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Evaluation of Lynch syndrome modifier genes in 748 MMR mutation carriers

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Key words: Lynch syndrome; MMR genes; cancer risk; modifier factors

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ABSTRACT

Several studies have reported that, in Lynch syndrome resulting from mutations of the mismatch repair (MMR) genes, a CA repeat ≤ 17 within the *IGF1* promoter, SNPs within the xenobiotic metabolizing enzyme gene *CYP1A1* and SNPs on 8q23.3 and 11q23.1 modify colorectal cancer (CRC) risk in MMR mutation carriers. We analysed the impact of these polymorphisms on CRC risk in 748 French MMR mutation carriers derived from 359 families. We also analysed the effect of the *Novel 1* SNP (18q21), which has recently been shown to increase CRC risk in the general population. We observed a significant difference in the CRC-free survival time between males and females, between *MSH2* and *MSH6* mutation carriers and between *MLH1* and *MSH6* indicating that this series is representative of Lynch syndrome. In contrast, the univariate log-rank test, as well as multivariate Cox model analysis controlling for familial aggregation and mutated MMR gene, year of birth and gender showed that the polymorphic alleles tested were not associated with a significant CRC risk increase, neither on the entire sample nor among males and females. This discrepancy with previous reports might be explained both by the genetic heterogeneity between the different populations analysed and the allelic heterogeneity of the MMR mutations. We conclude that genotyping of these polymorphisms is not useful to evaluate CRC risk in MMR mutation carriers and to optimise their clinical follow-up.

INTRODUCTION

Lynch syndrome or Hereditary Non Polyposis Colorectal Cancer (HNPCC), the most common form of inherited colorectal cancer (CRC), results from germline mutations within the genes of the mismatch repair system (MMR), *MSH2*, *MLH1*, *MSH6* and *PMS2* (for review see reference 1). In MMR mutation carriers, the main tumour risks are colorectal and endometrial cancers. The cumulative risk at 70 years of CRC has been estimated to be 47-78% in males²⁻⁴ and 30-57% in females²⁻⁴ and the risk of endometrial cancer to be 25-61%.^{2,3} Like in other Mendelian forms of cancer characterized by an incomplete penetrance, one of the main challenges is to identify modifier genetic factors able to modulate the mutation penetrance. Characterization of validated modifier genetic factors should have important clinical consequences in the future since it should be possible to adapt the follow-up of MMR gene mutation carriers, in terms of nature and timing of investigations, according to modifier alleles.

The modifier genes which have been reported so far in Lynch syndrome have been characterized according to two strategies: several studies have been focused on genes whose implication in CRC development had previously been suggested. One of the first modifier genetic factors identified in Lynch syndrome corresponds to a CA repeat polymorphism present within the *IGF1* promoter, 1 kb upstream from the transcriptional initiation site.^{5,6} In a study performed in 121 MMR mutation carriers from 59 families, mainly of Caucasian origin, Zecevic et al.⁵ reported that a CA repeat ≤ 17 was significantly associated to a higher CRC risk and an earlier age of tumour onset. This result was confirmed by an independent study performed in 443 Australian and Polish MMR mutation carriers originated from 269 distinct families.⁶ Another class of modifier genes reported in Lynch syndrome corresponds to genes encoding xenobiotic metabolizing enzymes involved in environmental carcinogen metabolism. In

129 subjects of South African origin and harbouring the same *MLH1* missense mutation, males harbouring the null genotype for the *GSTT1* and *GSTM1* genes developed cancer earlier than the males harbouring the other genotypes.⁷ This effect of the *GSTT1* and *GSTM1* null genotypes was not confirmed in a second study including 257 MMR mutation carriers from 130 families.⁸ Nevertheless, the authors reported that subjects heterozygous for the *CYP1A1* rs1048943 SNP (c.1384A>G; p.Ile462Val) developed CRC earlier than the individuals with the homozygous wild-type genotype and that subjects heterozygous for this polymorphism and an additional SNP rs4646903 (Msp1; g.6235T>C) had an increased CRC risk.⁸

The second strategy, which has recently allowed the detection of modifier genes in Lynch patients, originated from the numerous genome-wide association studies reporting SNPs associated with CRC risk in the general population. Starting from the hypothesis that SNPs acting as risk factors for CRC in the general population might act as risk modifiers in patients harbouring a highly penetrant mutation, Wijnen et al.⁹ recently reported a significant association of CRC risk with rs16892766 (8q23.3) and rs3802842 (11q 23.1) in 675 Dutch MMR mutation carriers from 127 families.

In this study, we investigated the impact of these different genetic factors on CRC risk in a large series of French MMR mutation carriers.

PATIENTS AND METHODS

Patients

The study included 748 unselected patients (Table 1) derived from 359 families with Lynch syndrome and recruited from Rouen (n = 494) and Lille (n = 254) University hospitals in France. All these individuals were confirmed carriers of a deleterious mutation of *MSH2*, *MLH1* or *MSH6* and were Caucasian. Among these 748 mutation

carriers, 329 (44%) had been diagnosed with CRC prior to inclusion in the study, with a mean age of CRC onset of 43 years (range: 18-82 years). Additionally, 51 mutation carriers (6.8%) had been diagnosed with another tumour belonging to the Lynch syndrome spectrum (endometrial carcinoma, tumour of urinary tract, ovarian carcinoma, cancer of the stomach and of the small intestine) and 368 (49.2%) had developed no tumour at the time of inclusion.

Genotyping

The *IGF1* CA repeat was PCR-amplified using dye labelled primers and the length of the PCR products was determined after migration on an Applied Biosystems model 3130 Genetic Analyser (PE Applied Biosystems, Foster City, CA) and analysed using the GeneMapper Analysis Software version 4.0 (Applied Biosystems). To calibrate the results, we sequenced, after cloning into a plasmid, the most frequent allele (n=19) and used the corresponding genomic DNA as a reference DNA. The rs16892766 (8q23.3), rs3802842 (11q23.1) SNPs and the rs1048943 (c.1384A>G; p.Ile462Val) and rs4646903 SNPs, both located within the *CYP11A1* gene (15q24.1), were genotyped using SNaPshot multiplex assays based on primer extension with dye labelled dideoxynucleotides (ABI PRISM SNaPshot Multiplex kit, Applied Biosystems, Foster, USA). We also analysed, by SNaPshot multiplex analysis, the *Novel 1* SNP located on 18q21 which had been shown to be associated with CRC risk in the general population by altering *SMAD7* expression.¹⁰ Labelled products were separated using a 25 min run on an ABI Prism 3100 DNA sequencer and data were analysed using the GeneMapper Analysis Software version 4.0 (Applied Biosystems). Primer sequences used for genotyping and conditions of the SNaPshot multiplex assays are available on request.

Statistical analyses

The genotype frequencies were tested for Hardy-Weinberg equilibrium. In all following analyses of time to CRC, first CRC onset was the event of interest, age (in years) was used at the time scale and retrospective follow-up started at birth for all subjects in the study and ended at either first CRC onset for incident CRC cases or age at last follow-up otherwise. These analyses were performed for all subjects and separately for males and females and were repeated for CRC cases only. The association of sex or mutated MMR gene with CRC risk was assessed using the log-rank test. The association of each polymorphism (*i.e.*, five SNPs and *IGF1* CA repeat length) and CRC risk was assessed using the log-rank test then the Cox proportional hazard model with stratification on gender (if applicable), mutated MMR gene (*MSH2*, *MLH1*, *MSH6*) and date of birth (<1940, 1940-1949, 1950-1959, 1960-1969, 1970-1979 and \geq 1980) and with control for familial clustering. In the Cox model analyses of each of the five SNPs, an overall comparison of the three genotypes was performed and a hazard ratio (HR) and corresponding 95% confidence interval were estimated for homozygous and heterozygous subjects, relative to subjects homozygous for the wild-type allele. Moreover, alternative Cox model analyses of each of the five SNPs considered the number of alleles with the SNP (0, 1, 2) and fitted a trend producing and alternative test of association with CRC risk and an estimate of the CRC hazard ratio per mutated allele. Statistical analyses were performed using the SAS software version 9.1 (SAS Institute, Cary NC).

RESULTS

Among the 748 MMR mutation carriers included into this study, 329 carriers (44%) developed CRC. As shown in Table 1, a significant difference was observed in the age

at first CRC occurrence between males and females, with males developing CRC earlier, between *MSH2* and *MSH6* mutation carriers, between *MLH1* and *MSH6* mutation carriers, with *MSH2* and *MLH1* carriers developing CRC earlier, but not between *MSH2* and *MLH1* mutation carriers (log-rank test, $P = 0.26$). The five SNPs analysed, located on 8q23.3, 11q23.1, 18q21 and within *CYP1A1* were in Hardy-Weinberg equilibrium. The distribution of the *IGF1* CA repeat alleles was the following: 12 repeats ($n=8$), 13-15 repeats ($n=0$), 16 repeats ($n=2$), 17 repeats ($n=28$), 18 repeats ($n=85$), 19 repeats ($n=976$), 20 repeats ($n=262$), 21 repeats ($n=114$) and 22 repeats ($n=21$) and alleles were dichotomised in ≤ 17 and ≥ 18 repeats for the statistical analyses, like in the previous studies.^{5,6}

In univariate analysis, age at first CRC occurrence did not significantly vary for the five SNPs considered or according to number of *IGF1* CA repeats whether for the whole sample or separately for males and females (Table 2). As indicated in Table 3, the multivariate Cox analysis performed in the total sample of mutation carriers revealed that, for rs16892766 (8q23.3), the homozygosity for the minor C allele present only in 3 mutation carriers was significantly associated to a decreased risk relative to subjects with AA alleles (HR = 0.267, $P = 0.0271$). For the *CYP1A1* SNPs, we observed a significant difference only in males for one of the SNPs analysed (rs4646903): the CC genotype, detected in 4 male mutation carriers, was associated with an increased risk relative to subjects with TT alleles (HR = 3.496, $P = 0.0033$). Considering that penetrance of *MSH6* mutation was found in this study to be lower than that of *MSH2* and *MLH1* mutations, we restricted the log-rank and Cox analyses to *MSH2* and *MLH1* carriers and this restriction did not modify the results (data not shown).

Since colonoscopy had not been performed in all the unaffected mutation carriers at the time of this study, we then focused the statistical analyses on the 329 MMR

mutation carriers with CRC, in order to avoid a bias in the phenotypic evaluation. As indicated in Table 4, the unrestricted multivariate Cox analysis showed that the only remaining significant association was a decreased risk associated with the 8q23.3 *CC* genotype but it should be emphasized that, because this genotype was only carried by two CRC cases, no HR estimate could be produced.

DISCUSSION

Like in other Mendelian predisposition to cancer with an incomplete penetrance, the characterization of modifier loci for CRC in Lynch syndrome might allow identification of a subset of mutation carriers who could benefit from a reinforced tumour detection program, such as annual chromocolonoscopy from 20 years of age. This prospect prompted us to evaluate in a large series of 748 MMR carriers the risk conferred by the allelic variants which have recently been reported as risk modifiers for CRC in MMR mutation carriers. Within this series, 44% of the MMR mutation carriers had developed CRC at the time of the analysis, the median age of CRC onset was 43 years among them and we observed a significant difference of CRC penetrance between males and females and between *MSH2* and *MLH1* mutation *versus* *MSH6* mutation carriers. These results are in agreement with the published studies which have estimated that the cumulative CRC risk at 70 years is higher in males than in females²⁻⁴ and is lower in *MSH6* mutation carriers as compared to *MSH2* or *MLH1* mutation carriers.^{2,3,11,12} These findings are in favour of the representativeness of our series with respect to Lynch syndrome.

The distribution of *IGF1* CA repeat length was similar to that reported by Zecevic et al.⁴ in 121 MMR mutation carriers, the frequency of the major allele (19 repeats) being 65% and 58% in the two studies, respectively, although we found a lower

proportion of alleles with length ≤ 17 (2.5% versus 5.1%). In the study published by Zecevic et al.⁵ on 121 MMR mutation carriers, the presence of a CA repeat ≤ 17 , detected in 12 subjects, was found to be associated with a higher CRC risk (HR 2.36) and an earlier median age of CRC onset (44 versus 56.5 years), but our study, performed on a larger series, did not allow us to reproduce these results.

For the rs1048943 (c.1384A>G; p.Ile462Val) and rs4646903 SNPs, located within the *CYP1A1* gene, we found respective allelic frequencies for the minor allele of 0.03 and 0.12, which correspond to the HapMap values estimated in the European population. Pande et al.⁸ had reported a higher MAF (0.074) for rs1048943 in 257 MMR mutation carriers, but it should be underlined that these carriers were from different ethnicities and included Hispanic and Asian subjects, in whom the allelic frequency of the *G* allele is known to be higher. In this series of 257 mutation carriers, CRC free survival statistically differed according to rs1048943 genotype, with a 1.78 fold increase of CRC hazard associated with the *AG* genotype, a result that we did not reproduce in our series of 748 MMR mutation carriers from Caucasian origin. For the second *CYP1A1* SNP (rs4646903), Pande et al.⁸ had reported that the *TC* genotype was associated with an increased hazard for earlier CRC and we detected no significant difference in our study for this genotype (Table 3). In contrast, we found that the *CC* genotype was associated in males with a 3.5 fold increased hazard ratio, but this observation should be interpreted with utmost caution, considering the limited number of male mutation carriers presenting this genotype (n=4). For rs16892766 (8q23.3) and rs3802842 (11q23.1) SNPs, we observed respective allele frequencies of 7% and 28%: these frequencies had been respectively estimated in the European population at 7%¹³ and 29%¹⁴ and, in 675 Dutch MMR mutation carriers at 10% and 24.9%.⁹ We did not detect in our series a modification of CRC risk in mutation carriers harbouring the 11q23.1

rs3802842 *CC* genotype, as previously reported in females. Among the 675 Dutch mutation carriers, the 8q23.3 rs16892766 *CC* genotype, detected in 9 subjects, was found to be associated with a 2.16 fold CRC risk increase.⁹ In contrast, we found in our study (Table 3) that this genotype was significantly associated to a decreased CRC risk (HR = 0.267), and this significant association was the only one remaining when we restricted the analysis to affected carriers, but the small number of subjects harbouring this genotype (n=3) and presenting a CRC (n=2) must be highlighted.

During the submission of this study, Talseth-Palmer et al.¹⁵ reported that in *MLH1* carriers, but not in *MSH2* carriers, the 11q23.1 *CC* and 8q23.3 *AC* genotypes were associated with an increased risk, but this significant association detected in 373 Australian mutation carriers was not found in 311 Polish mutation carriers analysed in the same study. We did not replicate either of these associations in the French sample. Indeed, the only association, that we detected for these two SNPs in the 267 French *MLH1* mutation carriers was a decrease of CRC risk with the 8q23.3 *CC* genotype (p<0.0001) but as this genotype was carried by only one CRC case, it was not possible to estimate the corresponding HR and due caution should be applied to interpret this finding.

To increase the power of the statistical analysis of the 8q23.3 and 11q23.1 SNPs impact on CRC risk in *MMR* mutation carriers, we performed a meta-analysis based on the study published by Wijnen et al.⁹ and our results, corresponding to 1423 *MMR* mutation carriers (Figure 1). In univariate analysis, age at first CRC occurrence significantly varied in male carriers according to the 8q23.3 genotype (*AA*, *AC*, or *CC*; P=0.0265 using Fisher's standard method to combine P-values from two log-rank tests), with increased CRC risk associated with the *AC* genotype and decreased risk associated with the *CC* genotype. In multivariate Cox analysis, as shown on the Forest plot for the

total sample (Figure 1), CRC risk did not significantly vary according to the 8q23.3 genotype. In males, the multivariate Cox analysis showed that the 8q23.3 *CC* genotype was associated with a CRC risk decrease (combined fixed-effect weighted sum HR = 0.61 relative to *AA*, P=0.0135). It remains however that there were very few males and even fewer male CRC cases carrying the *CC* genotype in both studies and thus the results of this meta-analysis should be interpreted with caution. Univariate and multivariate Cox analyses did not reveal any significant association between CRC risk and the 11q23.1 genotype (Figure 1).

In conclusion, the evaluation in a series of 748 MMR mutation carriers representative of Lynch syndrome of the *IGFI* CA repeat, *CYP1A1*, 8q23.3 and 11q23.1 SNPs, previously reported as modifier factors for CRC risk in MMR mutation carriers, did not allow us to reproduce the previously published results. The only significant association that we detected both in the whole sample and in the unbiased affected mutation carrier sample, was between the 8q23.3 *CC* genotype and a decreased CRC risk in males, whereas previously published studies had reported an increased CRC risk associated either with this genotype⁹ or with the heterozygous *AC* genotype.¹⁵ This discrepancy might be explained by the genetic heterogeneity between the different analysed populations. Considering that, in MMR mutation carriers, cancer development requires somatic mutation of several target genes containing repeated sequences, it is likely that numerous polymorphisms affecting either the DNA repair or target genes and / or that environmental factors playing a role in colorectal carcinogenesis will modify the penetrance of MMR mutations. It is also possible that a key factor modulating the penetrance is the allelic heterogeneity of the MMR mutations. Finally, another explanation of this discrepancy between our results and previous studies is that the previously reported associations were spurious associations due to too small samples.

Therefore, we conclude that, from the medical point of view, genotyping of these polymorphisms is not useful to evaluate CRC risk in MMR mutation carriers and to optimise their clinical follow-up.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1 Clinical characteristics of MMR mutation carriers

	Number of subjects (%) (n=748)	Number of incident CRC cases (n=329)	Log-rank test P-value
Gender			
Male	349 (46.7)	177	<0.0001
Female	399 (53.3)	152	
Index case			
Yes	290 (38.8)	259	
No	458 (61.2)	70	
MMR gene mutated			
<i>MSH2</i>	414 (55.4)	178	0.0495 (<i>vs MSH6</i>)
<i>MLH1</i>	267 (35.7)	115	0.0069 (<i>vs MSH6</i>)
<i>MSH6</i>	67 (8.9)	36	
Year of birth			
<1940	57 (7.6)	39 (11.8)	
1940-1949	106 (14.2)	73 (22.2)	
1950-1959	188 (25.1)	119 (36.2)	
1960-1969	165 (22.1)	66 (20.1)	
1970-1979	129 (17.2)	26 (7.9)	
≥1980	103 (13.8)	6 (1.8)	
Age			
With CRC	329 (44)	mean, min/max 43, 18/82	
Without CRC	419 (56)	38, 18/78	

Table 2 Allelic frequency of genetic variants and assessment of the associated colorectal risk by the log-rank test

Genetic variant	Allelic frequency	Log-rank test P-value	
		All subjects (n=748)	CRC patients (n=329)
- rs16892766 (8q23.3)			
MAF	0.07 (<i>C</i> allele)		
Total sample		0.2188	0.0946
Males		0.3146	0.5209
Females		0.7409	0.0585
- rs3802842 (11q23.1)			
MAF	0.28 (<i>C</i> allele)		
Total sample		0.6206	0.2316
Males		0.8345	0.7805
Females		0.7557	0.4081
- Novel 1 (18q21)			
MAF	0.47 (<i>C</i> allele)		
Total sample		0.9698	0.3560
Males		0.4676	0.3483
Females		0.8847	0.2349
- rs1048943 (<i>CYP1A</i>)			
MAF	0.03 (<i>G</i> allele)		
Total sample		0.7632	0.3442
Males		0.8957	0.5035
Females		0.9003	0.6082
- rs4646903 (<i>CYP1A</i>)			
MAF	0.12 (<i>C</i> allele)		
Total sample		0.9489	0.6490
Males		0.3701	0.7074
Females		0.6772	0.5724
- <i>IGF1</i> CA repeat			
MAF	0.025 (≤ 17)		
Total sample		0.5763	0.6002
Males		0.2918	0.1562
Females		0.9457	0.4249

Table 3 Evaluation in 748 MMR mutation carriers of the CRC risk associated with 8q23.3, 11q23.1, 18q21, *CYP1A1* and *IGF1* variants

Locus	Genotype	Total sample (N = 748)					Males (N = 349)					Females (N = 399)				
		N	CRC cases	Hazard Ratio#	95% CI	P*	N	CRC Cases	HR	95% CI	P*	N	CRC cases	HR	95% CI	P*
8q23.3	rs16892766															
	AA	641	277	1			290	142	1			351	135	1		
	AC	104	50	1.184	0.866-1.617	0.2892	57	34	1.266	0.836-1.916	0.2656	47	16	1.067	0.677-1.680	0.7802
	CC	3	2	0.267	0.083-0.861	0.0271	2	1	0.460	0.301-0.701	0.0003	1	1	/	/	<0.0001
	<i>Per allele</i>			1.032	0.778-1.368	0.8283			1.117	0.770-1.621	0.5602			0.899	0.575-1.405	0.6397
11q23.1	rs3802842															
	AA	380	167	1			178	90	1			202	77	1		
	AC	321	139	0.923	0.725-1.176	0.5165	141	71	0.844	0.611-1.167	0.3047	180	68	1.019	0.723-1.438	0.9125
	CC	47	23	1.107	0.684-1.791	0.6793	30	16	1.043	0.599-1.815	0.8814	17	7	1.222	0.543-2.750	0.6284
	<i>Per allele</i>			0.988	0.806-1.210	0.9058			0.947	0.735-1.220	0.6740			1.052	0.778-1.422	0.7428
18q21	Novel 1															
	CC	156	72	1			84	45	1			72	27	1		
	CG	395	165	0.956	0.704-1.296	0.7702	185	87	1.021	0.675-1.546	0.9215	210	78	0.865	0.546-1.370	0.5359
	GG	197	92	1.006	0.727-1.390	0.9729	80	45	1.088	0.706-1.677	0.7007	117	47	0.903	0.564-1.447	0.6714
	<i>Per allele</i>			1.007	0.859-1.181	0.9326			1.044	0.841-1.295	0.6962			0.964	0.765-1.215	0.7570
<i>CYP1A1</i>	rs1048943															
	AA	708	313	1			331	168	1			377	145	1		
	AG	40	16	1.026	0.601-1.752	0.9249	18	9	0.930	0.444-1.948	0.8480	22	7	1.168	0.583-2.338	0.6619
	GG	0	0	/	/	/	0	0	/	/	/	0	0	/	/	/
	<i>Per allele</i>			1.026	0.601-1.752	0.9249			0.930	0.444-1.948	0.8480					0.6619
<i>CYP1A1</i>	rs4646903															
	TT	598	265	1			279	143	1			319	122	1		
	TC	140	61	1.031	0.769-1.382	0.8400	66	32	0.909	0.601-1.375	0.6524	74	29	1.198	0.789-1.818	0.3956
	CC	10	3	1.163	0.387-3.495	0.7882	4	2	3.496	1.517-8.057	0.0033	6	1	0.507	0.071-3.640	0.4997
	<i>Per allele</i>			1.039	0.801-1.347	0.7730			1.001	0.680-1.474	0.9948			1.079	0.746-1.561	0.6874
<i>IGF1</i>	CA repeat															
	≥18; ≥18	710		1			329		1			381		1		
	≥18; ≤17	38		0.731	0.447-1.196	0.2127	20		0.662	0.372-1.179	0.1611	18		0.979	0.450-2.132	0.9579

Table 4 Evaluation in 329 MMR mutation carriers with CRC of the risk associated with 8q23.3, 11q23.1, 18q21, *CYP1A1* and *IGF1* variants

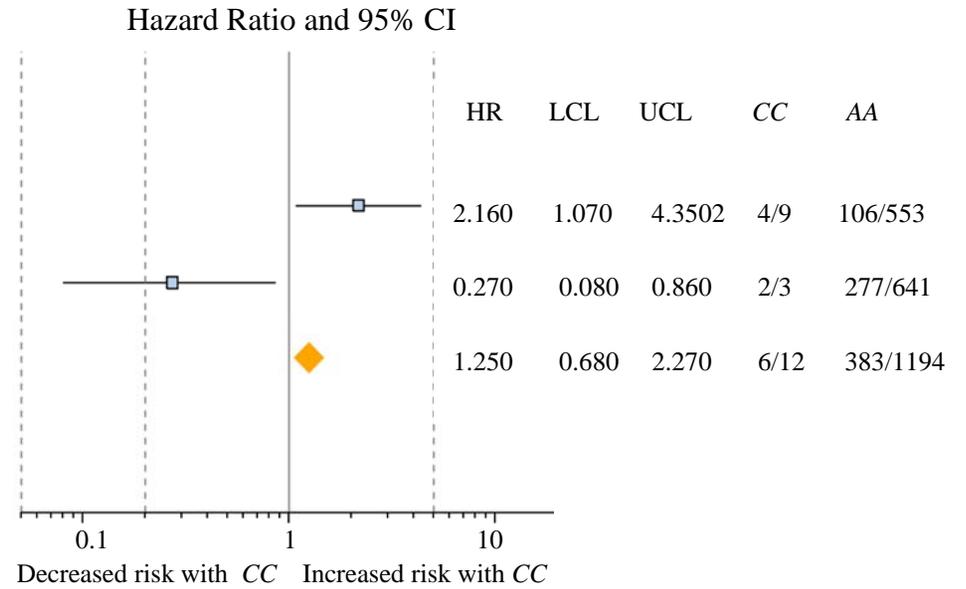
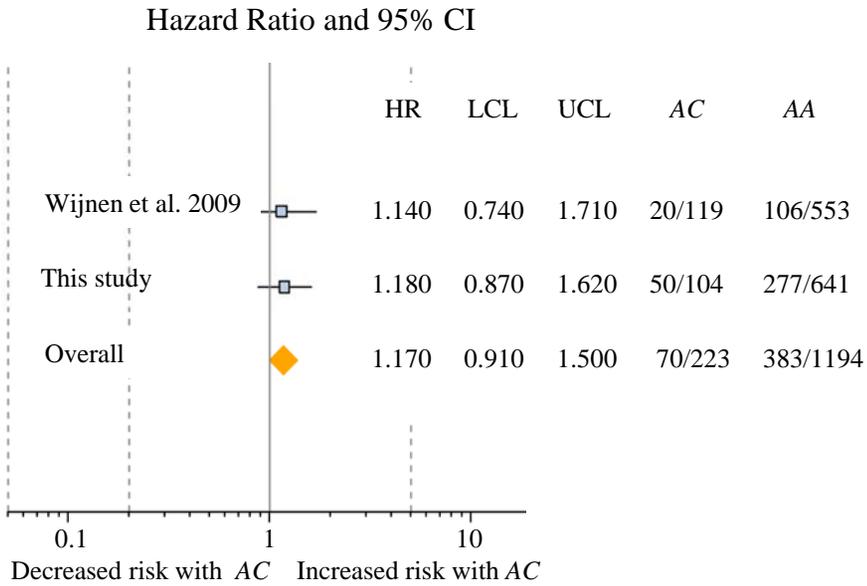
Locus	Genotype	Total sample (N = 329)				Males (N = 177)				Females (N = 152)			
		N	Hazard Ratio#	95% CI	P*	N	HR	95% CI	P*	N	HR	95% CI	P*
8q23.3	rs16892766												
	AA	277	1			142	1			135	1		
	AC	50	0.793	0.559-1.124	0.1920	34	0.768	0.478-1.234	0.2749	16	0.833	0.5041-1.376	0.4759
	CC	2	/	/	<0.0001	1	/	/	<0.0001	1	/	/	<0.0001
	<i>Per allele</i>		0.666	0.481-0.922	0.0144		0.647	0.414-1.009	0.0549		0.698	0.443-0.102	0.1227
11q23.1	rs3802842												
	AA	167	1			90	1			77	1		
	AC	139	0.941	0.738-1.199	0.6216	71	0.840	0.593-1.191	0.3274	68	1.067	0.751-1.515	0.7180
	CC	23	1.234	0.694-2.195	0.4736	16	1.034	0.548-1.950	0.9182	7	1.815	0.664-4.963	0.2457
	<i>Per allele</i>		1.028	0.831-1.272	0.9763		0.947	0.723-1.239	0.947		1.170	0.854-1.604	0.3289
18q21	Novel 1												
	CC	72	1			45	1			27	1		
	CG	165	0.828	0.588-1.166	0.2804	87	1.081	0.701-1.667	0.7249	78	0.571	0.315-1.035	0.0647
	GG	95	0.741	0.516-1.062	0.1029	45	0.867	0.554-1.358	0.5326	47	0.570	0.305-1.068	0.0793
	<i>Per allele</i>		0.865	0.726-1.031	0.1056		0.919	0.735-1.149	0.4607		0.796	0.595-1.065	0.1238
<i>CYP1A1</i>	rs1048943												
	AA	313	1			168	1			145	1		
	AG	16	1.107	0.609-2.011	0.7387	9	1.105	0.502-2.432	0.8045	7	1.110	0.447-2.754	0.8224
	GG	0	/	/	/	0	/	/	/	0	/	/	/
	<i>Per allele</i>		1.107	0.609-2.011	0.7387		1.105	0.502-2.432	0.8045		1.110	0.447-2.754	0.8224
<i>CYP1A1</i>	rs4646903												
	TT	265	1			143	1			122	1		
	TC	61	0.988	0.725-1.348	0.9412	32	1.070	0.696-1.647	0.7568	29	0.902	0.572-1.421	0.6558
	CC	3	1.741	0.925-3.278	0.0856	2	2.276	0.890-5.822	0.0861	1	1.241	0.759-2.029	0.3895
	<i>Per allele</i>		1.038	0.786-1.372	0.7919		1.139	0.773-1.678	0.5117		0.930	0.618-1.399	0.7277
<i>IGF1</i>	CA repeat												
	≥18; ≥18	314	1			166	1			148	1		
	≥18; ≤17	15	0.710	0.397-1.272	0.2502	11	0.562	0.297-1.063	0.0765	4	1.709	0.470-6.212	0.4160

#Hazard ratio relative to subjects not carrying the SNP on either allele for each of the five SNPs considered or to subjects with CA repeat length greater or equal to 18 on both alleles for the *IGF1* promoter and obtained from Cox proportional hazard regression stratified on gender (if applicable), mutated MMR gene (*MSH2*, *MLH1*, *MSH6*) and year of birth (<1940, 1940-1949, 1950-1959, 1960-1969, 1970-1979 and \geq 1980), and with control for familial aggregation. The per allele value refers to Cox regression with a trend fitted for number of alleles carrying the SNP (0,1,2).

*For each of the five SNP variants and each sample (total sample, males, females), the first two P-values refer to separate comparisons of heterozygous and homozygous subjects for the SNP considered with subject not presenting the SNP on either allele; the third P-value refers to the overall comparison of heterozygous, homozygous and SNP-free subjects (heterogeneity test); the final P-value refers to the assessment of the trend for the number of alleles carrying the SNP (0,1,2). For *IGF1* CA repeat length, the P-value refers to the comparison of subjects with CA repeat length less or equal to 17 on one allele to subjects with CA repeat length greater or equal to 18 on both alleles. All tests are Wald tests.

Figure 1. Forest plots of the meta-analysis of the *CRC* risk associated with 8q23.3 and 11q23.1 variants in 1423 MMR mutation carriers. HR: Hazard Rate; CI: Confidence Interval; LCL: Lower 95% Confidence Limit; UCL: Upper 95% Confidence Limit. For each panel, the last two columns display the number of subjects with colorectal cancer and the indicated genotype followed by the overall number of subjects with this genotype.

Forest plots for 8q23.3



Forest plots for 11q23.1

