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 (HNPPC) 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. HNPPC is characterised by
early onset CRC and, in some kindreds, endometrial, gastr-
ic, 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, HNPPC kindreds. Most (>90%) HNPPC
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-HNPPC
CRC.2–4 However, it is estimated that FAP and HNPPC
only account for <3% of CRC cases and studies of early
onset and non-HNPPC 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
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 has been unrewarding.7–9 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 suscepti-
bility 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.14–16 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.17 Germline
CDH1 mutations are associated with familial diffuse type
gastric cancer.18 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 instabil-
ity (MSI) status had been determined as described
previously46 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 analy-
sis of the 67 EOCRC and 20 familial CRC cases was car-
rried out using 10 sets of primers that amplified the entire
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SSCP analysis was performed using 20 cm 8% polyacry-
lamide, 5% glycerol, 0.5
°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 (Hybird Ltd). For exon 3, to prevent false positives caused by Tαq
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
HNPPC probands were screened for germline CDH1
mutations by PCR-SSCP analysis of all 16 exons as
described previously by Richards et al.21

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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 HNPPC cases or in CRC cases affected before 55
years of age.

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Figure 1 Chart to show total number of EOCRC patients analysed for
TGFβRII mutations and microsatellite instability status.
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 four 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. 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 homoyzgous C-C. This polymorphism has also been previously reported.

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. 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, further studies are required to exclude a role of infrequent germline CDH1 mutations in EOCRC cases.

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