Extracolonic features of familial adenomatous polyposis in patients with sporadic colorectal cancer

Citation for published version:

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published in:
British Journal of Cancer

Publisher Rights Statement:
BJC open

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and/or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Extracolonic features of familial adenomatous polyposis in patients with sporadic colorectal cancer

MG Dunlop1,2, SM Farrington1,2, VJ Bubb3, C Cunningham2,3, M Wright1, LJ Curtis3, ZA Butt4, E Wright4, BW Fleck4, D Redhead1, R Mitchell6, JB Rainey1, IMC Macintyre4, DC Carter1 and AH Wyllie3

1University of Edinburgh, Department of Clinical Surgery, Royal Infirmary, Edinburgh EH3 9YW, UK; 2MRC Human Genetics Unit, Western General Hospital, Edinburgh EH4 2XU, UK; 3Cancer Research Campaign Laboratories, Department of Pathology, University of Edinburgh, Edinburgh EH9 4AG, UK; 4University of Edinburgh, Department of Ophthalmology, Royal Infirmary, Edinburgh EH8 9AG, UK; 5Department of Clinical Radiology, Royal Infirmary, Edinburgh EH3 9YW, UK; 6Department of Maxillofacial Surgery, City Hospital, Edinburgh EH10 5SB, UK; 7Department of Surgery, St John’s Hospital, Livingston, West Lothian EH54 6PP, UK; 8Department of Surgery, Western General Hospital, Cawood Road, Edinburgh EH4 2XU, UK.

Summary We have investigated the occurrence of attenuated extracolonic manifestations (AEMs) of familial adenomatous polyposis (FAP) in patients with non-polyposis colorectal cancer. In a prospective case–control study, we observed that significantly more colorectal cancer patients exhibited AEM than did age- and sex-matched controls (19.5% vs 7.5%, P < 0.004). However patients with AEMs do not have occult FAP, as we found no heterozygous adenomatous polyposis coli (APC) gene mutations. Heterozygous constitutional DNA replication errors (RERs) occur in a proportion of colorectal cancers, particularly right-sided lesions, and in almost all tumours from hereditary non-polyposis colorectal cancer (HNPCC) patients. As AEMs have been reported in familial colon cancer cases, we investigated the relationship of AEM to tumour RER phenotype. There was indeed an excess of AEMs in patients with right-sided tumours (30.2% of 116 patients, P = 0.03) and in those with RER tumours (3 out of 12 patients with RER tumours vs none out of 21 patients with non-RER tumours, P < 0.05). Two patients with AEM were from HNPCC families compared with none of those without AEM (P < 0.05). The association of AEMs with colorectal cancer is intriguing, and we speculate that it may be a manifestation of mutational mosaicism of the APC gene, perhaps associated with a constitutional defect in DNA mismatch pair.

Keywords: colorectal cancer; genetic instability; APC gene

An association has been reported between sporadic colorectal cancer and two clinical features that are normally considered to be extracolonic manifestations of the autosomal dominant syndrome familial adenomatous polyposis (FAP) (Sondergaard et al., 1993; 1995a, b; Houlston et al., 1992; Dunlop, 1983; Hunt et al., 1994). These features, congenital hypertrophy of the retinal pigment epithelium (CHRPE) and mandibular osteomas, occur in 90% and 75% respectively of patients with classical FAP (Utsunomiya and Nakamura, 1975; Bulow et al., 1984; Traboulsi et al., 1987; Berk et al., 1988; Chapman et al., 1989; Giardiello et al., 1991; Hodgson et al., 1994; Wallis et al., 1994). The finding of such extracolonic features in patients with non-FAP colorectal cancer is of considerable interest because of the central, and probably initiating, role of the gene for FAP (APC) in colorectal tumorigenesis. Constitutional and heterozygous mutation of the APC gene is responsible for the FAP syndrome (Groden et al., 1991; Nishihio et al., 1991; Nagase and Nakamura, 1993), while a high proportion of non-FAP colorectal adenomas and cancers have somatic APC mutations (Powell et al., 1992; Ichii et al., 1993; Nagase and Nakamura, 1993). Inactivating APC mutations have also been noted in dysplastic aberrant crypt foci (Jen et al., 1994; Smith et al., 1984), which are believed to be the very earliest histological manifestation of colorectal neoplasia.

The available data suggest that the extracolonic manifestations of FAP, which we term attenuated extrachoroidal manifestations (AEMs), occur particularly in colorectal cancer patients with a family history of the disease (Sondergaard et al., 1985b; Houlston et al., 1992; Hunt et al., 1994). Thus we wished to determine whether there was any link with hereditary non-polyposis colorectal cancer (HNPCC), which is responsible for 2–5% of all colorectal cancer cases. HNPCC gene carriers develop few colorectal adenomas but are at high risk of colorectal cancer, particularly of the right colon, as well as other cancers (Lynch et al., 1993; Jass et al., 1994). Causative germline mutations in the DNA mismatch repair (MMR) genes hMSH2, hMLH1, hPMS1 and hPMS2 (Liu et al., 1996) have been identified in HNPCC families. Such mutations result in defective MMR (Parsons et al., 1993; Umar et al., 1994), manifest as instability of repetitive DNA in tumours (Ionov et al., 1993; Aaltonen et al., 1993; Thibodeau et al., 1993; Peltomaki et al., 1993; Lothe et al., 1993; Aaltonen et al., 1994; Bhattacharyya et al., 1994; Liu et al., 1995a) and termed replication error (RER) tumour phenotype. Somatic mutations in GTBP, another MMR gene, are responsible for the RER phenotype in some tumours (Drummond et al., 1995; Palombo et al., 1995; Papadopulous et al., 1995). However, germline GTBP mutations have not been identified in any of the highly penetrant HNPCC families analysed so far (Papadopoulous et al., 1995), although GTBP involvement in low-penetration colorectal cancer susceptibility has not been excluded. The RER tumour phenotype is present in about 15% of sporadic colorectal cancers (Ionov et al., 1993; Aaltonen et al., 1993; Thibodeau et al., 1993; Lothe et al., 1993; Liu et al., 1995a), 80–90% of which are in the proximal colon (Ionov et al., 1993; Thibodeau et al., 1993; Lothe et al., 1993).

We have investigated the prevalence of AEMs in a prospective case–control study. In order to elucidate the underlying basis of this association, we evaluated AEM in relation to tumour location, family history and tumour RER phenotype to ascertain any link with MMR deficiency and HNPCC. As we have excluded the possibility of unrecognised cases of FAP by extensive APC gene mutation analysis, our
observations are consistent with the hypothesis that AEMs are a manifestation of mutation mosaicism of the APC gene and may indicate genetically determined cases in a population of sporadic colorectal cancer patients.

Methods
Study population and pedigree ascertainment
In order to obtain an unselected patient population group, we enrolled 169 patients prospectively after surgery for colorectal cancer in four Lothian hospitals. The four hospitals together serve the entire population of Lothian and admit essentially all patients in the region with colorectal cancer on an emergency and elective basis. Thus, the study population should be reasonably free of bias. Known FAP cases or patients with colonic multiple polyposis were excluded. Paired age and sex-matched healthy community controls with no personal cancer history were enrolled from patients registered with local Edinburgh medical practices that refer patients to three of the same four hospitals from which the cancer patients were recruited. Family histories were documented in a standard proforma and verified from statutory Scottish General Register Office records for first- and second-degree relatives with cause of death, where appropriate. Disease status for live relatives in Scottish kindreds and members of families resident outside Scotland was verified from clinical or pathology records. All personal data, family history and retinal and mandible findings together with data on tumour histology, staging and site were collated in a computer database. Local ethics committee approval was granted for all aspects of this study, including mandibular radiography, ophthalmoscopy with mydriasis and blood sampling.

Retinal and mandible examinations
Bilateral retinal examination was performed in 167 of the patients and 160 of the controls described above by indirect ophthalmoscopy (Model Omega 100, Heine, Germany), using a Nikon 20D lens after mydriasis by one of two ophthalmologists. All efforts were made to ensure that the ophthalmologists were unaware of the subjects’ health status and did not discuss recent operations or family history with the patients. Retinal lesions were recorded and photographed. Thirty patients (with and without CHRPE) were assessed by both ophthalmologists, and this ensured inter-observer reproducibility. The diagnostic criteria we used for retinal pigmentation in non-polyposis colorectal cancer patients were the same as those taken as diagnostic of CHRPE in familial adenomatous polyposis, namely: 

1. Pigmented lesions with depigmented halo; 
2. Pigmented lesions over one optic disc diameter; 
3. Small bilateral pigmented lesions without a halo or >4 small unilateral lesions (Traboulsi et al., 1987; Chapman et al., 1988; Berk et al., 1988; Giardello et al., 1991; Hodgson et al., 1994; Wallis et al., 1994). Because of the variety of CHRPE lesions, we devised a scoring system derived from the relative importance ascribed to each feature in previous FAP studies. Pigmented haloed lesions and lesions >1 disc diameter scored 5; small lesions scored 1 and the total score for bilateral lesions was doubled.

Posteroanterior, occlusal and pantomographic radiographic views of the mandible were obtained using Roentgen 501, Siemens Heliodent MD and Morita Panex EC machines respectively on 163 patients and 159 controls, with immediate re-view and further oblique or intra-oral views for inconclusive cases. Radiographs were interpreted independently on two separate occasions by a radiologist and an oral surgeon blinded to the subjects’ disease status. Osteomas were defined as discrete homogeneous radio-opaque areas >2 mm in diameter with no surrounding radiolucent zone, distinct from the teeth and apices (Bulow et al., 1984; Sondergaard et al., 1985a, b; 1993).

APC gene screening
We wished to determine whether any of our findings could be explained on the basis of the constitutional and heterozygous APC mutations associated with a weakly expressed form of FAP. Hence, we searched for truncating APC mutations in all coding sequences from exon 9 to the termination signal at the 3’ end of exon 15, which includes all regions of APC known to be associated with expression of the CHRPE phenotype in FAP (Olschwang et al., 1993). Peripheral blood from all 33 patients exhibiting AEM was screened by an in vitro synthesised protein assay (IVSP) (Powell et al., 1993; Prosser et al., 1994) for translation-terminating APC gene mutations and by heteroduplex analysis (Prosser et al., 1994; Keen et al., 1991) for each of exons 9–14. Positive control samples from FAP patients and manufacturer’s control reactions were always run in parallel. The APC gene was amplified by polymerase chain reaction (PCR) in overlapping fragments from DNA and RNA purified from peripheral blood with forward PCR primers, including signals for transcription and translation. RNA was available for five patients in whom exons 1–14 were analysed by IVSP of reverse-transcribed complementary DNA as described (Prosser et al., 1994). Resultant PCR products were used in a coupled transcription—translation reaction (Promega, UK) generating radiolabelled synthetic poly peptide sequences, which were analysed by SDS–PAGE followed by autoradiography. Approximately 500 bp of the most 5’ part of APC exon 15 was analysed by heteroduplex analysis in overlapping fragments using the PCR primers as described (Grodan et al., 1991) to exclude any mutations occurring in the most 5’ region of exon 15, which would result in such a short peptide in the IVSP assay that a mutation could be masked. Exons 9–14 were analysed separately in all 33 patients by heteroduplex analysis of PCR-amplified leucocyte DNA with electrophoresis on MDE™ gels (JT Baker, USA) and ethidium bromide staining (Keen et al., 1991). Any heteroduplex variants between normal and tumour DNA were reamplified and sequenced.

Tumour DNA instability analysis
As microsatellite DNA instability (RER phenotype) has been observed in colorectal cancers and is the result of inherited or of somatic mutation in one of the DNA mismatch repair genes (Leach et al., 1993; Parsons et al., 1993; Liu et al., 1995a; Papadopoulos et al., 1995), we wished to correlate our findings of the AEM phenotype with such tumour DNA instability. We were able to obtain fresh surgical resection specimens form 33 of the patients recruited to the study at one of the participating hospitals (12 with and 21 without AEMs). DNA was purified from tumour and normal mucosa and RER status assessed by comparison of PCR-amplified paired tumour/control DNAs at six PCR-amplified (CA), repeat marker loci: D2S123, D2S119, D3S1293, D8S282, D13S160 (Gyapay et al., 1994) and at a polyadenine tract; BAT40 (Liu et al., 1995b). Formamide-denatured PCR products were electrophoresed on 6% denaturing acrylamide gels in 0.5 x TBE buffer and silver-stained (Bassam et al., 1991). All aberrant banding patterns indicative of RER phenotype were confirmed by repeat analyses. Tumours were considered to exhibit the RER phenotype when band alterations were present at two or more loci when compared with matched control DNA as described previously (Liu et al., 1995a, b).

Results
Study group characteristics and family history
Cancer patients and control subjects were well age and sex matched, although some paired control subjects did not attend. There were 71 females and 98 male patients (mean age 63.1 years, range 20–92 years) and 69 female and 91 male controls (mean age 62.4 years, range 32–86 years). One hundred and fifty-one patients (89%) and 141 controls (87%) were of Scottish descent. More patients than controls had
first-degree relatives with colorectal cancer (40/169, 24% vs 16/162, 10%; \( P < 0.003 \)). The index case was excluded to avoid bias in the ascertainment of HNPCC families, as, by definition, controls cannot contribute a colorectal cancer case. Two of the patient kindreds (1.2%) fulfilled HNPCC criteria (Vasen et al., 1991) but none of the control families did so. Sixteen patient (9.5%) and no control families \( (P < 0.0002) \) fulfilled more relaxed criteria for genetically determined cases \( (> 2 \) colorectal and \( > 1 \) other HNPCC cancers in a first-degree kinship) (Mecklin, 1987; Kee and Collins, 1991).

**Prevalence of AEM in colorectal cancer patients**

The prevalence of retinal and/or mandibular extracolonic manifestations of FAP was significantly higher in patients than in controls \( (33/169, 19.5\% \text{ vs } 12/160, 7.5\%; \ P < 0.004) \), and the major contribution to this excess was retinal pigmentation. Summary data of the proportion of each patient group with AEMs are shown in Table I. Although we assessed a reasonably large study population at the outset and the differences that we observed do reach statistical significance, it should be noted that the numbers in some of the subcategories are small. Nonetheless, there does appear to be an association of AEMs with colorectal cancer and, within the cancer group, with proximal tumour location, with an RER phenotype and with a family history of HNPCC.

A representative case of multiple small patches of retinal pigmentation is shown in Figure 1. Although the features were similar to that seen in FAP, they were of lower magnitude, being generally smaller, and there were none of the typical large ‘bear-track’ lesions seen in FAP (Berk et al., 1988; Hodgson et al., 1994). However, there was a substantial excess of patients with retinal pigmentation, as 22/167 patients (13%) vs 8/159 control subjects (5%) had lesions which fulfilled published diagnostic criteria for FAP patients (Berk et al., 1988; Chapman et al., 1988; Wallis et al., 1944; Hodgson et al., 1994) \( (P < 0.02) \). Retinal pigmentation expressed as CHRPE score for both patients and controls is shown in Figure 2. In a sign test comparison of matched patient/control pairs, patient CHRPE scores were significantly greater than those of controls (Figure 2). For this analysis, there were 160 pairs as not all control subjects attended, and so the corresponding paired patients were excluded. Patient scores exceeded controls in 45 and were less than controls in 22 comparisons \( (P < 0.01) \). Both patients from HNPCC families had CHRPE, and 32% of patients with CHRPE had a first-degree relative affected by bowel cancer.

One of the control subjects with striking retinal pigmentation had a father and aunt with colorectal cancer. In the control group as a whole, in which we were able to confirm a negative family history, CHRPE prevalence was substantially less than in the colorectal cancer patients \( (4.9\% \text{ vs } 19.5\%, \ P < 0.02) \).

Osteoma prevalence was almost 3-fold greater in patients than in control subjects, although osteoma prevalence alone did not reach statistical significance owing to the small numbers. Thirteen of 163 patients (8.0%) and 5 of 159 (3%) controls undergoing mandibular radiography had \( > 1 \) osteoma. An orthopantomogram from a patient with colorectal cancer is shown in Figure 3.

**Table I** Prevalence of extracolonic manifestations of FAP (AEMs) in patients with sporadic colorectal cancer and in healthy control subjects. Relationship of AEMs to patient family history of HNPCC, tumour location and tumour RER phenotype

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Individuals with AEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control subjects</td>
<td>160</td>
<td>12 (7.5%)</td>
</tr>
<tr>
<td>Colorectal cancer patients</td>
<td>169</td>
<td>33 (19.5%)</td>
</tr>
<tr>
<td>Right-sided tumour</td>
<td>53</td>
<td>16 (30.2%)*</td>
</tr>
<tr>
<td>Distal tumour</td>
<td>116</td>
<td>17 (14.7%)*</td>
</tr>
<tr>
<td>RER tumour phenotype</td>
<td>12</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Non-RER tumour</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>FH* of HNPCC</td>
<td>2</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>No FH* of HNPCC</td>
<td>31</td>
<td>0</td>
</tr>
</tbody>
</table>

*FH, family history. *\( P < 0.05 \).

**APC gene mutation analysis**

There were no constitutional truncating APC mutations identified in peripheral blood DNA for RNA in 33 patients with AEM (data not shown). The combination of IVSP assays of exon 15 and heteroduplex analysis of exons 9–14 for all patients, along with IVSP analysis of cDNA for exons 1–14 in five patients, excludes the presence of heterozygous truncating APC mutations in those patients with extracolonic features of FAP. Hence, unrecognised cases of FAP cannot explain these observations.

**Tumour clinicopathological characteristics**

There were 172 carcinomas in 169 patients, three patients (1.8%) having synchronous tumours. There were 14 Dukes'
stage A (8.1%), 85 stage B (49.4%), 73 stage C (42.5%). There was an association between the presence of AEMs and right-sided tumours (Table II). Right-sided tumours have also been shown to be associated with a RER phenotype (Ionov et al., 1993; Thibodeau et al., 1993; Lothe et al., 1993; Kim et al., 1994). Sixteen of 53 patients (30.2%) with right (i.e. caecal, ascending and hepatic flexure) colonic tumours had AEMs compared with 17 of 116 patients (14.7%) with more distal lesions (P < 0.032). Interestingly, two of the patients with synchronous tumours (66%) had AEMs while the third had two small CHRPE lesions.

Tumour DNA instability and AEM

We analysed DNA from a total of 33 tumours for the presence of genetic instability. 12 from patients with AEMs and 21 from patients without AEMs. In all, three tumours exhibited an RER phenotype, and all of these tumours were from patients with AEMs. Thus, three tumours from the 12 patients (25%) and none from the 21 patients without AEMs were RER tumours (P < 0.05). Representative silver-stained gels demonstrating the RER phenotype at the marker locus D13S160 in three of the four tumours are shown in Figure 4. Although we did not consider band shifts at only one locus to be diagnostic of RER phenotype, four of the seven patients (57%) with tumours exhibiting RER at ≥1 locus had AEMs, and the tumour was proximal in all of these cases.

Discussion

The results of this case–control study establish the association between colorectal cancer and extracolonic features normally associated with the autosomal dominant syndrome of FAP. Our observations support and extend previous reports that have noted extracolonic manifestations of FAP in patients with colorectal cancer (Sondergaard et al., 1985a; 1993; Houlston et al., 1992; Hunt et al., 1994). An association between sporadic colorectal cancer and mandibular osteomas has been reported in Scandinavia (Sondergaard et al., 1985a; b; 1993), and an excess of CHRPE in selected patients with familial non-polyposis colorectal cancer has been reported in the UK (Houlston et al., 1992; Hunt et al., 1994). Our findings accord well with UK study of Hunt et al. (1994), in which a lower patient prevalence of mandibular osteomas than that reported in Scandinavia (Sondergaard et al., 1985a; 1993) was observed. These population differences may indicate the involvement of environmental factors. In a recent study by the same Scandinavian authors, there was no evidence of an excess of CHRPE in a small cohort of 34 colorectal cancer patients (Hartvigsen et al., 1995). However, patients with a family history of colorectal cancer were specifically excluded from that study and so comparison with our findings is not possible. Nonetheless, taken together, these observations have substantial clinical relevance and suggest that currently healthy individuals with retinal pigmentation or craniofacial osteomas typical of AEMs may be at increased risk of colorectal cancer. Further studies are required to determine if the controls in this study who had evidence of AEMs have a higher than expected incidence of colorectal neoplasia.

The first of two lines of investigation that we pursued, to elucidate the molecular basis of the observed excess of AEM

Table II Location of 172 colorectal cancers in 169 patients and AEM status. There were three patients with a total of two synchronous tumours each, and these are discussed in the text

<table>
<thead>
<tr>
<th>Anatomical site</th>
<th>Number (%) of tumours (n = 172)</th>
<th>Number (%) of patients with AEMs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caecum</td>
<td>31 (18%)</td>
<td>6 (19.4%)</td>
</tr>
<tr>
<td>Ascending colon</td>
<td>15 (8.2%)</td>
<td>7 (46.7%)</td>
</tr>
<tr>
<td>Hepatic flexure</td>
<td>7 (4.1%)</td>
<td>3 (42.9%)</td>
</tr>
<tr>
<td>Transverse colon</td>
<td>10 (5.8%)</td>
<td>1 (10.0%)</td>
</tr>
<tr>
<td>Splenic flexure</td>
<td>6 (3.5%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>Descending colon</td>
<td>8 (4.7%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Sigmoid</td>
<td>34 (20%)</td>
<td>6 (17.6%)</td>
</tr>
<tr>
<td>Rectum</td>
<td>61 (35.7%)</td>
<td>10 (16.4%)</td>
</tr>
</tbody>
</table>

Figure 2 Plot of CHRPE scores from cases and controls demonstrating that a significantly greater proportion of patients had CHRPE scores ≥5, corresponding to previous CHRPE diagnostic criteria (22/167, 13% vs 8/159, 5%; P < 0.02).

Figure 3 Orthopantomogram from a patient with colorectal cancer with several radiodense regions in the body of the mandible (arrowed).

Figure 4 Representative silver-stained amplification products after acrylamide gel electrophoresis for the marker D13S160 from tumour and normal mucosa DNA from four patients. Novel, tumour specific bands are present in tumours A, B and D indicating tumour DNA instability. In tumour A there has been a contraction from normal in the repeat DNA at the D13S160 locus, whereas in tumour B there has been an expansion. Tumour and normal are identical at the D13S160 locus in patient C.
in colorectal cancer patients, was to exclude the presence of constitutional APC gene mutations, as some cases might be due to unrecognised FAP. We excluded such heterozygous APC mutations as far as is reasonably practical in all cancer patients with AEMs. APC mutations associated with expression of the CHRPE phenotype in FAP patients are restricted to a segment downstream of exon 8 (Olschwang et al., 1993). We employed sensitive mutation detection techniques to carry out a detailed analysis of this entire region in blood leucocyte DNA or RNA from all patients exhibiting the AEM phenotype (Powell et al., 1993; Prosser et al., 1994). Constitutional APC gene defects upstream of intron 4 can induce an attenuated FAP phenotype with very few polyps and the late onset of cancer (Spirio et al., 1993). Although mutations in exons 1–4 could be responsible for some cases of apparently sporadic colorectal cancer, CHRPE is never present in FAP cases with such mutations (Olschwang et al., 1993) and so cannot explain our findings. Another explanation that seems improbable concerns the possibility that constitutional APC mutations resulting only in amino acid alterations could be responsible for both AEM and colorectal cancer susceptibility. However, mutations resulting in protein truncation or in substantial reduction in intracellular APC protein concentration is an absolute requirement for the development of FAP (Nagase and Nakamura, 1993; Powell et al., 1993). In addition, truncating mutations are found almost universally in sporadic colorectal cancer (Nagase et al., 1992; Ichihara et al., 1992) adenomas (Powell et al., 1992; Ichihara et al., 1993) and even in some aberrant crypt foci (Jen et al., 1994; Smith et al., 1994). This argues strongly that mis-sense APC mutations are not responsible for our findings of an association of colorectal neoplasia and AEMs.

Next, we determined the proportion of patients with AEMs that exhibited genetic instability, as mutations in more than one anonymous repeat DNA fragment have been observed in normal tissues in a subset of HNPPC patients with a tumour RER phenotype (Parsons et al., 1995). Although the numbers of cases in some of the subcategories were small, we did observe a statistically significant association between AEMs and a familial history of HNPPC, a proximal tumour preponderance and tumour DNA instability, all of which are very suggestive of the involvement of a non-APC gene defect. In fact, not all patients with AEMs had a tumour with the RER phenotype may be due to failure of our RER assay to identify subtle DNA repair defects resulting from particular mutations in the known MMR genes hMSH2, hMLH1, hPMS1 and hPMS2. It is also possible that defects within other genes known to participate in DNA mismatch repair may induce a weak, constitutional mutator phenotype. One such obvious candidate is GTBP, for which no germline mutations have been found in large classical HNPPC families (Papadopoulos et al., 1995), but low-penetrance mutations in GTBP may be responsible for a proportion of apparently sporadic colorectal cancer. Several other genes homologous with the known MMR genes are also currently under investigation by a number of research groups. Such genes may result in a low level of genetic instability, which could explain the lack of tumour RER phenotype in all patients with AEMs.

There is already evidence to support the notion that defective DNA mismatch repair can result in a constitutional mutator phenotype and that such a phenotype results in predisposition to cancer. Genetic instability has been observed in normal tissue and sperm from transgenic mice with a homologous inactivation of MLH1 and MSH2 (de Wind et al., 1995; Baker et al., 1995). The constitutional mutator phenotype that has been observed in HNPPC patients with only a heterozygous MMR gene mutation (Parsons et al., 1995) suggests that, in some cases, a dominant-negative effect may be involved. These previous studies set the precedent that MMR deficiency could induce somatic mutational mosaicism at simple repeat arrays. We suggest that such somatic mosaicism could also involve mutation at the APC gene. Such mutational mosaicism would be expected to be associated with an elevated colorectal cancer risk in addition to the occurrence of other features of FAP, albeit less frequently and to a lesser degree than in true FAP cases. Thus, we propose that the association of AEM and DNA instability reported here is best explained on the basis of APC mutations occurring in a small proportion of all normal somatic cells, including cells contributing to colorectal mucosa, retinal pigment epithelium and osseous tissue. This explanation invokes the notion of APC mutational mosaicism induced by DNA instability.

Further support for a link between extracolonic features of FAP and susceptibility to colorectal cancer mediated by genetic instability comes from three different sources. One descriptive report documents a family in which several family members were affected by colorectal cancer (Maher et al., 1992). In this family, there were also a number of relatives with fibrous tumours typical of desmoid disease, a feature well described in classical FAP and affecting 5–7% of polyposis patients. Further supporting evidence concerns the association of brain tumours with colonic polyposis in Turcot’s syndrome. The genetic basis of Turcot’s syndrome in most cases is germline APC mutation, identical to the mutations seen in classical FAP (Hamilton et al., 1995). However, in a minority of cases, germline mutation of a mismatch repair gene, not APC, is responsible. If this is correct, then this is one indication that the RER phenotype does have an important influence from the very earliest stages of neoplastic transformation in the colorectum. Our own recent findings indicate that genetic instability has a marked influence on the occurrence of APC mutations during neoplastic transformation in the colorectal epithelium (Huang et al., 1996). In that study, we found that simple repeats within the APC gene, especially poly-A-transeptations, appear to be particular targets for replication slippage mutations in tissues that are defective in MMR. Thus normal tissues, including the germline, in patients with relative MMR deficiency might also acquire mutations in the APC gene. Somatic mutational mosaicism has been observed in rare cases in other heritable cancer syndromes, including Li–Fraumeni syndrome (Kovat et al., 1992), retinoblastoma (Greger et al., 1990) and Wilms’ tumour (Chao et al., 1991; Zaidi et al., 1992).

Mosaicism may explain why the retinal and mandibular changes that we observed in this study group were more subtle that those seen in FAP patients. Despite every cell carrying a mutant APC allele in FAP patients, only a small proportion of cells contribute to retinal and osseous lesions. Indeed, even in the colorectum, there are some normal cells; and some FAP patients have relatively few colonic adenomas. Our proposed model implies that only a small proportion of retinal and mandibular cells carry a mutant APC allele, and so the number and extent of the retinal, osseous and indeed colorectal lesions would be expected to be proportionately less. Such mosaicism could represent a mechanism by which mutations in expressed genes involved in cancer development could accumulate in stem cells in adult, or even in intrauterine, life without a requirement for induction of clonal expansion. Thus, the degree of mandibular and retinal changes would be indicative of the level of mosaicism and perhaps even cancer risk. It is intriguing that two of the three patients with synchronous tumours had AEM while the third also had two small CHRPE lesions. Definitive proof of somatic APC mosaicism in patients with AEM will require further extensive detailed molecular analysis.
exhibiting AEMs and the association of AEM with tumour DNA instability. The strong family history of colorectal cancer in a control subject with marked retinal pigmentation is also suggestive of a real association between AEM and familial cancer susceptibility.

In conclusion, we have demonstrated an excess of extracranial features of FAP in an unselected patient group with colorectal cancer but without the colonic polyposis characteristic of FAP or a constitutional mutation in one allele of the APC gene. Although the relationship is not exclusive, the phenotype does appear to be linked with proximal tumour location, the RER tumour phenotype and with a family history of HNPC, all of which are known to be associated with defective DNA mismatch repair. Further studies are required to investigate these novel observations and to elucidate this potential role for MMR deficiency in cancer predisposition. In addition, follow-up of healthy individuals found to have AEM in this study may determine whether there is indeed an associated increase in colorectal cancer risk and whether the presence of AEM merits colonooscopic surveillance.

Acknowledgements
We thank N Brown for clinical organisation, R De Mey and A Fordyce for genealogy, A Carothers for statistical advice, M Phillips for oral radiography and the general practitioners of Muirhouse and Blackhall for access to matched control subjects. In particular, we thank all patient and control subjects who participated in this study. This work was supported by grants from the Cancer Research Campaign (SP226/0101, SP1370/0501), Scottish Hospitals Endowment Research Trust (SHERT 1042), Edinburgh University Cancer Research Fund, Sir Stanley and Lady Davidson Fund and the Melville Trust.

References


