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No *TGFBR2* germline mutations in juvenile polyposis patients without *SMAD4* or *BMPR1A* mutation

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Juvenile polyposis (JPS) is an autosomal dominant disorder characterised by the presence of multiple gastro-intestinal juvenile polyps and an increased risk of colorectal cancer (CRC).¹ JPS is caused by germline mutation of *SMAD4* or *BMPR1A*, both involved in the transforming growth factor β /bone morphogenic protein (TGF β /BMP) signalling pathway. A recent study by van Hattem *et al*, published in this journal (Gut 2008;57:623–7), showed that a germline defect in one of these genes is found in approximately 50% of JPS patients, with 30–40% being a point mutation or small deletion and 10–15% a large genomic deletion. Since no germline defect is found in ~50% of JPS patients, it is likely that other genes exist which cause JPS.²

Several candidate genes, mostly involved in TGF β /BMP signalling, have been investigated for a role in JPS pathogenesis. No mutations have been found in these genes.^{3–6} (table 1) Recently, the TGF β co-receptor endoglin was proposed as a JPS susceptibility gene, but other studies could not confirm this.² Also, *PTEN*, the gene originally linked to Cowden syndrome (CS) and Bannayan–Riley–Ruvalcaba syndrome (BRRS), has been suggested as a JPS gene. The current consensus, however, is that *PTEN* mutations in patients with juvenile polyps likely represent CS or BRRS patients that have not (yet) developed extra-intestinal clinical features specific to these conditions.⁷ Lastly, the *CDX2* gene was investigated in juvenile polyposis, since mice with a heterozygous mutation of *CDX2* develop intestinal hamartomatous polyps, but no pathogenic mutations were found in 37 JPS families.⁸

The TGF β receptor type II (*TGFBR2*) is a component of the TGF β pathway and is mutated within a polyadenine tract in exon 3 in up to 90% of CRCs with microsatellite instability and in 15% of microsatellite stable malignancies.⁹ In addition, germline mutation of *TGFBR2* has been reported in a patient with hereditary CRC (944C>T, reference sequence NM_003242).¹⁰ Also, mice with conditionally knocked out *TGFBR2* in fibroblasts develop intra-epithelial neoplasia of the prostate and invasive squamous cell carcinoma of the forestomach and loss of *TGFBR2* in intestinal epithelium promotes invasion and malignant transformation of tumors in Apc^{1638N/wt} mice.^{11 12} Because of its role in TGF β signalling and in (colorectal) carcinogenesis, we investigated whether germline mutation or deletion of the *TGFBR2* gene is involved in JPS pathogenesis.

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Nineteen JPS patients from 18 families, in whom germline mutation or deletion of *SMAD4*, *BMPR1A*, *PTEN* or *ENG* was previously excluded,² were investigated for germline defects in the *TGFBR2* gene. JPS was defined according to accepted clinical criteria.¹ All exons and intron–exon boundaries of the *TGFBR2* gene were analysed by direct sequencing and the possibility of germline deletion of (parts of) the *TGFBR2* gene was investigated by multiplex ligation-dependent probe amplification (MLPA) (P065 MLPA kit, MRC-Holland BV, Amsterdam, The Netherlands). No pathogenic germline mutations or deletions in *TGFBR2* were found in this cohort. Known polymorphic variations were found in intron 3, intron 4, exon 4, and intron 7 (table 2).

TGFBR2 germline mutation is linked to Marfan syndrome type 2.¹³ Surprisingly, these patients do not have an increased risk of cancer.¹⁴ Possibly, diverging phenotypic effects of the different *TGFBR2* mutations are responsible for the absence of malignancies in Marfan patients carrying a *TGFBR2* mutation.¹³ Alternatively, the germline variation (944C>T) found in the patient with hereditary CRC could be a rare polymorphism without significance for CRC development. Although this alteration was not found in 119 control subjects,¹⁰ others found it at a similar frequency in normal controls (7 of 492) and individuals with sporadic CRC (6 of 228).¹³ Moreover, no additional germline mutations in *TGFBR2* have been found in patients with hereditary non-polyposis colorectal cancer (HNPCC) or in patients with familial or early onset CRC.^{15,16}

Because of its role in TGF β signalling and CRC pathogenesis we hypothesised that *TGFBR2* may be a JPS susceptibility gene. Linkage analysis could not be performed due to the lack of large JPS kindreds in our cohort. It is nevertheless felt that *TGFBR2* is unlikely to be involved in JPS pathogenesis since no germline mutations or deletions in *TGFBR2* were found in the current study. Still, about half of JPS patients remain without molecular diagnosis and the search for other JPS causing genes should continue apace. Candidate genes could include other, perhaps less obvious, components of the TGF β /BMP pathway.

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Table 1

Candidate genes investigated in the pathogenesis of juvenile polyposis

Gene	Patients studied/mutations found	Reference (first author and year)
<i>BMPR1B (ALK6)</i>	32/0	Howe 2004 ³
<i>BMPR2</i>	59/0 [*]	Howe 2004 ³ , van Hattem 2008 ²
<i>ACVR1 (ALK1)</i>	66/0 [†]	Howe 2004 ³ , Gallione 2004 ⁶ , van Hattem 2008 ²
<i>SMAD1</i>	30/0	Bevan 1999 ⁴
<i>SMAD2</i>	34/0	Bevan 1999 ⁴ , Roth 1999 ⁵
<i>SMAD3</i>	34/0	Bevan 1999 ⁴ , Roth 1999 ⁵
<i>SMAD5</i>	30/0	Bevan 1999 ⁴
<i>SMAD7</i>	34/0	Bevan 1999 ⁴ , Roth 1999 ⁵
<i>CDX2</i>	37/0	Woodford-Richens 2001 ⁸

^{*} 32 patients investigated by sequencing (Howe 3) and 27 by multiplex ligation-dependent probe amplification (MLPA) (van Hattem²).

[†] 39 patients investigated by sequencing (Howe³ and Gallione⁶) and 27 by MLPA (van Hattem²).

Table 2Polymorphisms found in *TGFBRII*

Location	Nucleotide	Amino acid change	Number of JPS patients	refSNP ID
Intron 3	c.338+7 A>G	Intronic	9/18	rs1155705
Intron 4	c.530-4 T>A	Intronic	7/18	rs11466512
Exon 4	c.1242 C>T	p.N414N	6/18	rs2228048
Intron 7	c.1600-8 C>T	Intronic	1/18	rs11466530

Reference sequence: NM_001024847.

JPS, juvenile polyposis; TGFBRII, transforming growth factor receptor type II.