ARTICLE

Germline *APC* variants in patients with multiple colorectal adenomas, with evidence for the particular importance of E1317Q

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Mendelian tumour syndromes are caused by rare mutations, which usually lead to protein inactivation. Few studies have determined whether or not the same genes harbour other, more common variants, which might have a lower penetrance and/or cause mild disease, perhaps indistinguishable from sporadic disease and accounting for a considerable proportion of the unexplained inherited risk of tumours in the general population. Germline variants at the *APC* locus are excellent candidates for explaining why some individuals are predisposed to colorectal adenomas, but do not have the florid phenotype of familial adenomatous polyposis. We have screened 164 unrelated patients with 'multiple' (3–100) colorectal adenomas for germline variants throughout the *APC* gene, including promoter mutations. In addition to three Ashkenazi patients with I1307K, we found seven patients with the E1317Q variant. E1317Q is significantly associated with multiple colorectal adenomas (OR = 11.17, 95% CI = 2.30–54.3, *p* < 0.001), accounting for ~4% of all patients with multiple colorectal adenomas. In addition, four patients with truncating *APC* variants in exon 9 or in the 3′ part of the gene were identified. Germline *APC* variants account for ~10% of patients with multiple adenomas. Unidentified predisposition genes almost certainly exist. We argue that it is worthwhile to screen multiple adenoma patients for a restricted number of germline *APC* variants, namely the missense changes E1317Q and I1307K (if of Ashkenazi descent), and, if there is a family history of colorectal tumours, for truncating mutations 5′ to exon 5, in exon 9 and 3′ to codon 1580.

INTRODUCTION

An inherited predisposition to cancer is often manifested as a family history of disease, early-onset tumours, or multiple lesions of one or several sites. Patients with a Mendelian predisposition to colorectal tumours usually present a strong family history of the disease, and often with multiple benign lesions. Examples include familial adenomatous polyposis (FAP; OMIM 175100), Peutz-Jeghers syndrome (OMIM 175200) and juvenile polyposis (OMIM 174900). Hereditary non-polyposis colon cancer (HNPCC; OMIM120435/6) is also caused by a single gene defect, but is typified less by multiple tumours than by a family history of early-onset cancers of specific sites. These Mendelian syndromes are rare, likely accounting for <5% of all colorectal cancers, but inherited factors may play a role in up to half of all cases of colorectal cancer (1).

It is suspected that outside the Mendelian colon cancer syndromes, there exists a genetic predisposition to bowel tumours, which is caused by common, low-penetrance alleles. Some patients, for example, have 'multiple colorectal adenomas'— a few or tens of benign colorectal neoplasms—and sometimes cancer; but they do not have the florid or pathognemonic phenotype of FAP. Their ages of presentation and family history are variable, and their disease may be considered unremarkable, failing to prompt genetic investigation or active management of other family members. The population prevalence of multiple adenomas is poorly described, but is probably higher than that of patients with a Mendelian predisposition to colorectal tumours.

There is no evidence that germline variants at the mismatch repair loci, *hMSH2* and *hMLH1*, predispose to multiple colorectal adenomas (2), and HNPCC patients have little, if any, tendency to develop more adenomas than the general population (3). Some patients with <100 colorectal adenomas and with a strong family history of multiple adenomas and colorectal cancer are, however, known to harbour germline *APC* mutations in the 5′ and 3′ regions of the gene (4,5). This disease variant is known as attenuated polyposis (AAPC). The spectrum of germline mutations known to be associated with AAPC is not fully defined, but is known to include nonsense and frameshift variants in exons 3, 4, 5 and 9 and in the region of the gene 3′ to codon 1580. It is not clear whether multiple adenoma patients outside AAPC families also harbour variants in the *APC* gene.

A low-penetrance, missense variant of *APC*, I1307K, predisposes to multiple colorectal adenomas in the Ashkenazi Jewish population (6). A further missense *APC* variant, E1317Q (7), is more common and widespread than I1307K and it too has been proposed as an adenoma predisposition allele (8). The existence of variants such as I1307K, suggests that similar *APC* variants, whilst rare individually, might overall contribute to the development of an important proportion of colorectal adenomas. Such variants might include (i) missense changes like I1307K; (ii) nonsense or frameshift changes in specific parts of the gene, such as those associated with AAPC; and (iii) mutations affecting other aspects of *APC* function, such as transcriptional regulation. Germline deletions of *APC* have occasionally been associated with mild FAP (9), but this association has been inconsistent and sometimes troubled by problems of methodology.

We have ascertained a set of 164 patients with multiple colorectal adenomas (3–96 tumours). The aims of this study were: (i) to find out what proportion of multiple adenoma patients have disease resulting from germline *APC* variants; (ii) to determine whether patients whose disease results from *APC* variants have any distinguishing clinicopathological features; and (iii) to indicate whether or not genetic testing for *APC* variants is warranted in people with multiple adenomas. The results of this study help to answer the question posed by Lynch and Smyrk (10), namely what is the contribution of germline *APC* variants to the risk of colorectal tumours in the general population?

RESULTS

The clinical features and family histories of the 164 patients were studied. Patients presented at the mean age of 54 years (median = 53, range = 16–77, mode = 52). At the date of the last colonoscopy, 134 patients had developed a mean of 14 adenomas in total (median = 6.5, range = 3–96, mode = 5). The remainder of the patients were simply reported as having 'multiple' adenomas, although none fulfilled the diagnostic criteria for FAP of 100 adenomas. The numbers of adenomas almost certainly underestimate the lifetime total developed by some patients: some polyps were fulgurized without histological assessment; most patients in the study were alive and at risk of developing further tumours; some tumours may have been missed at colonoscopy; and a small number of patients had undergone a partial or total colectomy. Many patients had also developed metaplastic polyps, although data on these were incomplete and have not been formally assessed. Of the 83 patients from whom the data were available, 42 (50%) had a family history (first or second degree relative) of colorectal adenoma(s) and/or carcinoma(s) and 41 had no such history. In 81 cases, family history was absent or incompletely reported in hospital records, and no reply was received when a questionnaire was sent to patients retrospectively. Evidently, family history is likely to be under-reported for several reasons, not least the presence of asymptomatic and unscreened colorectal adenomas in relatives. Four patients (1-14, SP/WW, 848HS, 1599AM) had family history strongly suggestive of Mendelian inheritance. Nineteen patients (12%) had developed one or more colorectal carcinomas and three (2%) had developed cancer of another site. No patient was known to have congenital hypertrophy of retinal pigment epithelium, desmoids, features of Gardner's syndrome or upper gastrointestinal tumours.

Fourteen (8.5%) of the 164 patients carried inherited *APC* variants with putative pathogenic effects (whether as the sole cause of their disease or as a contributory factor). No patient carried more than one such *APC* variant. The most common variant was E1317Q (Fig. 1a). This missense change was carried by seven (4.3%) patients. Of a total of 503 population-based UK controls studied [133 of whom were analysed by White *et al.* (7) and 80 by Frayling *et al.* (8)], just two carried E1317Q. The difference in E1317Q frequency between cases and controls is significant ($p < 0.001$, Fisher's exact test), with a relative risk of 11.17 (95% CI 2.30–54.3). The clinical features and family histories of the seven patients with E1317Q variants are shown in Table 1. The clinical features of these patients are largely unremarkable, although it is interesting that the number of adenomas varies widely among patients presenting at similar ages. Some E1317Q carriers reported a family history of colorectal neoplasia. No common haplotype shared among the E1317Q carriers could be identified (data not shown), suggesting multiple or ancient origins of this variant. Allelic loss at *APC* was analysed in 51 adenomas (median diameter = 0.5 cm, range = 0.2–2.0 cm) from eight E1317Q carriers. Only four tumours (8%) showed loss at any of the markers studied.

Three patients (1.8%) carried I1307K *APC* variants. All were of Ashkenazi origin and the details of their disease are shown in Table 1. This variant has been extensively studied in Ashkenazim and confers a relative risk of colon tumours of ~2, although the relative risk of multiple colorectal adenomas may well be higher. The clinical features and family histories of these patients did not distinguish them from the other patients in this study.
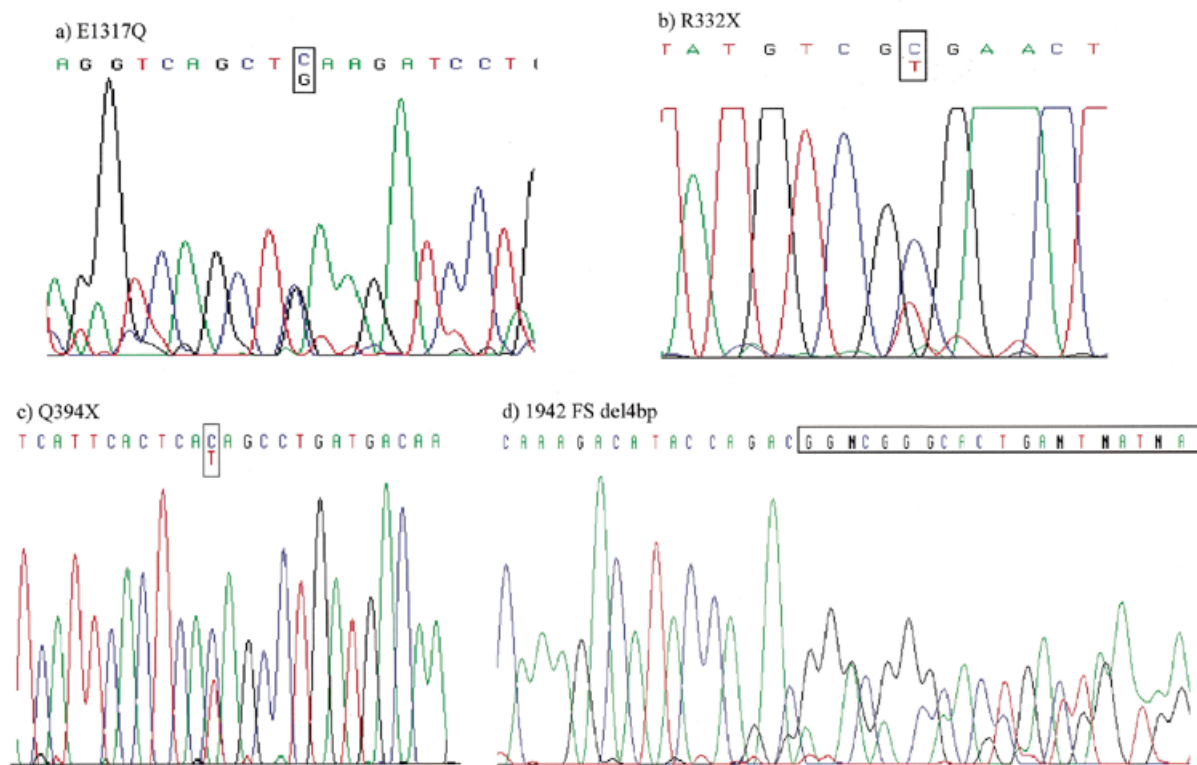
Figure 1. Germline *APC* variants: (**a**) E1317Q; (**b**) R332X; (**c**) Q394X; and (**d**) codon 1942 frameshift (5824delGACA).

Table 1. Clinical details of patients with germline *APC* variants

ID	Age[a]	No. of adenomas[b]	Colorectal cancer	Family history[c]	APC variant
1-14	39	3	Yes	Yes (?Mendelian)[d]	I1307K
2-33	57	6	Yes	M colon cancer	I1307K
2–49	63	17	No	No	I1307K
1308EM[d]	59	26	No	No	E1317Q
1310TW[d]	48	6	No	No	E1317Q
1252FG	45	5	No	Yes (no details)	E1317Q
701SR	60	70	No	Yes (no details)	E1317Q
SP/WW	?	14	Yes × 2	Yes (?Mendelian)	E1317Q
SM	58	49	Yes	B colon cancer, S adenomas	E1317Q
1612AB	63	96	No	No	E1317Q
848HS	42	5	?	Yes (?Mendelian)	R332X
1582RT	51	80	Yes × 2	F colon cancer	Q394X
1599AM	?	'Multiple'	No	Yes (?Mendelian)[a]	1924 frameshift (5770insA)
755HW	59	5	Yes	B adenoma	1942 frameshift (5824delGACA)

[a]Age (years) at presentation. All patients presented symptomatically, except for SP/WW who was screened as a result of family history of bowel tumours.
?, not known.
[b]Macroscopic polyps, confirmed by histology.
[c]Only colon tumours are considered: M, mother; F, father; B, brother; S, sister.
[d]Also see Frayling *et al*. (8).
[e]Also see Eccles *et al*. (14).

Two patients harboured nonsense germline *APC* variants in the 5′ part of the *APC* gene. Both mutations were in the alternatively spliced region of exon 9 (11). One variant, R332X (Fig. 1b), was in a patient (848HS) of Ashkenazi origin with a strong family history of colorectal adenomas. Affected individuals from this family had considerable variation in disease severity, which is not unusual for families with exon 9 mutations (11,12). The R332X mutation has been reported previously in an Ashkenazi family from Canada by Soravia *et al.* (5). Our family has members in Canada, but this branch appears to be unaffected by colorectal tumours, they live in a different part of the country from the family in Soravia *et al.*'s study (5) and the two kindreds have no known recent common ancestor. Another Swiss R332X carrier was reported by Andreutti-Zaugg *et al.* (13), but the family history and ethnic origins of this individual are unknown.

The second patient with a germline exon 9 *APC* mutation carried a Q394X allele (Fig. 1c). This patient (1582RT) had a family history of colorectal cancer (Table 1). He had developed 80 adenomas and two metachronous colorectal carcinomas by presentation.

Two patients harboured mutations in the 3′ part of the *APC* gene. One of these patients (1599AM) had a codon 1924 frameshift (5770insA) germline change. This mutation has previously been described by Eccles *et al.* (14) in a family with hereditary desmoid disease (HDD) and results in a truncated, and probably unstable, protein of 1947 amino acids. Genealogical investigation showed that the individual whom we studied was related to the same family reported by Eccles *et al.* (14). Multiple colorectal adenomas have now been found in two members of the HDD family. It is quite possible that other family members had adenomas that were not detectable by conventional colonoscopy. The codon 1924 mutation, therefore predisposes both to desmoids and colorectal adenomas and HDD represents a variant of FAP or AAPC, rather than a distinct disease. The second patient (755HW) with a 3′ *APC* mutation, carried a codon 1942 frameshift (5824delGACA) change (Fig. 1d), resulting in a truncated protein of 1969 amino acids. This patient had a family history of colorectal tumours and had developed five adenomas herself (Table 1).

No other truncating or missense germline *APC* variants were detected, apart from the known polymorphisms. A subset of 92 randomly chosen patients was screened for mutations in the putative *APC* promoter 1A, but no variants from the wild-type were detected.

The patients with germline *APC* variants (Table 1) presented at a mean age of 54 years, not significantly different ($P > 0.5$, Student's *t*-test) from patients in whom no pathogenic variants were detected. The patients with identified variants tended to have a stronger family history than other patients (71% positive compared with 46%), but this was not significant ($P > 0.1$, Fisher's exact test). All four patients in this study with a pedigree apparently showing Mendelian inheritance (three generations affected) had germline *APC* variants. Patients with identified germline *APC* variants, developed significantly more adenomas than the rest of the patient set (means of 29 and 14, respectively; $P < 0.05$, Student's *t*-test).

DISCUSSION

We have screened a large number of patients with multiple (3–100) colorectal adenomas for germline variants throughout the entire coding region of the *APC* gene (2843 amino acids), plus the promoter region in a subset of 92 randomly chosen cases. Our aim was to provide a comprehensive analysis of the contribution of germline *APC* mutations and subpolymorphic variants to patients with multiple adenomas. Such patients may comprise ~5–10% of all patients with colorectal tumours and may therefore be more common than both FAP and HNPCC patients. We found *APC* variants with probable pathogenic effects in 9% of our patients. Nevertheless, we believe that a small number of mutations may have been missed owing to the imperfect sensitivity of SSCP with silver staining, whole gene deletions, or mutations in introns. Whilst most patients with multiple adenomas therefore develop their disease owing to unknown genetic and/or environmental factors, an important minority do so, at least in part, as a result of inherited *APC* variants.

We have provided evidence to show that the E1317Q *APC* variant is associated with a predisposition to multiple colorectal tumours (estimated risk ratio = 11.17). E1317Q appears to be associated specifically with multiple colorectal adenomas and weakly with colorectal cancer (15). We have found no *APC* variants in linkage disequilibrium with E1317Q. Some patients with E1317Q developed large numbers of adenomas—a few members of the family of SP/WW even had ~100 adenomas, close to the threshold for classical FAP (J. Sampson *et al.*, unpublished data)—although other E1317Q carriers developed only a few tumours. It is possible that a standardized screening programme for E1317Q carriers would reveal a much more homogeneous phenotype.

Further evidence exists to suggest that E1317Q has a pathogenic effect. It has been reported as a somatic mutation in a sporadic colorectal adenoma (7), as a somatic mutation in a colorectal adenocarcinoma from an I1307K patient (6), and as one of the two detected mutations in the colon cancer cell line SKCO1 (16). Our study of the allelic loss also provides evidence for the functional effects of E1317Q. Sporadic colorectal adenomas of a similar size to those we have studied show allelic loss at a frequency of ~25% (17). In contrast, only 9% of the E1317Q adenomas showed loss ($P < 0.05$, exact binomial). The lower frequency of loss in the E1317Q carriers is more typical of FAP patients with germline mutations early in the gene (18,17) than sporadic cases [although, interestingly, FAP patients with truncating germline mutations close to codon 1317 show a high frequency of allelic loss in their tumours (18)].

The accumulated evidence suggests that E1317Q has some direct pathogenic effect, but how it exerts this is unclear. E1317Q (and I1307K) are in a region of *APC* in which colorectal tumours frequently harbour mutations (19), in which truncating germline mutations cause severe FAP (20) and which is associated with allelic loss as the 'second hit' in FAP polyps (18). The region around codon 1300 lies between the first and second β-catenin binding and degradation repeats of APC (21). Residue 1317 lies in a region without significant homology to other protein domains (data not shown), but this amino acid may play an important role in maintaining the structure of the β-catenin binding and degradation repeats and

hence in preventing tumorigenesis. An effect of E1317Q on mRNA stability or splicing is possible, but has not been demonstrated and is unlikely on theoretical grounds. It remains possible that residue 1307 has at least some functional effect (8).

The absence of further subpolymorphic variants like E1317Q and I1307K from our patient sample suggests that *APC* variants do not contribute a very large proportion of the population risk of colorectal tumours. On the reasonable assumption that E1317Q and I1307K are effectively selectively 'neutral', the absence of other subpolymorphic variants reflects chance (genetic drift) and/or the absence of pathogenic effects of missense mutations in other regions of *APC*. Nevertheless, I1307K and E1317Q have demonstrated that relatively common, reduced-penetrance alleles at Mendelian cancer loci may contribute to tumour risk. Missense variants in other Mendelian cancer genes, such as *hMLH1*, should be assessed fully for their effects on cancer risk (22).

The spectrum of truncating mutations in the 5′ and 3′ regions of *APC* which are associated with multiple adenomas (including AAPC) remains unclear. This reflects several factors: failure of clinicians to consider a diagnosis of AAPC in patients with multiple adenomas and without classical FAP; probable reduced penetrance of AAPC mutations compared with those causing FAP, leading to non-Mendelian pedigrees and absence of colon cancer; genetic heterogeneity [with the *CRAC1* locus (23), for example]; and the difficulty of screening a large gene such as *APC* for mutations. Proper assessment of genotype–phenotype associations for AAPC may also be confounded by differences in endoscopic techniques, which means that microadenomas and/or flat adenomas may be missed (24) and patients misclassified as AAPC when they actually have classical FAP (although such misassessment appeared not to have been a major factor in our set of patients, since none was found to carry an *APC* mutation expected to produce classical FAP). It is notable that, although each presented symptomatically, all four patients in our study with truncating *APC* mutations were discovered to have a family history of colorectal tumours. Thus, all these families could be classified as having AAPC on the basis of their germline mutations and family histories.

With the exception of the I1307K variant, most previous studies of the causes of the multiple adenoma phenotype have been limited to a relatively small number of AAPC families with a strong history of colorectal tumours. In contrast to these studies, our patients were largely ascertained through symptomatic presentation and were therefore more typical of the general population. Among the largest previous studies were those of Spirio *et al.* (4) and Soravia *et al.* (5). Spirio *et al.* (4) studied seven AAPC families and found 5′ *APC* mutations in four of these. Soravia *et al.* (5) screened for truncating mutations in 11 families in which most affected individuals developed 10–100 adenomas. They found the same exon 4 deletion in three kindreds (presumably of common ancestry) and the individual families with N263X (exon 4), R332X (exon 9), 338FS (exon 9) and 2047FS (exon 15) mutations, but their methods would not have detected missense mutations or changes resulting in an unstable protein. With the exception of R332X (see above), we have found none of the mutations reported by Spirio *et al.* (4) or Soravia *et al.* (5).

Pedemonte *et al.* (25) screened the whole coding region of the *APC* gene in 18 patients with multiple (3+) colorectal adenomas, all of whom had a family history of neoplasia (although not necessarily of colorectal tumours). Five patients carried germline changes. One variant was a splice acceptor mutation in intron 8, which produced a novel mRNA species, a frameshift change of uncertain effect was found at codon 2829 (14 amino acids from the end of the protein) and three missense variants were found (two of uncertain effect and one silent change). We have found none of the changes reported by Pedemonte *et al.* (25).

We conclude that ~10% of patients with multiple adenomas have inherited *APC* variants, which account for their disease either alone or in combination with other unknown genetic or environmental factors. These variants are not distributed throughout the *APC* gene, but are restricted to particular regions (specifically, missense variants close to codon 1300 and truncating mutations in exon 4, the alternatively spliced region of exon 9 and 3′ to codon 1580). Occasionally, studies have reported that germline deletions of the *APC* gene are associated with multiple adenomas (9), but other *APC*-deletion families have a classical FAP phenotype. Assessment of germline *APC* deletions can be problematic (9) and was not undertaken in this study.

Different *APC* variants may cause disease through different mechanisms (26). Truncating mutations in the 5′ and 3′ regions may lead to mRNA and/or protein instability. Exon 9 mutations may lead to alternative mRNA splicing to remove the mutant. I1307K apparently acts, at least in part, as a pre-mutation. The effects of E1317Q are unknown, but its position (like I1307K) in a critical region for *APC* function suggests a functional effect. It remains possible that E1317Q acts in concert with other (somatic) APC mutations—for example, by increasing their selective advantage or broadening the spectrum of selected changes (13)—and therefore acts as a susceptibility allele through a less direct mechanism. What is clear, however, is that most multiple adenoma patients do not carry germline *APC* variants in the coding region or promoter. Their disease may result from unidentified genes or from the environment, or both. Nevertheless, in clinical practice, we believe that it is worthwhile to screen multiple adenoma patients for a restricted number of *APC* variants, namely the missense changes E1317Q and I1307K (if of Ashkenazi descent), and—if there is also a family history of colorectal tumours—for truncating mutations 5′ to exon 5, in exon 9 and 3′ to codon 1580. Identification of these variants may allow a more intensive screening of gene carriers and testing of at-risk family members.

MATERIALS AND METHODS

Inclusion criteria for the study were 3–100 synchronous or metachronous colorectal adenomas, developed by an individual from birth to the date of study. Most patients (*n* = 130) were ascertained from individuals presenting to St Mark's Hospital (Harrow, London, UK) with symptoms resulting from their tumours, most of whom underwent one or more colonoscopies. A further set of patients (*n* = 24) was derived from Wexham Park Hospital (Slough, UK). A small number of patients (*n* = 10) was derived from colonoscopic screening of asymptomatic individuals, referred to genetics clinics at St Mark's Hospital or the Institute of Medical Genetics (Cardiff, UK). Of the set of 164 patients, 116 had previously been screened for variants in *APC* exon 15G (codons 1263–1377) only. Three germline

Table 2. Oligonucleotides designed specifically for this study

Primer name	Primer sequence (5′→3′)	Region
41F	TGGAATTTAAGCATCACCTGG	*APC* promoter
320R	AGCAGCTTAGTAGTGGAGAT	*APC* promoter
299F	ACCCAGACGTCCAGAGNTCT	*APC* promoter
580R	ATGGCAGTTGACACGCATAG	*APC* promoter
559F	CGGACCTCCCCCGACTCTTT	*APC* promoter
837R	CTGGCGGGCTGCACCAATAC	*APC* promoter
Exon 10aF	AATCATAACAGGAAGGGGAT	APC exon 10a
Exon 10aR	CAGTCACACCCCCAGAGG	APC exon 10a

I1307K variants and two E1317Q variants in this region were reported in this patient sample by Frayling *et al.* (8), and are included in the results of this larger and comprehensive study. For all patients, clinicopathological details were determined from hospital records. Family history was determined from hospital records and/or a retrospective questionnaire.

Patients provided 10–20 ml of peripheral blood, from which DNA was extracted using standard methods. All codons of the *APC* gene were screened using SSCP analysis to amplify specifically exons 0–14 (including 9a and 10a), and exon 15 in 23 parts (A–W). The oligonucleotides and the reaction conditions were essentially as described by Groden *et al.* (27), supplemented by additional oligonucleotides (Table 2) for more recently discovered exons and the promoter (28). SSCP–PCR products were detected by silver staining on midi-gels or on the Phast system, or by the ABI 310 sequencer (using a PCR product in which both oligonucleotides were dye-labelled). Any SSCP bandshifts were sequenced in forward and reverse orientations from a new PCR product. If any sequence was ambiguous, a further PCR product was cloned into pGEMTeasy (Promega, Southampton, UK) and multiple clones were sequenced. Screening for the known I1307K *APC* variant, which can be difficult to detect using SSCP analysis, was performed using the amplification refractory mutation system (ARMS)–PCR method described by Frayling *et al.* (8).

Allelic loss at *APC* was assessed using the closely linked microsatellite markers *D5S489*, *D5S656*, *D5S2117* and *D5S82*, as described by Lamlum *et al.* (18). Insufficient material was available for screening these tumours for truncating somatic *APC* mutations. The same markers were used to construct haplotypes for selected patients in order to assess the probability of common ancestry.

Statistical analysis was performed using the Fisher's exact test, the Student's *t*-test and the exact binomial. Odds ratios and confidence intervals were calculated using Woolf's method.

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REFERENCES

1. Houlston, R., Collins, A. and Morton, N. (1992) Dominant genes for colorectal cancer are not rare. *Ann. Hum. Genet.*, **56**, 99–103.
2. Beck, N.E., Tomlinson, I.P.M., Homfray, T.F.R., Frayling, I.M., Hodgson, S.V. and Bodmer, W.F. (1997). The frequency of germ-line HNPCC gene mutations in patients with multiple or early-onset colorectal adenomas. *Gut*, **41**, 335–338.
3. Jass, J.R. (1995) Colorectal adenomas in surgical specimens from subjects with hereditary non-polyposis colorectal cancer. *Histopathology*, **27**, 263–267.
4. Spirio, L., Olschwang, S., Groden, J., Robertson, M., Samowitz, W., Joslyn, G. *et al.* (1993) Alleles of the APC gene: an attenuated form of familial polyposis. *Cell*, **75**, 951–957.
5. Soravia, C., Berk, T., Madlensky, L., Mitri, A., Cheng, H., Gallinger, S. *et al.* (1998) Genotype-phenotype correlations in attenuated adenomatous polyposis coli. *Am. J. Hum. Genet.*, **62**, 1290–1301.
6. Laken, S.J., Petersen, G.M., Gruber, S.B., Oddoux, C., Ostrer, H., Giardello, F.M. *et al.* (1997) Familial colorectal cancer in Ashkenazim due to a hypermutable tract in APC. *Nature Genet.*, **17**, 79–83.
7. White, S., Bubb, V.J. and Wyllie, A.H. (1996) Germline APC mutation (Gln 1317) in a cancer-prone family that does not result in familial adenomatous polyposis. *Genes Chromosomes Cancer*, **15**, 122–128.
8. Frayling, I., Beck, N., Ilyas, M., Dove-Edwin, I., Goodman, P., Pack, K. *et al.* (1998) The APC variants I1307K and E1317Q are associated with colorectal tumors, but not always with a family history. *Proc. Natl Acad. Sci. USA*, **95**, 10722–10727.
9. Hodgson, S., Coonar, A., Hanson, P., Cottrell, S., Scriven, P., Jones, T. *et al.* (1993) Two cases of 5q deletions in patients with familial adenomatous polyposis: possible link with Caroli's disease. *J. Med. Genet.*, **30**, 369–375.
10. Lynch, H. and Smyrk, T. (1998) Classification of familial adenomatous polyposis: a diagnostic nightmare. *Am. J. Hum. Genet.*, **62**, 1288–1289.
11. Young, J., Simms, L., Tarish, J., Buttenshaw, R., Knight, N., Anderson, G. *et al.* (1998) A family with attenuated familial adenomatous polyposis due to a mutation in the alternatively spliced region of APC exon 9. *Hum. Mutat.*, **11**, 450–455.
12. Rozen, P., Samuel, Z., Shomrat, R. and Legum, C. (1999) Notable intra-familial phenotypic variability in a kindred with familial adenomatous polyposis and an APC mutation in exon 9. *Gut*, **45**, 829–833.
13. Andreutti-Zaugg, C., Couturier, A., Chappuis, P. and Hutter, P. (1999) Detection of protein truncating mutations in exons 1–114 of the APC gene using an *in vivo* fusion protein assay. *Hum. Mutat.*, **13**, 170–171.
14. Eccles, D., van der Luijt, R., Breukel, C., Bullman, H., Bunyan, D., Fisher, A. *et al.* (1996) Hereditary desmoid disease due to a frameshift mutation at codon 1924 of the APC gene. *Am. J. Hum. Genet.*, **59**, 1193–1201.
15. Popat, S., Stone, J., Coleman, G., Marshall, G., Peto, J., Frayling, I. *et al.* (2000) Prevalence of the E1317Q variant in colorectal cancer patients. *Cancer Lett.*, **149**, 203–206.
16. Rowan, A., Lamlum, H., Ilyas, M., Wheeler, J., Straub, J., Papadopoulou, A. *et al.* (2000) APC mutations in sporadic colorectal tumours: a new mutational 'hotspot' and interdependence of the 'two hits'. *Proc. Natl Acad. Sci. USA*, **97**, 3352–3357.
17. Spirio, L., Samowitz, W., Robertson, J., Robertson, M., Burt, R., Leppert, M. *et al.* (1998) Alleles of APC modulate the frequency and classes of mutations that lead to colon polyps. *Nature Genet.*, **20**, 385–388.
18. Lamlum, H., Ilyas, M., Rowan, A., Clark, S., Johnson, V., Bell, J. *et al.* (1999) The type of somatic mutation at *APC* in FAP is determined by the site of the germline mutation: a new facet to Knudson's 'two-hit' hypothesis. *Nature Med.*, **5**, 1071–1075.
19. Miyoshi, Y., Nagase, H., Ando, H., Horii, A., Ichii, S., Nakatsuru, S. *et al.* (1992) Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. *Hum. Mol. Genet.*, **1**, 229–233.
20. Nugent, K., Phillips, R., Hodgson, S., Cottrell, S., Smith-Ravin, J., Pack, K. *et al.* (1994) Phenotypic expression in familial adenomatous polyposis: partial prediction by mutation analysis. *Gut*, **35**, 1622–1623.
21. Polakis, P. (1997) The adenomatous polyposis coli (APC) tumor suppressor. *Biochim. Biophys. Acta*, **1332**, F127–F147.
22. Drotschmann, K., Clark, A. and Kunkel, T. (1999) Mutator phenotypes of common polymorphisms and missense mutations in MSH2. *Current Biol.*, **9**, 907–910.
23. Tomlinson, I., Rahman, N., Frayling, I., Mangion, J., Barfoot, R., Hamoudi, R. *et al.* (1999) Inherited susceptibility to colorectal adenomas and carcinomas: evidence for a new predisposition gene on 15q14–q22. *Gastroenterology*, **116**, 789–795.

24. Wallace, M., Frayling, I., Clark, S., Neale, K. and Phillips, R. (1999) Attenuated adenomatous polyposis coli: the role of ascertainment bias through failure to dye-spray at colonoscopy. *Dis. Colon Rectum*, **42**, 1087–1090.

25. Pedemonte, S., Sciallero, S., Gismondi, V., Stagnaro, P., Biticchi, R., Haeouaine, A. *et al.* (1998) Novel germline APC variants in patients with multiple adenomas. *Genes Chromosomes Cancer*, **22**, 257–267.

26. Ishidate, T., Matsumine, A., Toyoshima, K. and Akiyama, T. (2000) The APC-hDLG complex negatively regulates cell cycle progression from the G_0/G_1 to S phase. *Oncogene*, **19**, 365–372.

27. Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., Albertsen, H. *et al*. (1991) Identification and characterization of the familial adenomatous polyposis coli gene. *Cell*, **66**, 589–600.

28. Hiltunen, M., Alhonen, L., Koistinaho, J., Myohanen, S., Paakkonen, M., Marin, S. *et al.* (1997) Hypermethylation of the APC (adenomatous polyposis coli) gene promoter region in human colorectal carcinoma. *Int. J. Cancer*, **70**, 644–648.