Juvenile polyposis : aspects of molecular genetics and histology on the pathogenesis of a precancerous syndrome

van Hattem, W.A.

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Lodewijk A. A. Brosens, W. Arnout van Hattem, Marijke C. E. Kools, Chantal Ezendam, Folkert H. Morsink, Wendy W.J. de Leng, Francis M. Giardiello, G. Johan A. Offerhaus

Department of Pathology, Academic Medical Center, Amsterdam, The Netherlands;
Department of Pathology, University Medical Center, Utrecht, The Netherlands;
Department of Medicine, The Johns Hopkins School of Medicine, Baltimore, Maryland, USA.

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

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ABSTRACT Juvenile polyposis (JPS) is an autosomal dominant disorder characterized by multiple gastro-intestinal juvenile polyps and an increased risk of colorectal cancer. JPS is caused by germline mutation of SMAD4 or BMPR1A, both involved in TGF-β/BMP signaling. A germline defect in one of these genes is found in about half of JPS patients, suggesting that mutations in other genes may exist that predispose to JPS. TGFBRII is a member of the TGF-β signaling pathway and often somatically mutated in CRC. In this study, the role of TGFBRII in juvenile polyposis pathogenesis is investigated. **Methods**. Genomic DNA from 19 patients with juvenile polyps, without germline SMAD4 or BMPR1A mutation, was investigated for the presence of germline mutations in the TGFBRII gene. **Results**. No pathogenic TGFBRII variations were found in the germline of 19 patients with juvenile polys. **Conclusions**. No evidence was found for a role of TGFBRII in JPS pathogenesis, indicating that TGFBRII is unlikely to be a JPS susceptibility gene. Likely, other JPS causing genes exist in addition to SMAD4 and BMPR1A. Gut 2009 Jan;58(1):154-6

Juvenile polyposis (JPS) is an autosomal dominant disorder characterized by the presence of multiple gastro-intestinal juvenile polyps and an increased risk of colorectal (CRC).(1) JPS is caused by germline mutation of *SMAD4* or *BMPR1A*, both involved in the Transforming Growth Factor–ß/Bone Morphogenic Protein (TGF-ß/BMP) signaling pathway. A recent study 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 JPS causing genes exist.(2)

Several candidate genes, mostly involved in TGF-ß/BMP signaling, 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*

Table 1. Candidate genes investigated in the pathogenesis of juvenile polyposis

Gene	Patients studied/ Mutations found	Reference (first author and year)
BMPR1B (ALK6)	32/0	Howe 2004
BMPR2	59/0*	Howe 2004, van Hattem 2008
ACVR1 (ALK1)	66/0**	Howe 2004, Gallione 2004, van Hattem 2008
SMAD1	30/0	Bevan 1999
SMAD2	34/0	Bevan 1999, Roth 1999
SMAD3	34/0	Bevan 1999, Roth 1999
SMAD5	30/0	Bevan 1999
SMAD7	34/0	Bevan 1999, Roth 1999
CDX2	37/0	Woodford-Richens 2001

*32 patients investigated by sequencing, 27 by MLPA.
** 39 patients investigated by sequencing, 27 by MLPA.

mutations in patients with juvenile polyps likely represent CS or BRRS patients that have not (yet) developed extraintestinal 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-ß receptor type II (*TGFBRII*) is a component of the TGF-ß 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 *TGFBRII* has been reported in a patient with hereditary CRC (944C>T, reference sequence NM_003242).(10) Also, mice with conditionally knocked out *TGFBRII* in fibroblasts develop intraepithelial neoplasia of the prostate and invasive squamous cell carcinoma of the forestomach and loss of *TGFBRII* in intestinal epithelium promotes invasion and malignant transformation of tumors in Apc1638N/wt mice.(11, 12) Because of its role in TGF-β signaling and in (colorectal) carcinogenesis, we investigated whether germline mutation or deletion of the *TGFBRII* gene is involved in JPS pathogenesis.

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 *TGFBRII* gene. JPS was defined according to accepted clinical criteria.(1) All exons and intron-exon boundaries of the *TGFBRII* gene were analyzed by direct sequencing and the possibility of germline deletion of (parts of) the *TGFBRII* gene was investigated by MLPA (P065 MLPA kit, MRC-Holland BV, Amsterdam, The Netherlands). No pathogenenic germline mutations or deletions in *TGFBRII* were found in this cohort. Known polymorphic variations were found in intron 3, intron 4, exon 4, and intron 7 (Table 2).

TGFBRII 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 *TGFBRII* mutations are responsible for

Table 2. Polymorphisms found in TGFBRII

Location	Nucleotide	Amino acid change	Number of JPS patients	refSNP ID
Intron 3	c.338+7 A>G	intronic	01-09-18	rs1155705
Intron 4	c.530-4 T>A	intronic	01-07-18	rs11466512
Exon 4	c.1242 C>T	p.N414N	01-06-18	rs2228048
Intron 7	c.1600-8 C>T	intronic	01-01-18	rs11466530

Reference sequence: NM_001024847

the absence of malignancies in Marfan patients carrying a *TGFBRII* 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 *TGFBRII* have been found in HNPCC patients or in patients with familial or early onset CRC.(15, 16)

Because of its role in TGF-β signaling and CRC pathogenesis we hypothesized that *TGFBRII* 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 *TGFBRII* is unlikely to be involved in JPS pathogenesis since no germline mutations or deletions in *TGFBRII* 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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