BRITISH JOURNAL OF BIOMEDICAL SCIENCE, 2018
VOL. 75, NO. 1, 46–49
https://doi.org/10.1080/09674845.2017.1362729

BIOMEDICAL SCIENCE IN BRIEF

p53 and *miR-34b/c* genetic variation and their impact on ulcerative colitis susceptibility

Z Hosseinpour[a], Z Salehi[a], S Talesh Sasani[a] and K Aminian[b]

[a]Faculty of Sciences, Department of Biology, University of Guilan, Rasht, Iran; [b]Research Center for Gastroenterology and Liver Diseases, Razi Hospital, Guilan University of Medical Sciences, Rasht, Iran

ARTICLE HISTORY Received 6 June 2017; Accepted 27 July 2017
KEYWORDS *p53*; *miR-34b/c*; polymorphism; ulcerative colitis

Ulcerative colitis (UC), a chronic inflammatory bowel disease (IBD), is characterized by repeated flare-ups of inflammation that can contribute to the development of colorectal cancer [1]. The pathogenesis of UC remains largely unknown, but involves a complex interaction between immunological, environmental and genetic factors [2]. Among the genetic factors, *P53* plays an important role in the development of UC [3]. Of the known *TP53* polymorphisms, Arg72Pro (rs1042522) an amino acid substitution of arginine (Arg) to proline(Pro) at position 72 in exon 4, is one of the most widely studied polymorphisms. The Arg72 and Pro72 isoforms have different biochemical and biological properties, such as different binding to components of the transcriptional machinery and different activation of transcription [4]. The Arg72 isoform induces apoptosis faster and suppresses transformation more efficiently than the Pro72. In addition, the Arg form of the p53 protein is more vulnerable than Pro form to binding and degradation by the HPV-E6 oncoprotein [5]. More importantly, the Arg72Pro polymorphism of *TP53* influences the p53-mediated inflammatory response. P53 is a transcription factor that regulates a group of target genes such as miRNAs, especially the *miR-34* family members (*miR-34a, miR-34b* and *miR-34c*) [6].

MicroRNAs (miRNAs) are small, 21–25-nucleotide non-coding RNAs that regulate gene expression by binding to complementary sequences in the 3′-UTR of target mRNAs. A potentially functional common SNP rs4938723 T > C is present in the promoter region of pri-*miR-34b/c* [7]. Several independent studies suggest that genetic variants in the pri-miRNA sequence of *miR-34b/c* (rs4938723 T > C) can be used as possible biomarkers of disease development. As *p53* physically interacts with *miR-34b/c* gene, the *P53* Arg72Pro and *miR-34b/c* rs4938723 (T > C) polymorphisms were selected to test our hypothesis that these SNPs are associated with the risk of UC.

This case-control study comprises 180 UC cases recruited at the Department of Gastroenterology, Ghaem Hospital, Rasht, with disease documented by clinical symptoms, colonoscopic and pathological findings, from June 2013 to July 2015. Endoscopic UC activity at the time of the colonoscopy was categorized according to the Mayo criteria [8]. Histologic scores were graded mild, moderate or severe, and the extent of UC was defined according to Montreal Classification. The frequencies of E1, E2 and E3 were 61.1, 25.6 and 13.3%, respectively. All patients received Mesalazine. Two hundred and fifty healthy volunteers were selected by matching healthy individuals without IBD or known chronic, autoimmune disease based on gender and age. All participants gave written prior informed consent.

The genomic DNA was extracted from peripheral blood cells using the GPP solution kit (Gen Pajoohan, Iran) according to the manufacturer's instruction. Variants of codon72 of *P53* were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RLFP) analysis. Amplifications were carried out using specific primers designed by Oligo primer analysis software (Version 7.54, Molecular Biology Insights, USA). Primer sequences were F: 5′-CTTTTCACCCATCTACAGTCCC-3′ and R: 5′-AAACACTGACAGGAAGCCAAA-3′. PCR was performed with a Bio-Rad thermal cycler in 25 µl of PCR mixture containing approximately 30 ng of genomic DNA, 0.1 M deoxyribonucleotide triphosphates (dNPTs), 10 × PCR buffer (50 mM KCl, 10 mM Tris HCl and 0.1%Triton X-100), 1.5 mM $MgCl_2$, 0.5 units of Taq polymerase and 2 pmol of each primer. PCR cycling conditions were implemented with an initial denaturation step for 3 min at 94 °C, followed by 35 cycles for 35 s at 94 °C, 30 s at 57 °C, 30 s at 72 °C and with final extension at 72 °C for 5 min. A

CONTACT S Talesh Sasani sasani@guilan.ac.ir

416 bp DNA fragment was amplified and digested with *BstUI*. The Pro/Pro homozygote showed two bands of 279 bp and 137 bp, the Arg/Pro heterozygote showed three bands of 279 bp, 137 bp and 416 bp and the Arg/Arg homozygote showed only one band of 416 bp (Figure 1(a)).

miR-34b/c rs4938723 was genotyped using tetra primers amplification-refractory mutation system (tetra-ARMS-PCR) method. The primer sequences were: F: 5′-GGGAACCTTCTTTGACCTATT-3′,R: 5′-GTTAGTTACATCAAATGTGGT-3′(T allele) and F: 5′-GGGAACCTTCTTTGACCTATC-3′, R: 5′-GTAGCCTCTGACATATGGGA-3′ (C allele).The PCR product of the C allele was 314 bp; that of the T allele was 170 bp (Figure 1(b)). The amplification as follows: initial denaturation at 94 °C for 3 min, amplification for 35 cycles at 94 °C for 30 s, 54 °C (T allele) and 55 °C (C allele) for 30 s and 72 °C for 30 s, followed by a final elongation step at 72 °C for 5 min.

Allelic frequencies were calculated by the χ^2 tests. Odds ratios (ORs) and 95% confidence intervals (CIs) were used. We investigated the associations between two SNPs (TP53Arg72Pro and miR-34b/c rs4938723) and UC under the different genetic models: Genotypic Model (AA vs. Aa vs. aa), Dominant Model (AA + Aa vs. aa) and Recessive Model (AA vs. Aa + aa). Statistical significance was set at $p < 0.05$ level. All statistical analyses were conducted using the MedCalc version 12.1.4.0.

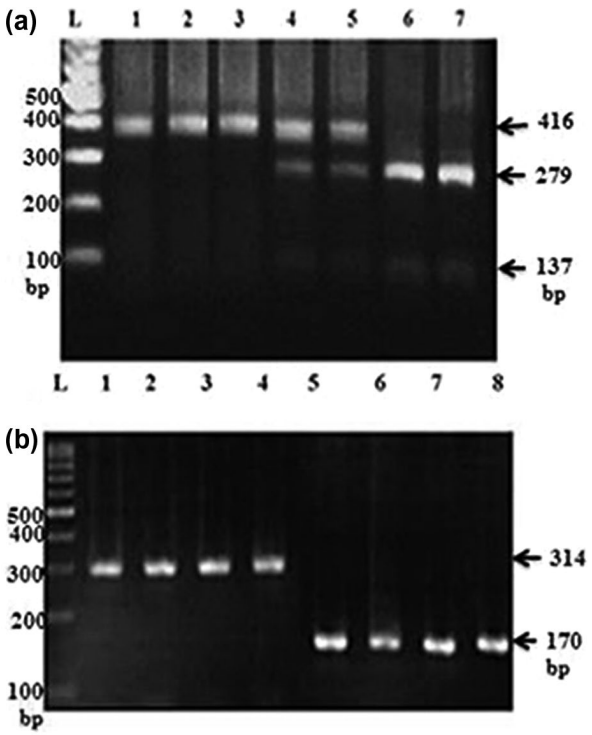

Figure 1. (a): RFLP-PCR pattern of *p53* in different genotypes after *BstU1* digestion. Lane L: DNA ladder; lanes1, 2 and 3: Arg/Arg genotype failed to cleaved by *BstU1*; lanes 4 and 5: Arg/Pro genotype produced three fragments (416, 279 and 137 bp); lanes 6 and 7: Pro/Pro genotype produced two fragments (279 and 137 bp). (b): Detection of *miR-34b/c* rs4938723 T>C polymorphism by ARMS-PCR. The molecular weight marker is shown in the left part of the gel. Lanes 1, 2, 3 and 4 show the C; lanes 5, 6, 7 and 8 represent T allele.

The UC patients were 106 men and 74 women; mean (SD) age: 32 ± 8.6 years old; controls were 140 men and 110 women; mean age: 33 ± 2.5 years old. There was no significant difference in the distributions of age and sex between the cases and the controls ($p = 0.31$, t-test, $p = 0.26$, chi-squared, respectively). Thirty-one (17%) patients had extra intestinal manifestation. Genotype and allele frequencies of *TP53Arg72Pro* in UC patients and controls are shown in Table 1. The variant genotype of *TP53* Pro/Pro was associated with a significantly increased risk of UC compared with GG genotype (OR = 7.1; 95% CI = 3.2–15.7; $p < 0.001$). In the recessive genetic model, we identified a significantly increased risk of UC in subjects with the variant homozygote Pro/Pro of *TP53Arg72Pro*, when compared with homozygote Arg/Arg and heterozygote Arg/Pro carriers (OR = 6.0; 95% CI = 2.9–12.4; $p < 0.001$). The allelic frequencies of case subjects (Arg, 0.48; Pro, 0.52) were significantly different from those of the control subjects (Arg, 0.65; Pro, 0.35, $p < 0.001$).

The distribution of *miR-34b/c* (rs4938723 T > C) polymorphism showed that the variant CC (OR = 5.5; 95% CI = 2.4–12.4; $p < 0.001$) and TC (OR = 2.5; 95% CI = 1.6–3.7; $p < 0.001$) genotypes were associated with an increased risk of UC compared with wild-type TT

Table 1. Genotype Frequencies of p53 Arg72Pro and miR-34b/c rs4938723 T>C Polymorphisms and combination analysis.

Models	Genotypes	Patients *n* (%)	Controls *n* (%)	*p*-value
P53 Arg72Pro				
Codominant	Arg/Arg	43 (23.9)	85 (34)	
	Arg/Pro	101 (56.1)	155 (62)	0.26
	Pro/Pro	36 (20)	10 (4)	<0.001
Dominant	Arg/Arg	43 (23.9)	85 (34)	
	Pro/Pro+Arg/Pro	137 (76.1)	165 (66)	0.02
Recessive	Arg/Arg+Arg/Pro	144 (80)	240 (96)	
	Pro/Pro	36 (20)	10 (4)	<0.001
Overdominant	Arg/Arg+Pro/Pro	79 (43.9)	95 (38)	
	Arg/Pro	101 (56.1)	155 (62)	0.22
miR-34b/c (rs4938723)				
Codominant	TT	54 (30)	135 (54)	
	TC	104 (57.8)	105 (42)	<0.001
	CC	22 (12.2)	10 (4)	<0.001
Dominant	TT	54 (30)	135 (54)	
	CC+TC	126 (70)	115 (46)	<0.001
Recessive	TT+TC	158 (87.8)	240 (96)	
	CC	22 (12.2)	10 (4)	0.002
Overdominant	TT+CC	76 (42.2)	145 (58)	
	TC	104 (57.8)	105 (42)	0.001
TP53	*miR-34b/c*			
Arg/Arg	TT	16 (8.9)	56 (22.4)	0.001
Arg/Arg	TC	25 (13.9)	25 (10)	0.21
Arg/Arg	CC	2 (1.1)	4 (1.6)	0.67
Arg/Pro	TT	29 (16.1)	77 (30.8)	<0.001
Arg/Pro	TC	69 (38.3)	75 (30)	0.07
Arg/Pro	CC	3 (1.7)	3 (1.2)	0.68
Pro/Pro	TT	9 (5)	4 (1.6)	0.05
Pro/Pro	TC	10 (5.6)	4 (1.6)	0.03
Pro/Pro	CC	17 (9.4)	2 (0.8)	<0.001

genotype. Significantly increased frequencies of the C allele were observed in patients with UC compared with T allele ($p < 0.001$). Subsequent grouping of the TT and CT genotypes in the recessive genetic model revealed a significantly increased risk of UC in CC genotype carriers when compared with that of the wild-type homozygous TT and heterozygous CT genotype carriers (OR = 3.3; 95% CI = 1.5–7.2; $p = 0.002$).

The heterozygote Arg/Pro combined with homozygote TT was found more frequently among controls. However, heterozygote Arg/Pro combined with TC was found more frequently in patients with UC. The homozygote TT coupled with Arg/Arg or Arg/Pro seemed to confer protective effects on UC risk (OR = 0.33; 95% CI = 0.18–0.61; $p < 0.001$; OR = 0.43; 95% CI = 0.26–0.69; $p < 0.001$). The Pro/Pro combined with CC genotype was associated with increased risk of UC (OR = 12.93; 95% CI = 2.94–56.72; $p < 0.001$). Based on the above data, we observed a statistically significant interaction between *TP53Arg72Pro* (G > C rs1042522) and *miR-34b/c* (T > C rs4938723) with the risk of UC (Table 1). A significant increase in UC risk was found among carriers of *TP53*Arg72Pro and *miR-34b/c* (T > C rs4938723) variant alleles.

Several groups have assessed the *p53* codon72 polymorphism in UC, with consistent results. One reported significant link between *p53* Pro homozygosity and the clinical course of UC [9]. Another reported an association between the Pro allele and the Pro/Pro genotype and UC (Pro allele: $p < 0.001$; OR = 7.9; 95% CI = 4.0–15.3; Pro/Pro: $p < 0.001$; OR = 35.2; 95% CI = 12.6–98.8) [10]. A case-control study including 461 IBD patients, 181 primary sclerosing cholangitis and 62 healthy controls, the *TP53Arg72Pro* was significantly associated with an increased IBD risk in homozygotes (Arg/Arg genotype) [3].

It has been generally accepted that the risk of developing colorectal cancer (CRC) is associated with the extent of inflammation in the colon, as well as the duration of disease. Various initiating factors have been found to be involved in cancer-related inflammation such as nuclear factor *κB*, tumour necrosis factor-*α*, interleukin *(IL)-1β, IL-6* and *p53*. Most important mutation occurs early in UC and involves *p53*. Overexpression of *p53* in colonic epithelia is usually detected in UC patients when no dysplasia is histologically seen and it is used as a discriminator between regenerative changes and intraepithelial neoplasia [11].

Corney et al. (2007) reported that the *miR-34b* and *miR-34c* cooperate in adhesion-independent growth and suppressing cell proliferation by targeting p53. Furthermore, p53 can bind to the promoter region of *miR-34b/c*, which led to an increase of *miR-34b/c* expression [12]. The methylation of *miR-124a* and *miR-34b/c* occurs early in colorectal carcinogenesis. The SNP rs4938723 (T > C) is located within the CpG island of pri-*miR-34b/c*. *MiR-34* has been shown to be associated with carcinogenesis, prognosis and survival of various cancers [13]. The results of present study revealed a significant association between the *miR-34b/c* (rs4938723) polymorphism and UC. Its homozygous variant (CC) manifested more than five-fold positive significant risk for UC relative to the TT variant. Moreover, subjects with the variant genotypes (CC + TC) showed an overall increased risk of UC more than two-fold relative to TT carriers.

Some studies have investigated the contribution of miR-SNPs and the risk of various cancers. Yadegari et al. has shown there is no association between *miR-146-a* (rs2910164) and gastric cancer [14]. Ren et al. observed that the *miR-21* expression was significantly increased in osteosarcoma tissues compared with the adjacent normal bone tissues and was correlated with osteosarcoma progression [15]. The *TP53* and *miR-34b/c* interaction analysis showed that the combined genotype of Pro/Pro with CC was associated with a 12-fold increased risk of UC in our population. Some limitations of our study are as follows. We only evaluated two SNPs in the *TP53* and *miR-34b/c*, which was inadequate to assess UC risk for gene studies. In addition, we assessed only the link between *TP53* and *miR-34b/c* polymorphisms with disease activity and severity. Whether these genetic variations may be able to predict disease courses and outcomes need to be evaluated in future studies.

This work represents an advance in biomedical science as it describes a link between *P53* and miR-34b/c with the risk of UC.

Acknowledgements

The authors thank the all people who participated in the research studies described in this article. This study was supported partly by University of Guilan.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Yashiro M. Ulcerative colitis-associated colorectal cancer. World J Gastroenterol. 2014;20(44):16389–16397. DOI:10.3748/wjg.v20.i44.16389.

[2] Maloy kJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature. 2011;474(7351):298–306. DOI:10.1038/nature10208.

[3] Volodko N, Salla M, Eksteen B, et al. TP53 codon 72 Arg/Arg polymorphism is associated with a higher risk for inflammatory bowel disease development. World J Gastroenterol. 2015;21(36):10358–10366. DOI:10.3748/wjg.v21.i36.10358.

[4] Pérez LO, Abba MC, Dulout FN, et al. Evaluation of p53 codon 72 polymorphism in adenocarcinomas of the colon

and rectum in La Plata. Argentina. World J Gastroenterol. 2006;12(9):1426–1429.

[5] Storey A, Thomas M, Kalita A, et al. Role of a p53 poly morphism in the development of human papillomavirus-associated cancer. Nature. 1998;393(6682):229–234. DOI:10.1038/30400.

[6] He L, He X, Lim LP, et al. A microRNA component of the p53 tumour suppressor network. Nature. 2007;447 (7148):1130–1134.

[7] Xu Y, Liu L, Liu J, et al. A potentially functional polymorphism in the promoter region of miR-34b/c is associated with an increased risk for primary hepatocellular carcinoma. Int J Cancer. 2011;128(2):412–417. DOI:10.1002/ijc.25342.

[8] Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med. 1987;317(26):1625–1629. DOI:10.1056/NEJM198712243172603.

[9] Vietri MT, Riegler G, Ursillo A, et al. p53 codon 72 polymorphism in patients affected with ulcerative colitis. J Gastroenterol. 2007;42(6):456–460. DOI:10.1007/s00535-007-2026-z.

[10] Vaji S, Salehi Z, Aminian K. Association of p53 codon 72 genetic polymorphism with the risk of ulcerative colitis in northern Iran. Int J Colorectal Dis. 2011;26(2):235–238. DOI:10.1007/s00384-010-1021-7.

[11] Triantafillidis JK, Nasioulas G, Kosmidis PA. Colorectal cancer and inflammatory bowel disease: epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies. Anticancer Res. 2009;29(7):2727–2737.

[12] Corney DC, Flesken-Nikitin A, Godwin AK, et al. MicroRNA-34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. Cancer Res. 2007;67(18):8433–8438. DOI:10.1158/0008-5472.CAN-07-1585.

[13] Bensen JT, Tse CK, Nyante SJ, et al. Association of germline microRNA SNPs in pre-miRNA flanking region and breast cancer risk and survival: the Carolina Breast Cancer Study. Cancer Causes Control. 2013;24(6):1099–1109. DOI:10.1007/s10552-013-0187-z.

[14] Yadegari ZS, Akrami H, Hosseini SV, et al. miR-146a gene polymorphism and susceptibility to gastric cancer. Br J Biomed Sci. 2016;73(4):201–203. DOI:10.1080/09674845.2016.1233790.

[15] Ren X, Shen Y, Zheng S, et al. miR-21 predicts poor prognosis in patients with osteosarcoma. Br J Biomed Sci. 2016;73(4):158–162. DOI:10.1080/09674845.2016.1220710.