Electronic letter

J Med Genet 2001;**38** (http://jmedgenet.com/cgi/content/full/38/2/e7)

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.[2–4] 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 4–6] 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) inhibits the growth of colorectal cancer cell lines through the type II 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.[9–11] In addition, somatic mutations of *TGFBR2* are frequent in microsatellite stable (MSS) colorectal cancers.[12] Although germline *TGFBR2* mutation has been described in familial CRC,[13] 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.[14–18] 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.[19] Germline *CDH1* mutations are associated with familial diffuse type gastric cancer.[20] In addition, we and others have reported EOCRC in patients with germline *CDH1* mutations.[21 22] 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 previously[4 6] 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 exon 7 coding region as described by Lu *et al*.[25] PCRs were carried out in standard buffer, 1.5-2.0 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs, 10-20 pmol of each primer, 1 unit of *Taq* polymerase, 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 Pfu 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*.[21]

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

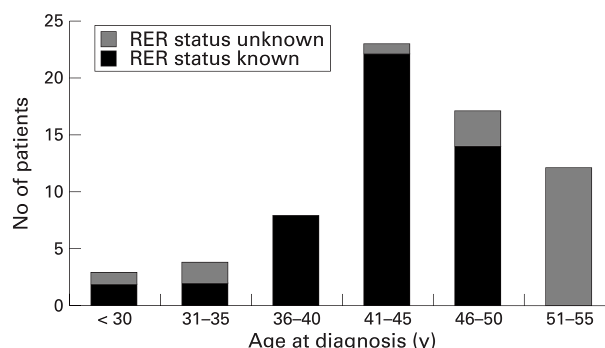

Figure 1 Chart to show total number of EOCRC patients analysed for TGFβRII mutations and microsatellite instability status.

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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, 11/17 were heterozygous, and 1/17 was homozygous C-C. 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,[21 22] further studies are required to exclude a role of infrequent germline *CDH1* mutations in EOCRC cases.

We thank the West Midlands NHS Executive, the Birmingham United Hospital Fund, and the British Digestive Foundation for financial support.

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