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PREVALENCE OF THE Y165C, G382D AND 1395delGGA GERMLINE MUTATIONS OF THE *MYH* GENE IN ITALIAN PATIENTS WITH ADENOMATOUS POLYPOSIS COLI AND COLORECTAL ADENOMAS

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Biallelic germline mutations in the base excision repair gene *MYH* have been reported in patients with multiple colorectal adenomas and cancer and in sporadic FAP patients not showing a detectable *APC* germline mutation. In this study, the prevalence of the common Y165C and G382D germline variants of the *MYH* gene was examined in 70 FAP/AAPC patients with no detectable *APC* mutation and a family history compatible with recessive inheritance. In addition, 141 normal-population adenoma patients (mean number of adenomas, 2.8; range, 1–9) and 52 clean colon controls were studied. The entire coding region of the *MYH* gene was analyzed in Y165C or G382D heterozygous patients. Since the same second mutational event (a 3 bp deletion in exon 14, 1395delGGA) was detected in 3 patients, the prevalence of this variant was also examined in all groups. In all, 14 of 70 patients in the FAP/AAPC group (20%; 95% CI = 11.7–31.6%) had biallelic germline *MYH* variants and 3 were heterozygotes (4.3%). None of the 141 normal-population adenoma patients carried biallelic germline *MYH* variants (95% CI = 0.06–4.1%) and 3 were heterozygotes (2.1%). In the control group, no *MYH* variants were detected. These results indicated that *MYH*-associated polyposis (MAP) is present in about 20% of Italian FAP/AAPC patients, in whom no germline *APC* mutation is detectable and showing a family history compatible with recessive inheritance, and in a small fraction of patients with colorectal adenomas in the general population. In addition, our data suggest that mutation 1395delGGA is a subpolymorphic *MYH* mutational event in some Caucasian populations.

Key words: MYH*; familial adenomatous polyposis coli; colorectal adenomas*

Familial adenomatous polyposis (FAP) is a clinically well-defined form of hereditary colorectal cancer characterized by the development of hundreds to thousands of colorectal adenomas, conferring a high risk of large bowel cancer at a young age. Although most FAP cases show a pattern of autosomal dominant transmission of disease, up to 30% of FAP cases are apparently sporadic (*i.e.*, no family history of colorectal cancer or adenomas is reported). A clinical diagnosis of FAP is made when a patient has 100 or more adenomatous polyps macroscopically counted along the colon-rectum, independently of family history of colorectal cancer or adenomas. FAP families are defined as attenuated (AAPC) when the number of polyps is less than 100 in some of the patients (belonging to the same family) and the mean age at diagnosis is older as compared to that recorded in the classical FAP patients.[1]

The gene responsible for the vast majority of FAP cases, the *APC* gene, was cloned in 1991.[2] Since then, over 826 germline mutations have been found in families with FAP.[3] However, the presence and nature of the germline defect remains unknown in up to 30% of FAP patients.[4] Missense and chain-terminating *APC* mutations were also reported in a small fraction of patients with multiple colorectal adenomas.[5–8]

Recently, it has been reported that a proportion of cases of *APC*-negative FAP/AAPC (7.5%) and cases with multiple colorectal adenomas (4–30%) might be caused by germline biallelic mutations in the base excision repair (BER) gene *MYH*.[9–11] As expected from a recessive trait, most of *MYH*-positive cases were apparently sporadic or with no history of disease in parents. The majority of Caucasian patients with *MYH* defects carried 1 of 2 mutations (Y165C and G382D) in at least one allele that were also found in population controls. These mutations affect *MYH* amino acid residues highly conserved throughout evolution and attenuate the enzymatic activity of the equivalent protein in *Escherichia coli*.[12,13]

To assess the role of *MYH* mutations in colorectal adenomas and cancer susceptibility, we examined the prevalence of the Y165C and G382D variants in *APC*-negative Italian FAP/AAPC patients, in patients with colorectal adenomas and in a group of subjects with clean colon at total colonoscopy (controls). The complete coding region of *MYH* (exons 1–16) was subsequently analyzed in patients heterozygous for one of these variants. Since a 3 bp deletion in exon 14 (1395delGGA) was detected in 3 of these patients as the second mutational event, the prevalence of this variant was examined in all groups.

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MATERIAL AND METHODS

In the present study, we evaluated FAP, AAPC and normal-population adenoma patients as well as a group of subjects with clean colon after total colonoscopy (controls) for the presence of germline *MYH* mutations. We selected patients with *APC*-negative FAP/AAPC and a family history of FAP compatible with recessive inheritance (only the proband or siblings affected by colorectal adenomatous polyposis). Patients were drawn from 2 sources: 4 cancer family clinics in northern Italy (Aviano, Genova, Milano) and 2 epidemiologic studies carried out in Bari, Genova and Torino.

FAP patients

A diagnosis of FAP was made when the patients showed a macroscopic count of ≥ 100 adenomatous polyps or a description of the polyposis phenotype compatible with classical FAP. Thirty-eight patients fulfilled these criteria, all of them recruited at the cancer family clinics. They were 24 males and 14 female; the mean age at diagnosis was 39.9 years (median, 40.5; range, 3–67 years).

AAPC patients

An AAPC diagnosis was made when the patients showed macroscopic count of adenomatous polyps ranging from 10 to 99. When the accurate number of adenomatous polyps was not available, a description compatible with attenuated polyposis was considered. Thirty-one patients fulfilled these criteria: 19 were recruited at the cancer family clinics and 12 were selected among patients enrolled in the 2 epidemiologic studies. They were 20 males and 11 females; the mean age at diagnosis was 51.8 years (median age, 50.0; range, 27–81 years).

Available clinical data on the polyposis phenotype did not make it possible to distinguish between FAP and AAPC in patient 4066. Two AAPC patients carried *APC* variants of uncertain significance: case 2451 showed the missense variant A2119V and case 2831 showed the splice acceptor variant 845-17A→G.[5]

Normal-population adenoma patients

In all, 141 patients with adenomatous polyps were analyzed. They were 104 males and 37 females; the mean age was 61.0 years (median 61.0; range, 38–87 years). The mean number of adenomas was 2.8 (median number, 2.0; range, 1–9). Fourteen patients (9.9%) had a synchronous colorectal cancer. Methods of studies and characteristics of the study populations were described in detail elsewhere.[8]

Group 1. They were 133 patients: 50 with 1 adenoma, 24 with 2 adenomas and 59 with 3 or more adenomas (mean, 4.2; median, 3; range, 3–9). They were identified in the course of a multicenter retrospective-prospective cohort study aimed at estimating the frequency of germline and somatic *APC* mutations in subjects with adenomas of the large bowel. Multiple synchronous adenomas were defined as adenomas diagnosed at a single endoscopy or at different examinations performed no more than 6 months apart.

Group 2. Eight patients with at least 3 colorectal adenomas diagnosed at a single endoscopy or at different examinations performed no more than 6 months apart (mean, 6.2; median, 6.0; range, 4–8) were identified from a consecutive series of patients who had large bowel adenomas diagnosed between 1992 and 1995 at the National Cancer Institute of Genova.

Controls

Controls were subjects with no adenomas at an index total colonoscopy (clean colon). They were identified between January 1994 and April 1999 from the colonoscopy files within the same centers where group 1 patients with adenomatous polyps were enrolled. Controls fulfilled all inclusion criteria as cases except for the presence of adenomas and 52 subjects have been enrolled in the study. They were 38 males and 14 females; the mean age at endoscopy was 61.4 years (median, 61; range, 42–77 years). Blood sample and detailed pedigree were available for both cases and controls.

Direct sequencing of the β-catenin region of the *APC* gene (exon 15D-15O, nucleotides 2780–6401[2]) revealed the presence of 4 germline *APC* missense variants of uncertain significance in 2 group 1 cases and 1 control. In case 3630 (a 61-years-old patient with 8 adenomas), S1010N variant was detected. In case 3765 (a 65-years-old patient with 3 adenomas, 1 of which with infiltrating carcinoma), E1317Q variant and the silent variant A1755A were detected.[8] In case 3786 (a 42-year-old control), 2 variants, A1002G and I1649V, were detected on the same chromosome. Case 2602 (group 2) carried a chain-terminating mutation (8489delA) at the 3′ region of the *APC* gene.[5]

Molecular analysis

DNA was extracted from peripheral blood using standard phenol-clorophorm extraction.

MYH

Exon 7, in which Y165C mutation is located, was examined in all samples by direct sequence and the amplification refractory mutation system (ARMS) described by Al Tassan *et al*.[12] Exon 13, in which G382D mutation is located, was examined in all samples by direct sequence or *Bgl*II digestion. Exon 14, in which 1395delGGA is located, was examined on an ABI Prism 310 Genetic Analyzer using GeneScan Analysis. The presence of 1395delGGA was confirmed by sequence analysis. The entire open reading frame (ORF) was examined by direct sequence in patients heterozygous for one of the above *MYH* variants. PCR reactions of exons 1–16 of *MYH* were performed using primer pairs described at http://www.uwcm.ac.uk/study/medicine/medical_genetics/research/tmg/projects/hMYH.html

Amplification products were purified using the High Pure Purification Kit (Roche, Mannheim, Germany). DNA sequence analyses in forward and reverse directions were performed using the dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Sequencing reactions were purified by ethanol precipitation and analyzed on an ABI Prism 310 Genetic Analyzer. All germline mutations were confirmed by sequencing at least 2 independent PCR products.

Statistical analysis

Prevalence estimates of *MYH* mutation are reported together with their 95% confidence interval calculated by exact methods for binomial data. Comparison of age between groups was performed by Mann-Whitney test.[14]

RESULTS

Biallelic *MYH* mutations were found in 14 of FAP/AAPC patients (20%; 95% CI = 11.7–31.6%): 5 of 38 FAP patients, 8 of 31 AAPC patients and 1 patient for whom available clinical data on the polyposis phenotype did not allow us to distinguish between FAP and AAPC. Clinical data are shown in Table I. No biallelic mutations were observed in 141 normal-population adenoma patients and in 52 controls.

FAP patients

Five of 38 patients (13.2%; 95% CI = 4.9–28.9%) carried biallelic *MYH* mutations: 1 was homozygous for the exon 7 missense mutation Y165C (DNA 4119), 1 was homozygote for exon 14 mutation 1395delGGA (DNA 3320), 1 was compound heterozygotes for Y165C/G382D (DNA 4500) and 2 were compound heterozygotes for G382D/1395del GGA (DNA 4103, 4120). Three of 38 FAP patients (7.9%; 95% CI = 2.1–22.5%) remained heterozygous after the analysis of the complete ORF of the *MYH* gene: 2 for Y165C (DNA 4105, 4107) and 1 for 1395delGGA (DNA4111).

The mean age at diagnosis of FAP was 48.6 years (median, 49; range, 37–58 years) in patients who carried biallelic *MYH* mutations (n patients = 5) and 38.3 years (median, 38; range, 3–67 years) in mutation-negative patients (n patients = 30; Mann-Whitney test, $Z = 1.51$; $p = 0.13$). Synchronous colorectal carci-

TABLE I – CHARACTERISTICS OF PATIENTS WITH FAP/AAPC AND GERMLINE *MYH* MUTATION

MYH mutation	Age at diagnosis	Polyp count	Extracolonic manifestations[a]	Colectomy	Colorectal cancer
FAP patients					
4119 Y165C/Y165C	37	> 100	Dental cysts and CHRPE	Yes	Yes
4500 Y165C/G382D	53	Numerous	No	Yes	Yes
3320 1395delGGA/1395delGGA	46	> 100	Dermoid cysts and CHRPE	Yes	Yes
4103 G382D/1395delGGA	49	Numerous		Yes	Yes
4120 G382D/1395delGGA	58	> 100	No	Yes	No
AAPC patients					
3010 Y165C/Y165C	27	> 30	No	No	No
3468 Y165C/Y165C	57	90	Osteomas	Yes	Yes
4102 Y165C/Y165C	56	Multiple		Yes	Yes
4502 Y165C/Y165C	47	Multiple	No	Yes	Yes
4083 Y165C/G382D	59	76	CHRPE	Yes	Yes
4115 Y165C/1395delGGA	37	90	Osteomas	No	No
4141 Y165C/1395delGGA	43	80		Yes	Yes
2852 G382D/1395delGGA	50	22		Yes	Yes
4066[b] Y165C/G382D	61			Yes	Yes

[a]No extracolonic cancer was reported.–[b]Available clinical data on the polyposis phenotype did not allow us to distinguish between FAP and AAPC.

TABLE II – CLINICAL CHARACTERISTICS AND *MYH* MUTATION STATUS IN RELATIVES

Proband	Relative	*MYH* mutation	Number of polyps	Age[a]	Colorectal cancer
4119		Y165C/Y165C	101	37	Yes
	Sister	Y165C/Y165C	101	36	Yes
	Son	Y165C/−		32	No
	Son	Y165C/−		27	No
3468		Y165C/Y165C	90	57	Yes
	Sister	no	0	56	No
	Sister	Y165C/−	0	58	No
	Daughter	Y165C/−	0	37	No
3010[b]		Y165C/Y165C	> 30	27	No
	Father[b]	Y165C/−	3	61	No
	Mother	Y165C/−	0	62	No
	Sister[b]	Y165C/Y165C	> 30	21	No
	Paternal Uncle	Y165C/−		59	No
	Paternal uncle	Y165C/−		61	No
	Paternal uncle	Y165C/−	0	62	No
2852		G382D/1395delGGA	22	50	Yes
	Brother	G382D/−	2	70	Yes
	Sister	G382D/−	0	60	Yes
	Son	G382D/−	0	28	No

[a]At diagnosis or last contact.–[b]Carrier of E1317Q *APC* variant.

noma was detected in 20 of 38 patients (52.6%): 14 of 30 (46.7%) without *MYH* mutation, 2 of 3 patients (66.7%) with single *MYH* mutation and 4 of 5 patients (80.0%) with biallelic *MYH* mutations.

AAPC patients

Eight of 31 patients (25.8%; 95% CI = 12.5–44.9%) carried biallelic *MYH* mutations: 4 were homozygous for the exon 7 missense mutation Y165C (DNA 3010, 3468, 4102, 4502), 2 were compound heterozygotes for Y165C/1395delGGA (DNA 4115, 4141), 1 was compound heterozygotes for Y165C/G382D (DNA 4083) and 1 was compound heterozygotes for G382D/1395delGGA. The mean age at diagnosis of AAPC was 47.0 years (median, 48.5; range, 27–59 years) in patients carrying *MYH* biallelic mutations (*n* patients = 8) and 53.5 years (media, 54.0; range, 34–81) in mutation-negative patients (*n* patients = 23). Synchronous colorectal carcinoma was detected in 16 of 31 patients (51.6%): 10 of 23 patients (43.5%) without *MYH* mutation and 6 of 8 patients (75.0%) with biallelic *MYH* mutations.

MYH mutation status in relatives

DNAs of relatives were available for 4 of the 14 FAP/AAPC patients who carried biallelic *MYH* variants. Results of relatives' screening for the variants carried by the proband are shown in Table II. In family 3010, previous molecular analysis of the *APC* gene detected the E1317Q variant in the proband as well as in his sister and father.[8]

Normal-population adenoma patients

None of 141 patients with adenomatous polyps (0%; 95% CI = 0.06–3.3%) carried biallelic *MYH* mutations and 3 (DNA 3965, 3514, 3529) remained heterozygous (2.1%; 95% CI = 0.5–6.6%) after the analysis of the complete ORF of the *MYH* gene.

Control group

No *MYH* variants were detected in 52 subjects (0%; 95% CI = 0.2–8.6%) with clean colon after total colonoscopy.

DISCUSSION

In the present study, 14 of 70 patients (20%) with a clinical diagnosis of FAP/AAPC but without a detectable *APC* mutation were homozygotes (*n* = 6) or compound heterozygotes (*n* = 8) for Y165C, G382D, or 1395delGGA germline mutations of the *MYH* gene. This finding confirms that biallelic Y165C and G382D mutations of the *MYH* gene may be responsible for a substantial proportion of FAP/AAPC cases in southern European Caucasian population. In addition, 3 of 70 (4.3%) patients were heterozygotes for one of the variants described above after the complete analysis of the ORF of the gene. These may represent false negative results

TABLE III – AGE AT DIAGNOSIS ACCORDING TO CLINICAL CLASSIFICATION AND TO GERMLINE *MYH* MUTATION STATUS

	Total	Polyposis phenotype[a]	
		FAP	AAPC
Mutation negative			
Number of patients	53	30	23
Mean age, years (median; range)	44.9 (43; 3–81)	38.3 (38; 3–67)	53.5 (54; 34–81)
Single mutation			
Number of patients	3	3	-
Mean age, years (median; range)	42 (45; 35–46)	42 (45; 35–46)	
Biallelic mutation			
Number of patients	14*	5	8
Mean age, years (median; range)	48.6 (49.5; 27–61)	48.6 (49; 37–58)	47 (48.5; 27–59)

[a]Available clinical data on the polyposis phenotype did not allow us to distinguish between FAP and AAPC in patient 4066.

(*i.e.*, a second *MYH* mutation is present but was not detected) or, alternatively, it is possible that monoallelic *MYH* mutation may represent one of the causative mutational events in at least some of these patients.

As a novel finding we found that, out of 20 unrelated Italian patients with an *MYH* mutation, 8 (7 FAP/AAPC patients and 1 patient with sporadic adenomas) carried the same 3 bp deletion in exon 14 (1395delGGA) that was recently described in 1 of 75 colorectal cancers patients from the United Kingdom.[15] This finding suggests that 1395delGGA is also a subpolymorphic *MYH* mutation in southern European Caucasian population. This variant results in the removal of a glutamic acid residue (codon 466) within a C-terminal domain that is highly conserved and was shown to play a role in 8-oxo-G recognition by biochemical studies.[13]

In our study, FAP patients with common biallelic *MYH* mutations were older than mutation-negative patients (mean age, 48.6 *vs*. 38.3 years). Mean age at diagnosis in *APC* mutation-positive FAP proband from the National Polyposis Registry of Milan is 30.5 years (median age, 28; range, 9–73 years). Hence, FAP patients carrying common biallelic *MYH* mutations had a late diagnosis, more than 10 years later than those who carried an *APC* mutation. *MYH* mutations-positive AAPC patients were slightly older than mutation-negative AAPC cases (Table III). Similar to previous report,[10] extracolonic manifestations such as osteomas and congenital hypertrophy of the retinal pigment epithelium were also reported in some of these patients (Table I).

A synchronous colorectal cancer at diagnosis was reported for 37 of 70 FAP/AAPC patients (52.8%): 23 of 53 *MYH* mutation-negative patients (43.4%), 2 of 3 *MYH* heterozygous patients (66.7%) and 11 of 14 biallelic *MYH* mutation carriers (78.6%). The frequency of cancer at diagnosis was high, possibly due to a delay in seeking medical care by these individuals, as for this study patients were selected as FAP/AAPC cases in which only the proband or siblings were affected by adenomatous polyposis. A slightly higher frequency of colorectal cancer among *MYH*-mutated patients was observed. A greater frequency of colorectal cancer among carriers of biallelic *MYH* mutations was also reported by Sieber *et al*.[10]

Among normal-population adenoma patients (1–9 adenomas), none was homozygous and 3 were heterozygous for *MYH* mutations (2.1%). This finding suggests that only a small fraction of patients with colorectal adenomas carry biallelic germline *MYH* variants in the Italian population. Similarly, it was recently reported that 4 of 1,042 (0.4%) population-based Finnish colorectal cancer patients had biallelic *MYH* mutations, suggesting that *MYH*-associated colorectal cancer is rare but as common as colorectal cancer associated with FAP.[16]

In conclusion, it can be estimated that 5% of Italian FAP/AAPC patients, and about 20% of those in whom no germline *APC* mutation is detectable and showing a family history compatible with recessive inheritance, carry common biallelic *MYH* mutations, while only a small fraction of patients with colorectal adenomas in the general population harbor these germline *MYH* variants. This finding suggests that a molecular-based diagnosis of classical or atypical adenomatous polyposis may have important implications in the management of at-risk individuals: *APC*-associated polyposis is a dominant condition (*i.e.*, first-degree relatives of a patient have an a priori 50% risk of inheriting the mutation), while *MYH*-associated polyposis is a recessive condition (*i.e.*, first-degree relatives of a patient are at risk only if both parents carry at least one *MYH* mutation). Also, our data indicate that at least 3 subpolymorphic variants (Y165C, G382D and 1395delGGA) are present within the southern European Caucasian population.

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