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Somatic Mutation of MutYH in Tunisian Patients With Sporadic Colorectal Cancer

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The *MutYH* gene is an adenine-specific DNA glycosylase that prevents G/T transversions. Germline mutation in this gene causes *MYH*-associated polyposis (MAP) that predispose to hereditary colorectal cancer (CRC). This study describes for the first time the association of the *MutYH* mutation with sporadic CRC. From the 48 Tunisian sporadic CRC cases analyzed, two patients showed somatic mutation of the *MutYH* gene. In addition, the two hotspot germline mutations *MutYH* Y165C and G382D seem to be infrequent in sporadic CRC. J. Clin. Lab. Anal. 21:372–374, 2007. © 2007 Wiley-Liss, Inc.

Key words: BER; *MYH*-associated polyposis; APC; FAP; transversion

INTRODUCTION

The *MutYH* gene, located on chromosome 1p34.3–p32.1 and identified in human in 1995 (1), is a member of the base excision repair (BER) system involved in oxidative DNA damage repair. The MutYH protein functions in a postreplication repair pathway and is responsible for recognition and removal of inappropriately inserted adenine in Ao8-oxoG mismatches. If unrepaired, the Ao8-oxoG mispairs can result in C:G to A:T transversions (2). Recently, biallelic mutations in the BER gene *MutYH* have been shown to be responsible for predisposing to multiple adenoma and colorectal cancer (CRC) (3,4). This defect is responsible for *MYH*-associated polyposis (MAP) and mostly account for 20% of *APC*-negative familial adenomatous polyposis (FAP) families (4,5).

Most candidate genes involved in certain hereditary cancers have been reported to be subject to somatic inactivation in the sporadic forms. This was the case for the *APC* gene that results in a very high risk of FAP when mutated in the germline and that is involved at somatic level in 80% of sporadic CRCs (6,7). Only two studies have addressed the question of whether somatic inactivation of *MutYH* plays a significant role in sporadic colorectal tumorigenesis. Both of them showed no evidence of implication of this gene in sporadic CRC (8,9).

The aim of this study was to investigate the possible implication of the *MutYH* gene mutation in Tunisian patients affected with sporadic CRC.

MATERIALS AND METHODS

A total of 48 unrelated patients (age range, 61 ± 13 years) were recruited from the Charles Nicolle Hospital of Tunis. Cases with inflammatory bowel disease or a known Mendelian cancer syndrome were excluded. The

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corresponding clinicopathological data were collected for each tumor (Table 1). For each patient, tumor DNA and normal control DNA were extracted from paraffin-embedded colon sections using the DNeasy® Tissue Kit (Qiagen, Valencia, CA). Only those areas containing >70% tumor cells were used for DNA extraction. The corresponding normal control tissue for each patient was checked by a histopathologist to ensure the absence of tumor cells in the sample and was used to confirm that the mutations were somatic. The *MutYH* hotspot region corresponding to exons 7, 8, and 13 were amplified by polymerase chain reaction (PCR). We performed direct sequencing of PCR product with BigDye Terminator v1.1 cycle sequencing kit using the ABI 3730 DNA sequencer according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Sequencing was carried out in both directions to confirm the findings.

RESULTS AND DISCUSSION

In our series of 48 sporadic CRCs we found one mutation present in two patients in a heterozygote state (c.1186_1187insGG; p.E396fsX41). This mutation was described at the germinal level in other studies (10,11). In our case this mutation was present only in tumors and was absent in the constitutional DNA (Fig. 1), indicating that the mutational event happened at somatic level in the *MutYH* gene. The first patient is a 75-year-old man with a right-side, moderately differentiated, T3N0Mx cancer, presenting a 1-cm polyp accompanied with colloid component of the mucosa (>50%) and lymphocytic infiltrates. The second patient is a 57-year-old woman with a left-side, moderately differentiated cancer, displaying a prominent infiltration of intraepithelial lymphocytes, with no polyps observed.

TABLE 1. Patient characteristics

Clinicopathological factors	Number of patients
Sex	
Male	23
Female	25
Localization	
Colon	
Left	18
Right	19
Rectum	11
Histological type	
Adenoma	6
Adenocarcinoma in low-grade dysplasia	6
Adenocarcinoma in middle-grade dysplasia	14
Adenocarcinoma in high-grade dysplasia	22
Classification	
Stage	
T1	1
T2	8
T3	23
T4	6
Invasion	
N0	23
N1	7
N2	6
Nx	2
Not available	10

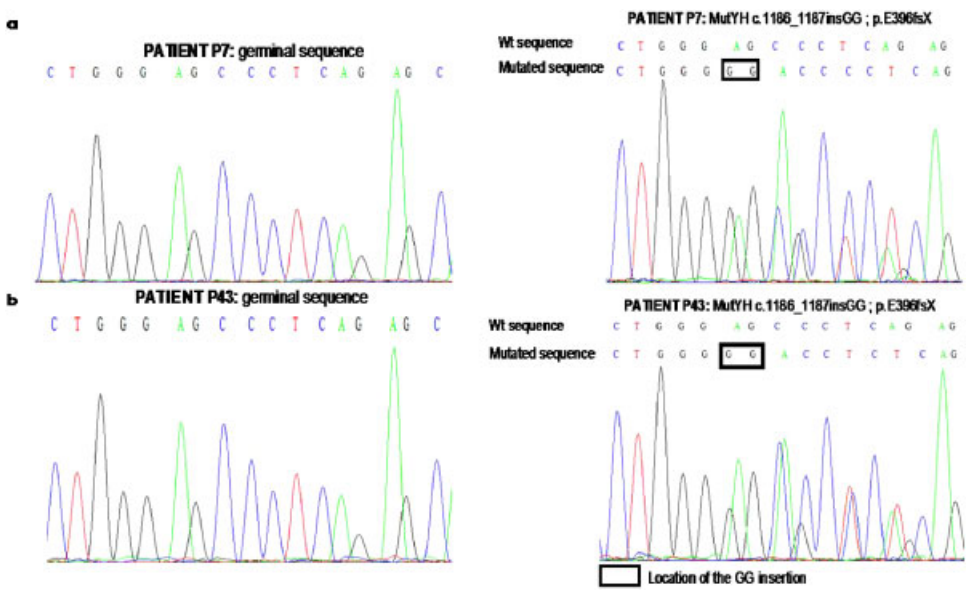

Fig. 1. Somatic mutation of the *MutYH* gene. **a:** Normal germinal sequence vs. mutated somatic sequence of patient 7. **b:** Normal germinal sequence vs. mutated somatic sequence of patient 43. (The squares show the GG insertion at the position 1186_1187 of the *MutYH* gene.)

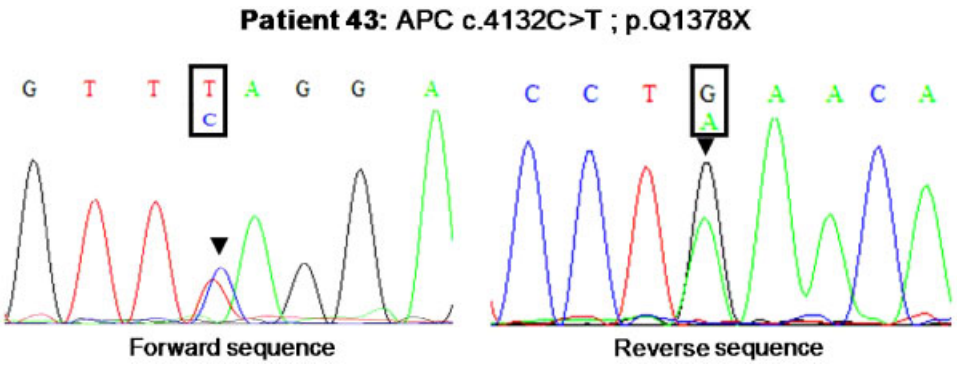

Fig. 2. Somatic APC T:C > G:A transversion at position 4132 of patient 43. (The squares show the mutation in the forward and reverse sequence.)

We also investigated whether these two patients had a somatic APC mutation that has been shown to be associated with *MutYH* inactivation. Al-Tassan et al. (3) showed that *MutYH* cancer have an increased frequency of G:C to T:A somatic transversion mutations of APC. The analyses of APC in these two patients mutated somatically on *MutYH* showed only for the first patient (Fig. 2) a c.4132C > T transversion giving rise to a truncated APC protein (p.Q1378X).

Germline mutations in *MutYH* gene seem to be less heterogeneous and their frequencies show marked ethnic differences (4,12). In this context, we studied the occurrence of the two *MutYH* hotspot germline mutations Y165C (c.494A > G) and G382D (c.1145G > A) that account for 85% of Caucasian MAP patients (4,12). We did not find any of these mutations in our series either in a homozygous or in a heterozygous state, in both normal and tumoral tissues. This result shows that these two frequent mutations in MAP do not occur in sporadic CRC.

In conclusion, our study shows for the first time, that the *MutYH* gene can be subject to somatic inactivation in sporadic CRC. Both patients presenting this mutation showed at the tumor site a pronounced lymphocyte infiltration that was described in other reports (13), and did not present a large number of polyps at the opposite of that described in MAP, which is in agreement with sporadic CRC. Our results indicate that the *MutYH* mutation detected functions as a dominant-negative, as has been proposed by some authors (9,14), since in our two patients the mutation has been detected in a heterozygous state. Moreover, we also showed in one case, the association of *MutYH* mutation with a somatic G to T transversion of the *APC* gene. Hence, somatic mutation of the *MutYH* gene seems to have the same effect as that reported in the *MutYH* germline associated polyposis patients.

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