Published in final edited form as:
Dis Colon Rectum. 2008 October ; 51(10): 1467–1474. doi:10.1007/s10350-008-9356-7.

Missense Polymorphisms in the *Adenomatous Polyposis Coli* Gene and Colorectal Cancer Risk

Sean P. Cleary, M.D., M.Sc.[1,2], **Hyeja Kim, M.Sc.**[1], **Marina E. Croitoru, M.Sc.**[1], **Mark Redston, M.D.**[3,4], **Julia A. Knight, Ph.D.**[1], **Steven Gallinger, M.D., M.Sc.**[1,2,3], and **Robert Gryfe, M.D., Ph.D.**[1,2,3]

[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

[2]Department of Surgery, University of Toronto, Toronto, Ontario, Canada

[3]Ontario Familial Colorectal Cancer Registry, Cancer Care Ontario, Toronto, Ontario, Canada

[4]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

Abstract

PURPOSE—Whereas truncating germline mutations of the adenomatous polyposis coli (*APC*) gene give rise to familial adenomatous polyposis, missense polymorphisms of *APC* may confer a weaker risk for colorectal cancer.

METHODS—We sequenced the entire open reading frame of the *APC* gene and tested for two common MYH mutations in a population-based series of patients with colorectal cancer and 5 to 99 adenomas. Missense adenomatous polyposis coli alterations identified in this colorectal cancer multiple-polyp population were analyzed in a population-based series of patients with colorectal cancer and healthy control subjects.

RESULTS—Germline *APC* or mutY human homologue (*MYH*) alterations were identified in 16 of 39 colorectal cancer-multiple polyp patients. Four missense *APC* gene alterations (S130G, E1317Q, Dl822V, G2502S) were observed in 13 individuals and 3 additional patients carried presumed pathogenic (*APC* Y94X, biallelic *MYH* Y165C and heterozygous *MYH* G382D) mutations. When independently assessed in 971 patients with colorectal cancer and 954 healthy control subjects, none of the identified missense *APC* alterations conferred a significantly increased risk for colorectal cancer, odds ratio (95 percent confidence intervals): S130G=3.1 (0.29–32.25), E1317Q= 1.08 (0.59–2.74), G2502S= 1 (0.65–1.63), D1822V (heterozygous)=0.79 (0.64–0.98), D1822V (homozygous) =0.82 (0.63–1.27).

CONCLUSIONS—Germline missense *APC* alterations observed in 33 percent of patients with multiple colorectal neoplasms seemed to play a limited role in colorectal cancer risk when independently assessed by a population-based, case-control analysis.

Keywords

APC gene; Polymorphism; Colorectal cancer; Adenomatous polyp; Risk; *MYH* gene

Address of correspondence: Robert Gryfe, M.D., Ph.D., Mount Sinai Hospital, Room 455, 600 University Avenue, Toronto, Ontario M5G 1X5, Canada. rgryfe@mtsinai.on.ca.

Approximately 20 percent of patients with colorectal cancer (CRC) or adenomatous polyps have a significant family history of CRC and as many as three-quarters of these familial cases demonstrate a dominant inheritance pattern.[1,2] Familial adenomatous polyposis (FAP) is caused by truncating germline mutations in the adenomatous polyposis coli (*APC*) tumor suppressor gene,[3] whereas hereditary nonpolyposis colorectal cancer (HNPCC) is associated most commonly with germline mutations in the *MLH1*, *MSH2*, *MSH6*, and *PMS2* mismatch repair genes.[4] These two syndromes account for <1 percent and approximately 2 percent of CRC cases, respectively.[4] More recently, biallelic germline missense mutations of the *MYH* base excision repair gene have been associated with the development of polyposis and CRC, [5] whereas monoallelic germline *MYH* mutations seem to be associated with a more modest increase in risk for CRC or polyps.[6] Thus, to date, the majority of cases in which a familial clustering of CRC has been observed cannot be explained by any known genetic predisposition. [4]

More than 95 percent of patients with FAP have truncating germline *APC* mutations that result in the premature termination of protein translation.[7] Interestingly, germline mutations in *APC* display a genotype-phenotype correlation because the location of a truncating mutation within the *APC* gene has an effect on the age of onset of disease, the severity of polyposis, and the pattern of extracolonic manifestations.[4,8] In the attenuated adenomatous polyposis coli (AAPC or AFAP) syndrome, truncating mutations at the extreme 5′ and 3′ ends of the gene are associated with a “milder” form of FAP, with the development of fewer polyps (<100) and a later age of onset of CRC.[9]

In contrast to the rare, highly penetrant phenotypes conferred by truncating *APC* mutations in FAP and AAPC, the presence of more common, less penetrant predisposition alleles provides an attractive and potentially more common mechanism for CRC risk in individuals who have a significant family history of CRC. In Support of this hypothesis, recent meta-analyses have demonstrated moderately increased CRC risk in carriers of the *TGFβR-1*(6A), *HRAS1**VNTR, and *APC* I1307K alleles.[10]

Although most previously characterized pathogenic *APC* mutations result in a truncated protein product,[11] it is plausible that other alterations in the gene may influence development of CRC. The relationship between *APC* gene dysfunction and the development of CRC is complex. The genotype-phenotype relationship of *APC* mutations in FAP, the possibility of a haploinsufficiency model of tumorigenesis,[7,12] and the relationship between the *APC* I1307K polymorphism and polyps and CRC all imply that simple loss of *APC* tumor-suppressor function is inadequate to fully describe the role of *APC* in CRC. Thus, missense *APC* polymorphisms may influence CRC risk by altering the functional, structural, and/or localization characteristics of the protein, thereby altering cellular physiology.

Although the possibility that nontruncating alterations in *APC* may contribute to CRC risk has been explored, previous studies have analyzed only a portion of the *APC* gene,[13-15] or the entire *APC* gene in relatively small populations of cancer or polyp patients with few if any unaffected control subjects.[16-19] In one moderate-sized, hospital-based series, no statistically significant *APC* alleles were identified in 91 high-risk Swedish CRC cases or in 247 sporadic CRC cases and 476 control subjects.[20] In contrast to these searches of the entire *APC* gene, a few case-control series have evaluated CRC risk associated with specific alleles, such as *APC* D1822V[21] or E1317Q,[13,22-28] and in the case of E1317Q results of these efforts remain inconclusive.

To identify germline *APC* missense alterations that may alter the risk of CRC, we have sequenced the entire open reading frame of the *APC* tumor suppressor gene in a population-based series of 39 individuals who did not fulfill clinical criteria for FAP, but in whom a CRC

resection included evidence of ≥5 synchronous adenomatous polyps. To account for the potentially confounding contribution of *MYH* alterations in this population, we also assessed for the germline status of the two common *MYH* mutations.[6] Finally, to specifically determine relative risk, all nonsynonymous *APC* allele alterations identified in our original screen of patients with CRC and attenuated polyposis were then assessed in a large population-based series of 971 patients with CRC and 954 healthy control subjects.

MATERIAL AND METHODS

CRC Cases: The Ontario Familial Colorectal Cancer Registry (OFCCR)

The OFCCR is one of six sites of the National Institute of Health/National Cancer Institute supported Cooperative Family Registries for Colorectal Cancer Studies. Detailed characteristics of the OFCCR have been published previously.[29] In brief, incident CRC patients aged 20 to 74 years, diagnosed between July 1, 1997 and June 30, 1998, and aged 20 to 70 years, diagnosed between July 1, 1998 and June 30th 2000, were identified by the population-based Ontario Cancer Registry and pathology reports reviewed by clerical staff. Other nonadenocarcinoma histologies (such as squamous-cell cancer, carcinoid, and sarcoma), recurrent and *in situ* malignancies, and cases of classical FAP were excluded. Permission was sought from primary physicians to contact the patient. Each patient was asked to complete a family history questionnaire from which a pedigree was constructed and the familial risk of each case was classified as high, intermediate, or low risk by using predetermined criteria.[29] Family history data were verified by genetic counselors. All high-risk and intermediate-risk cases and a 25 percent random sample of sporadic cases were selected to participate in the OFCCR, including completing a personal risk factor questionnaire and a diet questionnaire. Relatives of the proband also were invited to participate in the OFCCR. Blood samples and paraffin blocks of tumor tissues were collected.

Patients with CRC and Multiple Adenomatous Polyps

Incident cases from the OFCCR who were identified as having multiple synchronous polyps on pathology reports from CRC resections were selected for detailed pathology review. Polyps with villous, tubulovillous, tubular, and serrated histologic architecture were included in the polyp count; hyperplastic and inflammatory polyps were excluded. Cases with 5 to 99 adenomatous polyps in their colectomy specimen pathology report were selected for analysis. These subjects were excluded from the subsequent population-based CRC case-control analysis.

Unaffected Controls

Controls were obtained from two sources: 1) using a random selection of residential telephone numbers in Ontario, subjects without any history of CRC (n = 533 controls) were matched by five-year age and sex to the distribution of OFCCR cases, and 2) using a similar strategy, subjects in Ontario without any history of breast cancer were matched to a similar population-based breast cancer registry, the Ontario Familial Breast Cancer Registry (n =421 controls).[30,31] Family history questionnaires, diet, and personal risk factor questionnaires were administered to controls and blood samples were obtained.

As per local institutional review board approval, this research was conducted with all patients' informed, written consent.

APC and MYH Sequence Analysis in Patients with CRC and Multiple Adenomatous Polyps

Lymphocytes were isolated from venous blood samples by Ficoll separation and genomic DNA extracted by Phenolchloroform or Qiagen® prep kit (Qiagen, Valencia, CA). The entire open

reading frame of *APC* was divided into 28 PCR fragments for the purposes of sequencing. Exons 1–14, including all intron-exon boundaries were amplified individually in single PCR fragments (primer sequences and PCR conditions available on request). Exon 15 was divided into 14 overlapping segments of approximately 550 bp each. Aliquots of PCR products for each patient sample were electrophoresed on 2 percent agarose gels. Samples that ran as a single band on agarose were purified using Qiagen® PCR purification kit (Qiagen) and eluted in 30 μl of elution buffer. For aliquots with nonspecific bands, the entire sample was rerun on 2 percent agarose. The desired band was then excised, extracted using Qiagen® gel extraction kit (Qiagen) and eluted in 30 μl of elution buffer. Manual sequencing of each PCR product was performed using Thermosequenase® (Amersham, Piscataway, NJ) and ^{33}P-labeled di-deoxy dNTP terminators.[32] Each product was sequenced from the forward and reverse direction and loaded onto a denaturing urea-acrylamide gel.[33] Gels were run at 60W for 1.45 hours and 4.5 hours (if necessary), dried onto filter paper, and exposed to film. All nonsynonymous alterations were confirmed by sequencing the corresponding region in both the forward and reverse directions. Additionally, each patient was screened for two common *MYH* gene variants, Y165C and G382D, by dHPLC and sequence analysis as previously described.[6] In carriers of either of these mutations, the entire *MYH* gene was analyzed for other, possibly biallelic, mutations by dHPLC and sequence analysis as previously described.[6]

Allele Discrimination Assay in CRC Cases and Healthy Control Subjects

Once missense polymorphisms were identified and selected for case-control analyses, genotyping was performed using a 5′nuclease (Taqman®) assay. All probes and primers were designed using the Primer Express® software package (PE Biosytems, Foster City, CA) and synthesized by Operon (Qiagen). Allele specific probes were designed to bind to the wild-type and variant alleles at each locus and labeled with a different 5′ fluorescent label. Wild-type probes were labeled with HEX and variant probes were labeled with 6-carboxy-flourescein (FAM). Both probes were labeled on the 3′ end with Blackhole® quenchers (Operon®). DNA from each sample was diluted in duplicate into 96-well master plates. Duplicates of these master plates were made for each assay by aliquoting 15 ng of DNA from each well into a skirted-frosted 96-well plate suitable for the Taqman assay along with duplicates of the appropriate controls. Each reaction included 15 ng genomic DNA, 30 pmol of each primer, 12.5 pmol of each probe, and 5 μl of Quantitect PCR Probe master mix (Qiagen) in a final volume of 10 μl. Fluorescence was measured by using the ABI PRISM 7900HT Sequence Detection system. The genotype of each sample was then determined by using the graphic view of relative fluorescent intensities from the Sequence Detection System Version 2.0 (Applied Biosystems®).

Statistical Analysis

All means for continuous, normally distributed values are reported as means±standard deviations. Median values for nonnormally distributed variables are reported as medians and ranges. All categorical values (such as genotype, gender, family history, and epidemiologic variables) were compared by chi-squared or Fisher's exact test. Ordinal data (polyp and tumor numbers) were compared by Mann-Whitney *U* test and Kruskall-Wallis test. Continuous variables were compared by ANOVA and Student's *t*-test.

RESULTS

APC Gene Analysis in Patients with CRC and 5 to 99 Adenomas

During the first two years of recruitment by the OFCCR (July 1, 1997 to June 30, 1999), we identified 112 patients with CRC whose pathology reports were classified as having multiple synchronous polyps. The original pathology reports were re-reviewed, and only adenomatous polyps were included in the subsequent polyp count, whereas hyperplastic or inflammatory

polyps were excluded. Thirty-nine cases were identified with 5 to 99 synchronous adenomatous polyps in their colectomy specimen pathology report and who had provided a blood sample to the OFCCR by January 1, 2002. The characteristics of these patients with CRC and multiple polyps are provided in Table 1. The mean age (±SD) was 61.5 ± 9.4 years. Fourteen cases had a history of two or more synchronous or metachronous CRCs based on review of the personal history questionnaire. All had five or more histologically confirmed adenomatous polyps. Fifteen individuals had more than 5 polyps, and two had more than 50 polyps.

Manual sequencing of the entire 8,535 bp open reading frame of *APC* was performed in these 39 CRC-multiple adenoma patients. A total of five nonsynonymous germline *APC* single nucleotide variants were detected, including one predicted to lead to protein truncation and four predicted to encode amino acid alterations. A single patient was found to have a nonsense mutation, Y94X, which results in a tyrosine (TAT) to stop (TAA) substitution. This 59-year-old patient had two synchronous CRCs, >50 adenomatous polyps, and a strong family history of CRC. Given the phenotypic characteristics of this case as well as the nature of the mutation, this subject has been classified as an unrecognized case of AAPC. The Y94X mutation has not been previously reported in the FAP or *APC* databases.[34]

A summary of all the missense variants identified in patients with CRC and 5 to 99 adenomas is shown in Table 2. A previously unreported missense change, S130G, was observed at the 5′ end of the gene in a single patient, the result of an A to G (AGT>GGT) substitution. This variant was found in a patient who had CRC and seven synchronous adenomatous polyps at age 64 years. There was no history of CRC in first-degree or second-degree relatives of the S130G carrier. A glycine to serine substitution at codon 2502 was identified in two multiple polyp patients. One of the G2502S carriers developed 2 synchronous cancers with 11 adenomatous polyps at age 53 years and did not have a family history of CRC. The other G2502S carrier developed CRC at age 63 years and had five adenomatous polyps. We detected this polymorphism in two of this patient's relatives, one of whom had a history of polyps. The G2502S polymorphism has been observed in previous studies.[20] The E1317Q variant was observed in two multiple polyp patients. This variant has been described previously.[13,15,22-28] Finally, at codon 1822, we observed seven aspartate/valine heterozygotes and one valine/valine homozygote. This polymorphism has been described previously.[21]

All 39 multiple adenoma CRC patients were screened for *MYH* Y165C and G382D mutations. One subject was homozygous for the *MYH* Y165C mutation and had two synchronous colorectal cancers and 40 adenomatous polyps diagnosed at age 50 years. We also identified a heterozygous *MYH* G382D carrier who had two metachronous colorectal cancers at ages 53 and 59 years and more than 10 synchronous adenomatous polyps in each resection specimen. No other *MYH* mutations were detected in this subject by dHPLC analysis of the entire *MYH* coding region, including splice sites.[6] None of the previously described *APC* variants were detected in these two patients.

Germline APC Gene Analysis in a Population-Based CRC Case-Control Series

A total of 971 population-based CRC cases and 954 control subjects were genotyped for the four nontruncating *APC* alterations identified in our CRC-multiple polyp cohort. Characteristics of cases and controls are shown in Table 3. There were more women than men among the control subjects because approximately half of our controls were recruited through the Ontario Familial Breast Cancer Registry and were matched to breast cancer cases.[31] In addition, the CRC cases had stronger family histories of cancer compared with the control subjects at least in part because the OFCCR oversampled subjects with positive family histories of colorectal and other cancers.[29]

Genotyping results are shown in Table 4. The S130G variant seems to be rare and was found in three cases (0.3 percent) and one control (0.1 percent). Although potentially conferring an odds ratio of approximately 3.0, the current study was not powered to detect statistically significant differences in allele frequencies of such rare variants. Nonetheless, when the frequency of the S130G allele was compared among all three groups (2.6 percent CRC-multiple polyps *vs*. 0.3 percent CRC cases *vs*, 0.1 percent control subjects), a significant association (*P*=0.01, test for trend) with increasingly severe phenotype was observed. No other phenotypic associations were apparent when comparing S130G carriers and noncarriers (data not shown).

The E1317Q variant was observed at similar frequencies in both cases (1.8 percent) and controls (1.9 percent). Similarly, 4.6 percent of patients and 4.9 percent of control subjects were heterozygous for the G2502S variant, whereas one CRC patient and two control subjects were homozygous for this variant.

Heterozygous (36.4 *vs*. 32 percent) and homozygous (6.2 *vs*. 5.6 percent) carriers of the D1822V polymorphism were more common among control subjects than cases, respectively, Thus, D1822V heterozygous carrier status was associated with an odds ratio of 0.79 (95 percent confidence intervals (CI), 0.64–0.98; *P*=0.04) and homozygous carrier status with an odds ratio of 0.82 (95 percent CI, 0.63–1.27; *P*=0.35). If a dominant model is assumed, the combined heterozygous and homozygous variant genotypes are associated with an odds ratio of 0.82 (95 percent CI, 0.64–0.97; *P*=0.024). The Dl822V genotypes observed were in Hardy-Weinberg equilibrium in the control population, because their observed frequencies were not statistically different from the expected frequencies by chi-squared test (*P*=0.87). D1822V carrier status was not associated with a significant case-case difference in age of diagnosis of CRC with a mean age of diagnosis of 59.7, 60.5, and 60.2 years for wild-type, heterozygous, and homozygous variant carriers, respectively (*P*=0.43). Furthermore, multiple adenomatous polyps were observed in similar frequencies among wild-type and heterozygous D1822V carriers (3.7 and 3.9 percent, respectively). However, we did not observe any homozygous variant cases with multiple polyps. Furthermore, we did not observe any consistent relationship between the protective effect of the D1822V genotype and the age or dietary fat intake proftles of patients and control subjects (data not shown).

DISCUSSION

Approximately one in five patients diagnosed with a colorectal neoplasm has a family history of CRC, implying a significant hereditary contribution to CRC risk.[1] Although significant progress has been made in characterizing highly penetrant forms of hereditary CRC, such as FAP and HNPCC and *MYH*-associated polyposis,[4] we have postulated that some of the remaining hereditary cases may be the result of less penetrant genetic variants. The *APC* gene has been described previously as the “gatekeeper” of the colorerectal epithelium, and truncating mutations of this gene are observed in the germline of individuals with FAP, and somatic mutations are found in most adenomatous polyps and CRCs.[35] Missense variants of the *APC* gene represent more subtle and more common genetic changes, which may have varying impact on protein stability, structure, and function, leading to an increased, or even decreased, risk of neoplastic transformation and progression. The present study is the largest effort to date to both identify potential disease-associated germline *APC* substitution mutations and to ascertain risk association of these alterations in an independent case-control series. Furthermore, it is the first such study performed that used population-based patients and control subjects. Finally, it is one of the few studies to date that estimates the potential risk of *APC* alleles in a case series separate from the cases in which they were initially detected. This separate retesting avoids bias introduced where the same cases are used for both hypothesis generation (to *detect* an inventory of relatively rare, potential disease-related alleles) and hypothesis testing (to *estimate* the case-control risk of these alleles).[36] Despite the exclusion of patients with

phenotypic FAP by the OFCCR, 1 of 39 of the population-based cases with CRC and multiple synchronous adenomatous polyps did represent a case of AAPC (*APC* Y94X) and another case was caused by biallelic *MYH* mutations. A further CRC-multiple polyp patient was a heterozygous *MYH* G382D carrier, supporting our recent hypothesis of a possible codominant transmission pattern for *MYH* gene mutations.[6]

Our data suggest that the *APC* Sl30G variant may represent a CRC-risk allele. However, because this allele is very rare, it is likely that functional characterization, rather than case-control data, will be needed to further investigate this association.

APC El317Q has been reported previously as a CRC-risk associated allele[13,23] and was observed in two of our patients with CRC and multiple-polyps. However, similar to other smaller association studies,[15,22,24-28] our population-based data revealed that this allele was carried by a similar numbers of patients (1.8 percent) and control subjects (1.9 percent), providing strong evidence that this variant is not associated with an independent increased risk for CRC. Our large population-based, case-control series has sufficient power to exclude a significant odds ratio of 2.2 for an allele with the prevalence of *APC* E1317Q (alpha=0.05, power=0.8), and as such a smaller effect of this allele cannot be fully excluded. This underscores the importance of large population-based series to characterize relatively rare alleles with modest putative disease association and highlights the potentially erroneous conclusions of previous small studies.[13,23] Similarly, our case-control data were powered to exclude significant CRC-risk with an odds ratio of more than 1.7 for the G2502S variant.

D1822V is the most common missense *APC* variant described to date.[37] We observed that the heterozygous and homozygous variant genotypes were more common in control subjects than in patients (36.4 and 6.2 percent *vs*. 32 and 5.6 percent) respectively. This data yielded an OR of 0.79 (95 percent CI, 0.64–0.98) for heterozygotes and 0.82 (95 percent CI, 0.63–1.27) for homozygous variants, indicating that this variant likely confers a protective effect against CRC that is inherited in an autosomal dominant fashion. Our results are consistent with those previously published by Slattery and colleagues.[21] However, in contrast to this previous study, we did not observe any consistent relationship between the protective effect of D1822V specific to patients who reported a low-fat diet or at an advanced age.

The findings of our large, population-based study in combination with those of previously published smaller case series[14-16,18-20,22,24-27] suggest that it is highly unlikely that germline *APC* gene missense mutations confer a common, increased risk for CRC. The exception to this seems to be the *APC* 11307K variant,[10] identified almost exclusively in individuals of Ashkenazi Jewish origin and mechanistically leading to an increased rate of truncating somatic mutations of the *APC* gene.

Interestingly, previous studies similar to ours have not revealed common, risk-associated missense alleles of the *MSH2* or *MLH1* genes in CRC[28] or the *BRCA1* gene in patients with breast or ovarian cancer.[38,39] The fact that nonsense germline mutations of the *APC*, *MSH2*, *MLH1*, and *BRCA1* genes cause highly penetrant inherited cancer risk, yet presumably functionally deleterious germline missense alleles are rarely associated with even a weak cancer risk is difficult to reconcile. If a mutation of the *APC* gene were skewed to an “all or none” functional consequence, then we would not expect to identify moderate penetrance missense “hypomorphs.” However, in this scenario, one would expect missense alleles to frequently account for cases of FAP and they do not.[16,18] Nonetheless, the results of our study imply that the majority of genetic risk accounting for familial and even seemingly sporadic CRCs is likely to arise from as yet uncharacterized genes or, more likely, more complex gene-gene (digenic) or gene-environment interactions and not from monogenic inherited missense mutations of the “gatekeeper” *APC* gene.

Acknowledgments

Funding Support: S.P.C is a Research Fellow of the National Cancer Institute of Canada supported with funds from the Canadian Cancer Society. R.G. is a Charles H. Hollenberg Senior Fellow/Clinician Scientist supported with funds provided by Eli-Lilly Canada, Cancer Care Ontario, and the Canadian Institutes of Health Research. This work was supported by the National Cancer Institute, National Institutes of Health under RFA # CA-95–011, the Ontario Registry for Studies of Familial Colorectal Cancer (U01 CA074783), and through cooperative agreements with members of the Colon Cancer Family Registry (CFRs) and P.I.s, the Canadian Breast Cancer Research Initiative (grant # 9181 to J.A.K) and the National Cancer Institute of Canada (grant # 13304 to S.G.).

REFERENCES

1. Canon-Albright LA, Skolnick MH, Bishop DT, Lee RG, Burt RW. Common inheritance of susceptibility to colonic adenomatous polyps and associated colorectal cancers. N Engl J Med 1988;319:533–7. [PubMed: 2841598]
2. Houlston RS, Collins A, Slack J, Morton NE. Dominant genes for colorectal cancer are not rare. Ann Hum Genet 1992;56:99–103. [PubMed: 1503398]
3. Kinzler KW, Nilbert MC, Su LK, et al. Identification of FAP locus genes from chromosome 5q21. Science 1991;253:661–5. [PubMed: 1651562]
4. de la Chapelle A. Genetic predisposition to colorectal cancer. Nat Rev Cancer 2004;4:769–80. [PubMed: 15510158]
5. Sampson JR, Dolwani S, Jones S, et al. Autosomal recessive colorectal adenomatous polyposis due to inherited mutations of MYH. Lancet 2003;362:39–41. [PubMed: 12853198]
6. Croitoru ME, Cleary SP, Di Nicola N, et al. Association between biallelic and monoallelic germline MYH gene mutations and colorectal cancer risk. J Nat Cancer Inst 2004;96:1631–4. [PubMed: 15523092]
7. Laken SJ, Papadopoulos N, Petersen GM, et al. Analysis of masked mutations in familial adenomatous polyposis. Proc Natl Acad Sci USA 1999;96:2322–6. [PubMed: 10051640]
8. Nagase H, Miyoshi Y, Horii A, et al. Correlation between the location of germ-line mutations in the APC gene and the number of colorectal polyps in familial adenomatous polyposis patients. Cancer Res 1992;52:4055–7. [PubMed: 1319838]
9. Spirio L, Olschwang S, Groden J, et al. Alleles of the APC gene: an attenuated form of familial polyposis. Cell 1993;75:951–7. [PubMed: 8252630]
10. Houlston RS, Tomlinson IP. Polymorphisms and colorectal tumor risk. Gastroenterology 2001;121:282–301. [PubMed: 11487538]
11. Laurent-Puig P, Beroud C, Soussi T. APC gene: database of germline and somatic mutations in human tumors and cell lines. Nucleic Acids Res 1998;26:269–70. [PubMed: 9399850]
12. Yan H, Dobbie Z, Gruber SB, et al. Small changes in expression affect predisposition to tumorigenesis. Nat Genet 2002;30:25–6. [PubMed: 11743581]
13. Frayling IM, Beck NE, Ilyas M, et al. The APC variants 11307K and E1317Q are associated with colorectal tumors, but not always with a family history. Proc Natl Acad Sci USA 1998;95:10722–7. [PubMed: 9724771]
14. Boardman LA, Schmidt S, Lindor NM, et al. A search for germline *APC* mutations in early onset colorectal cancer or familial colorectal cancer with normal DNA mismatch repair. Genes Chromosomes Cancer 2001;30:181–6. [PubMed: 11135435]
15. Figer A, Irmin L, Geva R, Flex D, Slukes A, Friedman E. Genetic analysis of the APC gene regions involved in attenuated APC phenotype in Israeli patients with early onset and familial colorectal cancer. Br J Cancer 2001;85:523–6. [PubMed: 11506490]
16. Gayther SA, Sud R, Wells D, Tsiopura K, Delhanty JD. Rapid detection of rare variants and common polymorphisms in the APC gene by PCR-SSCP for presymptomatic diagnosis and showing allele loss. J Med Genet 1995;32:568–71. [PubMed: 7562975]
17. Pedemonte S, Sciallero S, Gismondi V, et al. Novel germline APC variants in patients with multiple adenomas. Genes Chromosomes Cancer 1998;22:257–67. [PubMed: 9669663]

18. Wallis YL, Morton DG, McKeown CM, Macdonald F. Molecular analysis of the APC gene in 205 families: extended genotype-phenotype correlations in FAP and evidence for the role of APC amino acid changes in colorectal cancer predisposition. J Med Genet 1999;36:14–20. [PubMed: 9950360]
19. Heinimann K, Thompson A, Locher A, et al. Nontruncating APC germ-line mutations and mismatch repair deficiency play a minor role in APC mutation-negative polyposis. Cancer Res 2001;61:7616–22. [PubMed: 11606402]
20. Zhou XL, Eriksson U, Werelius B, Kressner U, Sun XF, Lindblom A. Definition of candidate low risk APC alleles in a Swedish population. Int J Cancer 2004;110:550–7. [PubMed: 15122587]
21. Slattery ML, Samowitz W, Ballard L, Schaffer D, Leppert M, Potter JD. A molecular variant of the APC gene at codon 1822: its association with diet, lifestyle, and risk of colon cancer. Cancer Res 2001;61:1000–4. [PubMed: 11221825]
22. White S, Bubb VJ, Wyllie AH. Germline APC mutation (Gln1317) in a cancer-prone family that does not result in familial adenomatous polyposis. Genes Chromosomes Cancer 1996;15:122–8. [PubMed: 8834176]
23. Lamlum H, Al Tassan N, Jaeger E, et al. Germline APC variants in patients with multiple colorectal adenomas, with evidence for the particular importance of E1317Q. Hum Mol Genet 2000;9:2215–21. [PubMed: 11001924]
24. Popat S, Stone J, Coleman G, et al. Prevalence of the APC E1317Q variant in colorectal cancer patients. Cancer Lett 2000;149:203–6. [PubMed: 10737725]
25. Evertsson S, Lindblom A, Sun XF. APC 11307K and E1317Q variants are rare or do not occur in Swedish colorectal cancer patients. Eur J Cancer 2001;37:499–502. [PubMed: 11267860]
26. Gismondi V, Bonelli L, Sciallero S, et al. Prevalence of the E1317Q variant of the APC gene in Italian patients with colorectal adenomas. Genet Test 2002;6:313–7. [PubMed: 12537656]
27. Hahnloser D, Petersen GM, Rabe K, et al. The APC E1317Q variant in adenomatous polyps and colorectal cancers. Cancer Epidemiol Biomarkers Prev 2003;12:1023–8. [PubMed: 14578138]
28. Fearnhead NS, Wilding JL, Winney B, et al. Multiple rare variants in different genes account for multifactorial inherited susceptibility to colorectal adenomas. Proc Natl Acad Sci USA 2004;101:15992–7. [PubMed: 15520370]
29. Cotterchio M, McKeown-Eyssen G, Sutherland H, et al. Ontario familial colon cancer registry: methods and firstyear response rates. Chronic Dis Can 2000;21:81–6. [PubMed: 11007659]
30. Knight JA, Sutherland HJ, Glendon G, Boyd NF, Andrulis IL. Characteristics associated with participation at various stages at the Ontario site of the cooperative family registry for breast cancer studies. Ann Epidemiol 2002;12:27–33. [PubMed: 11750237]
31. Knight JA, Onay UV, Wells S, et al. Genetic variants of GPX1 and SOD2 and breast cancer risk at the Ontario site of the breast cancer family registry. Cancer Epidemiol Biomarkers Prev 2004;13:146–9. [PubMed: 14744747]
32. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977;74:5463–7. [PubMed: 271968]
33. Sanger F, Coulson AR. The use of thin acrylamide gels for DNA sequencing. FEBS Lett 1978;87:107–10. [PubMed: 631324]
34. Beroud C, Soussi T. *APC* gene: database of germline and somatic mutations in human tumors and cell lines. Nucleic Acids Res 1996;24:121–4. [PubMed: 8594558]
35. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996;87:159–70. [PubMed: 8861899]
36. Little J, Bradley L, Bray MS, et al. Reporting, appraising, and integrating data on genotype prevalence and genedisease associations. Am J Epidemiol 2002;156:300–10. [PubMed: 12181099]
37. Powell SM, Zilz N, Beazer-Barclay Y, et al. APC mutations occur early during colorectal tumorigenesis. Nature 1992;359:235–7. [PubMed: 1528264]
38. Durocher F, Tonin P, Shattuck-Eidens D, Skolnick M, Narod SA, Simard J. Mutation analysis of the BRCA1 gene in 23 families with cases of cancer of the breast, ovary, and multiple other sites. J Med Genet 1996;33:814–9. [PubMed: 8933332]
39. Janezic SA, Ziogas A, Krumroy LM, et al. Germline BRCA1 alterations in a population-based series of ovarian cancer cases. Hum Mol Genet 1999;8:889–97. [PubMed: 10196379]

Table 1

Summary of clinical data of patients with colorectal cancer and 5 to 99 adenomas*

	N=39	Range
Male/female ratio	31/8	
Age (mean±SD)	61.5±9.4	33–74
No. of cancers (mean±SD)	1.4±0.5	1–3
No. of polyps* (median)	5	5–68

* Adenomatous polyp counts were based on segmental (n=37) and subtotal (n=2) resection specimens. SD=standard deviation.

Table 2

APC variants identified in patients with colorectal cancer and 5 to 99 adenomas

APC variant	No. detected in 39 patients with CRC and multiple adenomas (percent)
S130G	1 (2.6%)
E1317Q	2 (5.1%)
D1822V Asp/Val	7 (17.9%)
Val/Val	1 (2.6%)
G2502S	2 (5.1%)

APC=adenomatous polyposis coli gene; CRC=colorectal cancer, Asp=aspartate; Val=valine.

Table 3

Summary of characteristics of cases and controls

	Patients (n=971)	Control subjects (n=954)	P value
Age (mean±SD)	59.9±9.1	52.9±10.7	<0.01†
Gender			
Male	526 (54.2%)	248 (25.9%)	
Female	445 (45.8%)	706 (74%)	<0.01‡
Family history of CRC			
First-degree relatives	27%	9%	<0.01‡
Second-degree relatives	26.4%	11.5%	<0.01‡
First-degree or second-degree relatives	43.7%	18.8%	<0.01‡
Family history of other cancer*			
First-degree degree relatives	26.2%	16.4%	<0.01‡
Second-degree relatives	32.6%	21.3%	<0.01‡
First-degree or second-degree relatives	49.8%	33.8%	<0.01‡

SD=standard deviation.

*Other cancers include: breast, endometrial, pancreatic, gastric, lung, and kidney cancers.

†Student's t-test.

‡Chi-squared test.

Table 4

Missense *APC* variant genotypes for CRC cases and controls in the OFCCR

Polymorphism Genotype	Patients (n=971)	Control subjects (n=954)	Adjusted OR* (95% CI)
S130G			
Wild-type (Ser/Ser)	968 (99.7%)	953 (99.9%)	
Heterozygous (Ser/Gly)	3 (0.3%)	1 (0.1%) 3.1	3.1 (0.29–32.25)
E1317Q			
Wild-type (Gln/Gln)	954 (98.2%)	936 (98.1%)	
Heterozygous (Gln/Glu)	17 (1.8%)	18 (1.9%)	1.08 (0.59–2.74)
D1822V			
Wild-type (Asp/Asp)	606 (62.4%)	548 (57.4%)	
Heterozygous (Asp/Val)	311 (32%)	347 (36.4%)	0.79 (0.64–0.98)
Homozygous (Val/Val)	54 (5.6%)	59 (6.2%)	0.82 (0.63–1.27)
G2502S			
Wild-type (Gly/Gly)	925 (95.3%)	905 (94.9%)	
Heterozygous (Gly/Ser)	45 (4.6%)	47 (4,9%)	1 (0.65–1.63)
Homozygous (Ser/Ser)	1 (0.1%)	2 (0.2%)	0.58 (0.03–10.1)

APC=adenomatous polyposis coli gene; Ser=serine; Gly=glycine; Asp=aspartate; Val=Valine; Glu=glutamine; Gtu=glutamic acid; OR=odds ratios; CI=confidence interval.

*Odds ratios adjusted for age, sex, and family history of CRC.