frequency in controls was obtained from ESP6500SI-V2 and pathogenecity was assessed by REVEL score >0.5. ESP6500SI-V2 public data was released by Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (http://evs.gs.washington.edu/EVS/).

[d]ACMG/AMP guidelines for variant classification (InterVar software; Li and Wang, 2017). A detailed description of the evidence used for variant classification is shown in Table S2.

[e]BRAF V600E and KRAS codons 12 and 13.

[f]All lesions (16 SP, 5 Ad., and 1 rectal cancer) from c.953−1G>A mutation carriers studied had either somatic LOH or mutations in RNF43.

[g]Results from functional assays performed by Quintana et al. (2018) were considered to classify this variant using InterVar.

[h]CIMP assessed using the ME042-C1 CIMP MS-MLPA kit (MRC-Holland, Amsterdam, the Netherlands). Methylated gene promoters (>20% methylation): *IGF2*, *RUNX3*, *NEURO*, *CDKN2A*, *CRABP1*. Unmethylated gene promoters (<12% methylation): *MLH1*, *SOCS1*, *CACNA1G*. DNAs from the patient's blood and a tumor with somatic *MLH1* promoter methylation were included as controls.

adenomas at age 48, and the other one, with two primary breast tumors at ages 47 and 50, bladder cancer at 66, and three synchronic CRCs, >15 adenomas, and five hyperplastic polyps at 67 years of age (Belhadj et al., 2017).

Taking into account our study and previous reports, where series of polyposis patients had been screened (Weren, Ligtenberg, et al., 2015; Belhadj et al., 2017), we may now conclude that the prevalence of *NTHL1* biallelic mutations among polyposis patients is of 1.9% (5/263 polyposis families).

7 | *MSH3* BIALLELIC MUTATIONS ARE VERY RARE AMONG UNEXPLAINED POLYPOSIS CASES

MSH3 is a DNA mismatch repair (MMR) gene that encodes a protein which, together with MSH2, initiates repair of DNA mismatches. Biallelic mutations in *MSH3*, c.[1148delA];[3001−2A>C] and c.[2319−1G>A];[2760delC], have been ;recently identified in two adenomatous polyposis patients in absence of germline mutations in *APC*, *MUTYH*, *NTHL1* or *POLE*, and *POLD1*, linking *MSH3* biallelic inactivation with high risk to adenomatous polyposis (Adam et al., 2016; Table S4). The mutational screening of *MSH3* in our cohort did not identify any biallelic carriers. Two predicted deleterious variants were identified in heterozygosity in three unrelated patients: c.1394A>G (p.Y465C), located in the ATPase region of the MutS domain V (C-terminal domain; Source: PFAM), and c.2732T>G (p.L911W) (Duraturo et al., 2011; Morak et al., 2017; Rohlin et al., 2017), affecting the MutS domain II (connector domain; Source: PFAM) and identified in two unrelated individuals (Table S1). Even if the two *MSH3* variants are located in domains that play a critical role in MMR, their clinical significance is most probably inexistent in the absence of a second mutation in the other allele (Adam et al., 2016). In conclusion, although clinically relevant, *MSH3* biallelic mutations are very rare among unexplained polyposis cases, at least in the populations studied (European).

8 | FINAL DISCUSSION AND CONCLUSIONS

Understanding the genetics of polyposis is clinically important as it helps discriminate between high- and low-risk groups. Over a decade ago, genome-wide high-throughput sequencing and copy number analyses opened a new window of opportunity to accelerate searches for genes associated with risk for polyposis, allowing the identification of new causal genes, such as *NTHL1*, *POLE*, and *POLD1*, or *MSH3* for adenomatous polyposis, *RNF43* for serrated polyposis and *GREM1* duplications for mixed polyposis. Several other candidate genes, such as *MCM9*, *FOCAD*, and *POLQ*, have been proposed; however, their true association with the disease has not been yet demonstrated, thus having so far no clinical relevance.

The assessment of the results obtained in our study, together with previously reported data and the frequency of mutations in cancer-free controls (both cases and controls series have been analyzed in European populations), indicates that neither heterozygous nor biallelic mutations in *MCM9* are associated with polyposis. On the contrary, the higher—although nonsignificant—frequency of mutations in *FOCAD* in polyposis patients compared to controls warrants the study of this gene in larger polyposis cohorts to provide a definitive answer about their causal involvement in polyposis predisposition. Currently, available evidence suggests that germline *POLQ* mutations are not associated with polyposis and that germline *RNF43* mutations exclusively cause serrated polyposis but do not explain other polyposis types.

Integration of our and previous findings indicates that in the European population, polyposis-causing biallelic *MSH3* mutations are extremely infrequent, and the prevalence of *NTHL1* mutations among genetically uncharacterized polyposes is ~2%. Of note, variation in the prevalence of variants may be found in different geographic areas or subpopulations.

Excluding *APC* and *MUTYH*, the other more recently described-polyposis genes, including *NTHL1*, *POLE*, and *POLD1*, *MSH3*, or *MLH3*, explain a very small fraction of adenomatous polyposes, with similar scenarios being observed for *RNF43* in serrated polyposis and *GREM1* duplications in mixed polyposis. Moreover, as shown in this review and meta-analysis, the involvement of other proposed genes in polyposis predisposition is most probably irrelevant. The causes of the low yield in the identification of causative genes in patients with relatively straightforward phenotypes remain unknown. Among the hypotheses that we may consider are (a) the presence of moderate- or low-risk alleles that, alone, but most probably in combination with environmental factors (lifestyle), predispose to polyposis; (b) the alteration of noncoding regulatory sequences that affect known or yet-to-be-discovered polyposis-predisposing genes; and (c) the existence of new high-penetrance genes with extremely low frequency of mutations, that might be identified as we perform genome-wide analyses in additional well-selected cohorts.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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