Germline mutation analysis of the transforming growth factor β receptor type II (TGFBR2) and E-cadherin (CDH1) genes in early onset and familial colorectal cancer

Editor—Genetic factors are clearly implicated in colorectal cancer (CRC) susceptibility, with 10% of all cases having an affected first degree relative and suggestions that up to 20% of all colorectal cancers occur in susceptible people. Identification of the molecular basis for familial adenomatous polyposis (FAP) and hereditary non-polyposis colon cancer syndrome (HNPCC) has provided insights into the mechanisms of colorectal cancer susceptibility and illustrated how somatic mutations in familial cancer genes are frequently implicated in sporadic cancers. FAP has a characteristic phenotype with profuse colorectal polyposis, and although variant attenuated forms of FAP are described, germline APC gene mutations are a rare cause of colorectal cancer. HNPCC is characterised by early onset CRC and, in some kindreds, endometrial, gastric, ovarian, pancreatic, and urinary tract cancers.1 Germline mutations in mismatch repair genes (MMR) such as MSH2, MLH1, PMS1, PMS2, and MSH6 account for many, but not all, HNPCC kindreds. Most (>90%) HNPCC kindreds with germline MMR mutations have a MSH2 or MLH1 mutation. Germline MMR gene mutations have also been described in isolated early onset colorectal cancer (EOCRC) or familial non-HNPCC CRC.2 3 However, it is estimated that FAP and HNPCC only account for <3% of CRC cases and studies of early onset and non-HNPCC familial cases have indicated that most such cases do not have germline MMR gene mutations or evidence of tumour microsatellite instability (MSI) (the hallmark of tumour MMR gene inactivation).2 4 46 Candidate gene approaches to identify further genes for early onset and familial CRC have been largely unsuccessful. Thus, mutation analysis of β-catenin and SMAD2, SMAD3, and SMAD4 have been unrewarding.7 8 Transforming growth factor β (TGF-β) receptor (TGF-β RII) encoded by the TGFBR2 gene. Inactivation of TGFBR2 is frequent in colorectal cancers with MMR gene inactivation because of MSI in a poly A tract within the gene.9 10 In addition, somatic mutations of TGFBR2 are frequent in microsatellite stable (MSS) colorectal cancers.11 Although germline TGFBR2 mutation has been described in familial CRC,12 the contribution of germline TGFBR2 mutations to colorectal cancer susceptibility is not well defined.

Somatic mutations in the E-cadherin gene (CDH1) are frequent in colorectal and other cancers, including gastric, breast, prostate, and ovary.13 14 E-cadherin is a homophilic cell adhesion molecule whose binding to β-catenin at adherens junctions prevents β-catenin mediated cell signalling. Loss of E-cadherin function leads to increased cell mobility and increased activity of the β-catenin/TCF transcription factor complex in the nucleus.15 Germline CDH1 mutations are associated with familial diffuse type gastric cancer.16 In addition, we and others have reported

EOCRC in patients with germline CDH1 mutations.17 18 However, the possible contribution of germline CDH1 mutations to familial and early onset CRC is unknown. We therefore investigated cohorts of EOCRC and kindreds with familial CRC for germline TGFBR2 and CDH1 mutations.

Genomic DNA from 67 patients with EOCRC at <55 years of age and probands from 20 familial CRC cases were investigated. The age distribution of the 67 cases is shown in fig 1. For 48 of 67 cases, tumour microsatellite instability (MSI) status had been determined as described previously4 4 and 39 of the 48 cases showed no evidence of MSI.23 All 20 familial CRC cases were from kindreds fulfilling the Amsterdam criteria for HNPCC,24 but in whom mutation screening by single stranded conformation polymorphism (SSCP) analysis for germline MSH2 and MLH1 mutations was negative. TGFBR2 mutation analysis of the 67 EOCRC and 20 familial CRC cases was carried out using 10 sets of primers that amplified the entire TGFBR2 gene and 100 ng DNA per 25 µl reaction, for 35 cycles (95°C for one minute, 55-57°C for one minute, 72°C for one minute), with a final elongation step of 72°C for 10 minutes, in an OmnE thermal cycler (Hybaid Ltd).

For exon 3, to prevent false positives caused by Taq polymerase slippage errors at the polyA repeat, the proof reading polymerase Phu Turbo (Stratagene) was used and all samples were directly sequenced as well as undergoing SSCP analysis.

Twenty three of the EOCRC aged <45 years and the 20 HNPCC probands were screened for germline CDH1 mutations by PCR-SSCP analysis of all 16 exons as described previously by Richards et al.23 SSCP analysis was performed using 20 cm 8% polyacrylamide, 5% glycerol, 0.5% × TBE gels, electrophoresed at 1 W for 12-15 hours at 4°C, followed by silver staining. For DNA sequencing, PCR products were purified using a presequencing reagent pack (Amersham Pharmacia Biotech), or Wizard PCR Preps (Promega, Southampton, UK) and sequenced using the PRISM dRhodamine dye terminator cycle sequencing kit (PE-Applied Biosystems) on an ABI 377 automated sequencer.

SSCP analysis did not show any TGFBR2 abnormality in the 87 patients tested, suggesting that germline TGFBR2 mutations are not frequent in MSH2/MLH1 mutation negative HNPCC cases or in CRC cases affected before 55 years of age.
years. As a small false negative rate may be associated with SSCP analysis, we proceeded to sequence exon 3 (a hot spot for somatic TGFBR2 mutations) in all 87 patients; however, no abnormality was detected in any case.

No germline mutation was detected in CDH1 in any of the 43 cases tested but several polymorphisms were identified. The exon 4 PCR product contained an intronic C(531+10)G SNP in three of 43 cases (two EOCRC and one HNPCC). We then examined 30 normal controls and detected a further seven heterozygotes for the CDH1 531+10 G variant. Thus, there were no differences between the frequency of the C(531+10)G SNP in EOCRC (2/23), familial cases (1/20), and normal controls. In addition, an exon 13 CDH1 C2076T variant was identified in 9/23 EOCRC and 9/20 HNPCC cases. This silent variant has been reported previously as a polymorphism.21 26 A further polymorphism was detected by sequencing the exon 13 PCR products: C/T (1937-13) in intron 12; 5/17 HNPCC patients were homozygous T-T, 1/7 were heterozygous C-C, and 1/17 was homozygous T-T. This polymorphism has also been previously reported.21 26

In summary, we did not detect evidence to suggest that germline TGFBR2 or CDH1 mutations are a frequent occurrence in patients with EOCRC or HNPCC. We did not detect germline TGFBR2 mutations in EOCRC and HNPCC cases without MSH2 or MLH1 mutations. Although a germline TGFBR2 mutation has been described previously in one HNPCC-like kindred, the onset of colon carcinoma in the three affected subjects in that family was >50 years in all cases.13 Hence, further studies of a large number of EOCRC and HNPCC cases will be needed to exclude a role for infrequent TGFBR2 mutations in these cases, and the role of TGFBR2 mutations should also be investigated in kindreds with familial late onset CRC who do not satisfy the Amsterdam criteria. Similarly, as EOCRC is a feature of a germline CDH1 mutation in some kindreds,27 further studies are required to exclude a role of infrequent germline CDH1 mutations in EOCRC cases.

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