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RESEARCH

TP53 Arg 72Pro and *MDM2* SNP309 Polymorphisms and Colorectal Cancer Risk: A West Algerian Population Study

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Abstract The tumor suppressor gene *TP53* and its regulator *MDM2* are both key players involved in multiple pathways including apoptosis, cellular transcriptional control and cell cycle regulation. Common germline polymorphisms in these genes may affect colorectal cancer (CRC) susceptibility. An arginine-to-proline substitution at codon 72 in the *TP53* gene is reported to decrease apoptotic potential, while a thymine-to-guanine polymorphism at nucleotide 309 (named SNP309) of murine double minute 2 *MDM2* gene increases its transcription. These two polymorphisms therefore may be of importance in colorectal carcinogenesis. The relation of these polymorphisms to colorectal cancer in the Algerian population was addressed in this study. DNA samples from 121 controls and 116 cases were genotyped for these two polymorphisms by PCR/RFLP then confirmed by sequencing. Unexpectedly no significant association was found between this potential marker *TP53* Arg72Pro and CRC ($p>0.05$). However, our findings reveal that individuals with the *MDM2* SNP309 GG genotype have a low risk of CRC as compared to the TT genotype (*OR*=0.49; 95 % CI: 0.24–0.98, $p=0.04$), with more significance for females (*OR*=0.16; 95 % CI: 0.06–0.41, $p<0.05$). Moreover, no significant association was observed between the combined *TP53* and *MDM2* genotypes and CRC. Contrary to initial expectations that the GG genotype with high *MDM2* levels would increase cancer risk, our results demonstrate that the *MDM2* SNP309 GG genotype is associated with decreased risk of colorectal cancer. This is suggesting that other mechanisms independent of increased *MDM2* levels can influence cancer susceptibility.

Keywords Polymorphism · *TP53* Arg72Pro · *MDM2* SNP309 · CRC · Algerian population · Case/Control study

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Introduction

Colorectal cancer (CRC) is a major world health plague. It develops from a polyp through an adenoma and dysplasia to a carcinoma with a metastatic potential [1]. The high mortality in developing countries is due to its late diagnosis. Because of its high frequency and severity, CRC is a serious public health problem in Algeria where it represents almost 13 % of cancers and is ranked third after lung, and breast cancers [2].

Previous epidemiological studies have identified dietary factors such as consumption of meat, especially red meat, and cigarette smoking as possible risk factors of the development of CRC [3, 4]. However, most individuals with these dietary risk factors never develop CRC; while many CRC cases develop among individuals without those known risk factors. The exact mechanism of CRC carcinogenesis is unclear.

The tumor suppressor *TP53* plays a critical role in defense against cancer development and progression. In response to

cellular stressors, p53 regulates the transcription of a wide range of genes including those involved in cell cycle, apoptosis, inhibition of angiogenesis and cellular senescence [5]. The Arg72Pro polymorphism (G > C change at nucleotide 215, rs1042522) of the gene *TP53*, has shown much interest in relation with cancer susceptibility and prognosis [5]. This polymorphism introduces a significant change in the structure as it occurs in the praline-rich domain of p53, which is necessary for this protein to fully induce apoptosis. Different studies have shown that, the p53 72Arg form is more efficient in apoptosis induction, whereas the p53 72Pro form was suggested to induce better G1 arrest and DNA repair but the role of this polymorphism in the development of human cancers remains uncertain. A number of studies have investigated the genetic effect of the *TP53* Arg72Pro polymorphism on CRC susceptibility, with contradictory results. Some studies [6–9] but not all [10–19]; support that the 72Pro allele was associated with an increased risk of CRC,

Otherwise, the murine double minute-2 (*MDM2*), a crucial negative regulator of the tumor suppressor *TP53*, has been implicated in a variety of cancers [20]. A single nucleotide polymorphism (SNP) in the promoter region of *MDM2*, SNP T309G (rs2279744), was demonstrated to increase the affinity of binding of the stimulatory protein Sp1 which result in increased *MDM2* expression and subsequent attenuation of the p53 pathway [21]. Following the discovery of the SNP309 polymorphism, conflicting evidence has linked the G-allele to enhanced cancer risk as well as early cancer diagnosis across different tumor types and ethnic groups [22]. It was reported that the increase in MDM2 protein results in direct inhibition of P53 transcriptional activity, enabling damaged cells to escape the cell-cycle checkpoint and become carcinogenic [23]. Hence, it is biologically reasonable to hypothesize a potential relationship between the *MDM2* SNP309 polymorphism and CRC risk. Whereas, the combined effect of SNP309 and *TP53* Arg72Pro polymorphisms on cancer risk has been addressed by few studies. SNP309 was not associated to risk of breast cancer [24] and bladder cancer [25] singly or in combination with the *TP53* Arg72Pro polymorphism. One small study of Lynch syndrome patients found no combined effect of SNP309 and *TP53* Arg72Pro polymorphisms on either the risk of colorectal cancer or age of diagnosis [26].

In this study, we examined the relation between *MDM2* SNP309 and *TP53* Arg72Pro polymorphisms and colorectal cancer risk, in the West Algerian population.

Material and Methods

Patients

This study includes only patients from the West of Algeria. Data from patient records were collected including patient gender and age at diagnosis. The control group was composed of 121 blood samples, obtained from healthy donors. The 116 blood samples of the CRC group was collected, from patients with sporadic CRC diagnosed at different stages, and confirmed after anatomopathological analysis, in oncology department of the University Hospital of Oran. The ethics committee of the Hospital approved the study and informed consent was obtained from all patients.

DNA Extraction

Genomic DNA from blood samples was prepared using a simple salting out procedure, as described in [27].

Genotyping

The *TP53* Arg72Pro polymorphism was determined by PCR-RFLP, using primers: sense: 5'CGTTCTGGTAAGGACAAG GGTT3' and antisense: 5'TCCATGAGACTTCAATGCCT GG3'. The product size expected was 441pb. 200 ng of DNA were used as template in a 25 μl PCR reaction mixture containing: 1.5 μmol MgCl2, 2 μmol of primers, 1 U Taq polymerase (Perkin Elmer Applied Biosystems, Weiterstadt, Germany). PCR cycling conditions were carried out with an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, A final extension step was performed at 72 °C for 5 min. PCR products were digested by 10 U of the endonuclease BtgI, which specifically cleaves the allele coding for Pro72, but not that for Arg72. Fragments were analyzed on 1 % agarose gel. Cleaved PCR products resulted in two fragments of 235 and 206 bp corresponding to the Pro allele, and a third one of 441 bp corresponding to the Arg allele.

Genotyping of the SNP309 polymorphism was determined according to the methods described elsewhere [28], using primers; sense: 5'CGGGAGTTCAGGGTAAAGGT3' and antisense: 5'AGCAAGTCGGTGCTTACCTG3'. The PCR product of 352 bp was digested with MspA1I, resulting in fragments of 187, 88, 46 and 31 bp for the G allele, and 233, 88 and 31 bp for the T allele.

Genotyping results were confirmed by sequencing. The PCR products generated were purified using a modification of the ExoSAP enzymatic clean-up method. 5 μl of PCR product was incubated with 1 U of Exonuclease I and 1 U of Shrimp Alkaline Phosphatase for 20 min at 37 °C then inactivated by incubating at 80 °C for 15 min. 7μL of each purified product was sequenced by using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) according to manufacturer's protocol. Genotyping of the products obtained sequencing was performed using capillary analyzer ABI PRISM® 3130 (Applied Biosystems). Sequence analyze was performed using the software: "Seqscanner" and "Multalin".

Statistical Analysis

For statistical analysis, we used EpiInfo ™ version seven to calculate the odds ratio (OR) and 95 % confidence interval (CI). AP value of less than 0.05 was used as significance criteria.

Results

This analysis included 116 CRC and 121 cancer-free control subjects. All samples were successfully genotyped for both polymorphisms. The compiled data analysis of the two groups is summarized in Table 1.

Frequency of TP53 Arg72Pro Polymorphism in CRC Group

RFLP analysis and sequencing showed that the distributions of the three different genotypes among CRC patients were as follows: 85.34 % homozygous for Arg/Arg genotype and 14.65 % for heterozygous Pro/Arg + Pro/Pro genotype (p=0.8). Pro and Arg alleles have almost similar frequencies in cases compared to controls (CRC group: Pro=9.91 %, Arg=90.08 % vs Control group: Pro=9.09 % and Arg=90.90 %). Distribution of alleles and genotypes in CRC group compared to their distribution in the controls, showed no significant difference (Table 2).

Frequency of MDM2 SNP309 Polymorphism in CRC Group

Distribution of T and G alleles in CRC group compared to their distribution in the controls showed a significant difference (Table 3). The TT, TG, and GG genotypes of the *MDM2* SNP309 were observed in 52.6, 30.2, and 17.2 % of patients. Using the logistic regression, a statistically significant and inverse association was observed between *MDM2* SNP309 genotypes and risk of colorectal cancer (TG versus TT: *OR*=0.47; 95 % CI: 0.26–0.84, p=0.01; GG versus TT: *OR*=0.49; 95 % CI: 0.24–0.98, p=0.04) (Table 3).

Frequency of MDM2 SNP309 Polymorphism and Arg72Pro in CRC Group

We next evaluated if P53 Arg72Pro modified the effect of *MDM2* SNP 309, but no association was found between these two polymorphisms (Table 4).

Table 1 Compiled data analysis of patients and controls

	Healthy controls	CRC
All	121	116
Gender		
Male	61	70
Female	60	46

Risk of Colorectal Cancer According to Genotypes of the TP53 Arg72Pro and MDM2 SNP309 Polymorphisms and the Gender

We found that women carrying the variant allele had statistically significant lower risk of colorectal cancer with an OR of 0.16 (95 % CI: 0.06–0.41) (p<0.05) compared to women carrying the wild type allele (see Table 5).

Discussion

This study evaluated the associations between the polymorphisms *MDM2* SNP309 and *TP53* codon Arg72Pro and the occurrence of CRC in the West Algerian population. Our results reject the hypothesis of the association of Arg72Pro of *TP53* with the risk of developing CRC in either men or women and reject the hypothesis of the GG genotype as a risk factor of CRC and suggest that women carrying at least one G allele might have a lower risk of developing CRC.

Since the identification of the *TP53* Arg72Pro polymorphism as a critical biomarker in modifying the risk of cancer [29], many studies have reported its association with CRC. This statement has been made for the majority of populations tested so far, such as Tunisian [30], European [31] and Japanese [32], with some exceptions for black African, African-American [33, 34] and central Chinese population [35]. Yet, Arg allele frequency reported here is the highest ever described, further supporting the idea that the allele frequencies of this polymorphism are ethnically related.

Our results reject its association with the risk of developing CRC. This result is in agreement with a study in a Chinese population [8], and in disagreement with two other studies on Greek-Caucasian and Korean populations, which reported respectively Arg or Pro alleles as predisposing to CRC [17, 9]. These conflicting results could be explained by the involvement of additional genetic and environmental factors. Still, meta-analysis on case-control studies (7414 cases and 9872 controls), showed no association between the *TP53* Arg72Pro polymorphism status and CRC risk in either men or women [36].

Like for *TP53* Arg72Pro polymorphism, a number of studies have investigated the genetic effect of *MDM2* SNP309 on CRC susceptibility but with contradictory results. A meta-analysis of 8 studies by Cao et al.,. in 2012 [37] showed that the *MDM2* SNP309 polymorphism might be a risk factor for CRC. In this study, the variant genotype was associated with a significant increased CRC risk among the overall populations (GT vs. TT: *OR*=1.19, 95 % CI: 1.06–1.35). Another meta-analysis including 7 studies by Fang et al., [38], drew an opposite conclusion. The authors revealed that the *MDM2* SNP309 polymorphism played a protective role in CRC

Table 2 Frequency distribution of *TP53* codon 72 polymorphism between cases and controls and its association with risk of colorectal cancer

	Controls (*n=121*) N. (%)	CRC Patients (*n=116*) N. (%)	OR (95 % CI)	*P Value*
Genotype				
Arg/Arg	102 (84.29)	99 (85.34)	1 [a]	
Arg/Pro + Pro/Pro	19 (15.69)	17 (14.65)	0.92 (0.45 to 1.87)	**0.82**
Allele				
Arg	220 (90.91)	209 (90.09)	1 [a]	
Pro	22 (9.09)	23 (9.91)	1.1 (0.6 to 2.03)	**0.75**

N number, % percentage, *OR* odds ratio, *CI*confidence interval, *p* significance, *a* genotype saved as reference category

susceptibility in Asians (GG vs. TT: *OR*=0.51, 95 % CI :0.41–0.64; GG vs. TG: *OR*=0.64, 95 % CI: 0.53–0.78; GG + TG vs. TT: *OR*=0.59, 95 % CI: 0.49–0.71; GG vs. TG+TT: *OR*=0.69, 95 % CI: 0.57–0.82). Finally, in 2013 an updated meta-analysis on the association of *MDM2* SNP309 polymorphism with colorectal cancer risk suggested that the *MDM2* is a candidate gene for CRC susceptibility [39]. The *MDM2* SNP309 polymorphism may be a risk factor for CRC in Asians and African populations, but not in Europeans. Currently there is only one study on *MDM2* SNP309 polymorphism and CRC risk among African population [40], and the genotype distributions in the control population of this study was deviated from HWE. Therefore, the positive results of the African population should be interpreted with caution.

In summary, we show here that the *MDM2* SNP309 GG genotype is associated with a protective effect of colorectal cancer in the West Algerian population. The presence of the *TP53* codon 72Pro allele did not lead to a statistically significant interaction affect the magnitude of this risk. The finding that the *MDM2 GG* genotype decreased risk was unexpected, since as described earlier, many studies that have reported associations have found increased risk with the GG genotype that are consistent with the impact of this genotype on *MDM2* RNA and protein levels, and hence, on inhibition of p53 [20]. In our study, we found a non-statistically reduced OR in relation to CRC. We cannot speculate on the basis of our results that increased levels of MDM2 protein might be associated with the occurrence of CRC.

Thus, it is possible that the association of the G or the T alleles with increased cancer risk may be influenced by ethnicity and by environmental factors unique to that particular population. More in depth larger scale studies are now required to further extend these findings.

How does the GG genotype lead to a decreased cancer risk?

It is at present not well understood, but there is evidence to suggest that the role of *MDM2* in tumorigenesis may vary in a gender-specific manner. Thus, it is not inconceivable that the mechanisms by which *MDM2* modulates colorectal cancer risk can differ between populations. The role of hormones in carcinogenesis is rapidly emerging as a complex, but important pathway. The estrogen-signaling pathway has been implicated in the role of SNP309 in accelerated formation of several cancers [22]. Besides, MDM2 protein may act as a strong contributor via the p53-independent pathway during the process of estrogen-induced cell proliferation [41]. A previous study showed that the SNP309 G allele was associated with the diagnosis of colorectal cancer at an earlier age for women, but not for men [42].

The G allele of SNP 309 of *MDM2* gene can induce the expression of p65 subunit of NF-kB which acts as an anti-apoptotic factor in neoplastic cells [43]. In addition, this same allele increases the binding affinity for Sp1, a receptor co-activator for multiple hormones including estrogen. It may affect the hormone-dependent transcriptional regulation of *MDM2* and results in elevation of the MDM2 protein level

Table 3 Frequency distribution of *MDM2* SNP309 between cases and controls and its association with risk of colorectal cancer

	Controls (*n=121*) N.(%)	CRC Patients (*n=116*) N. (%)	OR (95 % CI)	*P Value*
Genotype				
TT	42 (34.8)	61 (52.6)	1 [a]	
TG	51 (42.1)	35 (30.2)	0.47 (0.26 to 0.84)	**0.01**[*]
GG	28 (23.1)	20 (17.2)	0.49 (0.24 to 0.98)	**0.04**[*]
TG + GG	79 (65.2)	55 (47.4)	0.48 (0.28 to 0.80)	**0.005**[*]
Allele				
T	135 (55.8)	157 (67.7)	1 [a]	
G	107 (44.2)	75 (32.3)	0.60 (0.41 to 0.87)	**0.007**[*]

N Number, % Percentage, *OR* Odds ratio, *CI* Confidence Interval, *p* significance, *a* genotype saved as reference category

*: $p<0.05$ considered as statistically significant

Table 4 Analysis of joint effects for *TP53* Arg72Pro and *MDM2* SNP309 genotypes on colorectal cancer risk

Genotypes	Controls (*n=121*) N.(%)	CRC Patients (*n=116*) N. (%)	OR (95 % CI)	*P Value*
TP53 MDM2				
Arg/Arg TT	43 (35.5)	51 (44)	1 [a]	
Arg/Arg TG + GG	56 (46.3)	46 (39.7)	0.7 (0.39 to 1.21)	**0.20**
Arg/Pro + Pro/pro TT	10 (8.3)	9 (7.7)	0.75 (0.28 to 2.03)	**0.58**
Arg/Pro + Pro/pro TG + GG	12 (9.9)	10 (8.6)	0.7 (0.27 to 1.78)	**0.45**

N number, % percentage, *OR* odds ratio, *CI* confidence interval, *p* significance, *a* genotype saved as reference category

[44, 45]. Consistently, this polymorphism reveals gender-specific effects and enhanced in women with active estrogen signaling pathways [42]. In our study, when we stratified the groups into gender, we found an increased frequency of the GG genotype among women, which does not support the hypothesis of an active estrogen signaling via the SNP309 GG genotype.

In 2011, Knappskog and *al*., report a second SNP in position 285 (SNP285G > C) of *MDM2* promoter, which forms an haplotype with SNP309 (SNP285C//SNP309G) [46]. Furthermore, SNP285 C reduces the risk of both ovarian and breast cancer [46]. While the G allele enhances the transcription, the C allele reduces the binding of Sp1 transcription factor on the promoter. Thus, we can not statute about the influence of other modifying genes and/or environmental factors that can affect the implication of *MDM2* in the occurrence of CRC. It is necessary to study the interaction with the haplotypes SNP285 C/SNP309 G in future investigations.

Conclusion

In conclusion, our results suggest that the *MDM2* SNP309 GG genotype might be associated to a lower risk of developing CRC compared with TG or TT genotypes for the West Algerian population and especially for female subjects with at least one G allele.

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Table 5 Association between *TP53* Arg72Pro and *MDM2* SNP309 polymorphisms and risk of colorectal cancer divided by gender

	Controls	CRC patients	OR (95 % CI)	*P Value*
TP53 Arg72Pro Men				
Arg/Arg	50	58	1 [a]	
Arg/Pro + Pro/Pro	11	12	0.94 (0.38 to 2.31)	**0.89**
Women				
Arg/Arg	51	41	1 [a]	
ArgPro + Pro/Pro	9	5	0.69 (0.21 to 2.22)	**0.53**
MDM2 SNP309				
Men TT	26	40	1 [a]	
TG + GG	35	30	0.55 (0.27 to 1.11)	**0.09**
Women				
TT	18	26	1 [a]	
TG + GG	42	10	0.16 (0.06 to 0.41)	**0.00005***
TP53 Arg72Pro + MDM2 SNP309 *Men*				
Arg/Arg TT	21	26	1 [a]	
Arg/Arg TG + GG	25	30	0.96 (0.44 to 2.11)	**0.93**
Arg/Pro + Pro/Pro TT	8	9	0.90 (0.29 to 2.76)	**0.86**
Arg/Pro + Pro/Pro TG + GG	7	5	0.57 (0.15 to 2.08)	**0.39**
Women				
Arg/Arg TT	22	23	1 [a]	
Arg/Arg TG + GG	29	13	0.42 (0.17 to 1.03)	**0.05**
Arg/Pro + Pro/Pro TT	4	5	1.19 (0.28 to 5.04)	**0.80**
Arg/Pro + Pro/Pro TG + GG	5	5	0.95 50.24 to 3.76)	**0.94**

N number, % percentage, *OR* odds ratio, *CI* confidence interval, *p* significance, *a* genotype saved as reference category

* $p<0.05$ considered as statistically significant

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