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RESEARCH PAPER

The association of TP53 (Arg72Pro) rs1042522 C>G polymorphism and colorectal cancer susceptibility in Duhok individuals

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A B S T R A C T:

Background and aim: The *TP53* gene encodes an important class of cell cycle and tumor-suppressing factors that play critical roles in maintaining genomic stability. The *TP53* Arg72Pro (rs1042522 C>G) polymorphism has been reported to be associated with the risk of several types of adult cancers. Objective of this study was to investigate the possible association between TP53 Arg72Pro polymorphism with colorectal cancer and to examine its correlation with age groups, Tumor type, Nodal state, and Duck stage of individual.

Patients and methods: The study involved 50 patients with colorectal cancer (25 males and 25 females). This study was conducted to estimate the distribution of colorectal cancer within age groups, Tumor type, Nodal state, and Duck stage of individual also to investigate the distribution of TP53 Arg72Pro SNPs genotype in colorectal cancer, and determine whether TP53 Arg72Pro polymorphism is a possible relevance in susceptibility to colorectal cancer using RFLP-PCR analysis.

Results: The present study shows there were no statistically significant differences between the different age groups and Dukes states with gender. Additionally, tumor types and nodal states (either positive or negative) of colorectal cancer were significantly different with gender. Also, this study revealed that 10 (20.0%) colorectal cancer patients had a 152 bp undigested PCR product fragment representing homozygotes for proline, 8 (16.0%) had two 50 and 102 bp fragments representing homozygotes for arginine, and 32 (64.0%) had three 50, 102, and 152 bp fragments representing heterozygotes for proline. The results of control genotypes showed 14 (28.0%) people with a fragment of 152 bp indicating homozygotes proline, 20 (40.0%) with two fragments representing homozygotes arginine, and 14 (28.0%) with three bands showing heterozygotes for codon 72.

Conclusion: No substantial differences (P>0.05) between the frequency of Arg72allele and Pro72allele in colorectal cancer - affected males as opposed to the frequency of Arg72allele and Pro72allele in the control groups. In relation to the frequency of Arg72allele and Pro72allele in females, indicates substantial differences (P>0.05).

KEY WORDS: PCR- RFLP; TP53; Codon72; SNPs; colorectal cancer
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1. INTRODUCTION:

Cancer is categorized as a leading cause of mortality worldwide in the 21st century. According to the World Health Organization (WHO), cancer is the first or second leading cause of death before the age of 70 years in most countries in 2015(Bray et al., 2018).

Colorectal cancer (CRC) is the 3rd and 4th most commonly diagnosed cancer globally in women and men, respectively (Al-Sohaily et al., 2012; Wang et al., 2015; Siegel et al., 2016). Presently, CRC is considered one of the most prevalent malignant neoplasms. The review article by Sawicki et al., contains a concise consideration of genetic and environmental risk factors for colorectal cancer. Known risk factors associated with colorectal cancer include familial, hereditary factors, lifestyle-related and ecological factors. Lifestyle factors are significant because of the potential for improving our understanding of the

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disease. Physical inactivity, obesity, smoking, and alcohol consumption can also be addressed through therapeutic interventions (Sawicki et al, 2021). Approximately 1.4 million people worldwide were diagnosed with colorectal cancer in 2012, whilst it is estimated that 693,900 people died from the disease in the same year (Torre et al., 2015). According to data obtained from the World Health Organization mortality database and Eurostat, nearly 173,400 EU citizens died of colorectal cancer in 2016 (Malvezzi et al., 2016). The high mortality rate is due to the fact that CRC usually remains undetected until the disease progresses up to a point that it becomes difficult to cure (ASOC and RS, 2016). In addition, positive family history and inflammatory bowel diseases have significant roles in the growth of the tumor (Liu et al.,2019). It is known that the reasons for CRC cancer are either sporadic (70-80%) or heredity (20-30%) (Surget et al., 2014). A study by Marmol et al., reported that mutations target CRC oncogenes, tumor suppressor genes, and genes related to DNA repair mechanisms (Marmol et al., 2017). Colorectal cancer (CRCs) affects over a quarter of a million people. The risk of developing CRC in industrialized nations is approximately 5%. When the disease is localized, treatment success rates range from 70– 90%; however, advanced CRC has a high mortality rate, consistently ranking in the top three causes of cancer-related deaths. There is a large geographic difference in global distribution, and CRC is predominantly associated with developed countries and a Western lifestyle and diet (Hull et al., 2020).

Most colon cancers evolve from precancerous colorectal lesions called adenomas (Yan et al., 2014). Therefore, early detection and diagnosis could have a significant impact on the reduction of mortality from CRC. In 2020, it was estimated that there were 1.9 million new cases and 0.9 million mortalities caused by CRC. The incidence of the disease is higher in developing countries, and it is increasing in middle and low-income countries (Xi & Xu, 2021).

The p53 protein is a key tumor suppressor that has been widely studied in colorectal cancer, but no predictive or prognostic role in clinical practice has been proposed to date (Walther et al., 2009).

TP53, a well-known tumor suppressor gene located on chromosome 17p13.1, comprises 11 exons and 10 introns (Pietsch et al., 2006). TP53 is a critical tumor suppressor gene, which encodes a 53 kDa protein, p53. The p53 functions to protect against cancer by regulating cell cycle and apoptosis and maintaining DNA integrity. TP53 gene is highly polymorphic. Several TP53 gene polymorphisms are associated with cancer risk (Fu et al., 2017). The p53 gene mutations and single nucleotide polymorphisms (SNP) are essential in all types of human cancers (Børresen-Dale, 2003; Khan et al., 2005).

The fact that the Single Nucleotide Polymorphism Database hosts information regarding >1000 TP53 single nucleotide polymorphisms (SNPs) supports the highly polymorphic nature of this gene (Fu et al., 2017). Among those polymorphisms, Arg72Pro (rs1042522 C>G) in exon 4 is the most important and extensively investigated (Bergamaschi et al 2006). Genetic variations may alter the expression levels and structures of the tumor suppressor genes, consequently affecting their tumor suppressive function. In addition, TP53 genotyping is considered a useful inexpensive tool for predicting the risk of developing cancers, such as lung cancer, thereby contributing to earlier detection and management of the disease (Cavic et al., 2019).

In the TP53 gene, Arg72Pro polymorphism has been suggested to be associated with genetically determined susceptibility in various types of cancers including colorectal cancer. The human tumor suppressor gene TP53, located at the 17p13.1 locus, encodes a 393 amino acid-long protein and is 20 kb in humans (Surget et al., 2014). The association between TP53 Arg 72 Pro (rs1042522C<G) polymorphism and the risk of developing a number of types of cancer among adults has been reported in many studies (Yang et al., 2019). CRC is more likely to occur as a result of molecular changes caused by two major mechanisms of genetic instability: chromosomal instability and microsatellite instability (Marmol et al., 2017), (Muller et al., 2016). Modern orientation in management CRC: is the multidisciplinary team for diagnosis and treatment. The pathologist has a major role in most of the stages from diagnosis to treatment. The quality of histopathology reports is the cornerstone for diagnosis and predicting the prognosis of the tumor, so it helps determine chemotherapy and radiotherapy (Li et al., 2018),

(Fujita et al., 2003), (Kosmider & Lipton, 2007). This objective was to investigate the possible association between TP53 Arg72Pro polymorphism through polymerase chain reaction (PCR) test with colorectal cancer susceptibility and to examine its correlation with the clinic pathologic variables of cancer cases for the oncologic prognosis of patients.

2.MATERIALS AND METHODS

From February 2014 through June 2014, 50 colorectal cancer and normal samples with archival formalin-fixed, paraffin-embedded tissues were collected from the Central Laboratory of Pathology at Duhok (the Kurdistan region).

2.1.DNA extraction

The study was conducted in Scientific Research Center, Faculty of Science, University of Duhok. Both colorectal cancer and normal samples were transferred to laboratory Scientific Research Center and stored at -20°C until analysis.

The tissues in the paraffin were washed several times with xylene to remove the wax from the tissues, and then the removal of xylene was done by doing numerous washings with ethanol before isolating the DNA. From these tissues, the DNA of CRC was recovered. A kit is used to isolate DNA (Geneaid kit USA). NanoDrop 1000 Spectrophotometer was used for the determination of the DNA concentration and purity. For the samples that required it, DNA purification was carried out in order to calculate the quantitative concentration of the DNA samples. DNA was manually purified using ethanol and sodium chloride.

2.2.Primers for PCR

The primers used were oligonucleotides complementary to the sequence of the Arg72Pro polymorphism region found in the TP53 gene using primers shown in Table (1) previously used by Pinto *et al.*, (2008).

Table 1: Primers that used for amplification of TP53 gene codon72 Arg72Pro SNPs

Primer of gene TP53	Sequence (5'- 3')	Length bp	Amplicon bp
Arg72Pro-Forword	5′- GAA GAC CCA GGT CCA GAT GA-3′	20	152
Arg72Pro-Reverse	5′- CTG CCC TGG TAG GTT TTC TG-3′	20	

2.3.Polymerase chain reaction (PCR):

TP53 Arg72Pro polymorphism detection was performed by PCR-RFLP. Genomic DNA (100 ng) was used for amplification in 25 µl of reaction mixture. A Cinagen PCR ready master mix kit was used as follows: 12.5 µl Master mixture, 8.5 µl D.D.W, 1uL Forward primer, 1µl Reverse primer and 2 µl DNA sample. The amplification conditions were as follows; first step calling pre-PCR at 95 ° C for 5 min denaturation, then applying 35 cycles at 94 ° C for 30 s. denaturation, 54 ° C for 30 s. annealing, 72 ° C for 30 s. extension, and 5 min. extension as a final step within 72 ° C. After running the product bands visualized by UV light, PCR products were run on 2.5 percent agarose electrophoresis after adding 5 µL of saber-safe stain (Narina et al. 2011).

2.4.Analyzing Restriction Fragment Length Polymorphism:

The P53 Arg 72Pro SNP amplicons were overnight at 37°C digested with the BstU I restriction enzyme (Vilnius, Lithuania). finally, products were loaded onto 3% agarose gel and staining the gel with ethidium bromide.

2.5 Statistical analysis

The statistical package for social science (SPSS, version 26) was used for data entry and analysis. Descriptive statistical analysis (including frequency, percentage, mean, standard deviation, range, and ratio, and allele frequency) was used to describe the data; and Inferential statistical analysis was used to determine the association between variables by using Fisher's exact tests. The P-value ≤ 0.05 was considered statistically significant.

3.RESULTS

The study sample's mean age was 55.64 years, with an SD of 16.24. The age range is from 19 to 80. The sample's gender distribution was equal, with 25 (50%) males and 25 (50%) females.

According to Table 2, the age categories 59 to 68 years had the largest percentage of CRC patients (28%), followed by patients aged 39–48 and 49–58, at 16% and 16%, respectively.
According to this study's findings, patients with colorectal cancer have nodal states that are either negative (16 patients) or positive (34 patients), while their Dukes states are A (3 patients), B (23 patients), or C (23 patients). Table 2 shows that there were no statistically significant differences between age groups and Duke's state with gender as P = 0.6907 and P = 9.17E-48, respectively. Additionally, gender was significantly associated with different tumor types and nodal states of colorectal cancer, as indicated by P = 0.000282 and P = 0.0000111, respectively.

Table 2: Comparison of general characteristics of patients with colorectal cancer and controls

Characteristics	**Patients (n=50) gender**			**Percentage (%)**		**P-value**
	Males	**Females**		**Males**		
	Total			**Females**	**Total**	
1. Age groups						
≤28	2	2	4	4	4	8	
29-38	2	2	4	4	4	8	
39-48	0	8	8	0	16	16	0.6907
49-58	3	5	8	6	10	16	
59-68	8	6	14	16	12	28	
69-78	6	1	7	12	2	14	
79+	4	1	5	8	2	10	
2. Tumor types	**Patients (n=50) gender**			**Percentage (%)**		**P-value**
	Males	**Females**	**Total**	**Males**	**Females**	
Adenocarcinoma	16	14		30	28	
Mucinous adenocarcinoma	6	5		12	10	
Tubule villous adenoma	3	2		6	4	0.0003
Hemicolectomy	0	2		0	4	
Distal colon cancer	0	1		0	2	
Proximal colon cancer	0	1		0	2	
3. Nodal state	**Patients (n=50) gender**			**Percentage (%)**		**P-value**
	Males	**Females**	**Total**	**Males**	**Females**	
Negative	7	9	16	14	18	**0.0000**
Positive	18	16	34	36	32	
4. Dukes state	**Patients (n=50) gender**			**Percentage (%)**		**P-value**
	Males	**Females**	**Total**	**Males**	**Females**	
A	1	2	3	2	4	
B	8	15	23	16	30	9.17E-48
C	16	7	23	32	14	

3.1. PCR Analysis:
Both sample groups, 50 colorectal cancer patients (25 males and 25 females) and 70 controls, were successfully amplified and polymorphic alleles were analyzed for TP53 codon 72. The results of a 152bp amplicon are shown in Figure .

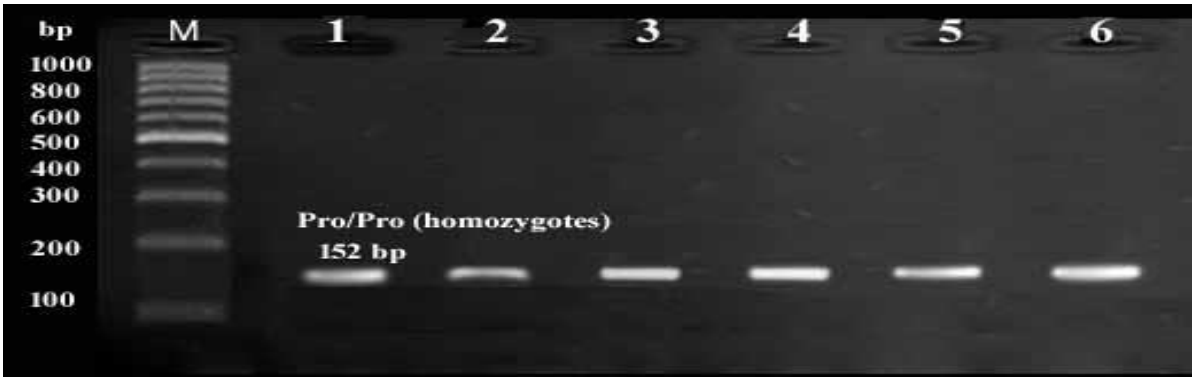

Figure 1: Represent the amplicon bands of TP53 Arg72Pro SNPs by 2% agarose gel electrophoresis.

3.2. Analysis of restriction fragment length polymorphism.

The TP53 Arg 72Pro SNP amplified fragments were digested with the BstU I restriction enzyme (Figure 2). The results showed that a single band (152 bp) indigested PCR product was identified as the Pro/Pro variant (CC) representing homozygotes proline, two fragments of 50 and 102 bp representing homozygotes arginine (Arg/Arg) wild (GG), and heterozygotes for codon 72 Arg/Pro variant (GC) identified by three fragments representing 50, 102, and 152 bp.

The current study revealed that 10 (20.0%) colorectal cancer patients had a 152 bp undigested PCR product fragment representing homozygotes proline (Pro/Pro), 8 (16.0%) had two 50 and 102 bp fragments representing homozygotes arginine (Arg/Arg), and 32 (64.0%) had three 50, 102, and 152 bp fragments representing heterozygotes for proline.

The results of control genotypes showed 14 (28.0%) people with a fragment of 152 bp indicating homozygotes proline (Pro/Pro), 20 (40.0%) with two fragments representing homozygotes arginine (Arg/Arg), and 14 (28.0%) with three bands showing heterozygotes (Arg/Pro) for codon 72 (Table 3). (Figure 2).

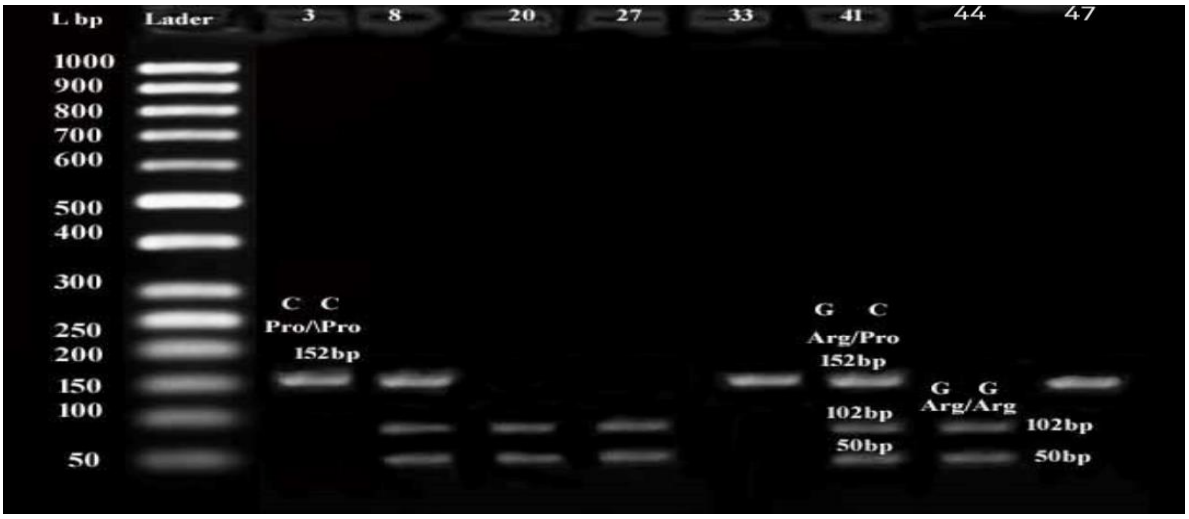

Figure 2: PCR- RFLP study illustrates TP53 codon 72 SNPs by electrophoresis of agarose gel (3%). After digestion, PCR products are shown: samples 3, 33 and 47, Pro / Pro (CC); samples 20, 27 and 44, Arg / Arg (GG); and samples 8 and 41, Arg / Pro (GC), markers of 50 bp DNA scale.

Table 3: Comparison of prevalence of allele and genotype between controls and patients with colorectal cancer.

	Genotypes	Study groups				P-value (two-sided)
		Controls (n=50)		Patients (n=50)		
	TP53 Arg72Pro					
	Arg/Arg	20	40.0	8	16.0	
	Arg/Pro	16	32.0	32	64.0	6.51E-64
	Pro/Pro	14	28.0	10	20.0	
	Allele frequency					
	Pro 72 allele frequency	22	44.0	26		
	Arg72 allele frequency	28	56.0	52.0		
				24		
				48.0		

Characteristics	Genotypes	Study groups				P-value (two-sided)
		Controls (n=25)		Patients (n=25)		
Male (p. n=25, c. n=25)	**TP53 Arg72Pro**					
	Arg/Arg	9	36.0	5	20.0	
	Arg/Pro	9	36.0	15	60.0	5.38E-06
	Pro/Pro	7	28.0	5	20	
	Allele frequency					
	Pro 72 allele frequency	12	44.0	13		
	Arg72 allele frequency	13	56.0	56.0		
				12		
				44.0		

Characteristics	Genotypes	Study groups				P-value (two-sided)
		Controls (n=25)		Patients (n=25)		
Female (p. n=25, c. n=25)	**TP53 Arg72Pro**					
	Arg/Arg	11	44.0	3	12	
	Arg/Pro	7	28.0	17	68	
	Pro/Pro	7	28.0	5	20	0.0045
	Allele frequency					
	Pro 72 allele frequency	11	22.0	13	26	
	Arg72 allele frequency	14	28.0	12	24	

4.DISCUSSION

The results of the current investigation indicate that there is no correlation between age groups, Duke state, or gender with the distribution of CRC. According to a study by Hoseini et al., CRC affects more women than men when it first appears in early adulthood, but the difference between the sexes in late adulthood is less noticeable (Hoseini, 2022). A significant risk factor for sporadic CRC is age. According to a study by Macrae, large bowel cancer is rare before the age of 40; its incidence rises between the ages of 40 and 50; and age-specific incidence rates rise in each decade following that (Macrae, 2022). White et al. investigated sex differences in incidence by conducting a cross-sectional review of the available national data for the UK. The incidence is higher overall in men, and their age distribution is earlier, according to the results, but the anatomical site still exhibits significant sex differences. In addition, there are no gender differences in the diagnosis of more severe disease (White et al., 2018). It has been recorded in a research that there was a link between obesity and a higher risk of CRC in more advanced clinical stages, particularly in men. According to the findings of the Brändstedt (2012) there were various ways to define obesity, as well as differences according to sex, the location of the tumor, and the stage of the tumor, which affect the risk of CRC.

The current study shows that there was an association between tumor types and nodal state with gender regarding the distribution of CRC. The study by Zhu et al. (2007) showed no significant link between TP53 R72P polymorphism and the Dukes' stage and also hasn’t been linked with tumor location, histologic grade, lymph node metastases, p53 positivity, or age at diagnosis. Results show that the TP53 R72P polymorphism has a significant link to tumor size.

The current study identified Pro/Pro variance (CC), representing homozygotes proline, in two fragments of 50 and 102 bp in the single band (152 bp). A case-control study by Yagublu et al. identified the heterozygous genotypes Arg and Pro as appearing more frequently in CRC patients than controls, but the association between the TP53 Arg72Pro polymorphism and risk of colorectal cancer was not significant in Azerbaijani (Yagublu et al., 2021**).** In a case-control study done in Malaysia by Aizat et al., peripheral blood samples of 202 sporadic CRC patients and 201 normal controls were collected, DNA extracted, and genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.

Results showed the frequency of the homozygous variant (Pro/Pro) genotype was significantly higher in cases compared to controls. The Pro/Pro homozygous variant genotype showed a significantly higher risk associated with CRC susceptibility. In addition, the study found individuals aged over 50 and carriers of the pro/pro genotype had a significantly higher risk of developing CRC (Aizat, 2011). A meta-analysis study carried out by Dong et al. (2018), involved 18 case-control studies, and the report gave data on TP53 genotype distribution from different Asian countries. Results found an association between TP53 and CRC; in addition, a study found that the risk of CRC was associated with the TP53 Pro allele and Pro/Pro genotype in Asian populations.

Although it is known that race plays a role in developing CRC, a meta-analysis study done by Economopoulos et al. reviewed 27 studies, of which 19 were conducted on Caucasians, 6 on Chinese populations, and 2 on mixed populations; the results showed p53 codon 72 Arg72Pro status does not seem to be associated with colorectal cancer risk (Economopoulos, 2010).

Concerning the prevalence of allele and genotype between patients and controls of CRC, the current study found that in men there was no association between patients and controls regarding both Arg/Arg and Arg/Pro, while in women there was a significant association. The study by Aizat et al. indicated Arg/Pro and Pro/Pro were significantly higher than Arg/Arg in males aged 50 and more. On the other hand, the differences between Arg/Pro and Pro/Pro compared with Pro/Pro in females aged 50 and more were not significant (Aizat, 2011).

In Slovak, a case-control study was carried out on 173 confirmed CRC patients and 303 healthy subjects. Genotyping was performed by PCR-RFLP methods. Tumor site genotype distribution revealed that female patients with localized colon cancer were significantly associated with the p53 Pro72Pro genotype. While gender-specific analysis showed a significant inverse association of the polymorphism EGF G61G with CRC risk only in male patients, whereas the cancer of the recto-sigmoid junction was associated with the EGF G61G genotype. By using logistic regression to find a combination of both p53 Arg72Pro and EGF A61G polymorphisms, the study indicated no link between both and the risk of CRC (Mahmood et al., 2014).

An updated meta-analysis based on 32 studies, done by Tian et al., suggests that the TP53 Arg72Pro polymorphism CC genotype may contribute to an increased risk of CRC, especially for rectal cancer and among Asians (Tian et al., 2017). Study of Fu et al., a sample of children younger than 18 years. In the stratified analysis, results indicated carriers of CG or GG genotypes had significantly higher susceptibility to the risk of Wilms' tumor, but the association was weak (Fu et al., 2017). According to a study by Zhu et al. (2007), the TP53 R72P polymorphism may contribute to the etiology of colorectal cancer in the Chinese population, particularly among alcohol-consuming patients. Doosti et al. (2011) found the *p53Arg/Arg* genotype may be correlated with a possible increased risk of CRC in the south-west of Iran.

A study by Kru ger et al., aimed to test whether codon 72 variation influences the age of onset of disease in hereditary non-polyposis colorectal cancer patients. Results suggested that p53 codon 72 genotypes are associated with the age of onset of colorectal carcinoma in a mismatch repair deficient background in a dose-dependent manner (Kru¨ger et al. 2005).

A study was done by Nerweyi in Duhok, Iraq, to investigate the distribution of the TP53 Arg72Pro SNP genotype in gliomas and determine whether the TP53 Arg72Pro polymorphism is of possible relevance in susceptibility to glioma using RFLP-PCR analysis. The sample involved 65 glioma patients and 70 controls. Results found that there were no significant differences between glioma

patients and controls regarding the distribution of alleles and genotypes (Arg/Arg, Arg/Pro, and Pro/Pro) in both males and females (Nerweyi, 2020).

In a meta-analysis and systematic review study done by Yu et al., a total of 30 case-control studies involving 5025 cases and 6680 controls were included. A significant correlation between TP53 rs1042522 polymorphism and cervical cancer was observed in two models (CC; CG vs. GG; GG vs CC) (Yu et al., 2022). Further study is needed to identify an association between the TP53 rs1042522 polymorphism and the risk of CRC.

5.Conclusion

The current study concluded that there was no significant association between the frequency of Arg72allele and Pro72allele in colorectal cancer-affected males as opposed to the frequency of Arg72allele and Pro72allele in the control groups. In relation to the frequency of Arg72allele and Pro72allele in females, the present study concluded that there are substantial differences ($P > 0.05$). Further study is needed for the association between the TP53 rs1042522 polymorphism and CRC.

References

Aizat, A. Shahpudin, S.N. Mustapha, M.A. Zakaria, Z. Sidek, A.S. Abu Hassan, M.R. et al. (2011). Association of Arg72Pro of P53 Polymorphism with Colorectal Cancer Susceptibility Risk in Malaysian Population, *Asian Pacific J Cancer Prev*, 12, 2909-13. DOI: 10.3748/wjg.v21.i36.10358

Al-Sohaily, S. Biankin, A. Leong, R. Kohonen-Corish, M. Warusavitarne, J.(2012). Molecular pathways in colorectal cancer, *J Gastroenterol Hepatol.* 27,1423–1431. doi: 10.1111/j.1440-1746.2012.07200.

American Society of Colon and Rectal Surgeons.(2014). Diseases and conditions, Available from: URL: https://www.fascrs.org/patients/disease-condition. [Cited in November, 2, 2022]

Bergamaschi, D. Samuels, Y. Sullivan, A. Zvelebil, M. Breyssens, H. Bisso, A. et al. (2006). I ASPP preferentially binds p53 proline-rich region and modulates apoptotic function of codon 72-polymorphic p53, *Nat Genet*,38(10),1133- 41. doi: 10.1038/ng1879.

Børresen-Dale, A.L. (2003). TP53 and breast cancer, Hum Mutat, 21(3),292-300.doi: 10.1002/humu.10174.

Brändstedt, J. Wangefjord, S. Nodin, B. Gaber, A. Manjer, J. Jirström, K.(2012). Gender, anthropometric factors and risk of colorectal cancer with particular reference to tumor location and TNM stage: a cohort study, Biology of Sex Differences, 3,23. doi: 10.1186/2042-6410-3-23

Bray, F. Ferlay, J. Soerjomataram, I. Siegel, R.L. Torre, L.A. Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J Clin* 68(6),394–424. Doi: 10.3322/caac.21492.

Cavic, M. Spasic, J. Krivokuca, A. Boljevic, I. Kuburovic, M. Radosavljevic, D. et al. (2019).TP53 and DNA-repair gene polymorphisms genotyping as a low-cost lung adenocarcinoma screening tool, *J Clin Pathol*, 72(1),75–80. DOI: 10.1136/jclinpath-2018-205553

Dong, Z. Zheng, L. Liu, W. Wang, C. (2018). Association of mRNA expression of TP53 and the TP53 codon 72 Arg/Pro gene polymorphism with colorectal cancer risk in Asian population: a bioinformatics analysis and meta-analysis, Cancer Management and Research,10, 1341–1349. DOI: 10.2147/CMAR.S164892

Doosti, A. Zamani, M. Dehkordi, P. G. Taheri, S. Mahmoudzadeh, M. (2011). Association of the *p53* codon 72 polymorphism with colorectal cancer in South West of Iran, *Scientific Research and Essays*, 6(15), 3148-3152, DOI: 10.5897/SRE10.1028

Economopoulos, K. Sergentanis, T.N. Zagouri, F. Zografos, G.C. (2010). Association between p53 Arg72Pro Polymorphism and Colorectal Cancer Risk: A Meta-Analysis, *Onkologie*,33(12), 666-74 DOI: 10.1159/000322210

Fu, W. Zhuo, Z.J. Jia, W. Zhu, J. Zhu, S. Lin, Z. et al. (2017). Association between TP53 gene Arg72Pro polymorphism and Wilms' tumor risk in a Chinese population, *Onco Targets Ther*,10,1149–1154. Doi: 10.2147/OTT.S131014

Fujita, S. Shimoda, T. Yoshimura, K. Yamamoto, S. Akasu, T. Moriya, Y. (2003). Prospective evaluation of prognostic factors in patients with colorectal cancer understanding curative, resaech, *J Surg Onco*, 84(3),127-31. DOI: 10.1002/jso.10308

Hoseini, B. Rahmatinejad, Z. Goshayeshi, L. Bergquist, R. Golabpour, A. Ghaffarzadegan, K. (2022). Colorectal Cancer in North-Eastern Iran: a retrospective, comparative study of early onset and late-onset cases based on data from the Iranian hereditary colorectal cancer registry, *BMC Cancer, 22(1),48.* doi: 10.1186/s12885-021-09132-5.

Hull, R. Francies, F.Z. Oyomno, M. Dlamini, Z. (2020). Colorectal Cancer Genetics, Incidence and Risk Factors: In Search for Targeted Therapies, Cancer Management and Research,12, 9869–9882. doi: 10.2147/CMAR.S251223

Khan, S.A. Thomas, H.C. Toledano, M.B. Cox, I. Taylor-Robinson, S.D. (2005). p53 Mutations in human cholangiocarcinoma: a review, *Liver Int*, 25, 704-16. DOI: 10.1111/j.1478-3231.2005.01106.x

Kosmider, S. Lipton, L. (2007). Adjuvant therapies for colorectal cancer, *World j Gastroenterol*, 13(28), 3799-805. DOI: 10.3748/wjg. v13.i28.3799.

Kru¨ger, S. Bier, A. Engel, C. Mangold, E. Pagenstecher, C. Doeberitz, M. (2005). The p53 codon 72 variation is associated with the age of onset of hereditary non-polyposis colorectal cancer (HNPCC), *Med Genet*, 42,769–773. doi: 10.1136/jmg.2004.028506

Li, S. Balmain, A. Counter, C.M. (2018). A model for RAS mutation patterns in cancers: finding the sweet spot, *Nat Rev Cancer J*, 18(12),767–77. DOI: 10.1038/s41568-018-0076-6.

Liu, J. Zheng, B. Li, Y. Yuan, Y. Xing, C (2019). Genetic Polymorphisms of DNA Repair Pathways in Sporadic Colorectal Carcinogenesis, *Journal of Cancer*,10(6), 1417-1433. doi: 10.7150/jca.28406.

Macrae, F. (2022). Colorectal cancer: Epidemiology, risk factors, and protective factors. Available in: Colorectal cancer: Epidemiology, risk factors, and protective factors. Cited in November 2, 2022.

Mahmood, S. Sivoňová, M.K. Matáková, T. Dobrota, D. Wsólová, L. Dzian, A. et al. (2014). Association of EGF and p53 gene polymorphisms and colorectal cancer risk in the Slovak population, Cent Eur. J. Med. 9(3), 405-416. DOI: 10.2478/s11536-013-0300-4

Malvezzi, M. Carioli, G. Bertuccio, P. Rosso, T. Boffetta, P. Levi, F.et al. (2016). European cancer mortality predictions for the year 2016 with focus on leukemias, *Ann Oncol* 27, 725-731. DOI: 10.1093/annonc/mdw022

Marmol, L. Sanchez-de-Diego, C. Dieste, A. Cerrada, E. Yoldi, M. (2017). Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer, *Int. J. Mol. Sci*,18(197), doi:10.3390/ijms18010197.

Muller, M.F. Ibrahim, A.E. Arends, M. (2016). Molecular pathological classification of colorectal cancer, *Virchows Arch J*, 469(2), 125-34. DOI 10.1007/s00428-016-1956-3.

Narina, S.S. d'Orgeix, C.A. Sayre, B. (2011). Optimization of PCR conditions to amplify microsatellite loci in the bunchgrass lizard (Sceloporus slevini) genomic DNA, *BMC Research Notes*, 4(1), 26. DOI: 10.1186/1756-0500-4-26

Nerweyi, F.F. (2020). THE RELATIONSHIP OF GLIOMAS WITH TP53 RS1042522 C and gt; G AND GLIOMAS IN DUHOK, *Journal of Duhok University*, *23*(2),157-165. https://doi.org/10.26682/sjuod.2020.23.2.17.

Pietsch, E.C. Humbey, O. Murphy, M.E. (2006). Polymorphisms in the p53 pathway, *Oncogene*, 25 (11),1602-1611. DOI: 10.1038/sj.onc.1209367

Pinto, G.R. Yoshioka, F.K. Silva, R.L. Clara, C.A. Santos, M.J. Almeida, J.R. et al. (2008). Prognostic value of TP53 Pro47Ser and Arg72Pro single nucleotide polymorphisms and the susceptibility to gliomas in individuals from Southeast Brazil, *Genet Mol Res*, 26,7(1), 207-16.doi: 10.4238/vol7-1gmr415..

Sawicki, T. Ruszkowska, M. Danielewicz, A. Nied´zwiedzka, E. Arłukowicz, T. Przybyłowicz KE. (2021). A Review of Colorectal Cancer in Terms of Epidemiology, Risk Factors, Development, Symptoms and Diagnosis Cancers, 13(9), 2025. DOI: 10.3390/cancers13092025

Siegel, R.L. Miller, K.D. Jemal, A. (2016). Cancer statistics, 2016, *CA Cancer J Clin*, 66 (1),7–30. DOI: 10.3322/caac.21332

Surget, S. Khoury, M.P. Bourdon, J.C. (2014). Uncovering the role of p53 splice variants in human malignancy: a clinical perspective, *Onco Targets Ther*, 7, 57-68. doi: 10.2147/OTT.S53876

Tian, X. Dai, S. Sun, J. Jiang, S. Jiang, Y. (2017). The association between TP53 Arg72Pro polymorphism and colortacl cancer: An updated meta-analysis based on 32 studies, *Oncotarget* 8(1),1156-1165. DOI: 10.18632/oncotarget.13589

Torre, L.A. Bray, F. Siegel, R.L. Ferlay, J. Lortet-Tieulent, J. Jemal, A.(2015). Global cancer statistics, 2012, *CA Cancer J Clin*, 65*(2)*, 87-108. DOI: 10.3322/caac.21262

Walther, A. Johnstone, E. Swanton, C. Midgley, R. Tomlinson, L. Kerr, D. et al. (2009). Genetic prognostic and predictive markers in colorectal cancer, *Nat Rev Cancer*, 9, 489-99. DOI: 10.1038/nrc2645

Wang, C. Zhang, J. Cai, M. Zhu, Z. Gu, W.Yu, Y. et al. (2015). DBGC: A Database of Human Gastric Cancer, *PLoS One*, 10, e0142591. doi: 10.1371/journal.pone.0142591

White, A. Ironmonger, L. Steele, R. Ormiston-Smith, N. Crawford, C. Seims, A. (2018). A review of sex-related differences in colorectal cancer incidence, screening uptake, routes to diagnosis, cancer stage and survival in the UK, *BMC Cancer*, 20,18(1), 906. doi: 10.1186/s12885-018-4786-

Xi, Y. Xu, P. (2021). Global colorectal cancer burden in 2020 and projections to 2040, *Transl Oncol*, 14(10), 101174. https://doi.org/10.1016/j.tranon.2021.101174.

Yagublu, V. Bayramov, B. Yuce, M, Aslanov, H. (2022).TP53 Codon 72 Polymorphism and the Risk of Colorectal Cancer in an Azerbaijani Population, *Turk J Gastroenterol*, 33(6), 485-90 doi: 10.5152/tjg.2022.21189

Yan, Z.H. Cui, L.H. Wang, X.H, Chao L., He, X. (2014). Comparative study of mutations in SNP loci of *KRAS*, hMLH1 and hMSH2 genes in neoplastic intestinal polyps and colorectal cancer, *World J Gastroenterol*, 28, 20(48), 18338-45. doi: 10.3748/wjg.v20.i48.18338

Yang, T. Wen, Y. Li, J. Yang, J. Tan, T. Pan, J. et al. (2019). Association of the *TP53* rs1042522 C>G polymorphism and hepatoblastoma risk in Chinese children, *J Cancer*, 10(15), 3444–49. doi: 10.18632/aging.101196. doi: 10.7150/jca.33063

Yu, M. Zhang, Q. Zhao, X. (2022). Associations of MDM2 rs2279744 and TP53 rs1042522 polymorphisms with cervical cancer risk: A meta-analysis and systematic review, *Frontiers in Oncology*. DOI 10.3389/fonc.2022.973077

Zhu, Z. Wang, A. Jia, H. Jin, X. He, X. Hou, L. et al. (2007). Association of the TP53 Codon 72 Polymorphism with Colorectal Cancer in a Chinese Population, *Jpn J Clin Oncol*, 37(5),385–390. doi:10.1093/jjco/hym034.