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 pathognomonic 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

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

aAge (years) at presentation. All patients presented symptomatically, except for SP/WW who was screened as a result of family history of bowel tumours.

bMacroscopic polyps, confirmed by histology.

Only colon tumours are considered: M, mother; F, father; B, brother; S, sister.

Also see Frayling et al. (8).

Also see Eccles et al. (14).
Two patients harboured nonsense germ line 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 Andreotti-Zaugg et al. (13), but the family history and ethnic origins of this individual are unknown.

The second patient with a germ line 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) germ line 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 germ line 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 germ line 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 germ line APC variants. Patients with identified germ line 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 germ line 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 germ line 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 (the 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
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, clinico-pathological 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 mid-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 the Phast system, or by the ABI 310 sequencer (using a PCR product in which both oligonucleotides were dye-labelled).

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