

MUTATION IN BRIEF**Germline MUTYH (MYH) Mutations in Portuguese Individuals with Multiple Colorectal Adenomas**

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Germline mutations in the base excision repair (BER) gene MUTYH (MYH) have recently been described in association with predisposition to multiple colorectal adenomas and cancer. In contrast to the classic dominant condition of familial adenomatous polyposis (FAP) due to germline mutations in the APC gene, the MYH polyposis is an autosomal recessive disease. The identification of individuals affected by MYH polyposis brings new and important implications for the diagnostic, screening, genetic counseling, follow up and therapeutic options in these patients. In this study, screening for germline mutations in the MYH gene was performed in 53 Portuguese individuals with multiple colorectal adenomas or classic adenomatous polyposis, in whom no mutation had been identified in the APC gene. The results revealed the presence of biallelic germline MYH mutations in 21 patients. In addition, we here report 3 mutations (c.340T>C [p.Y114H]; c.503G>A [p.R168H]; and c.1186_1187insGG [p.E396fsX437]) which, to our knowledge, have not been previously described. © 2004 Wiley-Liss, Inc.

KEY WORDS: APC; MYH; MUTYH; colorectal adenoma; FAP; polyposis; Portuguese

INTRODUCTION

Most known inherited predispositions to colorectal tumors are dominant and include tumor-suppressor genes or genes of the DNA mismatch repair pathway. Recently, it was described that Mendelian recessive mutations of the MYH gene (MUTYH; MIM# 604933; GenBank: U63329.1, AF527839.1; GDB: 9315115) predispose to multiple colorectal adenomas and carcinoma (Al-Tassan et al., 2002; Jones et al., 2002; Halford et al., 2003; Sampson et al., 2003; Cheadle and Sampson, 2003; Sieber et al., 2003). MYH takes part in the base excision repair (BER) pathway, which plays a basic role in the repair of mutations caused by reactive oxygen species generated during the aerobic metabolism, as well as by exogenous stimuli such as ionizing radiation and various chemical oxidants. In man, like in bacteria and yeast, the most stable product of oxidative damage is the guanine adduct 8-oxo-7,8-dihydroxy-2'-deoxyguanosine (8-oxoG), which tends to mispair with adenine, leading to G:C>T:A transversions

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after the next DNA replication (Shibutani et al., 1991; Kasai, 1997). The critical steps in DNA repairing by BER in man are carried out by a set of genes, the human orthologues of MutT, MutM and MutY in bacteria (Tajiri et al., 1995), that act synergistically to prevent mutagenesis induced by 8-oxoG. These are, the nucleoside triphosphatase hMTH1, which hydrolyses 8-oxoG-dGTP, limiting the incorporation of 8-oxo-G in DNA from the nucleotide pool (Sakumi et al., 1993) and the DNA glycosylases hOGG1 and hMYH (the human MutY homolog), which excise 8-oxoG:cytosine (Arai et al., 1997; Boiteux and Radicella, 2000) and 8-oxoG:adenine (A) mismatches respectively, in nascent DNA (Slupska et al., 1999; Shinmura et al., 2000).

Al Tassan and colleagues (Al-Tassan et al., 2002) described a family in which 3 siblings with multiple colorectal adenomas and cancer were shown to be compound heterozygotes for mutations in the MYH gene. Later, other groups have confirmed the existence of biallelic germinal mutations of this gene in a large series of unrelated patients with multiple colorectal adenomas (Enholm et al., 2003; Halford et al., 2003; Jones et al., 2002; Sampson et al., 2003; Cheadle and Sampson, 2003; Sieber et al., 2003), supporting the existence of recessive MYH polyposis (MAP) syndrome in approximately one third of the patients with between 15 and 100 adenomas (Sieber et al., 2003). Two mutational hot spots were identified in the MYH gene, p.Y165C and p.G382D (Al-Tassan et al., 2002), corresponding to approximately 78% of the mutations identified in affected Caucasians (Marra and Jiricny, 2003).

In this study, we examined the contribution of MYH germline mutations in a series of 53 APC mutation negative Portuguese patients with multiple colorectal adenomas or classic adenomatous polyposis.

MATERIALS

Fifty three Portuguese patients recorded in regional registries of colorectal polyposis, with multiple colorectal adenomas or classic adenomatous polyposis, with or without colorectal cancer (CRC), were selected for study of germinal mutations in the MYH gene, applying the following selection criteria: 1) no clear pathogenic germline mutation in the APC gene identified during genetic screening; 2) at least ten colorectal adenomas, with or without CRC. The clinical and family history of the selected patients is summarized in the Results and Discussion section. The number of adenomas was clearly specified for some patients while for others, counts were given as a range, e.g., <100, >100 and >1000.

To evaluate the frequency of p.Y165C and p.G382D in the population, at least 50 Portuguese healthy individuals were analyzed for these mutations.

To further characterize novel sequence variants/polymorphisms screening of at least 50 unrelated individuals of the Portuguese population was carried out (50 healthy and 53 affected with multiple adenomas, not pooled).

METHODS

Peripheral blood was drawn, after informed consent, from 53 unrelated Portuguese individuals. DNA was extracted using the Wizard DNA Extraction Kit (Promega) and the MYH gene was screened for mutations by SSCP. Exons 1-16 of MYH (GenBank accession: U63329.1 and AF527839.1, where +1 corresponds to the A of the ATG translation initiation codon) were PCR amplified using specific exon primers published in <http://www.uwcm.ac.uk/study/medicine/medical-genetics/research/tmg/projects/hMYH.html>, combining exons as follows: 3&4, 5&6, 7&8, 9&10, 11&12 and 13&14.

For the SSCP analysis (Orita et al., 1989), PCR products were separated on 6% polyacrylamide gels (acrylamide 59: bisacrylamide 1) at 4 °C, following denaturation. Bands were visualized by DNA silver staining. Exons showing abnormal SSCP patterns were sequenced on a repeated PCR product using the automatic sequencer ABI Prism® 3100 Genetic analyzer (Applied Biosystems). The p.Y165C and p.G382D mutations were often alternatively detected by restriction fragment length polymorphism (RFLP) analysis. The wild type allele of p.Y165C (p.Y165) is digestible with MwoI, generating fragments of 247, 49, 43, 29 and 14 base pairs (bp), whereas for the mutated allele p.C165, the 247 bp fragment is digested into 175 and 72 bp. As for the wild type allele, p.G382, it is undigestible with BglII, but the mutated allele, p.D382, is digested to two fragments of 423 and 82 bps. Samples with p.Y165C and p.G382D mutations, that had been previously sequenced, were also included as

controls. The RFLP analysis was done using 25 ul of a repeated PCR product, according to manufacturer's recommendations (New England Biolabs) and analyzed in a 2.5% (wt/vol) agarose gel (Fig. 1).

RESULTS AND DISCUSSION

Biallelic germline mutations of MYH are estimated to cause 1 to 3% of unselected colorectal cancers and appear to be preferentially associated with multiple adenomas (Halford et al., 2003). In this study biallelic germline mutations in MYH were identified in 21 of 53 Portuguese patients with multiple colorectal adenomas or classic adenomatous polyposis, who tested negative for APC germinal mutations (Table 1). In other studies, performed in the same class of patients, the frequency of MYH biallelic mutation varied between 7 and 33.8 % (Sieber et al., 2003; Sampson et al., 2003; Jones et al., 2002). Although ethnic or geographic differences cannot be excluded, the differences possibly reflect the criteria used in selecting patients for study (Sampson J et al., 2003; Cheadle and Sampson, 2003). Our results show a slightly higher overall biallelic mutation frequency (39.6%), with the following distribution: 10 in 26 patients without evidence for familial transmission of the disease, 9 in 15 belonging to families with affected siblings only, in line with the autosomal recessive mode of inheritance of the disease and 2 in 12, of families that showed evidence for vertical transmission. In both patients one of the parents had died of CRC, which might be suggestive that persons carrying a single MYH mutation have a somewhat increased susceptibility for CRC, as has been suggested by others (Sieber et al., 2003; Enholm et al., 2003). No heterozygous carriers for a single MYH mutation were identified among the patients here analyzed. As found in other studies (Enholm et al., 2003; Sieber et al., 2003), the frequency of biallelic mutations was higher in patients with <100 adenomas (15/32) than in those with 100-1000 (5/19). Only two patients with >1000 adenomas were analyzed and one of them presented biallelic mutations. With the exception to one patient who in addition to CRC, also presented breast cancer, no relevant extracolonic manifestations were observed in any of the individuals found to be carriers of biallelic MYH mutations.

Three novel mutations were identified in various patients, either in homozygosity or in compound heterozygosity with known mutations (Table 1). The frameshift mutation p.E396fsX437 (c.1186_1187insGG), which accounts for 19% of the mutations found, was present in homozygosity in two patients from an isolate in Madeira Island and in one patient from the mainland and as a compound heterozygote in two others. It was not detected in the analyzed 100 unrelated healthy individuals picked at random and not pooled (50 from Madeira Island and 50 from the mainland).

As to the two other novel mutations, p.R168H (c.503G>A) was present in a patient as compound heterozygote with p.Y165C, while p.Y114H (c.340T>C) was found in homozygosity in both a patient and her sister both affected with CRC. None was found in the 50 unrelated healthy Portuguese individuals analyzed.

Mutations p.Y114H and p.R168H consist on a change from a polar uncharged R group to a positively charged one and from a branched to a cyclic R group, respectively. Both mutations affect a conserved amino acid residue from bacteria to man. Besides, p.R168 is closely located to p.Y165, a mutation hotspot, the *Bacillus stearotheophilus* equivalent residue of which, Tyr88, is known to play a critical role in the recognition of the 8-oxoG base (Fromme et al., 2004).

Further elucidation of the functional role of the altered residues, together with the analysis of other family members, will help to establish a more definitive correlation of these novel mutations with its mechanisms of pathogenicity.

Among the 21 patients with MYH biallelic germline mutations, 10 were either homozygous or compound heterozygotes for the hotspot missense changes p.Y165C and p.G382D, while an additional seven patients were heterozygotes for one of them. Overall, the frequency of these alleles among MYH polyposis patients was 64.3%. For the general population, the observed frequency for p.G382D in 50 healthy individuals from Madeira Island and in 50 from the mainland was 1% and 2%, respectively. As for p.Y165C it was 1% in 50 healthy individuals from the mainland. These observations are compatible to what has been described in previous studies (Al-Tassan et al., 2002; Jones et al., 2002; Sieber et al., 2003) and confirm that MYH mutations are not enriched in the general population.

In addition, several reported and unreported polymorphisms were identified among the patients (Table 2).

This study contributes to support the existence of MYH polyposis syndrome 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 and the Portuguese population.

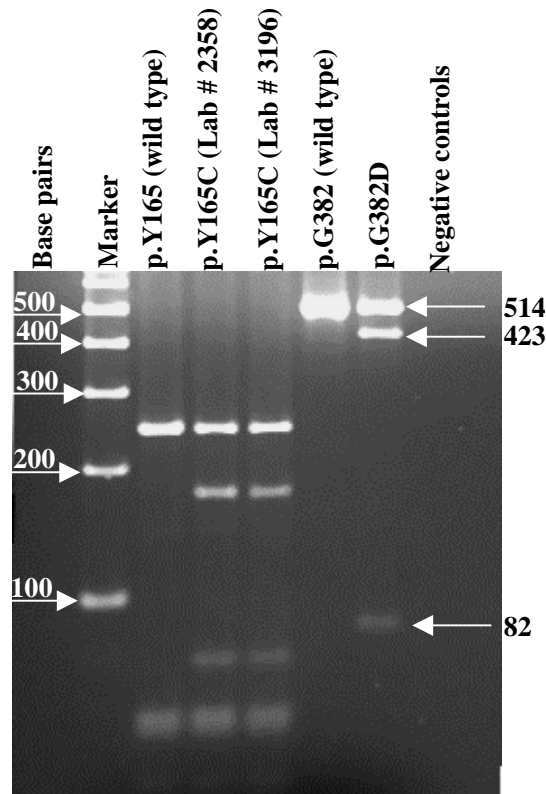


Figure 1. RFLP analysis for MYH p.Y165C and p.G382D using a 2.5% (wt/vol) agarose gel. The p.Y165 (wild type) allele was digestible with *Mwo*I generating the fragments: 247 and 49 + 43 bps, whereas the p.C165 (mutated allele) the 247 bp fragment is digestible into 175 and 72 bp. As for the p.G382 (wild type) allele it is undigestible with *Bg*/III but the mutated allele (p.D382) is digestible giving rise to the two fragments: 423 and 82 bps.

Table 1. Characteristics and Mutations in Portuguese Patients with MYH Polyposis

Lab. #	Age of Diagnosis	Number of adenomas	CRC	Family History	Biallelic MUTYH mutations ^(1,2)	
0056	43	<100	Yes	IC	c.1145G>A p.G382D	c.1145G>A p.G382D
0197	43	>100	No	Affected sister	c.494A>G p.Y165C	c.494A>G p.Y165C
0313	57	<100	Yes	Proband also w/ breast cancer; Affected sister	c.340T>C p.Y114H	c.340T>C p.Y114H
0497	54	20	Yes	2 affected sisters, 1 affected niece	p.E396fsX437	c.494A>G p.Y165C
0501	45	140	Yes	IC	c.1145G>A p.G382D	c.494A>G p.Y165C
0854	60	<100	No	Affected sister and affected father	c.494A>G p.Y165C	c.494A>G p.Y165C
1117	51	80-100	Yes	IC	c.494A>G p.Y165C	c.503G>A p.R168H
1373	57	<100	No	IC	c.1186_1187insGG p.E396fsX437	c.494A>G p.Y165C
1606	50	>1000	Yes	IC	c.1145G>A p.G382D	c.679C>T p.R227W*
1674	47	>100	No	Affected brother	c.1186_1187insGG p.E396fsX437	c.1186_1187insGG p.E396fsX437
2108	47	<100	No	Affected brother	c.1145G>A p.G382D	c.1103delC p.L369fsX393
2462	68	10	Yes	Affected mother	c.1145G>A p.G382D	c.494A>G p.Y165C
2535	67	50	Yes	Affected brother	c.347-1G>C	c.494A>G p.Y165C
2602	50	20	Yes	Affected brother	c.1145G>A p.G382D	c.1145G>A p.G382D
2905	54	<100	No	IC	c.1186_1187insGG p.E396fsX437	c.1186_1187insGG p.E396fsX437
3021	46	70	Yes	Three affected brothers	c.494A>G p.Y165C	c.494A>G p.Y165C
0239	50	>100	Yes	Four affected brothers	c.1186_1187insGG p.E396fsX437	c.1186_1187insGG p.E396fsX437
1556	36	>100	No	IC	c.494A>G p.Y165C	c.494A>G p.Y165C
2358	43	70	Yes	IC	c.494A>G p.Y165C	c.1145G>A p.G382D
3196	37	30	No	IC	c.494A>G p.Y165C	c.347-1G>C
0610	54	<100	Yes	IC; Sister w/ breast cancer	c.494A>G p.Y165C	c.1145G>A p.G382D

1-GenBank reference sequence MYH: U63329.1, where +1 corresponds to the A of the ATG translation initiation codon;

2- Nomenclature according to Antonarakis et al., (1998) and den Dunnen J and Antonarakis (2000).

IC – Index case, without evidence of family history of the disease.

Novel mutations shown in bold.

* Reported by Fleischmann C et al., 2004 while manuscript was under going peer review.

Table 2. MUTYH Polymorphism Frequencies among 53 Portuguese Patients

Polymorphism ^{1,2}	Frequency of each allele
c.64G>A (p.V22M)	97.73 % G and 2.27 % A
c.972G>C (p. Q324H)	82.60 % G and 17.40 % C
c.1435-40G >C*	91.67 % G and 8.30 % C
c.462+35G >A *	37.80 % G and 62.2 % A **
c.648+21C>A*	98.48 % C and 1.52 % A
c.648+61T>A*	98.48 % T and 1.52 % A
c.648+58G>A*	98.48 % G and 1.52 % A
c.1145-57G>T*	98.48 % G and 1.52 % T

* Novel polymorphisms.

** Only 30 patients analyzed.

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2- Nomenclature according to Antonarakis et al., (1998) and den Dunnen J and Antonarakis (2000).

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