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Reporting an Iranian Female Patient Suffering from Colorectal Cancer with a Missense Mutation in Exon 2 of *MEN1* Gene: An Unusual Case

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ABSTRACT

Background: Multiple endocrine neoplasia type 1 (MEN1) is a hereditary condition manifesting as an autosomal dominant genetic pattern. This genetic anomaly is marked by the formation of tumors in diverse endocrine glands, leading to a complex array of clinical manifestations. The correlation between colorectal cancer (CRC) and *MEN1* mutations remains unknown. This study aimed to report an Iranian female CRC patient with a *MEN1* genetic mutation. Material and method: A 37-year-old female presented to a physician complaining of rectal lesions, tubular adenoma, and rectal adenocarcinoma, suggesting moderately differentiated colorectal adenocarcinoma symptoms. HLA typing was tested for her. Finally, whole exome sequencing (WES) was performed using the patient's DNA. Based on a standard pipeline, the variant was identified and interpreted. Results: The findings demonstrated a known heterozygous missense mutation in the exon 2 of the *MEN1* gene, g.64577373T-G, c.209A>C, p.Asp70Ala in the patients which has a variant uncertain significant (VUS) pathogenicity. She also had no celiac disease (HLA-DQ2/DQ8 genotypes are negative). Conclusion: The correlation between the identified genotype and the anatomical phenotype of the patient suggested that the identified variant can lead to colorectal abnormalities in the patient, contributing to the distinctive features of MEN1 disease. Understanding the underlying genetic mechanisms is important for diagnosing and treating this condition.

Camera Ready

Introduction

Multiple endocrine neoplasia type I (MEN1) is an autosomal dominant familial tumor syndrome (Kimura et al., 2023); Guru et al identified its involved gene named multiple endocrine neoplasia type 1 (*MEN1*) or Menin 1 in 1997 (Guru et al., 1998). The *MEN1* gene, mapped on 11q13.1 and has 14 exons, produces the protein menin (Clark et al., 2016), which is recognized as a tumor suppressor whose functions and interactions are diverse. A wide range of partners is associated with

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menin, including histone deacetylase complexes and transcription factors (Yang et al., 2013). Menin regulates gene transcription through chromatin reorganization. Disruption of this gene makes individuals susceptible to multiple endocrine and non-endocrine tumors (Canaff et al., 2012). Considering that menin's crystal structure classifies it as a scaffold protein, it may be possible to explain why it interacts with such a wide range of molecules. This protein exhibits activity within the nucleus where it plays a significant role in gene transcription, binding both to organelles as well as cell membranes (Matkar et al., 2013).

The MEN1 prevalence is 1 in 30,000 (Concolino et al., 2016). The disorder impacts both genders uniformly, irrespective of ethnic, racial, or geographical factors; 76% of patients under 21 years and 63% above 16 years will experience this condition. Men, however, tend to develop macroadenomas more frequently, particularly during youth. Pituitary adenomas are the initial manifestation for 17% of patients with MEN1, and 21% of the subgroup are under 21 years of age (Kommlnoth et al., 2017).

For MEN1 disease more than 2100 mutations in the *MEN1* gene are described in the ClinVar database (Clark et al., 2016; Tsukada et al., 2009). According to present data indexed in the UMD-MEN1 database (http://www.umd.be/MEN1/), the majority of mutations identified in the *MEN1* gene are pathogenic mutations (1306 of 1763 records). Also, all involved mutations in the *MEN1* gene can be categorized into three types: 1) small rearrangements (90.8%) consisting of point mutations (42.2%) (missense mutations (25.1%), small deletions (21.3%), nonsense (17.0%)), small insertions (14.7%), and splice sites (10.8%); 2) large rearrangements (6.2%) consist of large deletions (6.0%) and large duplications (0.2%); and 3) mid-intronic variations (3.0%) (Mele et al., 2020). *MEN1* mutations lead to the appearance of endocrine tumors, which mainly contribute to the formation of multiple neoplastic endocrine growths in various organs, including the parathyroid glands, endocrine pancreas, duodenum, anterior pituitary, and occasionally the stomach, adrenal cortex, thymus, and lungs. Additionally, non-endocrine lesions may manifest in the skin, central nervous system (CNS), and soft tissues (Goudet et al., 2010).

Colorectal cancer (CRC) is the sixth most common cancer worldwide, and its patients account for 6% of cancer-related deaths (Manfredi, 2021). Mucosal epithelial tissue in the colon that protrudes into the intestinal cavity is a colorectal polyp. Neoplastic polyps are considered potentially malignant and should be examined pathologically (Shinya & Wolff, 1979). Despite progress over the past decades, the pathogenesis, diagnosis, and treatment of CRC remain largely unknown (Rawla et al., 2019).

Whole-exome sequencing (WES) has been used to detect variants in the exons of protein-coding genes, and also enables the identification of novel variants in untranslated regions and non-coding regions and approaches could help identify the causative genes (Brandi et al., 2001; Guo et al., 2012). Detection of related genes is one of the identification methods and somatic gene mutations can cause CRC (Komor et al., 2018). Our study aimed to identify variants that correlate with clinical manifestations in the patient using the WES assay.

Materials and Methods

Case Presentation

In 2023, a 37 years-old female patient with rectal lesions, tubular adenoma, and rectal adenocarcinoma symptoms and pathologically diagnosed as moderately differentiated colorectal adenocarcinoma was referred by the endocrinologists to a genetics laboratory for further evaluation to identification putative genetic mutation. Her colorectal examination revealed an ulcerative mass in the rectum and there were a few polyps in the colon; in addition, her pathological report suggested that she is in the moderately differentiated adenocarcinoma stage of CRC.

physical examiniation

Physical examination revealed that the patient's body temperature was 36.8 °C, heart rate 78 bpm/min, respiratory 16 bpm/min, and blood pressure 130/80 mmHg. Until now, the patient did not have any confirmed history of diabetes, hypertension, tuberculosis, or hepatitis.

Imaging examiniations

Abdominal computed tomography scan M.D.C.T (Multi detector computed tomography) scan with intravenous (IV) contrast of liver, spleen, pancreas, pelvic and kidney was of normal size and density.

There was a hyper dense mass lesion originating on the left side of the rectum measuring 26 × 30 mm with multiple regional lymph nodes which were further evaluated by ultrasound. In abdominal and pelvic ultrasonography (USG) evidence of increased thickness of the wall along with irregularity in the rectum and a few lymph nodes around 8 mm were observed. The gallbladder, spleen, kidney, liver, and bladder with normal volume, thickness, dimensions, and uniform and homogeneous parenchyma were located in them.

There were no lesions in the ovaries and uterus and no free fluid was reported in the abdominal cavity and pelvis but an increase in thickness with dimensions of 19 × 24 mm was in the rectal wall. The results of colonoscopy showed polyps in the recto sigmoid, colon, sigmoid, descending colon, hepatic, and splenic flexure in sizes of 8-10 mm. A 2 cm mass was observed in the rectum. No lesions were observed in other parts of the ascending and middle transverse colon, cecum, or terminal ileum. Then, the colon biopsy containing pieces of soft tissue with dimensions of 0.8 × 0.6 × 0.4 cm was placed in formalin and labeled.

Final Pathological Diagnosis

The histological and pathological findings confirmed the diagnosis of invasive moderately differentiated adenocarcinoma of CRC.

DNA Isolation

The patient provided 5 mL of her whole blood sample in an EDTA tube, which was stored at -20°C for DNA testing. A standard salting-out protocol was utilized to isolate genomic DNA from peripheral blood sample, followed by 1% agarose gel electrophoresis to examine DNA quality. A nanodrop instrument was also employed for determining DNA concentration and purity.

Mutation testing

Exome capture was performed with Agilent SureSelect Human All Exon V6 Kit (Agilent, Sata Clara, CA, USA) using 2 µg of genomic DNA from the patient. Afterward, the fragments were sequenced using Illumina HiSeq 2500 (Illumina, San Diego, CA, USA) to yield sequences of more than 100 bases from each end (Novogene, Cambridge, United Kingdom). To execute sequence mapping to the GRCh37/UCSC hg19 reference genome, a GATK-based workflow was followed. Using VarScan version 2.2.5, SNVs were detected, and SNVs were filtered based on population frequency using Greater Middle East (GME), gnomAD, Iranome, MuTec, and Variome Project databases. The WES workflow used in the study is provided in **Fig. 1**.

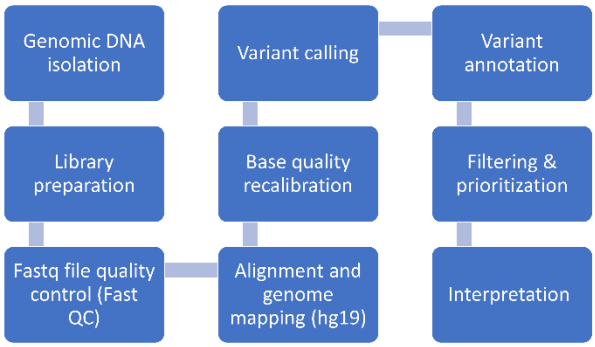


Fig. 1- Schematic representation of identifying mutations using the WES-based workflow.

Results

Clinical assessment

In 2023, an Iranian Baluch woman 37 years of age had gastrointestinal symptoms and after various examinations in the last two years was referred to a genetic laboratory by a gastroenterologist in Zahedan City, Iran. A blood test report of the patient is presented in **Table 1**. As shown in this table, the patient has a high level of alkaline phosphatase, SGOT, and CRP, and a low level of RBC.

According to **Fig. 2**, the proband's parents (case IDs: 18 and 19), and her sibling (case IDs: 25 and 26) were apparently healthy. However, there was a report of CRC in her mother's ancestry (case ID: 20, he died at age 25). Also, some cancers had happened in her familial pedigree (Case IDs: 1 and 13 with breast cancer; Case ID: 16 with oral squamous cell carcinoma; Case IDs: 21 (she died at age 40) and 22 with gastric cancer; Case IDs: 29 and 30 with celiac disease. Hence, an HLA typing test was conducted on the proband due to the presence of two individuals with celiac disease in the pedigree; the results for the DQ2 and DQ8 genes were negative, which means she has no celiac disease. A description of the findings of the proband's colonoscopy is provided in **Fig. 3**.

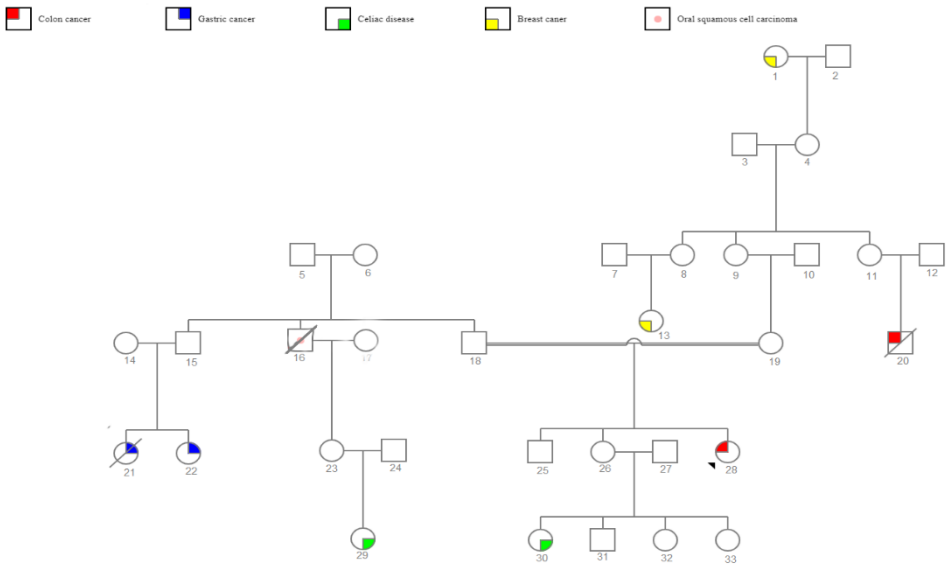


Fig. 2- Expanded pedigree of the patient. Proband (case ID: 28) was marked with an arrow.

Table 1 - Blood test to evaluate various factors.

	Result	Unite	Normal range
Hematology Test			
WBC	7.1	$10^3/\mu L$	4-10
RBC	3.65❋	$10^6/\mu L$	4.2-5
Hb	10	g/dL	12-16
PLT	214	$10^3/\mu L$	150-400
Blood Biochemistry			
FBS	86	mg/dL	90-110
Calcium	10	mmol/L	8.6-11
Sodium	139	mmol/L	135-145
Potassium	1.4	mmol/L	3.5-5.5
Phosphate	3.6	mmol/L	2.6-5
SGOT(AST)	80*	U/L	Up to 39
SGD(ALT)	120	U/L	2-31
Urea	26	mg/dL	10-50
Alkaline phosphatase	881*	U/L	70-260
Bilirubin	0.6	mg/dL	0.1-1.2
Creatinine	0.6	mg/dL	0.4-1.5
Blood urea nitrogen	12	mg/dL	7-27
Serology			
CRP	32*	mg/dL	up to 10
Hormone Analysis			
CEA(ECL)	1.3	ng/mL	up to 4.7
TSH(ECL)	2.8	m/µL	20-50 years 0.3-4.8

Abbreviations: WBC = White blood cells, RBC = red blood cell, Hb = Hemoglobin, PLT = platelet count, FBS = Fasting blood glucose, SGOT = serum glutamic-oxaloacetic transaminase, SGD = Neutrophil-specific granule deficiency, CRP = C-reactive protein, CEA = carcinoembryonic antigen, TSH = thyroid stimulating hormone.

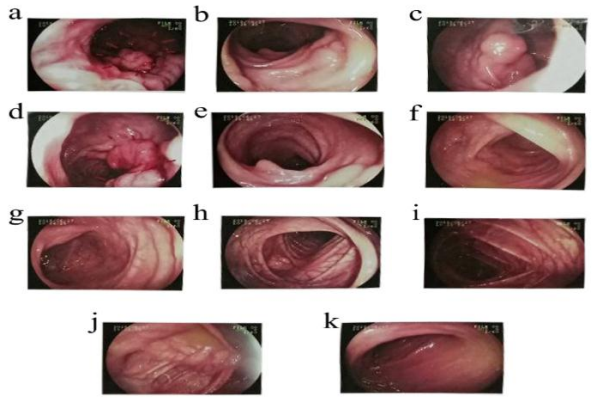


Fig. 3- Colonoscopy images of different parts of the colon. a = Anus; b, c, and d = Rectum; e = Rectosigmoid colon; f = Sigmoid colon; g = Descending colon; h = Middle Transverse colon; i = Ascending colon; J = Cecum; k = Terminal ileum.

Genetic analysis

WES analysis revealed a known heterozygous missense mutation (rs1220436193) in exon 2 of *MEN1* gene mapped to NM_001370251, g.64577373T-G, c.A209C, p.Asp70Ala. This indicates that Alanine at codon 70 of the menin protein has been replaced by Aspartic due to a missense mutation in one of the alleles. The allelic frequency for allele G of this variant in dbSNP is 0.01150 and 0.00382 in two Japanese studies and for a Korean study is 0.0182, while the frequency of the allele in in Global population is 0.000. Also, in the dbSNP database, two other potential substitutions are indexed for rs1220436193 (T>A and T>C); there is no report for T>A frequency, and the frequency of allele C in T>C is 0.000.

The protein-protein interactions for *MEN1* are provided using the STRING database (https://string-db.org/) (**Fig. 4**).

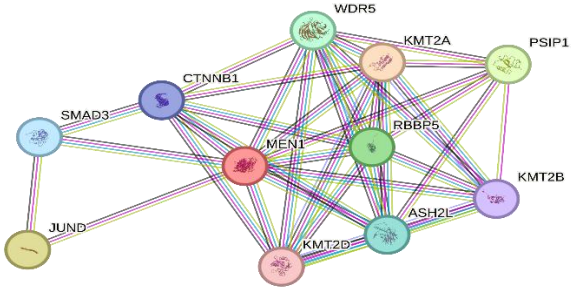


Fig. 4- The protein-protein interactions for the MEN1 gene.

Discussion

There are several different types of MEN syndromes (MEN1, MEN2, MEN2A, MEN4), which occur due to genetic mutations and are inherited in families (Khatami & Tavangar, 2018). Since MEN1 is an autosomal dominant disorder, individuals with a mutation in one copy of the *MEN1* gene have a 50% chance of passing the mutated gene to their offspring. Genetic testing and counseling are important for individuals with a family history of MEN1 or symptoms suggestive of the syndrome (Thakker et al., 2012). Ninety-five percent of patients with MEN1 disorder will display clinical symptoms by their fifth decade. Additionally, approximately 10% to 30% of MEN1 patients do not have *MEN1* gene mutations (Lemos & Thakker, 2008). The sporadic form of MEN1 may also affect 8-14% of patients with MEN1 (Syro et al., 2012). The results of a cohort investigation utilizing data from the Dutch MEN1 database indicated that individuals with a positive mutation for MEN1 and those without mutations experience distinct clinical trajectories. Patients without mutations tend to exhibit MEN1 manifestations at a later age and display a median survival akin to that of the general population, surpassing that of patients with *MEN1* mutations by 10 years (de Laat et al., 2016).

Fig. 4 presents a molecular analysis of the MEN1 gene that may provide a better understanding of its pathophysiological functions**.** According to one study, patients with CRC have higher levels of the JunD proto-oncogene (*JUND*) protein (Chang et al., 2023); this protein interacts with *MEN1*. Another study suggested that menin can inhibit JunD-mediated gastrin gene activation through its cooperation with the histone deacetylase complex (Mensah-Osman et al., 2011). *SMAD3* is involved in the *TGF-β* pathway, and its mutation is linked to CRC (Kaji et al., 2001). By inactivating menin, the *SMAD3*-inducing and *TGF-β* pathways can be suppressed (Fleming et al., 2013). In cultured cells, menin expression has a negative effect on cell proliferation, and several menin-interacting proteins involved have been identified (Agarwal et al., 2003).

Our patient has mutations in exon 2 of the *MEN1* gene, which are similar to those reported in some studies. Langer et al. found *MEN1* mutations in exon 2 in two patients with macronodular hyperplasia, indicating that *MEN1* mutations in

exon 2 are more common than other mutations in this study (Langer et al., 2002). According to the findings of a study involving a large number of MEN1 families using SSCP assays, 47 mutations (14 in exon 2) were identified in the *MEN1* gene, of which 80% produce truncated or non-functional proteins (Bassett et al., 1998). There was a study conducted on some Italian families and sporadic cases to investigate mutations in the *MEN1* gene. The results revealed several mutations with both sporadic and familial patterns; some of these mutations are located in exon 2 such as the missense mutation in Glu45Lys, the frameshift mutations in 311ins5(GCCCC), and 359del4(GTCT) (Morelli et al., 2000). Several mutations in introns and exons of the *MEN1* gene were identified by Klein et al. in 400 individuals from the United States and Canada. Exon 2 or intron 2 of the gene was characterized by the following mutations: missense mutations in Met1Val and Phe144Val; nonsense mutations in 29, 77, and 126 codons; deletions which caused frameshifts in codons 70, 83-84, 103, 111-112, 131, 134, 139, and 148; insertions/duplications causing frameshifts in codons 66-67, 107, and 114; and deletions within the codon 120. Additionally, somatic mosaicism decreases the likelihood of sporadic patients testing positive compared to familial patients (Klein et al., 2005). Chavoshi et al. reported an Iranian female patient with a delayed diagnosis of MEN1 who possessed a pathogenic mutation in exon 2 of *MEN1* (p.11e85Serfs Ter33) (Chavoshi et al., 2022).

According to our assessment, mechanisms that maintain menin at normal levels may be effective in treating MEN1-related diseases. For instance, an Iranian female patient with MEN1 who had been treated monthly with Sandostatin LAR for four years has achieved a significant level of health improvement (Mohajeri-Tehrani et al., 2022). Based on a recent study by Nyul et al., LXR-target genes, including ABCG1 and ABCA1, are inhibited by menin in both colonic epithelium and CRC cell lines. Conversely, menin inhibition is associated with the upregulation of LXR-target genes and reduction of total cellular cholesterol levels, demonstrating that menin inhibition may have a significant role in modulating cholesterol-dependent cellular processes, contributing towards the development of CRC (Nyul et al., 2023).

Management of MEN1 often involves regular surveillance and early intervention to address the specific tumors that may develop. This can include surgery, medical management, and other targeted treatments depending on the nature of the tumors involved. Regular follow-up is essential to monitor for any new tumor development or changes in existing tumors (Pieterman & Valk, 2022).

Limitations and challenges

The present study was restricted to the WES of the proband with no Sanger sequencing confirmation. Also, her family recommended that be tested for the identified variant. Moreover, gene expression and protein analysis of *MEN1* may provide insight into its role in CRC. We recommended full sequencing of the *MEN1* gene and other genes related to MEN1 disease and CRC with higher sequencing depth and involving the regulatory elements that are omitted in the WES technique. Furthermore, the pedigree was provided based on the patient's consultation and may have some bias. Our decision was based on the information provided to us by our referring physician and genetic counselor.

Conclusion

Currently, to the best of our knowledge, no clear research has been published indicating that mutations in the *MEN1* gene are associated with CRC. However, our research suggests that the MEN1 mutation may be associated with the patient's endocrine lesions. The *MEN1* molecular mechanism, associated factors, and environmental influences in the presence of mutation should be further explored through functional studies. In addition, the variant has not yet been documented in Iran. Mutational screening of the *MEN1* gene is useful for detecting high-risk individuals for *MEN1*-associated conditions. Our study provides new insights regarding the potential roles of mutations in the *MEN1* gene.

Informed Consent Statement

For participation in the study and the acquisition of pathological images and data, informed consent was obtained from the female patient (proband).

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to convey their sincere appreciation for the assistance of Dr. Kordi's Genetics Laboratory and the patient who participated in this study.

Abbreviations

MEN1 = Multiple endocrine neoplasia type 1

CRC = Colorectal cancer

WES = Whole exome sequencing

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