Journal of science faculty at Sirte University
JSFSU
2021
Peer-review Periodical Journal

جامعة سرت
Sirte University
1989

ISSN INTERNATIONAL STANDARD SERIAL NUMBER

eISSN: 2789-858X

SCIENTIFIC JOURNAL FOR THE FACULTY OF SCIENCE – SIRTE UNIVERSITY

SJFSSJ

DOI: 10.37375/issn.2789-858X - Indexed by Crossref, USA

VOLUME 3 ISSUE 2 OCTOBER 2023

Bi-annual, Peer- Reviewed, Indexed, and Open Accessed e-Journal

Legal Deposit Number@NationaL Library (Benghazi): 990/2021

1.02/2022

jsfsu@su.edu.ly
journal.su.edu.ly/index.php/JSFSU

Scientific Journal for the Faculty of Science-Sirte University Vol. 3, No. 2 (2023) 162-166

Scientific Journal for the Faculty of Science-Sirte University

Journal home page: http://journal.su.edu.ly/index.php/JSFSU/index

DOI: 10.37375/issn.2789-858X

Detection of *MUTYH* gene mutations in hereditary colorectal cancer Libyan families

Asma A. Abudabbous[1], Ehsan M. Idris[1], Afaf M. Aburwais[2], Halima A. Haded[3], Eman M. Kerwad[1], Fatima A. Aldeeb[1], Hajer M. Elshibani[3], Fatima A. Abulsharud[3], Mustafa M. Drah[1]

[1]*Genetics and Biotechnology Department, Science Faculty, Misurata University, Libya.*

[2]*Libyan Biotechnology Research Center, Misurata, Libya.*

[3]*Zoology Department, Science Faculty, Misurata University, Libya.*

DOI: https://doi.org/10.37375/sjfssu.v3i2.1596

ARTICLE INFO:

Received: 15 July 2023

Accepted: 02 October 2023

Published: 26 October 2023

Keywords: Colorectal Cancer, *MUTYH*, mutation, PCR, direct sequencing

A B S T R A C T

Colorectal Cancer (CRC) has become one of the most dangerous yet spreadable cancers, as a result of many wrong behaviors people do, in addition to being a hereditary disease. However, Libya was not included in a lot of studies due to the lack of adequate studies on infected CRC Libyan patients, thus this study aims to detect mutations in the *MUTYH* gene in Libyan families who heredity colorectal cancer. The study included 20 blood samples collected from (10 patients with hereditary colorectal cancer and 10 healthy people) all of them had a family history of this disease. Genomic DNA was extracted, amplified by PCR, and analyzed for *MUTYH* mutational status by direct sequencing. *MUTYH* mutations were present in 50% (10\20) of all analyzed samples. A total of 10 patients had *MUTYH* mutations in different positions; of which 5\10 (50%) had a deletion A in c.1140, 1\10 (10%) a patient had a substitution mutation in c.1149 C>N, 1\10 (10%) a patient had two type mutations, substitution mutation in c.1154 C>T and insertion CT inc.1153, 2/10 (20%) they had substitution mutation in c.1237 G>R, 1\10 (10%) a patient had substitution mutation in c.1140 A>C. This study concludes that indicates that analysis of the MYH gene should be performed in patients with multiple colorectal adenomas, On the other hand, it helped to clarify the type and frequency of MYH mutations among colorectal polyposis patients in Misurata. This study believes that an enlargement of the MUTYH mutation spectrum resulting from these types of studies will contribute to early detection and the prevention of secondary cancer development.

1 Introduction

Colorectal cancer (CRC) is the second most common cancer in women and the third in men. It ranked second in mortality and third in incidence among cancers worldwide in 2020 (Olovo, *et al.*, 2021). Most cases (about 95%) of the CRC are sporadical, Familiar CRCs are less common (about 5%) and occur when gene mutations are passed within a family from one generation to the other. In these cases, mutated genes (germline mutation) are inherited (Centelles, 2010). *MAP* is characterized by the presence of adenomatous polyposis of the colorectal and an increased risk of CRC (Jasperson *et al.*, 2010). The majority of changes found in the *MUTYH* gene are missense mutations, of which Y179C and G396D (previously annotated as Y165C and G382D) represent approximately 73% of *MUTYH* mutations. *MYH*-associated polyposis (MAP) is an autosomal recessive condition, associated with the development of multiple adenomas and carcinomas of the colon and rectum (Hitchins *et al.*, 2005). It is caused by biallelic mutations in the *MUTYH* gene (also referred to as *MYH*) (Jasperson *et al.*, 2010). *MUTYH* gene consists of 16 exons and is located on chromosome 1p34.3-p32.1. *and it* encodes a DNA glycosylase involved in base excision repair (BER) of 8-oxoG:A mismatches caused by oxidative

***Corresponding author*: E-mail:** asma.abodabous@gmail.com

DNA damage (Jansen, *et al.,* 2014). The Occurrence of such mutations in oncogenes and tumor suppressor genes drives colorectal carcinogenesis and is associated with the development of colonic polyps (Pitroski,, *et al*., 2011). Functionally, *MUTYH* helps prevent G:C to T:A transversion caused by oxidative stress to highly mutagenic DNA bases (Jasperson *et al.,* 2010). Study between 2014-2015, which aimed to detect the presence of genetic mutations of the *MUTYH* gene with an increased risk of developing CRC It was also observed that the frequency of *MUTYH* p.Y179C mutation is higher among CRC patients than *MUTYH* p.G396 mutation (Jansen, *et al.,* 2014). In 2011 a study was conducted in North Africa that aimed to determine the occurrence of a mutation c.1228dup - 1227 in a group of *MUTYH* patients and assessment of a founder effect within a group of 36 families had MAP, 11 families were found to have a homozygous mutation c.1228dup-1227.These families came from different countries (Algeria, Tunisia, Morocco, and Portugal). The frequency of c.1228dup_1227 is the highest (6.78% vs. 5.4%) of the mutations, and several studies also have shown two closed mutants (Y179C and G396D) are similar to about 80% of MUTYH allelic variants of Europeans Ethnic and geographic differences have been observed in the spectrum of mutations in this gene (Levre, *et al.,* 2011). In the study (2019), samples were collected from 150 Jordanian patients with colorectal adenoma and 150 individuals without cancer with no previous history of polyps. Sanger DNA sequencing of the *MUTYH* gene was performed. The data showed a high prevalence of two mutations in the germline (g.87C>T and c.1264G>C) while *MUTHY* among the affected Jordanians *(Mahasneh, et al., 2019).*

The c.1227-1228dup GG mutation, in a homozygous state, was also found in 13 patients with CRC in the study which evaluated a germline variant of the MUTYH gene, and is an indication to be considered in the testing of the MUTYH gene in patients with hereditary familial polyposis (Kdissa, *et al.,* 2020).

Libya was not included in a lot of studies due to the lack of adequate studies on infected CRC Libyan patients, thus this study aims to: Detection of mutations in the *MUTYH* gene in Libyan families have heredity colorectal cancer.

2 Materials and Methods

Sample Collection

The blood samples were collected from (10 patients with hereditary colorectal cancer and 10 healthy people) all of them had a family history of colorectal cancer. The samples were collected between May and June 2022 in Misurata Central Laboratory. Those samples were stored at -20 ˚C until DNA was extracted.

DNA Extraction

Genomic DNA was extracted from blood samples, using (MagicPure^{TM}Blood Genomic DNA Kit, TransGen, Chinese) according to the manufacturer's instructions.

The Assessment of DNA Quality and Quantity

The DNA concentration was spectrophotometrically assessed using thermo scientific NanoDrop machine (Thermo Fisher Scientific, America). The quality of extracted genomic DNA was examined by loading on agarose gel, to check the integrity of DNA.

Gel Electrophoresis

The electrophoresis was run at 50V for one and half hours. The gel was exposed to UV light using Bio spectrumTM 500 imaging system (UVP) and then photographed using a Multidock-it digital imaging system.

Polymerase Chain Reaction (PCR) Conditions

After extracting the genomic DNA from the samples, the specific region in exon 14 of the target gene (*MUTYH*) was amplified by PCR using the primers (Forward.:3-GGCAGTGGCATGAGTAACAA-5 and Reverse 3-AGAGCAGCTTCAGCGCAAG-5). The PCR reaction was run with the following program: 95˚C initial denaturation for activating the Taq DNA polymerase for 3 minutes in 1x cycle, denaturation at 95˚C for 30 seconds in a 35x cycles, annealing of the primers to the template at 60˚C for 30 seconds in 35x cycles, then extension at 72˚C for 1 minute in a 35x cycles and Step 5final extension at 72˚C for 7 minutes in 1x cycle. The PCR reaction was cooled down for 4˚C. All PCR reactions were done in the Scientific Research Unit at the Misurata Central Laboratory.

Sequencing Analysis

PCR products were sent to the Artha Genomincs Advanced Technologies in Tunisia, for sequencing by Sanger sequencing 7500. The sequencing results were analyzed by using Finch TV and CLC software and Genome browser website to identify mutations in the *MUTYH* gene. A codon number of the mutant gene and it's changed the amino acid sequence were determined by referring to the NCBI/BLAST website.

3 Results

To examine the integrity of the extracted genomic DNA, 5 g of DNA was added to an agarose gel (figure1). Genomic DNA concentration of the 10 specimens included in the study group was between (16.4 ng/µl – 121.8 ng/µl) and 260 nm/280 nm ratios were between (1.90 -1.75)**.**

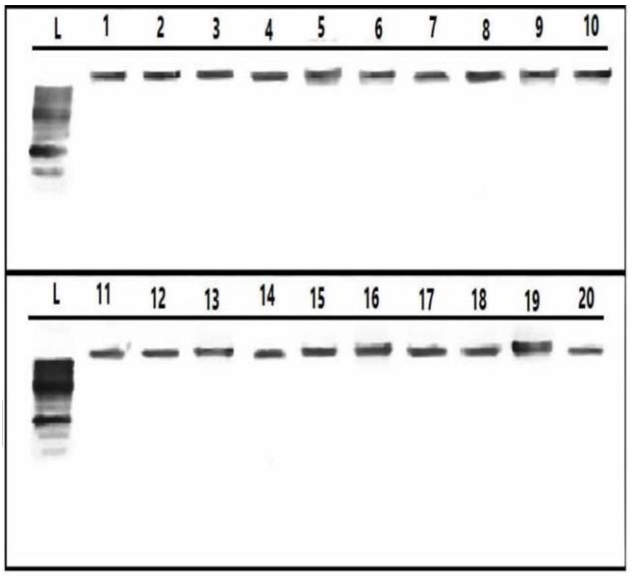

Figure (1): Gel electrophoresis for genomic DNA in 1% agarose gel. L: Ladder 1Kp

The *MUTYH* gene was amplified by PCR using sets of primers described earlier in Materials and Methods. Agarose gel electrophoresis (1%) was run to visualize PCR products and to verify that the products are of the expected size. The PCR products revealed a band of the expected size of *the MUTYH* gene at 149 bp (figure 2).

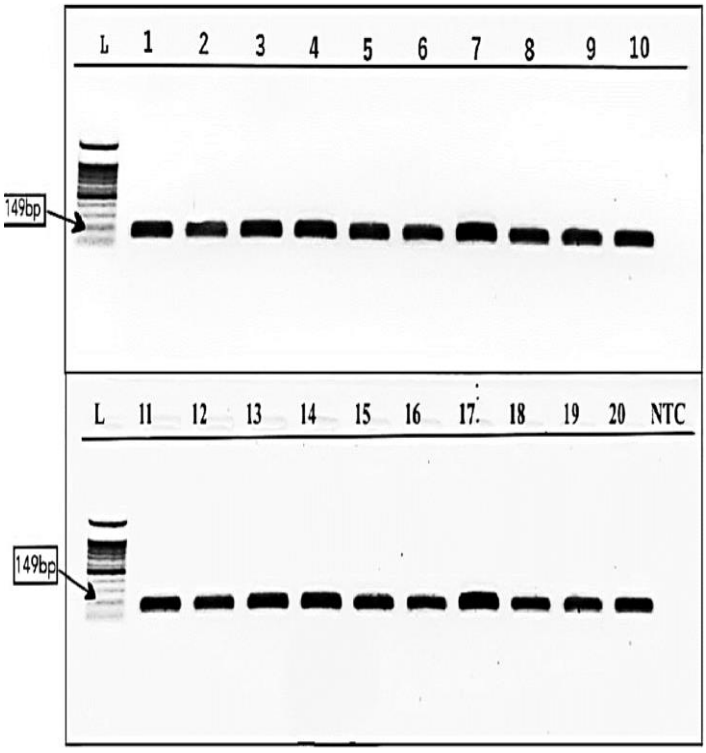

Figure (2): Gel electrophoresis of PCR samples product, the arrow indicates the product with 149bp, NTC: No templet control, L: Ladder 100bp

The type and frequency of *MUTYH* gene mutations are detailed in table (1), *MUTYH* mutations were present in 50 % (10\20) of all analyzed samples. A total of 10 patients had a *MUTYH* mutations in different positions; of which 5\10 (50%), had deletion A in c.1140, 1\10 (10%) a patient had substitution mutation in c.1149 C>N, 1\10 (10%) a patient had two type mutations, substitution mutation in c.1154 C>T, and insertion C inc.1153, c.1140 A > C and c.1237 G > R in proportion (1\10) 10% and (2\10) 20% (Figure 3).

Table (1): Distribution of MUTYH mutation types

N. of samples	Normal sequence	Mutation sequence	Mutation position
1	GCA	GAA	c.1140 A>C
5	GAA	G-A	c.1140 del A
1	CCT	NCT	c.1149 C>N
1	TCC CTC	T**C**CC TTC	c.1153 Ins C c.1154 C>T
2	ARA	AGA	c.1237 G>R

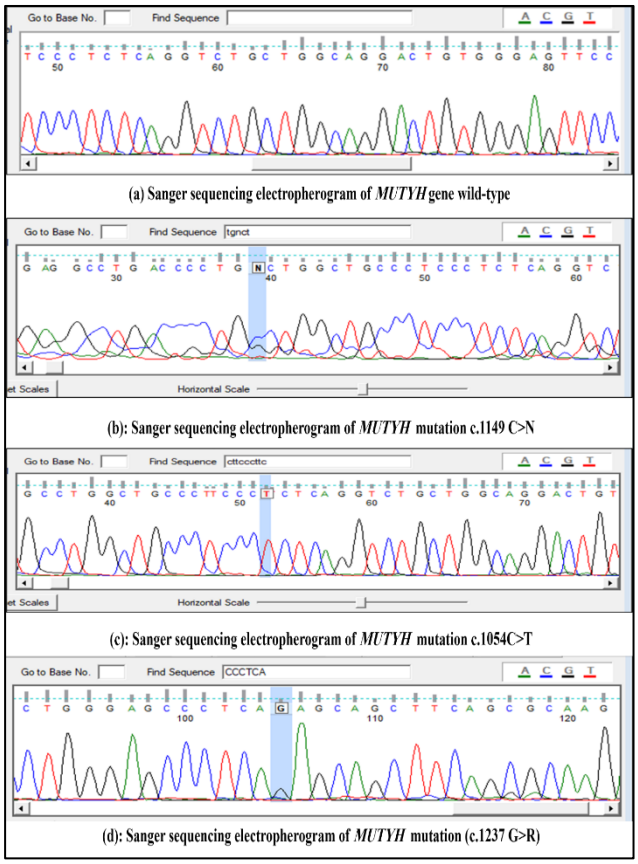

Figure (3): Sanger sequencing electropherogram of *MUTYH* gene.

4 Discussion

This study analyzed *MUTYH* mutations in heredity CRC of Libyan patients in whom CRC incidence and mortality are one of the highest in the Middle East and North Africa, and it is still increasing (Lefevre, et al., 2011). In this study, samples extracted from blood of 20 Libyan patients with CRC were screened for mutation in *MUTYH* gene by using PCR direct sequencing. *MUTHY* mutations were identified in 50% of all heredity CRCs in this study group of patients with CRC. This finding was in a good agreement with previous reports which identified *MUTHY* mutations in 73 % of heredity CRC patients (Sampson, *et al.,* 2005). The frequency of *MUTYH* mutations in the study group did not differ when compared with those of most other studies in the British, Italian, American, Portuguese, and Dutch populations (Sampson, *et al.,* 2005, Cheadle and Sampson, 2007). Previous Arabian studies that enrolled a relatively large number of patients and included patients with different stages of the disease, reported *MUTYH* mutation rates of 80%, 30% in Tunisia (Mahasneh, *et al.,* 2019, Abdelmaksoud-Dammak *et al.,* 2012), 40 % in Portugal (Isidro, *et al.,* 2004), and 95% in Egypt (Elsaid, *et al.,* 2017).

In the study conducted by Afaf Elsaid *et.al.* in 2016 to detect the presence of genetic mutations of *MUTYH* gene with an increased risk of developing CRC, samples extracted from blood of 120 Egyptian patients with CRC, shown that 15 samples had the *MUTYH* mutation and 105 samples negative for *MUTYH* mutation (Elsaid, *et al.,* 2017), where 15 patients also had a family history of CRC thus, a strong association between *MUTYH* mutation and an increased risk of CRC cancer among Egyptian patients, which agree with this study. It was also observed that the frequency of MUTYH p.Y179C (c.536A) mutation is higher among CRC patients than *MUTYH* p.G396D (c.1187G) mutation (Elsaid, *et al.,* 2017), in this study was detected mutation (c.1149C) in same exon.

In 2004 a study was conducted in Portugal in which the biallelic germline strain in the *MUTYH* gene was studied in 53 Portuguese patients with multiple or classic colorectal adenomas and 21 patients had a biallelic germline mutation. New Portuguese-specific mutations in different patients (c.1186 -1187insGG), (c.503G& A) and (c. 340T&C) (Isidro, *et al.,* 2004). These results agree with this study but different position of mutations.

There could be a relatively large number of factors that could be linked to the variations in the observed frequencies of *MUTYH* mutations in the different studies. Among these are using different methodologies for detection of the mutations with different ranges of sensitivity and specificity. Environmental factors have a major impact on different populations with diverse lifestyles, dietary habits, and variable exposures to carcinogens. In addition, using different numbers and kinds of samples, which might serve as another reason for such diversity. Couples with this in the variation in the carcinogen metabolizing genes in different population groups.

5 Conclusion

This study contributes to support the existence of Heredity Colorectal Cancer and indicates that analysis of the MYH gene should be performed in patients with multiple colorectal adenomas, particularly in those with family history of horizontal transmission of the disease. On the other hand, it helped to clarify the type and frequency of MYH mutations among colorectal polyposis patients in Misurata. Although this study showed these mutations in a small region in the MUTYH gene, whole gene sequencing screening procedure in MUTYH is highly recommended in familial colorectal cancer patients. Additional studies will be helpful in identifying new mutations.

Disclaimer: The article has not been previously presented or published and is not part of a thesis project.

Conflict of interest: There are no financial, personal, or professional conflicts of interest to declare.

References

Abdelmaksoud-Dammak, R., Miladi-Abdennadher, I., Amouri, A., Tahri, N., Ayadi, L., Khabir, A., ... & Mokdad-Gargouri, R. (2012). High prevalence of the c. 1227_1228dup (p. Glu410GlyfsX43) mutation in Tunisian families affected with MUTYH-associated-polyposis. *Familial cancer*, 11(3), 503-508

Centelles, J. J. (2012). General aspects of colorectal cancer. *International Scholarly Research Notices, 2012*

Cheadle, J. P., & Sampson, J. R. (2007). MUTYH-associated polyposis—from defect in base excision repair to clinical genetic testing. *DNA repair*, 6(3), 274-279.

Elsaid, A., Elshazli, R., El-Tarapely, F., Darwish, H., & Abdel-Malak, C. (2017). Association of monoallelic MUTYH mutation among Egyptian patients with colorectal cancer. *Familial cancer*, 16(1), 83-90.

Hitchins, M., Williams, R., Cheong, K., Halani, N., Lin, V. A., Packham, D., Ku, S., Buckle, A., Hawkins, N., Burn, J., Gallinger, S.,Goldblatt, J., Kirk, J., Tomlinson, I., Scott, R., Spigelman, A., Suter, C., Martin, D., Suthers, G., & Ward, R. (2005). MLH1 germline epimutations as a factor in hereditary nonpolyposis colorectal cancer. *Gastroenterology*, *129*(5), 1392–1399

Isidro, G., Laranjeira, F., Pires, A., Leite, J., Regateiro, F., e Sousa, F. C., ... & Boavida, M. G. (2004). Germline MUTYH (MYH) mutations in Portuguese individuals with multiple colorectal adenomas. *Human mutation*, 24(4), 353-354.

Jansen, M., Menko, F. H., Brosens, L. A., Giardiello, F. M., & Offerhaus, G. J. (2014). Establishing a clinical and molecular diagnosis for hereditary colorectal cancer syndromes: Present tense, future perfect?. *Gastrointestinal endoscopy*, *80*(6), 1145–1155.

Jasperson, K. W., Tuohy, T. M., Neklason, D. W., & Burt, R. W. (2010). Hereditary and familial colon cancer. *Gastroenterology*, *138*(6), 2044–2058.

Kdissa, A., Brusgaard, K., Ksiaa, M., Golli, L., Hallara, O., Ousager, L. B., ... & Gribaa, M. (2020). c. 1227_1228dupGG (p. Glu410Glyfs), a frequent variant in Tunisian patients with MUTYH associated polyposis. *Cancer Genetics*, 240, 45-53.

Lefevre, J. H., Colas, C., Coulet, F., Baert-Desurmont, S., Mongin, C., Tiret, E., ... & Parc, Y. (2011). Frequent mutation in North African patients with MUTYH-associated polyposis. *Clinical genetics*, 80(4), 389-393.

Mahasneh, A., Al-Shaheri, F. N., & BaniHani, M. N. (2019). Association of a new germline variant in the MUTYH DNA glycosylase gene with colorectal adenoma transformation into malignancy. *Iranian Biomedical Journal*, 23(6), 412.

Olovo, C. V., Huang, X., Zheng, X., & Xu, M. (2021). Faecal microbial biomarkers in early diagnosis of colorectal cancer. *Journal of cellular and molecular medicine*, *25*(23), 10783–10797.

Pitroski, C. E., Cossio, S. L., Koehler-Santos, P., Graudenz, M., Prolla, J. C., & Ashton-Prolla, P. (2011). Frequency of the common germline MUTYH mutations p.G396D and p.Y179C in patients diagnosed with colorectal cancer in Southern Brazil. *International journal of colorectal disease*, *26*(7), 841–846.

Sampson, J. R., Jones, S., Dolwani, S., & Cheadle, J. P. (2005). MutYH (MYH) and colorectal cancer. *Biochemical Society Transactions*, 33(4), 679-683.

SCIENTIFIC JOURNAL FOR THE FACULTY OF SCIENCE – SIRTE UNIVERSITY

Scientific Journal Impact Factor

TOGETHER WE REACH THE GOAL

jsfsu@su.edu.ly

journal.su.edu.ly/index.php/JSFSU