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ORIGINAL ARTICLE

Attenuated polyposis of the large bowel: a morphologic and molecular approach

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Abstract Attenuated polyposis could be defined as a variant of familial adenomatous polyposis (FAP) in which synchronous polyps of the large bowel range between 10 and 99. We analysed all cases of attenuated polyposis observed over the last 30 years with the objectives: (A) to classify the disease according to different type and proportion of polyps; (B) To ascertain the contribution of APC and MutYH genes; (C) to discover features which could arise the suspicion of mutations; (D) To obtain indications for management and follow-up. 84 individuals in 82 families were studied. Polyps were classified into four groups as adenoma, hyperplastic, other serrated lesions or others; APC and MutYH mutations were assessed. Mean age at diagnosis was 54 ± 14 years in men and 48 ± 13 in women ($P = 0.005$). Polyps were more numerous in women (37 ± 26 vs 29 ± 22). Sixty % of patients underwent bowel resection, mainly for cancer; the remaining were managed through endoscopy. A total of 2586 polyps were detected at diagnostic endoscopy: 2026 (80 %) were removed and analysed. Adenomas were diagnosed in 1445 (70 %), hyperplastic polyps in 541 (26 %), other serrated lesions in 61 (2.9 %). Adenomas and hyperplastic lesions were detected in the majority of patients. In 68 patients (81 %) in whom studies were executed, APC mutations were found in 8 and MutYH mutations in 10. Genetic variants were more frequent in women (12 vs 6, $P = 0.039$). Taking into consideration the prevalent (>50 %) histology and presence of mutations, patients could be subdivided into four groups: (1) APC mutated polyposis (AFAP), when adenomas were >50 % and APC mutations detected (no. 8, 10 %); (2) MutYH mutated polyposis (MAP), adenomas >50 % and biallelic MutYH mutations (no. 10, 12 %); (1) attenuated polyposis without detectable mutations, prevalence of adenomas, 48 cases (57 %); (1) hyperplastic-serrated polyposis, with prevalence (>50 %) of hyperplastic/other serrated lesions and no constitutional mutation (no. 18, 21 %). Aggregation of tumors, cancer in probands, distribution of polyps and other clinical characteristics showed no difference among the four groups. In conclusions, AFAP and MAP, the polyposis labeled by constitutional mutations, represented about 25 % of all attenuated polyposis. Mutation-associated cases showed an earlier age of onset of polyps and were more frequent in the female sex.

Keywords Cancer · Tumor · Polyps · Adenoma · Hyperplastic · Serrated · FAP · AFAP

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Introduction

In a small fraction of cases (<1.0 %) colorectal malignancies develop in patients with familial adenomatous polyposis (FAP), a rare autosomal dominant condition in which the colon and rectum are usually carpeted by hundred or thousand polyps of various dimensions [1, 2]. In most patients the FAP phenotype is associated with constitutional mutation in the APC gene, or, to a lesser

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fraction, in the MutYH gene [3, 4]. Diagnostic criteria, management and follow-up of FAP patients have been well characterized, and guidelines were published [5].

Attenuated polyposis (AFAP) is a phenotypic variant of FAP, in which, according to some authors, the number of polyps ranges between 10 and 99 [6, 7]. Patients with AFAP show an attenuated course, with development of adenomas and carcinoma at a more advanced age than classical FAP, and a low frequency of extracolonic manifestations [8, 9]. Moreover, constitutional mutations in either APC or MutYH genes can be found in no more than 20–30 % of all cases [6–9]. It is likely that the widespread use of colonoscopy and the diffusion of colorectal cancer screening will induce an increase in the detection of attenuated polyposis. At variance with FAP, guidelines on diagnostic criteria, time and type of optimal treatment, search of lesions in other organs, extent of surveillance and use of prevention agents remain undefined [10–12].

In addition, other types of attenuated polyposis await a further definition, and in particular hyperplastic/serrated polyposis [13–15]. In these patients, polyps tend to be hyperplastic or "serrated" (saw-teethed), but confusion still exists, since for some authors serrated polyposis includes hyperplastic polyposis [16], whereas for others [10] "serrated polyps were previously called hyperplastic polyps". Moreover, lack of consensus includes not only histologic criteria, but also management and follow-up. Finally, in a recent paper Gill and collaborators showed an almost exponential increase, in their Pathology Unit, in the diagnosis of sessile serrated polyposis over a very short period of time: no case diagnosed in 2009, 134 confirmed cases in 2012 [13].

In the present investigation, through a careful analysis of all cases of attenuated polyposis presented to our observation over 30 years, we purposed the following main objectives: (1) to classify attenuated polyposis mainly on the basis of the observed histology and to ascertain the contribution, to the proposed classification, of constitutional mutations in the two cancer-related genes APC and MutYH; (2) to find out possible clinical and morphological characteristics which could raise the suspicion of mutations; (3) to discuss clinical management of attenuated polyposis, with particular attention to time and type of surgery and to endoscopic follow-up.

Materials and methods

Patients

The study group included 84 patients, all of Italian ancestry (of 82 different families), with an endoscopic diagnosis of attenuated polyposis. This was defined as the presence at endoscopy (the first endoscopic evaluation in the majority of patients) of a number of synchronous polyps, of any histological type, ranging from a minimum of 10 to a maximum of 99 in at least two colorectal segments (IDC-0 Classification) [17]. Patients were usually referred to our Unit with suspicion of classical FAP, but an evaluation of clinical charts changed the diagnosis into an attenuated clinical form (i.e., less than 100 adenomas were present at the index endoscopy). Only 1 patient (in the APC+ group, Table 2) was less than 25 years old; in this specific case the diagnosis of classical FAP could not be excluded a priori.

Most subjects were "single" cases, since no other family member with polyposis was referred by history or detected. In two families (both with MutYH mutations) two siblings both showed attenuated polyposis, and were included in the study. In two families with APC mutations, one of the parents was affected by "polyposis" by history, so that only the proband could be included in the study, owing to lack of detailed information.

In some patients, the evaluation of the exact number of polyps was rather troublesome, owing to the frequent use, at endoscopy, of terms such as "numerous", "many", or "multiple". In these cases, endoscopists were recontacted, and the examinations (medical records and, where available, videos) re-evaluated in order to establish the most likely number of polyps at index endoscopy. Despite this, we remain aware of the difficulty in establishing the correct number of polyps, at least in some cases.

Families with Peuts–Jeghers syndrome (no. 8), Cowden/Bannayan disease (no. 2) and Juvenile polyposis (no. 3), all with less than 10 colorectal polyps, in our experience, were not included in the study.

Location and histological features

Besides their number at the diagnostic endoscopy, the distribution of polyps in the various large bowel segments and their dimensions were carefully recorded. As far as location was concerned, we subdivided polyps distribution into three categories: (1) polyps scattered in the "whole large bowel", (2) polyps predominantly (>50 %) located in the "Left colon" (Descending, Sigmoid and Rectum) and (3) polyps predominantly (>50 %) located in the "Right colon" (from Cecum to Splenic Flexure).

Histologic examination allowed us to classify polyps into four main categories:

1. "Adenoma", when pseudostratification of nuclei was evident, together with other classical features of adenomas, including dysplasia [18];
2. "Hyperplastic", in presence of a single layer of enterocytes lining the polyp, with no pseudostratification and no architectural or cytologic features of sessile

serrated adenoma (SSP/A) or traditional serrated adenoma (TSA);
3. "Serrated", in presence of SSP/A or TSA. Since in many instancies we retrieved a diagnosis of "serrated polyp" no otherwise specified, and we were not able to review in our Institutions all 61 polyps diagnosed as serrated, we used the definition of Serrated lesions without any further subdivision;
4. "Other" histological types (15 lesions) included a miscellanea of inflammatory polyps, mixed lesions, ganglioneuroma and hamartoma (1 polyp).

Molecular biology, APC and MUTYH gene mutations

Molecular studies were carried out and completed in 68 out of 84 patients (81 %); of the remaining 16, 4 refused genetic testing, in 6 the study was not possible for various reasons, and in 6 molecular definition was under study.

Molecular analysis of the APC gene was executed following standard procedures, as previously described [19, 20]. Briefly, DNA was extracted either from peripheral white blood cells or from Epstain–Barr virus-transformed cell lines that were obtained from probands. DNA was amplified by PCR, screened by Protein Truncation Test for exon 18, and directly sequenced for exons 4–18. Negative tests were sequenced for exon 18. The promoter and the entire open reading frame were analysed for the presence of deletions or rearrangements by using the SALSA PO43 APC MLPA Kit from MRC-Holland (Amsterdam, the Netherlands) according to the manufacturer's protocol.

MutYH constitutional alterations were studied as already reported [21, 22]. After DNA extraction and amplification, the entire open reading frame was sequenced. Amplification products were obtained from primers located in the flanking intron regions approximately 50 base pairs from the respective exons, in order to find out all possible splice-junction mutations.

Statistical analysis

Clinical characteristics of the investigated patients were expressed as mean ± standard deviations (SD), or as frequencies. The statistical significance of difference between groups, based on sex or presence of constitutional mutations, was assessed using Chi-Square, Fisher's exact, Kolmogorov–Smirnov or Kruskal–Wallis tests, as appropriate.

Multiple regression was carried out in order to justify the variability in the number of observed polyps during colonoscopy. Multinomial regression analysis was executed for evaluating the possible role of clinical and morphological parameters in predicting the presence of constitutional mutations. Logistic regression analysis aimed at identifying patients with major risk to develop colorectal cancer or to die. All analyses were performed with STATA 12 software.

Results

Main clinical and morphologic data are summarized in Table 1. Mean age at diagnosis was 54.0 ± 14 years in males and 48 ± 13 in females ($P = 0.005$). Men and women were almost equally distributed in the study group. The average number of polyps at the diagnostic endoscopy was 33 ± 24, range 10–92; polyps were more numerous in females (37 ± 26 vs 29 ± 22, $P = 0.025$). In the majority of patients there was no evidence of malignancy at diagnosis (54, 64 %); however colorectal cancer was detected in 30 (36 %) patients, in most cases at first colonoscopy. It is rather intriguing that in 5 individuals attenuated polyposis developed 1–5 years after intestinal resection for colorectal carcinoma. In approximately half of patients polyps were distributed in all colorectal segments; in 20 (24 %), lesions were predominantly located in the Left colon, in 19 (23 %) in the Right colon. Fifty patients (60 %) underwent large bowel resections, at diagnosis or during follow-up. Cancer was the main reason for surgery, though in 20 patients the approach was chosen because of polyposis not complicated by malignancy. Thirty-four (40 %) patients were managed through endoscopic removal of polyps. Nearly half of the patients (no. 37, 44 %) underwent colonoscopy because of screening (FOBT, family history of cancer); in 47 (56 %) symptoms had developed (the most frequent being rectal bleeding or abdominal pain). In two cases extracolonic manifestations (retinal pigmented spots or osteomas) were the first clinical sign leading to the diagnosis. The large majority of patients were alive at January 2015; of the 13 patients who had deceased, 10 died of colorectal cancer and 1 of pancreatic neoplasm, the remaining two for reasons unrelated to neoplasms.

A total of 2586 polyps were detected at the diagnostic endoscopy, of these 2062 (80 %) were removed and analysed. The histologic diagnosis was adenoma in 1445 (70 %), hyperplastic polyps in 541 (26.2 %), other serrated lesions in 61 (2.9 %) and other histologic types in 15 (1 %). As shown in Table 1B, adenomas and hyperplastic polyps were detected in the majority of patients.

In the 68 patients (81 %) in whom molecular analysis could be executed and completed, 8 showed mutations in the APC gene (11.7 % of the sample) and 10 additional subjects in the MutYH gene (14.7 %). Genetic testing was negative in 50 individuals. Table 2 shows details of the

Table 1 Main clinical and histological features in the whole study group

	Mean age at diagnosis	Average no. polyps	Average no. analyzed polyps	Distribution of polyps (total/left/right)	Surgery (yes/no)
Clinical features (A)					
Total (no. 84)	51 ± 14	33 ± 24	26 ± 23	45/20/19	50/34
Males (no. 46)	54 ± 14*	29 ± 22#	25 ± 22	26/11/9	27/19
Females (no. 38)	48 ± 13*	37 ± 26#	26 ± 25	19/9/10	23/15

	Reasons of colonoscopy (screening/symptoms)	Patient status (alive/dead)	Familial aggregation of tumors (yes/no)	Execution of genetic testing (yes/no)	Colorectal cancer (yes/no)
Total (no. 84)	37/47	71/13	59/25	68/16	30/54
Males (no. 46)	20/26	38/8	30/16	37/9	16/30
Females (no. 38)	17/21	33/5	29/9	31/7	14/24

	Polyps detected at endoscopy	Analyzed polyps	Types of polyps. adenoma/hyperplastic/other serrated/other (absolute values and percentage of total)	Type of polyps per patient	
Morphologic features (B)					
Total (no. 84)	2586	2062 (80 %)	1445/541/61/15 (70, 26.2, 3, 0.7 %)	Adenoma	79 of 84
Males (no. 46)	1246	1143 (92 %)	765/347/27/4 (67, 30, 2.4, 0.3 %)	Hyperplastic	45 of 84
Females (no. 38)	1340	919 (69 %)	680/194/34/11 (74, 21, 0.4, 0.1 %)	Serrated	16 of 84
				Hyperplastic + Serrated	47 of 84

* $P = 0.005$; # $P = 0.025$

mutations, which were more frequent in women (12 vs 6, $P = 0.039$ by χ^2 test).

As shown in Fig. 1, we attempted to classify attenuated polyposis taking into consideration, besides the number of polyps, their prevalent histological type and the presence of constitutional mutations. In 66 cases of 84 (79 %), adenoma was the prevalent histology, in the sense that more than 50 % of the lesions showed adenomatous changes. Of these 66, 18 (27.3 %) showed mutations and 48 (73 %) were negative. The group with mutations could be further subdivided according to the involved gene.

The remaining 18 cases of 84 (21 %) showed predominantly (>50 %) hyperplastic/and other serrated polyps histology; no mutation in either APC or MutYH could be detected in this group. More in particular, hyperplastic/and other serrated lesions were 80 % of the total, adenomas 17 %, other 3 %.

Tentatively, the four resulting groups could be labeled as follows:

(A) APC mutated familial adenomatous polyposis (AFAP),
(B) MutYH mutated adenomatous polyposis (MAP),
(C) Attenuated polyposis without detectable mutations (in the two genes more frequently involved in polyposis syndromes) and,
(D) Hyperplastic/serrated polyposis.

Table 3 summarizes the main clinical and histologic features of the four groups of patients. Statistical evaluation (univariate analysis) showed a significant difference ($P < 0.001$ by Kruskal–Wallis test) in the age of polyp onset, which was the earliest in the APC mutated group and the latest for hyperplastic/serrated polyposis. Female sex was significantly ($P = 0.005$) more frequent in AFAP, whereas male sex in attenuated polyposis without mutations. Four of 8 patients with APC mutations and 6 of 10 with MutYH mutations had adenomatous polyps as the only histological type. None of the patients with hyperplastic/other serrated lesions had exclusively polyps of this histological type, i.e., concomitant adenomas were always present.

Hyperplastic/Serrated polyposis syndrome has been defined as the presence of one of the following criteria: (1) At least 5 hyperplastic/other serrated polyps proximal to the sigmoid colon, 2 of which of at least 1.0 cm in diameter; (2) any number of hyperplastic/other serrated lesions occurring proximal to the sigmoid in a subject who has a first-degree relative with the syndrome; (3) More than 20 hyperplastic/other serrated polyps spread throughout the colon (23). Of the 18 patients of the present study, 6 showed criterion 1, 14 criterion 3, five more than 1 criterion, no subject displayed features of criterion 2.

By mean of multivariate analysis, we evaluated the possible association of the most relevant clinical and

Table 2 Constitutional mutations in MUTYH and APC genes detected in 18 individuals

Family	MutYH mutation Allele 1/Allele 2	Family history of polyposis/cancer	No. of polyps	Age at diagnosis
4 (A)	p.(Glu480del)/p.(Glu480del)	Yes	50	47
4 (B)	p.(Glu480del)/p.(Glu480del)	Yes	10	43
5 (A)	p.(Tyr179Cys)/p.(Gly396Asp)	Yes	24	51
5 (B)	p.(Tyr179Cys)/p.(Gly396Asp)	Yes	30	50
38	p.(Gln141Argfs*5)/p.(Arg182His)	Not	22	32
39	p.(Arg245His)/p.(Arg245His)	Not	30	66
47	p.(Tyr179Cys)/p.(Tyr179Cys)	Not	30	29
48	p.(Glu480del)/p.(Glu480del)	Not	35	34
71	p.(Tyr179Cys)/p.(Tyr179Cys)	Not	90	49
61	p.(Tyr179Cys)/p.(Tyr179Cys)	Not	11	49
Family	APC mutation	Family history of polyposis/cancer	No. of polyps	Age at diagnosis
9	p.(Glu1538Ilefs*5)	Not	25	18
19	p.(Lys226Ilefs*66)	Yes	30	35
20	p.(Asn1113Serfs*4)	Not	20	33
35	g.(113797-?_151992+?del)	Not	30	44
46	c.(-30434-?_-30079+?del)	Not	50	27
57	p.(Leu710Serfs*8)	Yes	40	34
58	p.(Pro1324G1nfs*91)	Yes	20	33
59	p.(Cys110Phefs*12)	Not	15	51

morphologic variables (age, sex, presence of mutation, distribution of polyps, presence or development of cancer, surgical treatment, endoscopic approach, history of cancer, symptoms of onset) with: (A) the specific subgroups in which patients were subdivided (Fig. 1), (B) the development of cancer in probands and (C) clinical outcome (dead or alive). As shown in details in Tables 4, 5 and 6, none of the investigated variables was associated with any of the three main end-points. Similarly, no association was ascertained between clinical variables and total number of polyps at the index endoscopy.

Discussion

The results of the present investigation can be summarized as follows:

First, the clinical condition in which 10–99 synchronous colorectal polyps are detected at endoscopy can tentatively be classified into four main groups on the basis of histologic features of polyps and presence of constitutional mutations in either APC or MutYH genes. Second, two of these groups (AFAP and MAP), which represent nearly 25 % of the total, are labelled by constitutional mutations; "attenuated polyposis" and hyperplastic/serrated polyposis (Fig. 1) do not show mutations in the above mentioned genes, and their diagnosis is based on the prevalent histological type. Third, polyps are usually mixed, in the sense that adenomatous and hyperplastic/other serrated lesions tend to cohexist in the majority of these patients, it is the prevalence (>50 %) of a given histology, or the presence of germline mutations, which defines each subgroup, at least with the present approach. Finally, no other relevant clinical features seem to be specifically associated with any of the four subgroups, in the sense that (a) a strong familial aggregation of tumors was present with approximately the same frequency, (b) cancer occurrence in probands was in the same order of magnitude (and colectomy was similarly more frequent than endoscopy as treatment of choice), and (c) the distribution of polyps in the large bowel was not different among groups. It is worth noting that mutation associated cases, especially those with APC gene alterations, showed an early age of onset of polyps (Table 3, $P < 0.001$). Since genetic cases require a more intensive clinical research (possible extracolonic manifestations, including duodenal and ampullary adenomas, desmoid tumors and thyroid nodules) the search of constitutional mutations is particularly indicated in individuals with attenuated polyposis with prevalence of adenomas in their third or fourth decade of life. Furthermore, constitutional mutations were detected more frequently in the female sex (especially in the APC+ group).

Attenuated polyposis can be defined as a variant of FAP, at least taking into account the phenotypic appearance. As a

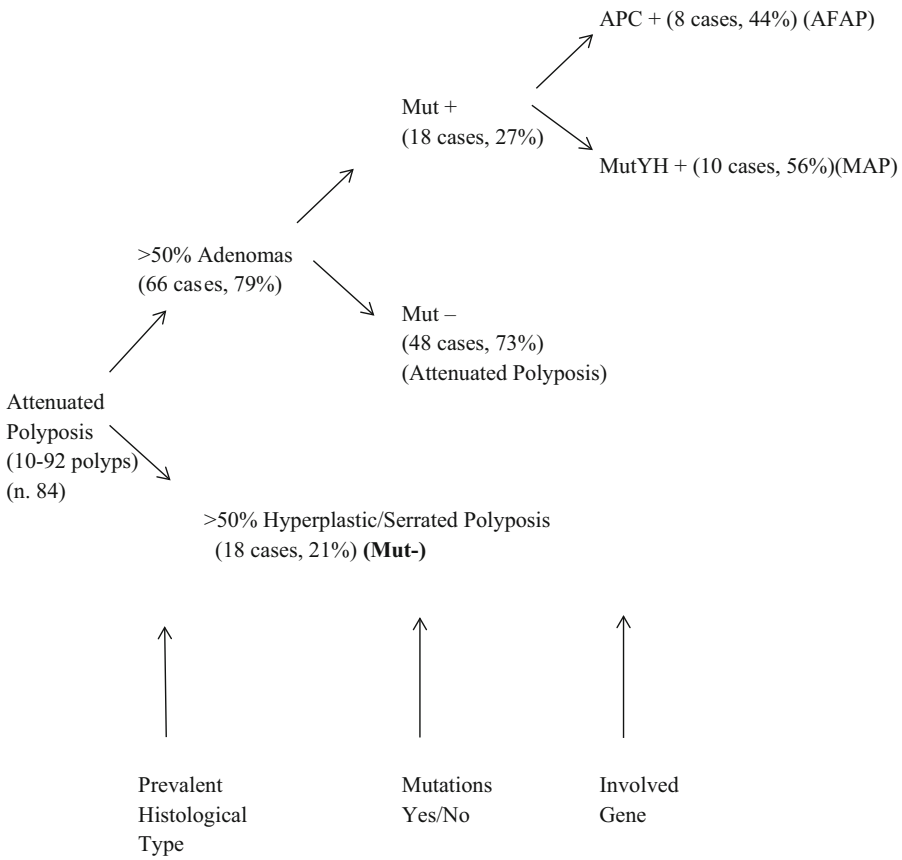

Fig. 1 Tentative classification of attenuated polyposis

Attenuated Polyposis	48	(57%)
Polyposis APC + (AFAP)	8	(10%)
Polyposis MutYH + (MAP)	10	(12%)
Hyperplastic/Serrated Polyposis	18	(21%)

general impression, the condition is increasing in frequency, presumably as a consequence of a greater awareness among physicians, but also owing to the widespread diffusion of colonoscopy and screening procedures. Due to the variability of clinical and histologic features, the diagnostic criteria have not been established and the disease can be defined in more or less complex ways. Thus, while for some authors [11] it can simply be defined as the presence of 10–20 to 99 polyps in the large bowel, Nielsen et al. [6] proposed the presence of the following 2 criteria: (A) at least two first-degree relatives with 10–99 adenomas diagnosed at age >30 years or (B) one patient with 10–99 adenomas at age >30 and a first-degree relative with colorectal cancer with a few adenomas, and, in neither case, family members with more than 100 polyps before the age of 30 years. And the definition can be made even more complex if we take into consideration presence or absence of germline mutations, early onset colorectal cancer or severe dysplasia, extracolonic manifestations, and a family history of multiple polyps.

Similarly, besides the histologic classification of hyperplastic and serrated lesions, diagnosis of hyperplastic/serrated polyposis remains cumbersome, although most recent investigations tend to include hyperplastic polyposis within serrated polyposis [11, 14]. The above mentioned criteria represent the reference definition for the syndrome [23]. At somatic level, serrated polyps show an increased rate of BRAF mutations, CpG island hypermetilation and DNA microsatellite instability [24–26]; however, these markers cannot be used with purposes of defining or classifying the syndrome.

In our opinion, the two reported definitions [6, 23] and other attempts to classify attenuated polyposis syndromes [11] miss an important point, which is also the main message of the present study: in most patients, polyps do not show a single histological type, but rather a mixture of adenomatous, hyperplastic and other serrated lesions, and still other histologies in a few cases only. Consequently, our attempt to classify attenuated polyposis on the basis of

Table 3 Tentative classification of attenuated polyposis into four main groups

	Familiality Yes/no	Age	Sex M/F	Living/deceased	No. polyps average	A/H/S/O % of total	Distribution (T/R/L)	Colorectal Cancer Yes/no	Colorectal cancer mean age of onset	Disease onset Screening/ symptoms	Surgery/ endoscopy
APC+ (no. 8) (AFAP)	6/2	34 ± 10**	1/7*	7/1	29 ± 12	92/7/1/0	6/0/2	2/6	47 ± 5	3/5	5/3
MutYH+ (no. 10) (MAP)	8/2	45 ± 11**	5/5*	10/0	33 ± 23	88/12/0/0	7/2/1	4/6	40 ± 8	4/6	7/3
Attenuated polyposis (no mutation) (no. 48)	32/16	53 ± 14**	31/17*	38/10	31 ± 26	87/11/2/0	18/16/14	16/32	51 ± 12	22/26	29/19
Hyperplastic/serrated polyposis (no. 18)	13/5	58 ± 8**	9/9*	16/2	39 ± 24	17/64/16/3	14/2/2	8/10	60 ± 7	8/10	9/9
Total (no. 84)	59/25	51 ± 14	46/38	71/13	33 ± 24	72/23/4/1	45/20/19	30/54	49.5 ± 8.3	37/47	50/34

A adenoma, *H* hyperplastic, *S* other serrated polyp, *O* other, *T* total colon, *R* right colon, *L* left colon

** $P < 0.001$; * $P = 0.05$

the prevalent (more than 50 % of the analysed polyps) histology seems to us rather sound.

In the majority of our patients, no constitutional mutations in either APC or MutYH genes were detected. This could mean that most of these cases are truly sporadic, and dependent on some unidentified exogenous factors. However, other possibilities can be taken into account; for instance, different genes could be involved. In a large series of 603 patients selected on the basis of at least one hamartomatous or hyperplastic/other serrated polyp in the large bowel—many of them with features of attenuated polyposis—Ngeow et al. [27] reported that in 77 (13 %) constitutional mutations in either ENG, PTEN, STK11, BMPR1A or SMAD4 could be detected. Moreover, specific constitutional variants in POLE or POLD1 genes were recently reported among 15 patients selected for the presence of at least 10 colorectal adenomas in families with history of colorectal cancer [28]. Clearly, Next Generation Sequencing could lead to the discovery of several genes potentially associated with the attenuated polyposis phenotype. Finally, other possible explanations (for lack of germline mutations) include APC or MutYH alterations which are difficult to detect with routine techniques, and APC mosaicism.

There were two sex-related differences that reached the statistical significance, and for which we cannot offer any plausible explanation. The first was the higher (37 vs 29) average number of polyps in the female sex; the second the increased rate of mutations (for both genes, but more evident for APC) again in females. More studies are needed to further clarify the issue.

A family history of cancer was reported in the majority of patients (59 of 84, 70 %). As shown in Table 3, this familiality was present in all four groups, approximately at the same extent. Although a wide tumor spectrum was observed, including all commonly occurring malignancies of humans, colorectal cancer was by far the most frequently referred neoplasm. These findings might suggest that in some family members intestinal tumors developed over a background of colorectal polyposis, but a direct proof of this contention is lacking. As a matter of fact, the large majority (80 of 84) of investigated individuals were "single" cases, and in no other members of the family there was evidence of attenuated polyposis.

What about clinical recommendations and management of attenuated polyposis? Our prolonged observations allow some prudent suggestions, still awaiting for further studies in larger and different groups of individuals. Management is undoubtedly more complex than in classical FAP, a disease known from the Nineteen century and for which general guidelines have been accepted [29, 30]. We strongly recommend for attenuated polyposis of any type an individualized approach which takes into consideration

Table 4 Variables potentially associated with any of the specific subgroup of Fig. 1 (AFAP, MAP, attenuated polyposis, hyperplastic/serrated polyposis) (multinomial regression)

	Relative risk ratio	CI	(95 %)
Mut−	Reference category		
Hyperplastic polyposis			
Age at diagnosis	1.02	0.95	1.09
Sex (M vs F)	2.64	0.61	11.47
Colon DX versus SX	4.20	0.24	72.84
Colon all versus SX	6.73	0.54	83.68
Cancer in families	0.36	0.08	1.75
Colorectal carcinoma (probands)	3.32	0.28	40.12
Symptoms of onset	0.42	0.10	1.79
Surgery	0.22	0.02	2.41
MutYH+			
Age at diagnosis	0.94	0.87	1.00
Sex	2.95	0.57	15.42
Colon DX versus SX	2.23	0.09	56.64
Colon all versus SX	6.21	0.50	77.08
Cancer in families	1.12	0.16	7.99
Colorectal carcinoma (probands)	1.17	0.14	10.08
Symptoms of onset	1.21	0.22	6.59
Surgery	2.03	0.23	18.17
APC+			
Age at diagnosis	0.79	0.63	1.00
Sex	0.000	0.00	–
Colon DX versus SX	0.00	0.00	–
Colon all versus SX	4.21	0.04	468.36
Cancer in families	0.35	0.01	23.48
Colorectal carcinoma (probands)	0.04	0.00	8.20
Symptoms of onset	1.26	0.03	46.43
Surgery	11.58	0.03	4849.07

Table 5 Variables potentially associated with the development of Colorectal Cancer in probands (logistic regression)

	Odds ratio	CI	(95 %)
Age at diagnosis	0.98	0.92	1.05
Sex (M vs F)	2.22	0.36	13.63
MutYH+ versus Mut−	2.90	0.36	23.28
APC+ versus Mut−	0.22	0.01	4.03
Colon DX versus SX	0.50	0.05	5.41
Colon all versus SX	0.27	0.04	2.00
Iperplast versus adenomatous histology	3.94	0.31	49.63
Other versus adenomatous histology	0.000		
Cancer in family	0.25	0.05	1.18
Symptoms of onset	2.53	0.38	16.89

Table 6 Variables potentially associated with the clinical outcome (alive or deceased) (logistic regression)

	Odds ratio	CI	(95 %)
Age at diagnosis	1.15	0.97	1.36
Sex (M vs F)	2.29	0.08	62.84
MutYH+ versus Mut−	1.00		
APC+ versus Mut-	15.21	0.20	1129.36
Colon DX versus SX	0.96	0.04	20.78
Colon all versus SX	0.08	0.00	2.40
Cancer in family	5.12	0.36	71.89
Colorectal carcinoma (probands)	1.07	0.11	10.73
Symptoms of onset	1.18	0.07	19.20
Surgery	2.36	0.06	100.03

age of patients, their willingness to undergo colonoscopy, prevalent histology of the resected polyps, molecular characterization, presence of a strong family history of colorectal cancer and distribution of polyps in the large bowel. For instance, although colectomy or hemicolectomy were executed in the majority of patients (50 of 84, 60 %),

a relevant fraction of individuals with attenuated polyposis can presumably be managed through endoscopy; this approach can be considered as appropriate when: (A) the patient is willing to undergo repeated endoscopies for many years, and is aware of the possible risk of polyp degeneration into infiltrating lesions; (B) polyps are relatively few (10–40, approximately) or in any case not so numerous or large to require immediate surgery; (C) the endoscopist is confident to obtain a clean colon after each examination; (D) histology shows low-grade dysplasia in all lesions. Time interval between endoscopies can also be individualized, but for most patients ranges between six and 12 months, although with time larger intervals could be adequate, at least in some patients. On the other hand the presence of other criteria favor the execution of surgery, in particular: (1) poor compliance or reluctance of patients to undergo frequent colonoscopy; (2) presence of high-degree dysplasia in one or more polyps; (3) presence of more than 50 polyps, especially when scattered in the various large bowel segments. Subtotal colectomy with ileorectal anastomosis appears as the technique of choice [31], though in cases with polyp localization in one or few (and contiguous) large bowel segments hemicolectomy or segmental resections can be taken into consideration.

Finally, according to the suggestion of recent studies [32, 33] we usually recommend to patients with attenuated polyposis to reach and maintain their ideal body weight, to do regular physical exercise at least three times per week, and to reduce their intake of meat and animal fat.

Rather interestingly, colorectal cancer occurred with approximately the same frequency in all four categories in which the study group was subdivided (Fig. 1; Table 3). This indicates that the risk of cancer is presumably the same for each type of attenuated polyposis, including hyperplastic/serrated polyposis [34], and that patients with constitutional mutations do not seems to carry an additional risk. It follows that genetic testing is undoubtedly useful, especially for other family members, and should be recommended in these patients; however, management of individual patients seems to be rather independent from the presence of mutations.

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References

1. Scott RJ (2003) Familial adenomatous polyposis (FAP) and other polyposis syndromes. Hered Cancer Clin Pract 1:19–30
2. Galiatsatos P, Foulkes WD (2006) Familial adenomatous polyposis. Am J Gastroenterol 101:385–398
3. Wäsch R, Robbins JA, Cross FR (2010) The emerging role of APC/CCdh1 in controlling differentiation, genomic stability and tumor suppression. Oncogene 29:1–10
4. Pezzi A, Roncucci L, Benatti P, Sassatelli R, Varesco L, Di Gregorio C, Venesio T, Pedroni M, Maffei S, Reggiani Bonetti L, Borsi E, Ferrari M, Martella P, Rossi G, Ponz De Leon M (2009) Relative role of APC and MUTYH mutations in the pathogenesis of familial adenomatous polyposis. Scand J Gastroenterol 44:1092–1100
5. Vasen HF, Möslein G, Alonso A, Aretz S, Bernstein I, Bertario L, Blanco I, Bülow S, Burn J, Capella G, Colas C, Engel C, Frayling I, Friedl W, Hes FJ, Hodgson S, Järvinen H, Mecklin JP, Møller P, Myrhøi T, Nagengast FM, Parc Y, Phillips R, Clark SK, de Leon MP, Renkonen-Sinisalo L, Sampson JR, Stormorken A, Tejpar S, Thomas HJ, Wijnen J (2008) Guidelines for the clinical management of familial adenomatous polyposis (FAP). Gut 57:704–713
6. Nielsen M, Hes FJ, Nagengast FM, Weiss MM, Mathus-Vliegen EM, Morreau H, Breuning MH, Wijnen JT, Tops CM, Vasen HF (2007) Germline mutations in APC and MUTYH are responsible for the majority of families with attenuated familial adenomatous polyposis. Clin Genet 71:427–433
7. Filipe B, Baltazar C, Albuquerque C, Fragoso S, Lage P, Vitoriano I, Mão de Ferro S, Claro I, Rodrigues P, Fidalgo P, Chaves P, Cravo M, Nobre Leitão C (2009) APC or MUTYH mutations account for the majority of clinically well-characterized families with FAP and AFAP phenotype and patients with more than 30 adenomas. Clin Genet 76:242–255
8. Wang L, Baudhuin LM, Boardman LA, Steenblock KJ, Petersen GM, Halling KC, French AJ, Johnson RA, Burgart LJ, Rabe K, Lindor NM, Thibodeau SN (2004) MYH mutations in patients with attenuated and classic polyposis and with young-onset colorectal cancer without polyps. Gastroenterology 127:9–16
9. Ponz de Leon M, Urso ED, Pucciarelli S, Agostini M, Nitti D, Roncucci L, Benatti P, Pedroni M, Kaleci S, Balsamo A, Laudi C, Di Gregorio C, Viel A, Rossi G, Venesio T (2013) Clinical and molecular features of attenuated adenomatous polyposis in northern Italy. Tech Coloproctol 17:79–87
10. Schlussel AT, Gagliano RA Jr, Seto-Donlon S, Eggerding F, Donlon T, Berenberg J, Lynch HT (2014) The evolution of colorectal cancer genetics-Part 2: clinical implications and applications. J Gastrointest Oncol 5:336–344
11. Shussman N, Wexner SD (2014) Colorectal polyps and polyposis syndromes. Gastroenterol Rep 2:1–15
12. Brosens LA, van Hattem A, Hylind LM, Iacobuzio-Donahue C, Romans KE, Axilbund J, Cruz-Correa M, Tersmette AC, Offerhaus GJ, Giardiello FM (2007) Risk of colorectal cancer in juvenile polyposis. Gut 56:965–967
13. Gill P, Wang LM, Bailey A, East JE, Leedham S, Chetty R (2013) Reporting trends of right-sided hyperplastic and sessile serrated polyps in a large teaching hospital over a 4-year period (2009–2012). J Clin Pathol 66:655–658
14. Mongin C, Coulet F, Lefevre JH, Colas C, Svrcek M, Eyries M, Lahely Y, Fléjou JF, Soubrier F, Parc Y (2012) Unexplained polyposis: a challenge for geneticists, pathologists and gastroenterologists. Clin Genet 81:38–46
15. Hazewinkel Y, Tytgat KM, van Eeden S, Bastiaansen B, Tanis PJ, Boparai KS, Fockens P, Dekker E (2014) Incidence of colonic neoplasia in patients with serrated polyposis syndrome who undergo annual endoscopic surveillance. Gastroenterology 147:88–95
16. Chetty R, Hafezi-Bakhtiari S, Serra S, Colling R, Wang LM (2015) Traditional serrated adenomas (TSAs) admixed with other

serrated (so-called precursor) polyps and conventional adenomas: a frequent occurrence. J Clin Pathol 68:270–273
17. ICD-O, Classificazione Internazionale delle malattie, Oncologia, WHO (1983) A cura di F. Rilke. Istocitopatologia 5
18. Ponz de Leon M (2014) What clinicians wish to know about benign colorectal polyps: an operative classification. Pathol Res Pract 210:645–648
19. Ponz de Leon M, Benatti P, Percesepe A, Cacciatore A, Sassatelli R, Bertoni G, Sabadini G, Varesco L, Gismondi V, Mareni C, Montera M, Di Gregorio C, Landi P, Roncucci L (1999) Clinical features and genotype-phenotype correlations in 41 Italian families with adenomatosis coli. Ital J Gastroenterol Hepatol 31:850–860
20. Venesio T, Balsamo A, Sfiligoi C, Fuso L, Molatore S, Ranzani GN, Risio M (2007) Constitutional high expression of an APC mRNA isoform in a subset of attenuated familial adenomatous polyposis patients. J Mol Med 85:305–312
21. Venesio T, Molatore S, Cattaneo F, Arrigoni A, Risio M, Ranzani GN (2004) High frequency of MYH gene mutations in a subset of patients with familial adenomatous polyposis. Gastroenterology 126:1681–1685
22. Nielsen M, Joerink-van de Beld MC, Jones N, Vogt S, Tops CM, Vasen HF, Sampson JR, Aretz S, Hes FJ (2009) Analysis of MUTYH genotypes and colorectal phenotypes in patients With MUTYH-associated polyposis. Gastroenterology 136:471–476
23. Burt RW, Jass J (2000) Hyperplastic polyposis. In: Hamilton SR, Aaltonen LA (eds) World Health Organization classification of tumours pathology and genetics. Springer, Berlin, pp 135–136
24. Spring KJ, Zhao ZZ, Karamatic R, Walsh MD, Whitehall VL, Pike T, Simms LA, Young J, James M, Montgomery GW, Appleyard M, Hewett D, Togashi K, Jass JR, Leggett BA (2006) High prevalence of sessile serrated adenomas with BRAF mutations: a prospective study of patients undergoing colonoscopy. Gastroenterology 131:1400–1407
25. Kambara T, Simms LA, Whitehall VL, Spring KJ, Wynter CV, Walsh MD, Barker MA, Arnold S, McGivern A, Matsubara N, Tanaka N, Higuchi T, Young J, Jass JR, Leggett BA (2004) BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. Gut 53:1137–1144
26. Iino H, Simms L, Young J, Arnold J, Winship IM, Webb SI, Furlong KL, Leggett B, Jass JR (2000) DNA microsatellite instability and mismatch repair protein loss in adenomas presenting in hereditary non-polyposis colorectal cancer. Gut 47:37–42
27. Ngeow J, Heald B, Rybicki LA, Orloff MS, Chen JL, Liu X, Yerian L, Willis J, Lehtonen HJ, Lehtonen R, Mester JL, Moline J, Burke CA, Church J, Aaltonen LA, Eng C (2013) Prevalence of germline PTEN, BMPR1A, SMAD4, STK11, and ENG mutations in patients with moderate-load colorectal polyps. Gastroenterology 144:1402–1409
28. Palles C, Cazier JB, Howarth KM, Domingo E, Jones AM, Broderick P, Kemp Z, Spain SL, Guarino E, Salguero I, Sherborne A, Chubb D, Carvajal-Carmona LG, Ma Y, Kaur K, Dobbins S, Barclay E, Gorman M, Martin L, Kovac MB, Humphray S; CORGI Consortium; WGS500 Consortium, Lucassen A, Holmes CC, Bentley D, Donnelly P, Taylor J, Petridis C, Roylance R, Sawyer EJ, Kerr DJ, Clark S, Grimes J, Kearsey SE, Thomas HJ, McVean G, Houlston RS, Tomlinson I (2013) Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. Nat Genet 45:136–144
29. Vasen HFA, Tomlinson I, Castells A (2015) Clinical management of hereditary colorectal cancer syndromes. Nat Rev Gastroenterol Hepatol 12:88–97
30. Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW, American College of Gastroenterology (2015) ACG clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes. Am J Gastroenterol 110:223–262
31. Lubbe SJ, Di Bernardo MC, Chandler IP, Houlston RS (2009) Clinical implications of the colorectal cancer risk associated with MUTYH mutation. J Clin Oncol 27:3975–3980
32. Giovannucci E, Ascherio A, Rimm EB, Colditz GA, Stampfer MJ, Willett WC (1995) Physical activity, obesity, and risk for colon cancer and adenoma in men. Ann Intern Med 1(122):327–334
33. Wallace K, Baron JA, Karagas MR, Cole BF, Byers T, Beach MA, Pearson LH, Burke CA, Silverman WB, Sandler RS (2005) The association of physical activity and body mass index with the risk of large bowel polyps. Cancer Epidemiol Biomarkers Prev 14:2082–2086
34. Boparai KS, van den Broek FJ, van Eeden S, Fockens P, Dekker E (2011) Increased polyp detection using narrow band imaging compared with high resolution endoscopy in patients with hyperplastic polyposis syndrome. Endoscopy 43:676–682