Gene 496 (2012) 55–58

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short communication

The first mutations in the *MYH* gene reported in Moroccan colon cancer patients

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ARTICLE INFO

Article history:
Accepted 15 December 2011
Available online 10 January 2012

Keywords:
MAP
Colorectal cancer
MYH gene

ABSTRACT

Background: Biallelic germline mutations in the *MYH* gene cause MYH-associated polyposis (MAP) disease, an autosomal recessive form of inherited colorectal cancer. People with MAP tend to develop attenuated multiple adenomatous colon polyps during their lifetime and will have an increased risk of colorectal cancer. Contrary to familial adenomatous polyposis, the number of adenomas is often lower in MAP (from 5 to 100), and even some patients have recently been reported with no identified adenomas.
There have been many investigations into MAP that have been conducted in many different countries. Currently there is limited data on MAP in Morocco, and it is reasonable to think, that the prevalence of this form of genetic predisposition is as high as other autosomal recessive genetic diseases found in countries with high rates of consanguinity.
The aim of this study is to examine the frequency of *MYH* mutations in colorectal cancer and/or attenuated polyposis in Moroccan patients.
Patients and methods: The study population consisted of 62 patients; 52 with colorectal cancer, three of them had attenuated polyposis (2 to 99 adenomatous polyps). 10 other patients were referred to our department for polyposis without colorectal cancer.
We carried out DNA analysis in 62 patients to screen for the three recurrent mutations c.494A>G (p.Tyr165Cys), c.1145 G>A (p.Gly382Asp) and c.1185_1186dup, p.Glu396GlyfsX43, whereas 40 subjects were screened for germline *MYH* mutations in the whole coding sequence of the *MYH* gene by direct DNA sequencing. All these 40 patients, except two, had colorectal cancer without polyposis.
Results: Three patients with colorectal cancer and attenuated polyposis carried biallelic mutations in the *MUTYH* gene one with the c.494 A>G mutation, one with the c.1105delC mutation, one with the c.1145 G>A mutation. One patient with 25 adenomas without colorectal cancer carried the c.1145 G>A mutation at a homozygote state and one patient with 3 polyps was heterozygote for the mutation c.1145 G>A. No biallelic mutations of *MYH* gene were detected in colorectal cancer patients and in patients with small number (<5) of polyps without colorectal cancer.
Conclusion: We report the first biallelic *MYH* mutations in four Moroccan patients with clinical criteria of MAP; three of them had colorectal cancer with attenuated polyposis. No *MYH* mutations were found in colorectal patients without polyposis.
Despite the relatively small sample size of the current study, our findings suggest that the MAP is not a frequent cause of colon cancer in Morocco as we had expected, and the molecular analysis of *MYH* gene should be restricted to patients displaying the classical phenotype of MAP.

1. Introduction

Approximately 30% of all colorectal cancer (CRC) cases identified to be due to inherited susceptibility in different populations (Lichtenstein et al., 2000). Germline mutations in the adenomatous polyposis coli *APC* gene, the mismatch repair genes, *MUTYH*/*MYH*,

Abbreviations: CRC, colorectal cancer; FAP, familial adenomatous polyposis; FFPE, formalin-fixed paraffin embedded; HNPCC, hereditary non polyposis colorectal cancer; MAP, MYH-associated polyposis; *MUTYH*, MutY Homolog.

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doi:10.1016/j.gene.2011.12.024

SMAD4, *ALK3* and *STK11/LKB1* which confer a high risk of CRC, are rare and account for less than 5% of patients, with much of the remaining variation in genetic risk likely to be explained by combinations of lower penetrance variants. Familial adenomatous polyposis (FAP) is one of the hereditary colorectal cancers, and represents 1% of all cases of CRC (Bisgaard et al., 1994). FAP is caused by mutations of the *APC* gene located on human chromosome region 5q21–22 (Bodmer et al., 1987). Al Tassan et al. described in 2002 the first autosomal recessive inherited disorder which is known to result in an increased risk for developing colorectal adenomas and carcinoma (Al Tassan et al., 2002). MUTYH-associated polyposis is clinically comparable, in his classical phenotype, to attenuated familial adenomatous polyposis with a variable number of adenomas and a low incidence of colonic manifestations (Nielsen et al., 2005). Different authors reported that *MUTYH* (MutY Homolog) mutations could be associated with colorectal cancers without polyps (Cleary et al., 2009). MAP is associated with mutations in the *MUTYH* gene with two mutational hot spots, c.494A>G and c.1145 G>A which give amino acid change p.Y165C and p.G328D respectively (p.G382D) (Cleary et al., 2009), corresponding to approximately 90% of the mutations identified in affected Caucasians (Marra and Jiricny, 2003).

Colorectal cancer is a frequent cause of digestive cancer mortality in Morocco and little is known about MAP in the Moroccan population. The only mutation reported, until now, in Moroccan patients is the c.1185_1186dup (p.Glu396GlyfsX43) within exon 13, and was identified in two unrelated Moroccan patients living abroad (Nielsen et al., 2005). There is a high level of consanguinity found in Morocco (Jaouad et al., 2009), thus favouring the occurrence of autosomal recessive genetic diseases, and considering the estimated prevalence of MAP related to only the three recurrent mutations in Morocco (Laarabi et al., 2010), it is likely that mutations in the *MYH* gene may be a contributing factor in a large proportion of colorectal cancer and adenomatous polyposis cases.

To assess the prevalence of *MYH* mutations in Moroccan patients suffering from colorectal cancer and attenuated polyposis, we carried out DNA analysis in 62 patients referred to the Department of Medical Genetics in Rabat for colorectal cancer and/or attenuated polyposis, we performed whole-gene sequencing of the *MYH* gene in 40 patients, and we screened for the three recurrent mutations c.494A>G, c.1145 G>A and c.1185_1186dup in 22 subjects.

2. Patients and methods

2.1. Patients

This study included 62 unrelated Moroccan patients referred to our department for *MYH* gene analysis and genetic counselling (Table 1). Colorectal cancer was confirmed in 52 patients by colonoscopy and histology. Among 52 patients with colorectal cancer, three had adenoma and cancer. All of them have complete colonoscopy. Some patients were screened for extracolonic manifestations by duodenoscopy and gastroscopy when there is a symptom suggesting a duodenal or gastric affection. Any patient had cutaneous manifestations. Patients with colorectal cancer were originated from different regions of the north of Morocco. Their median age was 42.6 years (22–67). The pedigree of the family was made and 5 ml of peripheral blood was drawn into EDTA from each patient. 49 patients had only colorectal cancer without polyposis, and 3 patients had colorectal cancer associated to adenomas (<100 adenoma). Additionally, 10 patients with attenuated polyposis (number of polyps under 100) were enrolled in this study. Two of them had a family history of colorectal cancer. The MAP was suspected on the basis of the number of polyps under 100 and/or a young age and/or familial cases (Fig. 1). The instability phenotype was not tested.

Table 1
The mean features of the included population.

Study population	Ages	Number of adenoma	Positive family history
Patients with colorectal cancer (N=49)	22–67 years	0–1	10
Patients with colorectal cancer and attenuated polyposis (N=3)	17 years–40 years–45 years	2–99	1
Patients with polyposis (N=10)	17–70	<100	2 patients

3. Methods

DNA was extracted from blood samples from patients after obtaining informed consent for DNA testing. Total genomic DNA was extracted using salt extraction methods. The three recurrent mutations c.494A>G, c.1145 G>A and c.1185_1186dup were screened by direct sequencing in the Department of Medical Genetics in Rabat. The reference sequence of *MUTYH* gene was obtained from a public database (NM_001048171.1). The whole gene exon-sequencing was carried out on an ABI prism 3130 in the Department of Genetics of Rouen University Hospital. The detected mutations were confirmed using a second independent DNA sample.

4. Results

Three patients with colorectal cancer and attenuated polyposis carried biallelic mutations in the *MYH* gene (one with the c.494A>G mutation, one with the c.1105delC mutation, one with the c.1145 G>A mutation). One patient with 25 adenomas without colorectal cancer carried the c.1145 G>A mutation at a homozygote state and one patient with 3 polyps was heterozygote for the mutation c.1145 G>A (Table 2). No colorectal cancer patients were found to be biallelic for the *MYH* mutations, and no biallelic mutations were detected when patients without colorectal cancer had polyposis with small number of adenoma (<5).

Patient II-7 (Fig. 1-A), a 53-year old man, born of non-consanguineous healthy parents, was referred to our department for genetic counselling. Fibroscopy and pathological examination showed the presence of 80 adenomatous polyps, and pathological examination found the biopsied polyps to be adenocarcinoma of high grade. This patient carries the mutation c.494 A>G (p.Tyr165Cys) at a homozygous state. The second patient, III-14 (Fig. 1-B), was a 36 year old woman, born of non-consanguineous healthy parents. Her brothers and sisters have never shown any digestive symptoms and are all healthy. The pathological examination showed tubulous adenoma with signs of dysplasia of low grade and absence of invasive elements. This patient carried the mutation c.1105delC (p.Ala371-ProfsX23) at a homozygous state.

Patient II-11 (Fig. 1-C), a 71 year old man, was referred for genetic counselling because of a familial history of colorectal cancer. Fibroscopy identified 25 polyps throughout the colon, without colorectal cancer. He is carrying the mutation c.1145 G>A (p.Gly382Asp) at a homozygous state. After that, we have demonstrated that the patient II-9 (Fig. 1-C) died from adenocarcinoma in colon, was carrying the familial mutation c.1145 G>A (p.Gly382Asp) at a homozygote state (data not shown). This molecular diagnosis was made in Formalin-fixed paraffin embedded (FFPE) blocks from surgical biopsies of the patient. This result allowed us to propose a genetic counselling to the offspring of this patient.

Patient IV-8, (Fig. 1-D), a 50 year old man with a familial history of attenuated polyposis, had 20 polyps throughout the colon, with 8 transformed polyps. Pathological examination showed tubulous adenoma. This patient carries the mutation c.1145 G>A (p.Gly382Asp) at a homozygous state. This result allowed us to carry out genetic testing for presymptomatic diagnosis in the patient's sister IV-8 (Fig. 1-D) and we showed that she was heterozygote for the familial mutation. Besides,