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ARTICLE

Genetic variant in the telomerase gene modifies cancer risk in Lynch syndrome

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Lynch syndrome (LS) is an inherited cancer-predisposing disorder caused by germline mutations in the mismatch repair (*MMR*) genes. The high variability in individual cancer risk observed among LS patients suggests the existence of modifying factors. Identifying genetic modifiers of risk could help implement personalized surveillance programs based on predicted cancer risks. Here we evaluate the role of the telomerase (*hTERT*) rs2075786 SNP as a cancer-risk modifier in LS, studying 255 and 675 *MMR* gene mutation carriers from Spain and the Netherlands, respectively. The study of the Spanish sample revealed that the minor allele (A) confers increased cancer risk at an early age. The analysis of the Dutch sample confirmed the association of the A allele, especially in homozygosity, with increased cancer risk in mutation carriers under the age of 45 (relative $risk_{LSca<45\_AA} = 2.90$; 95% confidence interval = 1.02–8.26). Rs2075786 is associated with colorectal cancer (CRC) risk neither in the general population nor in non-Lynch CRC families. *In silico* studies predicted that the SNP causes the disruption of a transcription binding site for a retinoid receptor, retinoid X receptor alpha, probably causing early telomerase activation and therefore accelerated carcinogenesis. Notably, cancer-affected LS patients with the AA genotype have shorter telomeres than those with GG. In conclusion, *MMR* gene mutation carriers with *hTERT* rs2075786 are at high risk to develop a LS-related tumor at an early age. Cancer-preventive measures and stricter cancer surveillance at early ages might help prevent or early detect cancer in these mutation carriers.
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INTRODUCTION

Lynch syndrome (LS) (MIM 120435) is an autosomal-dominant inherited disorder caused by germline mutations in the DNA mismatch repair (*MMR*) genes. It is characterized by early-onset colorectal cancer (CRC) and an increased risk of upper gastrointestinal, urologic and gynecologic cancers.[1] Several studies have estimated the cancer risks associated with mutations in the *MMR* genes, but these estimates vary substantially across the studies (20–80% lifetime risk).[2–9]

There is a considerable variation in LS expression and no obvious gene-specific genotype/phenotype correlations have been demonstrated nor does there appear to be any relationship between the location of a mutation and type of disease. In addition to environmental factors, there is evidence suggesting the existence of genetic factors that somehow explain the variability in individual cancer risk.[10] Identifying the genetic modifiers of risk can lead to an efficient stratification of mutation carriers based on their predicted risk, implying thus, a more appropriate clinical management based on personalized surveillance programs.

Most of the attempts to identify cancer-risk modifiers in LS have been based on the study of candidate genes and most of them have not been validated when tested in larger sample sizes.[11] In contrast, two genetic variants previously identified in CRC genome-wide association studies (GWAS), rs16892766 and rs3802842, might modify cancer risk in LS families.[12–14]

Telomeres are located at the end of chromosomes and protect the chromosome ends from nucleolitic degradation, end-to-end fusions and irregular recombination, being thus critical for genome stability and integrity. Telomeres progressively shorten with each cell replication cycle. Telomere length anomaly appears to be one of the earliest and most prevalent genetic alterations in the multistep process of malignant transformation.[15,16] Telomerase catalyzes the *de novo* addition of telomeric repeat sequences onto chromosome ends and it is usually inactive in normal somatic cells, whereas its expression has been linked to increased susceptibility to tumorigenesis.[17–19] Genetic variants located in genes involved in telomere maintenance, and in particular in *hTERT* (MIM 187270), the gene encoding the catalytic subunit of telomerase, have been associated with increased risk to cancer.[20–27] Among the better characterized cancer variants, the *hTERT* rs2075786 (c.2654 + 269G/A) SNP has been associated with lung cancer risk.[28,29] However, no studies have reported any

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Table 1 Characteristics of the *MMR* mutation carriers included in the study

		Spain			*The Netherlands*		
		Affected n *(%)*	*Unaffected* n *(%)*	*Total* n *(Fam)*	*Affected* n *(%)*	*Unaffected* n *(%)*	*Total* n *(Fam)*
No. of MMR carriers	LS cancer	147	108	255 (101)	201	474	675 (127)
	CRC	134	121		146	529	
Gene							
MLH1	LS cancer	82 (55.8)	69 (63.9)	151 (54)	73 (36.3)	175 (36.9)	248 (43)
	CRC	77 (57.5)	74 (61.2)		62 (42.5)	186 (35.2)	
MSH2	LS cancer	49 (33.3)	30 (27.8)	79 (34)	85 (42.3)	166 (35.0)	251 (57)
	CRC	43 (32.1)	36 (29.8)		61 (41.8)	190 (35.9)	
MSH6	LS cancer	11 (7.5)	9 (8.3)	20 (9)	42 (20.9)	133 (28.1)	175 (26)
	CRC	9 (6.7)	11 (9.1)		22 (15.1)	153 (28.9)	
PMS2	LS cancer	5 (3.4)	0 (0.0)	5 (4)	1 (0.5)	0 (0.0)	1 (1)
	CRC	5 (3.7)	0 (0.0)		1 (0.7)	0 (0.0)	
Gender							
Male	LS cancer	75 (50.3)	43 (41.7)	118	87 (43.3)	213 (44.9)	300
	CRC	73 (54.5)	45 (37.9)		79 (54.1)	221 (41.8)	
Female	LS cancer	73 (49.7)	64 (58.3)	137	114 (56.7)	261 (55.1)	375
	CRC	61 (45.5)	76 (62.1)		67 (45.9)	308 (58.2)	
Age[a] (average ± SD)	LS cancer	44.6 ± 12.0	36.8 ± 13.1		45.1 ± 10.8	46.2 ± 13.6	
	CRC	44.4 ± 12.3	39.1 ± 14.4		44.8 ± 11.5	47.6 ± 14.1	
Polypectomy	LS cancer	NA	NA		71 (42.8)	95 (57.2)	166
	CRC				58 (34.9)	108 (65.1)	101

Abbreviations: CRC, colorectal cancer; Fam, number of families; LS cancer, colon, rectum, endometrium, stomach, ovary, urinary tract and small intestine; MMR, mismatch repair; NA, not available information.
[a]Age at diagnosis for cancer-affected subjects, or age at last follow-up or at death for unaffected subjects.

association between variants located at the *hTERT* locus and the risk of CRC.

Here we evaluate *hTERT* rs2075786 as a modifier of cancer in LS patients in 255 MMR gene mutation carriers from Spain. Also, 675 mutation carriers from the Netherlands were analyzed. We also assessed its role as cancer-risk factor in a population-based case–control series and in non-LS familial cases.

MATERIALS AND METHODS

Subjects

Spanish sample. A total of 255 individuals from 101 LS families whose mutation carrier statuses were known were included in the study. They were assessed through the Hereditary Cancer Program of the Catalan Institute of Oncology from 1998 to 2010. All families were of Caucasian origin. Informed consent was obtained from all individuals. *MMR* mutation analysis was performed on genomic DNA extracted from peripheral blood lymphocytes. Large genomic alterations were studied using multiplex ligation-dependent probe amplification (SALSA MLPA Kits, MRC-Holland, Amsterdam, The Netherlands). Mutation screening was performed by direct sequencing after PCR amplification (primers and conditions available upon request). In all, 147 (57.6%) *MMR* gene mutation carriers had been diagnosed with a LS-related tumor, of whom 134 with CRC. The clinical characteristics of the subjects are detailed in Table 1.

Also, DNA from 277 sporadic CRC cases and 280 controls were obtained from a hospital-based case–control study (Bellvitge Colorectal Cancer Study). Cases were consecutive patients with a first diagnosis of colorectal adenocarcinoma attending the Bellvitge University Hospital in Barcelona. For the group of patients, the male/female ratio is 1.2 and the mean age is 66.7 (range: 23–91), and for the controls, 1.1 and 65.5 (range: 24–92), respectively. Details about the study population were published elsewhere.[30]

Dutch sample. A total of 675 *MMR* gene mutation carriers from 127 different families from the Dutch LS Registry were studied. Detailed description of the registry, individuals and DNA extraction method was published before.[12] Information on the subjects has been recently updated and thus, follow-up data added. In all, 201 (29.8%) *MMR* gene mutation carriers had been diagnosed with a LS-related tumor, of whom 146 are with CRC. Table 1 summarizes the updated clinical characteristics of the subjects.

The Dutch case–control series consists of 324 CRC cases and 785 controls. Cases are probands assessed through a familial cancer clinic; therefore, they are suspected of genetic CRC susceptibility but with no germline mutations identified in the *MMR* genes. Regarding controls, 475 are healthy blood donors and 310 are healthy spouses of *MMR* gene mutation carriers.

MMR gene mutation carriers are derived from multiple-case families selected for genetic counseling and testing. In this situation, the disease status clearly affects the likelihood of testing, causing an overrepresentation of affected individuals. This observation is evident in the Spanish but not in the Dutch sample. The Dutch registry has been running longer (it was established in 1985) and follows large and complete LS families, whereas the Spanish series comes from a cancer hospital-based genetic counseling unit established in 1998, where a clear ascertainment bias towards the assessment of cancer-affected family members exists. Because of the differences in affected *vs* unaffected ratios, the studies were performed independently for the Spanish and Dutch samples.

Genotyping

The rs2075786 SNP was genotyped using the commercially available TaqMan assay C_15824034_10 (Applied Biosystems Inc., Foster City, CA, USA) following the manufacturer's instructions. Reactions were performed in duplicate in the LightCycler 480 real-time PCR detection system (Roche Diagnostics GmbH, Mannheim, Germany). Genotype calling was performed automatically by the LightCycler 480 II software (Roche Diagnostics GmbH, Mannheim, Germany). Genotyping failed in 78 of the 2596 (3%) samples included in the study.

In silico analyses

Public genome browsers (Ensembl, NCBI and USCS genome browsers) were checked out to investigate the existence of transcription of noncoding RNAs from the region where rs2075786 is located. The presence

of microRNA-binding sites was studied using the microRNA target-prediction algorithms provided by http://www.microrna.org. The strength of the pseudoexon splice sites was analyzed using three different prediction programs: www.fruitfly.org/seq_tools/splice.html, http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html and http://ast.bioinfo.tau.ac.il/SpliceSiteFrame.html, as previously described.[31] Putative binding sites for transcription factors were identified using PROMO 3.0, a web-based program that employs the TRANSFAC database version 8.3 to construct specific binding-site weight matrices for prediction of transcription factor-binding sites.[32,33]

Relative telomere length assessment

Telomere length quantification was performed using a monochrome multiplex quantitative PCR method, as described by Cawthon with slight modifications (available upon request).[34]

Statistical analyses

Evaluation of the deviation of rs2075786 from the expected Hardy–Weinberg equilibrium was performed in controls from each population. The SNP was in Hardy–Weinberg equilibrium in controls from both populations ($P_{Spanish} = 0.48$; $P_{Dutch} = 0.93$).

The association between genotypes and risk of CRC or LS-related tumors was evaluated using conditional logistic regression adjusting for familial clustering. The end points considered were: age at diagnosis of the LS-related cancer or CRC for affected individuals and age of last follow-up for unaffected individuals. Likelihood ratio tests were applied to assess statistical significance. In general, relative risks (RRs), 95% confidence intervals (CIs) and associated *P*-values were estimated for different models of inheritance and the best model was selected using the Akaike information criterion. The interaction of genotypes with age stratified at 45 years was assessed to identify anticipation effects. For case–control studies, odds ratios (ORs) and 95% CI were calculated using logistic regression adjusted for gender. Kruskal–Wallis rank-based test was used to compare telomere length among rs2075786 genotype groups, adjusting for age and gender. Statistical analyses were performed using R.

RESULTS

Spanish study

No differences in rs2075786 genotype distributions were observed when comparing *MMR* gene mutation carriers affected with cancer, either LS-related or only CRC, and unaffected mutation carriers (Supplementary Table S1).

We further analyzed rs2075786 genotype distributions within two age intervals in the cancer group. For this aim, we established a cut off of 45 years of age based on the median age of all individuals included in the study. *MMR* gene mutation carriers diagnosed with cancer before 45 years of age were compared with those diagnosed after 45 years of age and those who were cancer-free after 45 years of age. Under this condition, the A allele conferred an increased risk of cancer in LS carriers under the age of 45 compared with those diagnosed with cancer or cancer-free after the age of 45 (Table 2).

Although borderline and not fulfilling the required standards for declaring an association, the results obtained in the Spanish sample led us to analyze another series (Dutch study) in a more comprehensive manner, thanks to the larger sample size and the availability of follow-up and polypectomy information.

Table 2 Genotype distributions within age intervals in Spanish *MMR* mutation carriers

Genotype	*LS ca. <45* n *(%)*	*LS ca. ≥45* n *(%)*	*RR (95% CI)*[a]	P
GG	31 (38.3)	43 (50.0)		
GA	43 (53.1)	29 (33.7)	4.69 (0.92–23.88)	0.062
AA	7 (8.6)	14 (16.3)	3.76 (0.41–34.31)	0.241
LRT (2 df)				0.126
GA + AA	50 (61.7)	43 (50.0)	4.66 (0.92–23.71)	0.064
LRT (1 df)				0.044
Per allele			1.98 (0.70–5.65)	0.199
LRT (1 df)				0.192

Abbreviations: CI, confidence interval; df, degrees of freedom; LRT, likelihood ratio test; LS ca., colon, rectum, endometrium, stomach, ovary, urinary tract or small intestine cancer; MMR, mismatch repair; RR, relative risk.
Young cancer-affected *MMR* mutation carriers (cancer diagnosed before 45 years of age) are compared with *MMR* mutation carriers who developed a LS-associated tumor after 45 years of age or who are cancer-free after 45 years of age.
[a]RRs were estimated using conditional logistic regression adjusting for familial clustering.

Dutch study

A total of 675 *MMR* gene mutation carriers from the Dutch hereditary CRC registry were included in the analysis. As previously observed for the Spanish *MMR* gene mutation carriers, no effect was detected on the general risk of cancer between cancer-affected and unaffected individuals (Supplementary Table S2).

To study the effect on cancer risk related with age intervals, cancer-affected *vs* unaffected *MMR* gene mutation carriers were compared within the groups under 45 years and within the groups ≥45. For this purpose, the two age groups were created as: group age <45, *MMR* gene mutation carriers diagnosed with cancer under the age of 45 and unaffected *MMR* gene mutation carriers under age 45 and group age ≥45, cancer-affected and unaffected *MMR* gene mutation carriers >45. Because of the larger sample size and the fact that unaffected carriers had a similar or even higher average age than cancer-affected carriers (Table 1), this type of distribution in age groups could be performed for the Dutch *MMR* gene mutation carriers.

In this series, the AA genotype confers an increased risk of LS-related cancers in individuals <45 years of age (RR = 2.76; 95% CI = 1.02–7.50). When only CRC was considered, an RR of 2.46 (96% CI = 0.78–7.82) was found for the AA homozygotes, but this observation was not statistically significant, probably because of the effect on the risk of all LS-related tumors. No association of rs2075786 with cancer risk was observed in the ≥45 groups (Table 3).

MMR gene mutation carriers are usually under strict clinical surveillance and undergo colonoscopies on a routine basis, implying that whenever detected, polyps are removed. As polypectomy affects subsequent CRC risk, we included this information in the analysis. Within the group age <45, all unaffected subjects with a polypectomy were excluded. Moreover, within the group age ≥45, all cancer-affected subjects with a polypectomy were performed under the age of 45 and all unaffected individuals with polypectomies were excluded from the analysis. A total of 100 individuals were removed. In this scenario, the increased cancer risk conferred by AA in the <45 group is maintained or even increased ($RR_{LSca<45\_AA} = 2.90$; 95% CI = 1.02–8.26) (Table 4). Slight random differences from the previous analysis dependent on the genotype of the cases excluded were observed.

No effect of gender on cancer risks was observed (data not shown). Regarding the *MMR* gene affected, the analysis was performed considering *MLH1* and *MSH2* separately (Supplementary Table S3). Although differences did not reach significance and larger series should be studied to draw definitive conclusions, the results suggest that the effect of rs2075786 on cancer risk occurs for both *MLH1* and *MSH2* mutation carriers under the age of 45 ($RR_{MLH1<45\_AA} = 3.86$; 95% CI = 0.70–21.33 and $RR_{MSH2<45\_AA} = 2.36$; 95% CI = 0.42–13.39).

Table 3 Risk of cancer associated with *hTERT* rs2075786 in Dutch *MMR* mutation carriers within age intervals (<45 and ≥45 years)

Genotype	*LS ca.* n *(%)*	*LS ca.-free* n *(%)*	*RR (95% CI)*[a]	P	*CRC* n *(%)*	*CRC-free* n *(%)*	*RR (95% CI)*[a]	P
Group age <45								
GG	32 (36.8)	112 (47.1)	1		27 (40.3)	113 (46.7)	1	
GA	41 (47.1)	109 (45.8)	1.12 (0.59–2.12)	0.735	30 (44.8)	112 (46.3)	0.92 (0.45–1.91)	0.829
AA	14 (16.1)	17 (7.1)	2.76 (1.02–7.50)	0.046	10 (14.9)	17 (7%)	2.46 (0.78–7.82)	0.126
Group age ≥45								
GG	53 (48.6)	94 (42.3)	1		37 (48.7)	114 (42.1)	1	
GA	41 (37.6)	102 (45.9)	0.86 (0.46–1.61)	0.644	30 (39.5)	121 (44.6)	0.93 (0.47–1.83)	0.830
AA	15 (13.8)	26 (11.7)	0.67 (0.27–1.69)	0.398	9 (11.8)	36 (13.3)	0.50 (0.17–1.45)	0.201
LRT (5 df)				0.259				0.397
Interaction with age (χ^2 test)				0.112				0.088
Per allele								
Age <45			1.46 (0.92–2.31)	0.109			1.31 (0.76–2.23)	0.328
Age ≥45			0.84 (0.54–1.28)	0.413			0.76 (0.47–1.22)	0.260
LRT (3 df)				0.163				0.445
Interaction with age (χ^2 test)				0.070				0.120

Abbreviations: CI, confidence interval; df, degrees of freedom; LRT, likelihood ratio test; LS ca., colon, rectum, endometrium, stomach, ovary, urinary tract or small intestine cancer; MMR, mismatch repair; RR: relative risk.
[a]RRs were estimated using a conditional logistic regression adjusting for familial clustering.

Table 4 Risk of cancer associated with hTERT rs2075786 in Dutch MMR mutation carriers within age intervals (<45 and ≥45 years) considering polypectomies

Genotype	*LS ca.* n *(%)*	*LS ca.-free* n *(%)*	*RR (95% CI)*[a]	P	*CRC* n *(%)*	*CRC-free* n *(%)*	*RR (95% CI)*[a]	P
Group age <45								
GG	32 (36.8)	104 (48.6)	1		27 (40.3)	105 (48.4)	1	
GA	41 (47.1)	94 (43.9)	1.19 (0.61–2.34)	0.604	30 (44.8)	96 (44.2)	1.03 (0.48–2.20)	0.938
AA	14 (16.1)	16 (7.5)	2.90 (1.02–8.26)	0.047	10 (14.9)	16 (7.4)	2.28 (0.70–7.40)	0.170
Group age ≥45								
GG	50 (48.1)	64 (41.3)	1		35 (48.6)	79 (41.1)	1	
GA	40 (38.5)	74 (47.7)	0.96 (0.45–1.91)	0.898	29 (40.3)	89 (46.4)	1.05 (0.49–2.25)	0.898
AA	14 (13.5)	17 (11.0)	0.52 (0.18–1.54)	0.239	8 (11.1)	24 (12.5)	0.33 (0.10–1.10)	0.070
LRT (5 df)				0.038				0.098
Interaction with age (χ^2 test)				0.082				0.040
Per allele								
Age <45			1.51 (0.93–2.44)	0.096			1.34 (0.77–2.30)	0.298
Age ≥45			0.79 (0.48–1.30)	0.354			0.68 (0.40–1.16)	0.155
LRT (3 df)				0.017				0.118
Interaction with age (χ^2 test)				0.064				0.071

Abbreviations: CI, confidence interval; df, degrees of freedom; LRT, likelihood ratio test; LS ca., colon, rectum, endometrium, stomach, ovary, urinary tract or small intestine cancer; MMR, mismatch repair; RR: relative risk.
[a]RRs were estimated using a conditional logistic regression adjusting for familial clustering.

Rs2075786 and CRC risk in the general population and in non-Lynch CRC families

To evaluate whether rs2075786 is associated with CRC risk in the general population and in non-Lynch familial CRC cases, we performed an association study in a case–control series from Spain and in non-Lynch CRC familial cases from the Netherlands. Rs2075786 was in Hardy–Weinberg equilibrium in both the Spanish ($P = 0.59$) and Dutch ($P = 0.88$) controls. No statistically significant association between rs2074786 and CRC was detected in any of the two situations, with ORs close to 1 (Supplementary Table S4).

Also, when separating by age groups (<45 and ≥45) in the non-Lynch familial CRC study (The Netherlands), no statistically significant differences were identified in either group (Supplementary Table S5). The age stratification could not be performed for the sporadic case–control series (Spain) because of the scarcity of sporadic CRC patients with <45 years of age present.

In silico studies to unravel rs2075786 functionality

We performed an *in silico* search of potential effects of the variant on gene splicing, transcription factor or microRNA binding, as well as of noncoding RNAs being transcribed from that genomic region. Public databases showed inexistence of noncoding RNAs, and microRNA target-prediction algorithms found no microRNA-binding sites when either allele (A or G) was present. Likewise, gene splicing was not

affected. *In silico* search for putative binding sites of transcription factors revealed that when the rare allele (A) of rs2075786 is present, the elimination of a binding site for retinoid X receptor alpha (RXR-α) is predicted.

Rs2075786 and telomere length

To test the hypothesis that telomere length is compromised by the presence of the A allele of rs2075786, we measured the relative telomere length in blood DNA of the cancer-affected LS patients from Spain. Individuals with the AA genotype have shorter telomeres than individuals with the GG ($P=0.011$) and GA ($P=0.058$) genotypes (Figure 1). No association of relative telomere length with rs2075786 genotypes was found in sporadic CRC patients (Supplementary Figure S1).

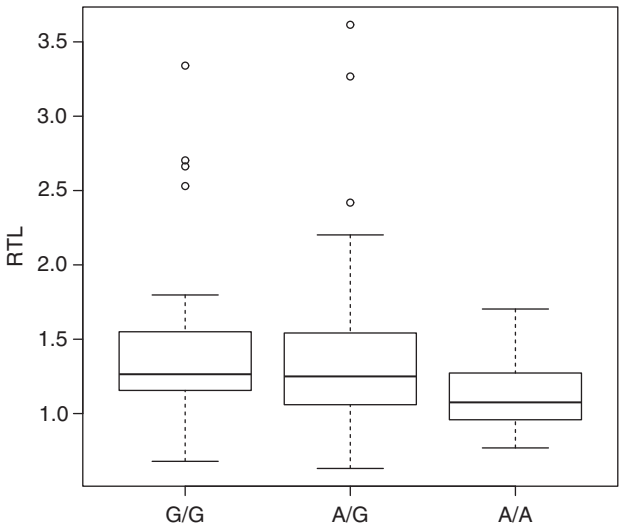

Figure 1 Blood relative telomere length (RTL) in 146 cancer-affected LS patients according to rs2075786 genotype distribution ($n_{GG}=66$, $n_{AG}=64$ and $n_{AA}=16$). The boxes represent the interquartile range of distributions (25th–75th percentile); the horizontal lines within the boxes, the medians; and the vertical lines, the 5th and 95th percentiles.

DISCUSSION

There remains a considerable variability in LS disease expression that cannot be readily explained by genetic variance occurring solely within the *MMR* genes. Several studies have tried to determine whether there are any genetic modifying factors that could be associated with an increased likelihood of developing cancer. In this study, we show that the AA genotype of the variant rs2075786 of the telomerase gene (*hTERT*) is associated with an increased risk of LS-related cancers in *MMR* gene mutation carriers under 45 years of age, indicating anticipation in the age of onset of cancer. Moreover, when information about polypectomies, a therapeutic intervention that affects CRC risk and might lead to false negatives, was incorporated to the analyses, the results obtained improved, strengthening the observation ($RR_{<45\_AA}=2.90$; 95% CI = 1.02–8.26).

The field of cancer-risk modifiers of LS is very active and has provided in the recent years controversial results. First, candidate genes that have some plausible biological role in the disease were evaluated. Except for rare exceptions, the initial findings identified in small-sized samples were not validated in larger series.[11] More recently, some polymorphisms identified through GWAS of unselected CRC patients and controls have been shown to modify cancer risk in LS families. Wijnen *et al*[12] first studied six GWAS CRC susceptibility SNPs in 675 *MMR* gene mutation carriers from 127 LS families. They found that two of them, at 8q23.3 and 11q23.1, were associated with CRC risk in mutation carriers, particularly in females.[12] These results were partly confirmed in a cohort of Australian and Polish *MMR* mutation carriers, but were not replicated in a French cohort.[13,14]

We selected the *hTERT* variant rs2075786 by somehow combining candidate gene and GWAS-based approaches. First, *hTERT* has an important role at very early stages of carcinogenesis, its expression has been linked to increased susceptibility to tumorigenesis, and rs2075786 has been associated with cancer risk.[15,16,18,19,28,29] And second, through GWAS, genetic variants located in genes involved in telomere maintenance, and in particular in *hTERT*, were also associated with increased risk to cancer.[20–27] In contrast to previous studies examining the role of modifier genes,[11] our study includes two independent series of *MMR* gene mutation carriers, of 255 and 675 individuals, the latest being among the largest series used to study genetic modifiers of LS. In view of our results with rs2075786, it may be relevant to study the role of other *hTERT* polymorphisms as cancer-risk modifiers in LS, in particular those variants with functional implications and/or with strong levels of evidence of being associated with cancer risk.[35]

In order to unravel the functional effect of rs2075786, *in silico* analysis predicted that when the minor allele (A) is present, a binding site for RXR-α is eliminated. Already existent evidence indicates that retinoids, through retinoid receptors, inhibit telomerase activity and downregulate hTERT expression.[36,37] These observations suggest that in the presence of the rs2075786 minor allele A, where a binding site for retinoid receptors is absent, natural retinoids cannot efficiently restrain hTERT expression, causing accelerated tumor cell growth.

It is nevertheless intriguing that rs2075786 does not influence CRC risk in sporadic or in other non-Lynch familial cases. It is well known that the carcinogenic pathway of MMR-deficient tumors clearly differs from that involved in most sporadic and non-LS familial CRC. It has been observed that MMR-deficient cell lines and colon tumors show high mutation frequencies at telomere ends, leading to accelerated telomere shortening.[38–41] The effect of an increased telomere-shortening rate is likely to require early activation of telomerase in such tumors. As hypothesized above, the presence of the A allele of rs2075786 might imply even earlier activation of telomerase. Although the effect might be very subtle to have an impact in MMR-proficient cells, it could trigger important consequences in a MMR-deficient context, such as in LS tumors. In this line of research, it may be interesting to evaluate the role of rs2075786 as a risk allele for sporadic CRC with microsatellite instability.

A possible explanation for the rs2075786 acting as modifier of cancer risk at early ages but not later in life might be that the effect the variant has on telomere shortening (Figure 1) is not efficient at late ages where telomeres are already physiologically shortened due to the aging process.[42] Nevertheless, further functional assays are required to provide experimental evidence about this matter.

When assessing small variations in cancer risk, the issue of personalizing follow-up immediately arises. A potential applicability of rs207586 genotyping in genetic counseling for clinical management of LS patients can be envisioned. Based on our results, young *MMR* gene mutation carriers with the rs2075786 AA genotype might benefit from an even more intensive clinical surveillance of CRC and other LS-related tumors than the one suggested by the standard LS surveillance protocols. Validation of our observation in additional series is warranted prior to translation to routine clinical practice.

Nevertheless, both *MMR* gene mutation carriers and clinicians should be conscious of the higher risk that rs2075786 carriers have at early ages, observing or even increasing the screening frequency for colorectal, endometrial and other LS-related tumors. Also, lifestyle changes that affect environmental modifier risk factors might help reduce the risk of developing cancer. Likewise, the recent promising results of aspirin-based chemoprevention obtained in LS patients suggest that MMR gene mutation carriers with the rs2075786 AA genotype might benefit from this chemopreventive treatment starting at early age.[42]

In conclusion, we observe an association that might help identify *MMR* gene mutation carriers with higher risk to prematurely develop a LS-associated tumor. Rs2075786 adds up to the already known LS modifiers, increasing thus the opportunity to incorporate the information of modifier factors into clinical practice.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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