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Can resistant starch and/or aspirin prevent the development of colonic neoplasia? The Concerted Action Polyp Prevention (CAPP) 1 Study

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Loss of function of the adenomatous polyposis coli (*APC*) tumour suppressor gene through truncating mutations or other means is an early event in most colo-rectal cancer (CRC). The *APC* gene encodes a large multifunctional protein that plays key roles in several cellular processes, including the wnt signalling pathway where an intact APC protein is essential for down regulation of β-catenin. The APC protein also plays a role in regulation of cell proliferation, differentiation, apoptosis, cell–cell adhesion, cell migration and chromosomal stability during mitosis. Acquisition of a non-functional *APC* gene can occur by inheritance (in the disease familial adenomatous polyposis (FAP)) or by a sporadic event in a somatic cell. Whilst there is strong epidemiological evidence that variation in diet is a major determinant of variation in CRC incidence, conventional adenoma recurrence trials in sporadic cases of the disease have been relatively unsuccessful in identifying potentially protective food components. Since the genetic basis of CRC in FAP and in sporadic CRC is similar, intervention trials in FAP gene carriers provide an attractive strategy for investigation of potential chemo-preventive agents, since smaller numbers of subjects and shorter time frames are needed. The Concerted Action Polyp Prevention (CAPP) 1 Study is using a 2×2 factorial design to test the efficacy of resistant starch (30 g raw potato starch–Hylon VII (1:1, w/w)/d) and aspirin (600 mg/d) in suppressing colo-rectal adenoma formation in young subjects with FAP. Biopsies of macroscopically-normal rectal mucosa are also being collected for assay of putative biomarkers of CRC risk.

Adenomatous polyposis coli: Colo-rectal cancer: Familial adenomatous polyposis: resistant starch: Asprin

Genetic basis of colon cancer

At its most fundamental, colo-rectal cancer (CRC) is a genetic disease due to loss of function of tumour suppressor genes and the activation of oncogenes. These genetic defects give the tumour cell competitive advantages (Hanahan & Weinberg, 2000) including: ability to replicate faster and/or indefinitely; self-sufficiency in growth signals; reduced sensitivity to apoptotic signals; resistance to anoxia; ability to evade immunosurveillance. Tumour suppressor genes may be inactivated by mutations in, or chromosomal loss of, both alleles or by hypermethylation of the promoter region of the gene. The latter process is potentially reversible. This loss of function results in failure to regulate key cellular processes, including proliferation, apoptosis and differentiation. Oncogenes (derived from normal cellular genes known as proto-oncogenes) encode oncoproteins, which can transform cells to malignancy. Mutations result in the oncoprotein having a novel structure (and, therefore, function), being present where it is normally absent or being expressed at higher than normal levels. In the latter two cases the mutation results in altered transcriptional control.

Approximately 3–5% of CRC cases have a known dominantly-inherited predisposition, another 5% of CRC

Abbreviations: APC, adenomatous polyposis coli; CRC, colo-rectal cancer; EB1, end-binding protein 1; FAP, familial adenomatous polyposis.
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families have highly-penetrant predisposing genes yet to be identified and in a further 5–10% there is a considerable family history (Burn *et al*. 2001) with ≥ 75% of cases being described as 'sporadic'. The best-characterised inherited predispositions to CRC are familial adenomatous polyposis (FAP) and hereditary non-polyposis colo-rectal cancer (HNPCC), but other rare syndromes include Juvenile polyposis, basal cell naevus syndrome (Gorlin's syndrome), Peutz-Jeghers syndrome and Turcot's syndrome (Burn *et al*. 2001). FAP is caused by germline mutations in the adenomatous polyposis coli (*APC*) gene (see p. 54), whilst hereditary non-polyposis HNPCC results from an inherited defect in one of the genes encoding the DNA mismatch repair system. From the perspective of CRC aetiology and prevention, the key finding is that the gene defects responsible for these relatively rare inherited syndromes are acquired in somatic cells and give rise to 'sporadic' CRC.

Diet in the aetiology and prevention of colo-rectal cancer

There is an approximately 15-fold range in age-standardised incidence of CRC across the world and good epidemiological evidence that much of this variation can be attributed to variation in food intake patterns (Department of Health, 1998). However, it has proved rather difficult to identify which foods or food components, in what quantities and over what time periods, are protective and which confer increased risk. The UK Working Group on Diet and Cancer concluded that there 'is moderately consistent evidence that diets with less red and processed meat and with more vegetables and fibre are associated with lower risk of colo-rectal cancer. Evidence is inconsistent for vitamins A, C and E, and β-carotene.' (Department of Health, 1998). The strongest evidence for protection (or, indeed, adverse effect) comes from suitably designed intervention trials but there have not been, to our knowledge, any nutritional intervention trials in CRC in which cancer has been the endpoint. Most of the trials to date have used surrogate end points, including putative biomarkers of risk such as altered mucosal cell dynamics or histological changes such as adenoma recurrence. Several recent trials using adenoma recurrence as the primary end point have shown no benefits of increased dietary fibre intake alone (for systematic review, see Asano & McLeod, 2002) or in combination with increases in intakes of fruit and vegetables and reduced fat intake (Schatzkin *et al*. 2000) despite the encouraging evidence from observational epidemiological studies (Department of Health, 1998). There appear to be no intervention studies with meat or meat products as the intervention agent. As only about one in ten adenomas in the large bowel will become a carcinoma and the factors that cause progression to carcinoma may be different from those that initiate polyp growth, it is possible that increased intakes of dietary fibre and other food components could prevent carcinoma development, with such benefits being difficult to detect in adenoma recurrence trials.

On the basis of the evidence that *APC* mutations are important in the early development of most CRC tumours (Powell *et al*. 1992) and that mutations in *APC* are causal for FAP, it is argued that FAP subjects are good models of *APC*-driven carcinogenesis and would be good models for understanding the impact of exogenous materials (drugs and dietary agents) on tumour development in CRC.

The adenomatous polyposis coli gene and its protein

The *APC* gene located at chromosome *5q21* was cloned in 1991 (Groden *et al*. 1991; Kinzler *et al*. 1991), spans an estimated 98 kb genomic DNA (Thliveris *et al*. 1996) and is normally composed of fifteen exons, which combine to give a coding sequence of 8972 bp. The most 3′ exon is unusually large at 7 kb (Fig. 1).

APC contains at least twenty-one exons, of which seventeen are presumed to be coding because they are downstream of an in-frame initiation codon (Santoro & Groden, 1997). Several splice variants have been described, including those in the non-coding exons 1A and 1B (Horii *et al*. 1993) and in codon 9 (Groden *et al*. 1991) and Sulekova & Ballhausen (1995) reported a novel coding exon of 54 bp between exons 10 and 11. The proteins encoded by alternatively-spliced variants of *APC* have been detected in neural tissue (Pyles *et al*. 1998), and other tissue specific variants are probable. A brain-specific *APC* homologue (designated *APCL*) has been located on chromosome *19p13.3*, which appears to have similar functions to *APC* in that particular tissue (Nakagawa *et al*. 1998).

The *APC* gene encodes a 2843-amino acid protein with a molecular mass of approximately 310 kDa (Groden *et al*. 1991). This large protein includes several structural and functional domains (see Fig. 2), through which the APC protein interacts with other cellular proteins.

Domains at the NH_2 terminal of the protein include the coiled-coil region, which mediates homo-oligomerisation. Probably the most intensively studied domains are the three fifteen amino acid repeats (between amino acids 1020 and 1169) and the seven twenty amino acid repeats (between amino acids 1262 and 2033), which are responsible for binding (Su *et al*. 1993) and down regulation (Rubinfeld *et al*. 1997) respectively of β-catenin, the key role played by APC in the wnt signal transduction pathway (described p. 52). APC is found in the cytoplasm of the cell, where it interacts with axin via its seven armadillo repeats (a forty-two-amino acid repeated structural motif; Kinzler *et al*. 1991) and its three Ser-Ala-Met-Pro repeat motifs (Kishida *et al*. 1998).

Functions of the adenomatous polyposis coli protein

The APC protein is best known as a tumour suppressor in the human colon, a property due, apparently, to the key role played by APC in destabilization or down regulation of β-catenin in the wnt signalling pathway (Fig. 3).

In the absence of wnt several proteins, including APC, form a multi-protein complex, which results in degradation of β-catenin. When the wnt receptor known as 'frizzled' binds a wnt signal peptide, a cascade is initiated, which prevents β-catenin degradation through the ubiquitin–proteasome pathway (Klingensmith & Nusse, 1994). The key step in the pathway is believed to be binding of glycogen synthase kinase 3β by the second messenger protein 'dishevelled', so preventing it from phosphorylating β-catenin, which is degraded only after phosphorylation.

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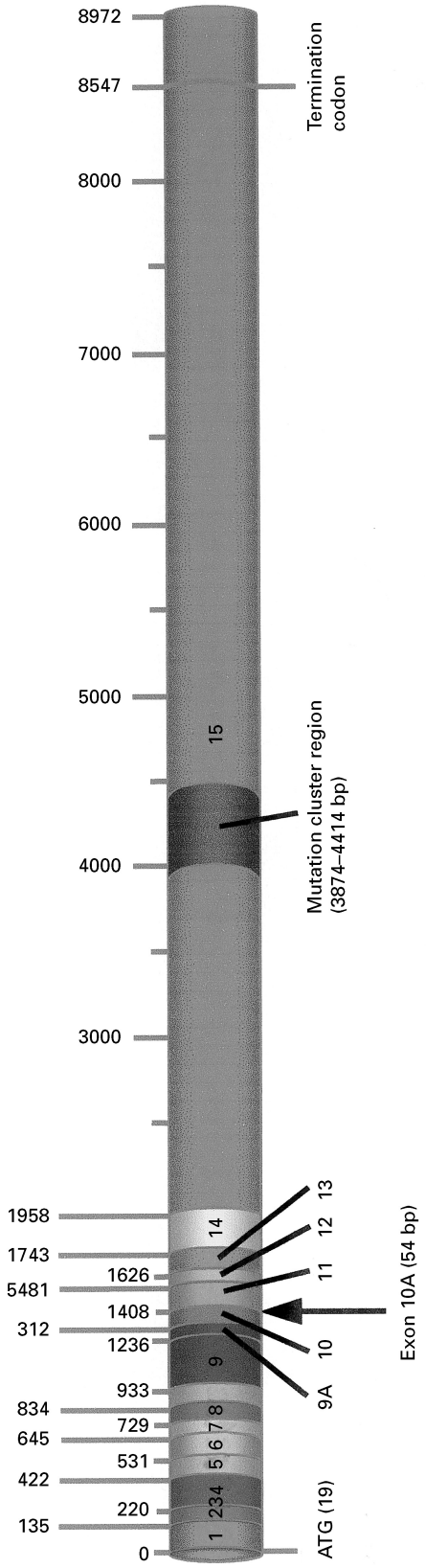

Fig. 1. Exon structure of the adenomatous polyposis coli gene. Numbers shown above the exon structure are exon boundaries in base pairs. The initiation (ATG (19)) and termination codons are indicated. Note also the mutation cluster region which is important in sporadic truncating mutations.

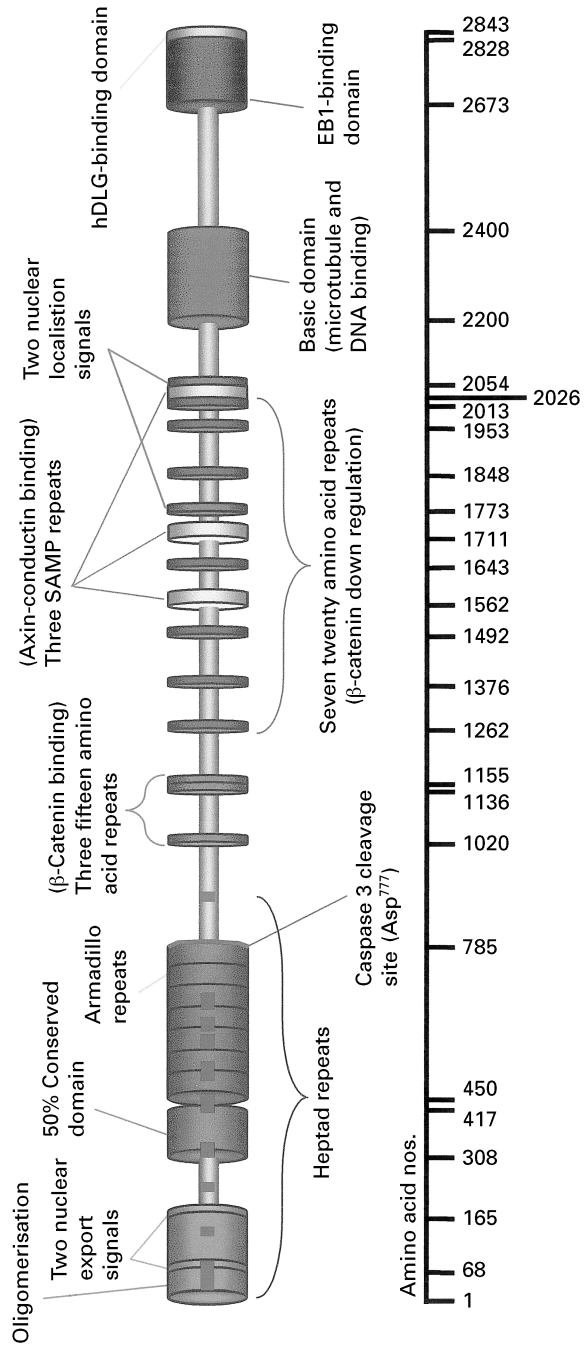

Fig. 2. Structural and functional domains of the adenomatous polyposis coli protein. The boundaries of longer domains and the start of shorter domains are indicated in amino acids shown below the protein structure. SAMP, Ser-Ala-Met-Pro; EB1, end-binding protein-1; hDLG, human homologue of discs large protein.

When β-catenin accumulates in the cytoplasm, it forms a complex with the transcription factor LEF-1 (Tcf-4), translocates to the nucleus and activates transcription of target genes (Behrens *et al.* 1996). However, APC proteins are involved in regulating many other aspects of cell function (Table 1).

Over-expression of *APC* inhibits cell proliferation (Baeg *et al.* 1995) through cell arrest in G_0 or G_1 (Groden *et al.*

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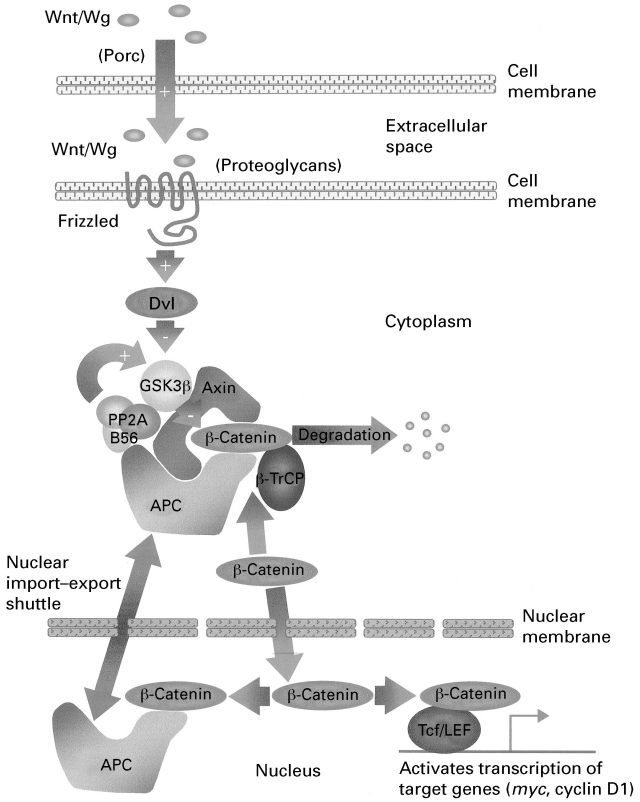

Fig. 3. Overview of the Wnt signalling pathway. Proteins shown include: Porc (Porcupine), Wnt/Wg (Wingless), Dvl (Dishevelled), GSK3β (glycogen synthase 3β) and PP2A (protein phosphatase 2A). APC, adenomatous polyposis coli; Tcf/LEF, T cell factor/lymphocyte enhancing factor; β-TrCP, β-transducin repeat-containing protein

Table 1. Putative functions of the adenomatous polyposis coli protein

1. Down regulation of β-catenin
2. Regulation of cell proliferation, differentiation and apoptosis
3. Regulation of cell–cell adhesion and of cell migration
4. Maintenance of chromosomal stability during mitosis
5. Regulation of the crypt fission cycle

1995), probably as a result of direct inhibition of cyclin-dependent kinases (Trzepacz *et al*. 1997). A lower level of apoptosis was observed in the macroscopically-normal colonic mucosa of patients with FAP compared with normal controls (Bedi *et al*. 1995), suggesting that the loss of even one allele of the *APC* gene reduces apoptosis. Direct evidence in support of the idea that APC is important in the induction or regulation of apoptosis comes from studies in which *APC* has been transfected into cell lines that do not express full-length APC protein, including SW480 and DLD-1 (Groden *et al*. 1995) and HT-29 (Morin *et al*. 1996). In the latter studies the transfected *APC* gene was placed under the control of a Zn-inducible promoter so that, after establishing stable transfectants, the effect of APC expression could be investigated by exposure of the cells to high Zn concentrations. APC expression was accompanied by a suppression of cell growth and a dramatic increase in the numbers of floating cells, apoptotic cells that had detached from the surface of the culture vessels (Morin *et al*. 1996). In the normal colonic mucosal crypt APC is expressed in post-replicative cells towards the lumen surface (Midgley *et al*. 1997), where it may play a role in initiating apoptosis, possibly through release from the extracellular matrix or breakdown of cell–cell contacts (Fearnhead *et al*. 2001).

APC plays an important role in chromosomal stability through its binding with end-binding proteins (EB1), which are ubiquitous microtubule-associated proteins involved in microtubule search and capture, cell polarity and chromosome stability (Rehberg & Graf, 2002). EB1 co-localises with APC (via the EB1-binding domain at the carboxy terminus of the protein; Fig. 2) to the spindle fibres and adjacent to the kinetochores during mitosis so that the EB1–APC complexes at the kinetochores form a physical link between the end of the spindle fibres and the centromeres of chromosomes (Fodde *et al*. 2001*a*). APC is found in many subcellular compartments, including the nucleus, to where it is directed by classical nuclear localisation signals (Zhang *et al*. 2000). Whilst in the nucleus, APC collects β-catenin and shuttles it out into the cytoplasm for degradation (Neufeld *et al*. 2000), a process aided by the nuclear export domains close to the NH_2 terminus of the APC protein (Henderson, 2000).

Given the multiple and apparently disparate cellular functions of APC it remains a major challenge to determine whether APC proteins have a single molecular function that can explain the various biological effects of this fascinating protein (Bienz, 2002).

Adenomatous polyposis coli gene and colo-rectal tumorigenesis

Mutations in the *APC* gene occur in the majority of colo-rectal neoplastic lesions, with no difference in the frequency of mutations from the smallest benign adenomas to carcinomas, suggesting that *APC* mutations play a major role in the early development of CRC (Powell *et al*. 1992). Support for the idea that *APC* is a tumour suppressor gene comes from studies of *APC* mutations in tumours from patients with FAP and from Min mice (which carry a germ-line mutation at codon 850 in one allele of the *Apc* gene) in which inactivating mutations were detected in the majority of human tumours (nineteen of twenty-four) and in all Min tumours (Levy *et al*. 1994). Loss of the second *Apc* allele could be detected in lesions containing as few as two dysplastic crypts (Levy *et al*. 1994), which confirms Knudson's two-hit hypothesis about the molecular function of tumour suppressor genes (see Strachan & Read, 1999). Somatic mutations in the *APC* gene in colo-rectal tumours are found primarily in the proximal half of the gene, with a preponderance in the so-called mutation cluster region ranging from codons 1250–1550 (Fearnhead *et al*. 2001; see Fig. 1). Mutations in this region are believed to be tumorigenic because they prevent β-catenin down regulation and result in the expression of: oncogenes such as *c-myc* (He *et al*. 1998), which give the initiated cell a selective advantage; cyclin D1; the multidrug resistance 1 gene, which may initiate tumorigenesis by suppressing cell death

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pathways in colonocytes (Yamada *et al*. 2000); the dominant negative helix-loop-helix regulator Id2 (Rockman *et al*. 2001). Loss of function of *APC* can also occur through gene inactivation or silencing as a result of hypermethylation of the promoter region of the gene. This hypermethylation occurs in about 18 % of the sporadic CRC in both adenomas and carcinomas and appears to be an early event in tumorigenesis (Esteller *et al*. 2000). In summary, *APC* is the gatekeeper gene for the colonic epithelium (Fodde *et al*. 1999), and loss of function of both *APC* alleles underlies both initiation and promotion of CRC (Fodde *et al*. 2001*b*).

Genetically-targeted intervention studies in colo-rectal prevention

The Concerted Action Polyp Prevention 1 Study

With the identification of germline mutations (such as those in *APC* causing FAP), which predispose to CRC, it has become possible to design intervention studies to investigate the efficacy of potential chemo-prevention agents using carriers of the mutated gene as subjects. The benefits of such genetically-targeted interventions include: confidence that the genetic basis of the disease is known; shorter time frames because subjects with FAP are on a fast-track to CRC; a smaller number of subjects than would be needed with studies using healthy volunteers (since only a small proportion of the latter will develop gastrointestinal neoplasia); potential recruits are known to the clinical genetics teams; expectation of better compliance (families with FAP are strongly motivated to help with such research because the direct benefit to them is potentially large).

However, when such studies were being planned about a decade ago, other researchers argued that the genetic predisposition in FAP was so strong that the effects of dietary or pharmaceutical agents would be, at best, difficult to detect. Subsequent studies have shown that non-steroidal anti-inflammatory drugs, such as sulindac (Giardiello, 1994; Debinski *et al*. 1995) and the cyclooxygenase-2 selective inhibitor celecoxib (Steinbach *et al*. 2000), can suppress polyp development in subjects with FAP. Indeed, there is recent evidence that celecoxib may also help suppress duodenal polyposis in FAP (Phillips *et al*. 2002), a condition that is much more difficult to manage clinically than is colonic polyposis. In addition, there is now a wealth of evidence from mouse models carrying *Apc* mutations that it is possible to manipulate tumour multiplicity by both pharmaceutical (Beazer-Barclay *et al*. 1996; Barnes & Lee, 1998; MacGregor *et al*. 2000) and dietary agents (Kennedy *et al*. 1996; Wasan *et al*. 1997; Williamson *et al*. 1999). There is also growing evidence that other environmental factors such as bacterial infection can modify the severity of colonic neoplasia in Min mice (Newman *et al*. 2001).

The Concerted Action Polyp Prevention 1 Study was designed to recruit 400 mutant *APC* gene carriers with intact colons who were randomised to one of four treatments: (1) double placebo; (2) aspirin (600 mg/d); (3) resistant starch (30 g raw potato starch–Hylon VII (1:1, w/w)/d); (4) aspirin and resistant starch. These intervention agents were chosen because (1) they were considered to be safe for prolonged use in healthy subjects (very important in a chemo-prevention study); (2) there was epidemiological and experimental evidence of potential efficacy (for overview, see Burn *et al*. 1998); (3) a putative mechanism of action for each agent was known. The anti-neoplastic actions of aspirin seem to be due mainly to its cyclooxygenase-inhibitory properties, which result in suppression of tumour cell growth, enhanced apoptosis and inhibition of angiogenesis (Elder & Paraskeva, 1997; Sunayama *et al*. 2002). It is probable that resistant starch functions as a pro-drug, delivering the active agent butyrate by bacterial degradation of the polysaccharide in the colon (Mathers *et al*. 1997). There is extensive evidence that, at physiological concentrations, butyrate suppresses proliferation and increases apoptosis of CRC cells (Hague *et al*. 1993; Chai *et al*. 2000).

Each recruit to the study participated in the intervention for a minimum of 1 year, with endoscopic examination of the large bowel before intervention and annually thereafter. The primary outcome measure was the number of adenomatous polyps, and biopsies of the macroscopically-normal rectal mucosa were taken for investigation of putative biomarkers of CRC risk, including cell proliferation, apoptosis and crypt fission. Currently, analysis of both primary and secondary outcome data is underway.

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