Apparition of Germline Mutation c.1395-1397del of MUTYH in Algerian Consanguineous Family with Colorectal Cancer

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Abstract

MUTYH is a glycosylase that removes adenine opposite 8-oxo guanine (OG) during base-excision repair of DNA. Variants of MUTYH defective in functional activity lead to MUTYH-associated polyposis (MAP), autosomal recessive predisposition to colorectal cancer (CRC). MAP combines clinical features with other hereditary CRC syndromes and exhibits phenotypic overlap, particularly with Lynch syndrome (LS). To determine the impact of MUTYH mutations in colorectal adenomas and cancer susceptibility, this prospective objective screens the MUTYH gene in seventeen Eastern Algerian families with clinically suspected LS but without mismatch repair (MMR) mutations. Methods: We examined the presence of the mutations in the probands and their relatives using direct sequencing of the entire coding region of the MUTYH gene. Results: The biallelic and monoallelic pathogenic MUTYH mutations, c.1395_1397del, were discovered in a consanguineous family with CRC and gastric cancer as a novel finding in our Eastern Algerian population. Conclusion: High rates of consanguinity in the Algerian population increase the risk of CRC caused by biallelic mutations in the MUTYH gene. In our families, the LS and MAP phenotypes might coexist. The inclusion of MUTYH testing in the diagnostic strategy of LS-suspected patients should make carriers of mutations in this gene, as well as their first-line relatives, aware of their increased risk and encourage them to undergo early screening.

Keywords

Algerian families; colorectal cancer; MUTYH-associated polyposis; MUTYH gene;
1 Introduction

There are two entities of tumour predisposition syndromes with high penetrance, which contribute to approximately 5% to 10% of colorectal cancers (CRC) (Aretz et al., 2006): familial adenomatous polyposis (FAP) (MIM #175100) and hereditary nonpolyposis colorectal cancer (HNPPC or Lynch syndrome, LS) (MIM #114500). Both these two diseases have autosomal dominant conditions and result from the family history of early onset CRC: typical FAP is characterized by the appearance of hundreds to thousands of colorectal adenomas. In LS, there is usually only a few adenomas, and tumors present high microsatellite instability (MSI-H) (Lynch & de la Chapelle, 2003). The phenotype with fewer (10–100) colorectal adenomas represents a heterogeneous and yet poorly characterized group, between FAP and LS, known as multiple colorectal adenomas (MCA).

On the Genetic profile, the FAP is caused by germline mutations in the tumour suppressor gene APC. HNPPC or LS is diagnosed by the detection of a germline mutation in one of the mismatch repair (MMR) genes: MLH1, MSH2, and MSH6. However, in 10-15% of cases clinically suspected of LS, the presence of genealogical criteria of selection (Amsterdam or Bethesda guidelines), MSI-H and abnormal immunohistochemistry (IHC) results in the tumours (clinical criteria) cannot be explained by MMR germline mutation analyses (Morak et al., 2014). The Germline mutations in the human Mut Y homolog (MUTYH; MIM# 604933) also known as MYH can be probably the cause of these predispositions to colorectal cancer. When the first pathogenic variant of the MUTYH gene was described By Al-Tassoc (Al-Tassan et al., 2002).

This pathology, called, MUTYH-associated polyposis (MAP) (MIM # 608456) is an autosomal recessive inheritance disorder that was also found to correlate with a predisposition to MCA and colorectal carcinomas (Al-Tassan et al., 2002; Raetz & David, 2019). The clinical aspect of MAP gives frequently a spectrum of expression from severe colorectal polyposis to attenuated forms. It can be present at the late age of onset or with few adenomas in CRC, which creates a partial phenotypic overlap with LS and can cause recognition problems (Morak et al., 2014; Sutcliffe et al., 2019). Castillejo and collaborators reported that MAP patients show some phenotypic similarities to LS patients (Castillejo et al., 2014). In this regard, the extracolonic tumour spectrum is similar in both groups and CRC can be diagnosed in the absence of adenomas or associated with a small number of adenomas (Gu et al., 2002).

The MUTYH gene is localized on the short arm of chromosome 1(1p32.1- p34.3), contains 16 exons and encodes a DNA glycosylase belonging to the base excision repair pathway (Nielsen et al., 2022). The major function of MUTYH is to prevent 8-oxodG (8-hydroxyguanine), a base lesion brought on by oxidative stress, from having mutagenic effects. To start the base excision repair process, it removes the adenine that was accidentally incorporated into the opposing template, 8-oxodG (8-hydroxyguanine) (Isidro et al., 2004). Base excision repair pathway-mediated resynthesis generates C: 8-oxodG base pairs, which serve as substrates for OGG1-mediated removal of the oxidized purine. Thus the action of MUTYH prevents the formation of G:C to T:A transversion mutations (Ricci et al., 2017; Nielsen et al., 2022). At the protein level, The MUTYH protein structure is made up of many functional domains that are involved in mispair recognition, DNA binding, base flipping, excision, and interaction with other DNA replication and MMR (MSH2, MSH6) protein (Markkanen et al., 2013). MUTYH is physically linked to MSH2/MSH6, and the MSH2/MSH6 complex enhances MUTYH’s DNA binding and glycosylase activity by o xo G:A mispairs (Castillejo et al., 2014).
To date, the impact of MUTYH mutations has been examined in patients with different phenotypes including FAP, MCA, LS, and sporadic CRC (Nielsen et al., 2022). Two missense mutations p.Tyr179Cys and p.Gly396Asp in the MUTYH gene are the most frequently observed in European populations (Grandval et al., 2014). The frequency of mutations in the MUTYH gene in Oriental and Arabic populations is much less well documented. Furthermore, the mutational spectrum is clearly distinct (rarity of p.Tyr179Cys and p.Gly396Asp mutations).

Because of its autosomal recessive inheritance, MAP is less frequent than LS or FAP. However, this type of genetic predisposition may become more common in Arabic countries, where certain families with many marriages between relatives, exhibit high rates of consanguinity. Here, we present the findings of a comprehensive mutation screening for the MUTYH gene in Algerian consanguineous patients from the same family, with clinically suspected LS but no MMR mutation, and we discuss the clinical implications (Nielsen et al., 2011; Sampson & Jones, 2009; Cheadle & Sampson, 2007).

2 Materials and Methods

This study included a series of seventeen families from the Eastern region of Algeria who were suspected of having LS because they fulfilled its clinical criteria (Amsterdam or revised Bethesda guidelines) (Vasen et al., 2013). All probands of these families showed both absences of germline pathogenic mutations and were negative for deletions or duplications of MLH1, MSH2, MSH6, EPCAM, and PMS2 genes before this study. Based on the family survey, we discovered a family, F11, in our group that did not have vertical transmission of disease and met Bethesda guidelines. We were attracted by the existence of consanguineous marriages between family members of F11, which made us consider a disorder with recessive inheritance.

Since pathogenic mutations in the MUTYH gene occur in patients with the MAP type of some familial colorectal malignancies, we postulated that MUTYH is involved in colorectal carcinogenesis in this family. All patients provided their informed consent for the cancer genetics search. Medical histories were obtained through an interview with the proband. Pedigrees were constructed for four generations and data from affected subjects (type, number, and localization of tumors and age at diagnosis) were documented and verified through clinical and pathological records where possible (Nielsen et al., 2009; Vogt et al., 2009; Ali et al., 2008; Mohammed, 2016).

Molecular analysis

Total Genomic DNA was isolated manually from peripheral blood lymphocytes from the probands and members of the families by using standard protocols following the salt extraction procedure (Miller et al., 1988). We investigated the families without mutations in MMR genes, by direct sequencing of the complete coding region of the MUTYH gene. All exons were sequenced in both the forward and reverse directions. We used for this, the Big Dye Terminator Chemistry v1.1 and an automated fluorescent sequencer, an ABI Prism 16 Capillary Array Sequencer (Applied Biosystems 3130 XL). The primers were designed using Primer 3Plus (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). All germline mutations were confirmed by sequencing at least two independent PCR products.

Bioinformatic tools


3 Results and Discussions

In our study, for the first time in Algeria, the biallelic pathogenic MUTYH mutation, c.1395_1397del was found in the proband of the family F11 (Fig.1). This 3-bp deletion (1395-7 del GGA) resulted in the removal of a glutamic acid residue, p.Glu466del, within a C-terminal domain which is a highly conserved region of
MUTYH. It has been shown to play a role in 8-oxo-G recognition by biochemical studies (Cheadle & Sampson, 2003).

This deletion was discovered in a man F11.1, who was diagnosed with well-differentiated liberkanian type adenocarcinoma in his transverse colon at the age of 46 (excluding familial adenomatous polyposis). Three years later, he developed a recurrence of the left colonic tumour process, which was treated with gastric abdominal wall extension. The index case, F11.1, belongs to an Algerian family from the East. The family pedigree analysis confirms that the family’s history is compatible with autosomal recessive inheritance (only the proband or siblings affected by colon cancer). This family has three consanguineous unions, one of which was the proband’s parents, who are first cousins (Sakurada et al., 2018; Win et al., 2014; Jones et al., 2009).

Proband F11.1 had five relatives with colon and gastric cancers, according to family history. Two members are of the same generation, and one is a first-degree relative who was diagnosed with colon cancer when he was 60 years old. The common ancestor died of an unidentified cancer at the age of 70. In this family, consanguinity increases the rate of acquiring two copies of the defected recessive alleles from the common ancestor. Therefore, it increases the probability of having homozygosity (Fig.2).

The c.1395_1397del variant of the MUTYH gene was found in four members of the same family, F11, among our Algerian patients. Two of them (the proband and one brother) had the biallelic form, while the two remaining members had this mutation in only one allele. The last ones appear to be healthy and disease-free. Their cousin, who came from a first-cousin marriage, was diagnosed with colon cancer at the age of 49. Although he was not tested, he appears to be a homozygous carrier of the same pathogenic variant. When c.1395_1397del of the MUTYH gene is homozygous, it co-segregates with cancer in this family (Fig. 2).

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Faced with the task of identifying the MUCYH gene, which is most commonly involved in recessive hereditary predisposition to colorectal cancer, MAP. Here, we investigated 17 unrelated Algerian families with MCA and LS without mutations or defaults in MMR genes (Ziada-Bouchaar et al., 2017). For the first time in Algeria, we found that the germine mutation c.1395_1397del in exon 14 of MUCYH was identified in 5, 88% of unrelated Algerian families fulfilling LS clinical criteria. Our result agrees with Castillejo’s research which revealed the overlapping phenotypes between Lynch and MAP syndromes (Castillejo et al., 2014).

The recurrent pathogenic MUCYH mutation, c.1395_1397del, is rare. It has been previously reported in only one of 75 colorectal cancer patients from the United Kingdom (Halford et al., 2003). The same variant has been also described in the Italian population, which suggests that it is a sub-polymorphic MUCYH mutation in the southern European Caucasian population (Gismondi et al., 2004).

Molecular epidemiology of MUCYH alterations demonstrates that the defects of this gene are often represented by the two initials c.536A4G/p.Tyr179Cys and c.1187G4A/p.Gly396Asp (previously c.494A4G/p.Tyr165Gys and c.1145G4A/p.Gly382Asp) missense mutations (Out et al., 2010). These two mutations were found to be the most common (Cleary et al., 2009), accounting for approximately 80% of all reported MUCYH variants in Caucasian populations, and is particularly characteristic for Europe (Lv, 2017; Nielsen et al., 2022).

However many countries, including different parts of Europe, have a unique spectrum of regional founder mutations. These mutations have been detected in specific ethnic groups that is Y90X, E466X, and 1187insGG in subjects of, Pakistani, Indian, and Portuguese descent, respectively (Croitoru et al., 2004; Nielsen et al., 2022); p.R245H in Hungary, p.480delE in Italy and p.P405L in the Netherlands (Yanus et al., 2018). In families of North African ancestry, Lefevre and colleagues reported a novel mutation, c.1227_1228dup (Lefevre et al., 2011). This mutation has been described together with another more prevalent one, p.E410GfsX43 in Tunisia (Abdelmaksoud-Dammak et al., 2012).

The increased risk of colorectal cancer and polyposis among biallelic MUCYH gene mutation has been confirmed by several groups (Jones et al., 2002; Win et al., 2011). However, the cancer risk associated with germline variants in individuals carrying only one MUCYH defective allele is controversial (Lubbe et al., 2009), and based on studies involving few samples (Croitoru et al., 2004; Olschwang et al., 2007).

Jenkins and colleagues discovered an increased risk in monoallelic carriers only in those over the age of 55 in a large study (Jenkins et al., 2006). These findings were recently supported by Patel’s study, which included 249 young people with monoallelic MUTYH pathogenic mutations but neither a high adenoma rate nor CRCs were found (Patel et al., 2021). This supports our findings that the proband and one sibling are biallelic carriers of the c.1395_1397del, whereas the other two carry the mutation in only one allele and are healthy individuals without any outward signs of disease.

Our patient’s follow-up has revealed that patients with biallelic mutations in the MUCYH gene developed colon cancer, followed by gastric cancer a few years later, the only extracolonic cancer found. Numerous studies and case reports have reported extracolonic tumors in monoallelic or biallelic MUCYH mutation carriers, including gastrointestinal and gastric cancers (Win et al., 2016; Nielsen et al., 2022). While some recent studies found MUCYH mutations to be associated with an increased risk of breast cancer (Fonfria et al., 2021; Nunziato et al., 2022), and endometrial cancer (Magrin et al., 2022). Others found no link between MUCYH mutations and these cancers (Beiner et al., 2009; Thompson et al., 2022).

Recently, Barreiro’s results indicated that MUCYH deficiency in heterozygosity can cause cancer via a process known as Loss of Heterozygosity (LOH) of the functional MUCYH allele. It has been verified that cancer patients have a greater prevalence of damaging MUCYH monoallelic variant carriers than the general population (Barreiro et al., 2021). However, the risks of extracolonic malignancies for carriers with monoallelic mutations have not yet been determined. In our investigation, the individuals with the
c.1395_1397del variant came from a consanguineous union between first cousins; hence, consanguinity increases the probability of having homozygosity of the defective variant in the MUTYH gene and the emergence of the cancer risk in this family. The married couple in this case shares 12.5% of the genes (Bener et al., 1996; Kakaje et al., 2020).

Worldwide, there is a variation in the prevalence of consanguineous marriages. When the rate is less than 1% in Europe and North America (Kakaje et al., 2020), the results of a recent study in the Algerian population show a high rate of consanguinity of around 38.33% (Benkou et al., 2020). It is about 36.3% in Syria (Kakaje et al., 2020), and it can reach up to 50% of the general population in Arab countries (Tadmouri et al., 2009), for instance, the rate can reach up to 56% in Saudi Arabia (Warsy et al., 2014), and 68% in Egypt (Mokhtar & Abdel-Fattah, 2001). The risk estimate for consanguinity may be slightly inflated when first-cousin marriages are widespread and encouraged (Thompson & Thompson, 2016).

4 Conclusion

Biallelic mutations in the MUTYH gene cause the autosomal recessive condition MAP, which increases the risk of colorectal cancer. A high rate of consanguinity contributes to the homozygosity of defective variants in the MUTYH gene and the apparition of the cancer risk in Algerian families. Cancer risks are unknown for people who have inherited a MUTYH mutation from only one parent. Because of the degree of consanguinity, a larger study is required to address the issue of potential risk.

The LS phenotype and the MAP phenotype may overlap. Taking into consideration the presence of biallelic MUTYH mutations among LS patients, the inclusion of MUTYH testing in the diagnostic strategy of LS-suspected patients should make aware carriers of mutations in this gene, as well as their first-line relatives, of this increased risk and encourage them to undergo early screening.

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References


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