Diploma thesis

Manifestations of Juvenile Polyposis Syndrome in SMAD4 Mutation Carriers of a Kindred

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Affidavit

Herewith I, Verena Schwetz, declare that I have written the present diploma thesis fully on my own and without any assistance from third parties. Furthermore, I confirm that no sources have been used in the preparation of the thesis other than those indicated in the thesis itself.

Graz, July 2010 Signature

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Abstract

Background/Aims: Juvenile polyposis syndrome (JPS) is an autosomal dominantly inherited syndrome leading to the development of multiple hamartomatous polyps of the entire gastrointestinal tract. Causative of JPS are germline mutations of three genes – SMAD4, BMPR1A and ENG. All three genes encode for proteins involved in the TGF-ß pathway, a molecular mechanism regulating a variety of biological processes such as cell proliferation and immune modulation. On the basis of juvenile polyps adenomas with different grades of intraepithelial neoplasia and subsequently adenocarcinoma can develop in the gastrointestinal tract. Family members of affected JPS patients should therefore undergo genetic testing. Regular upper and lower gastrointestinal endoscopy are recommended for mutation carriers. Based on a SMAD4 mutation positive family, the hypothesis was postulated that juvenile polyps occur not only in the stomach, small intestine and colon but also in the biliary tract and the gallbladder – a manifestation not yet described in the literature.

Methods: In the course of this prospective case study 8 affected SMAD4 mutation positive family members were screened for manifestations of JPS by means of endoscopy, sonography, and further imaging. Consequently, adequate therapy was carried out.

Results: Of these 8 affected family members, two were symptomatic at the time of diagnosis, six were asymptomatic – independent of the severity of the manifestation. The time span between the occurrence of the first symptoms and the correct diagnosis was six months for the index patient and 3 years for her relative. Each gene carrier presented with juvenile polyps in the colon, 5/8 with gastric manifestation in addition. In one patient early gastric cancer was detected, another showed a villous adenoma with high-grade intraepithelial neoplasia in the colon – both patients were asymptomatic. 2/8 patients presented with polyps in the gallbladder – in one case histological evaluation revealed cholesterol polyps – and 1/8 with a bile duct hamartoma confirmed in biopsy.

Significance: (1) All SMAD4 mutation carriers showed manifestations of JPS, the severity of which differing substantially. (2) 6/8 patients had an asymptomatic course of disease and were diagnosed only by genetic screening. (3) The two symptomatic patients had their correct diagnosis confirmed six months resp. three years after the occurrence of the first symptoms. (4) It remains controversial whether JPS occurs in the gallbladder and biliary tract – a manifestation not yet described in the literature, but if verified crucial for surveillance of SMAD4 mutation carriers.

Zusammenfassung

Einleitung: Das Juvenile Polyposis Syndrom (JPS) ist ein autosomal dominant vererbtes Tumorprädispositionssyndrom, das die Entstehung von multiplen hamartomatösen Polypen im gesamten Gastrointestinaltrakt hervorruft. Ursächlich sind Keinbahnmutationen dreier Gene – SMAD4, BMPR1A und ENG. Auf dem Boden von juvenilen Polypen können sich intraepitheliale Neoplasien, Adenome und in weiterer Folge Adenocarcinome im Gastrointestinaltrakt entwickeln. Genetische Untersuchungen sowie regelmäßige endoskopische Kontrollen bei bestätigtem Mutationsträgerstatus werden empfohlen. Anhand einer SMAD4 positiven Familie (acht Betroffene) wurde die Hypothese aufgestellt, dass juvenile Polypen nicht nur im Magen, Dünndarm und Colon auftreten, sondern auch die Gallenblase und Gallenwege betreffen können– eine bis dato noch nicht beschriebene Manifestation.

Methodik: Im Rahmen dieser prospektiven Fallstudie wurden 8 betroffene Angehörige einer Familie mit SMAD4 Mutation auf Manifestationen der JPS mittels Endoskopie und bildgebenden Verfahren untersucht und anschließend entsprechend behandelt.

Ergebnisse: Von den acht betroffenen Patienten waren zwei zum Zeitpunkt der Diagnosestellung symptomatisch, sechs asymptomatisch, unabhängig vom Ausprägungsgrad der Erkrankung. Die Zeitspanne vom Auftreten der Erstsymptome bis zur Diagnosestellung betrug 6 Monate bei der Indexpatientin, beziehungsweise 3 Jahre bei ihrer Familienangehörigen.
Jeder der Mutationsträger zeigte juvenile Polypen im Colon, 5/8 Patienten wiesen Magenmanifestationen auf. Bei einer Patientin wurde ein Magenfrühcarcinom diagnostiziert, bei einem Patienten ein villöses Adenom mit hochgradiger intraepithelialer Neoplasie im Colon – beide waren asymptomatisch. Bei 2/8 Patienten wurden Polypen in der Gallenblase nachgewiesen – in einem Fall ergab der histologische Befund das Vorliegen von Cholesterinpolypen – und bei 1/8 wurde ein Gallengangshamartom bioptisch bestätigt.

Diskussion: (1) Alle SMAD4-Mutationsträger wiesen Manifestationen des JPS auf – allerdings in äußerst unterschiedlicher Anzahl und Ausprägung. (2) 6/8 Patienten zeigten einen asymptomatischen Verlauf des JPS, wodurch die Diagnose erst im Rahmen des Screenings gestellt wurde. (3) Bei den zwei symptomatischen Patienten wurde die Diagnose erst 6 Monate bzw. 3 Jahre nach Symptombeginn gestellt. (4) Es bleibt strittig, ob das JPS auch in Gallenblase und Gallenwegen vorkommt – eine bisher noch nicht beschriebene Manifestation, die, falls bestätigt, von großer Wichtigkeit für das Screening von SMAD4-Mutationsträgern sein könnte.

Table of Contents

1 INTRODUCTION 11

1.1 Colorectal Cancer 11

1.2 Hereditary Hamartomatous Polyposis Syndromes 11

1.3 Juvenile Polyposis Syndrome 15
1.3.1 History 15
1.3.2 Diagnosis 16
1.3.3 Pathogenesis and Genetic Conditions 16
1.3.3.1 JP1 16
1.3.3.2 PTEN 17
1.3.3.3 BMPR1A 18
1.3.3.4 SMAD4 19
1.3.3.5 Large Genomic Deletions 23
1.3.3.6 ENG 24
1.3.3.7 Summary of Mutations 26
1.3.3.8 The TGF-ß Pathway 26
1.3.3.9 Tumourigenesis 30
1.3.3.10 Hamartoma – Adenoma – Carcinoma Sequence 33
1.3.3.11 Anticipation 33
1.3.4 Histology 34
1.3.5 Manifestations and Symptoms 37
1.3.5.1 Generalized Juvenile Polyposis and Juvenile Polyposis Coli 37
1.3.5.2 Juvenile Polyposis of Infancy 38
1.3.5.3 Gastric Polyposis 39
1.3.5.4 Hereditary Haemorrhagic Telangiectasia (HHT) 40
1.3.6 Malignant Potential 43
1.3.7 Surveillance Scheme 43
1.3.8 Therapy 45

2 MATERIAL AND METHODS 49

2.1 Aims 49

2.2 Hypotheses 49

2.3 Methods 49

3 RESULTS 51

3.1 Index Patient (III/2) 53

3.2 Patient II/6 56

3.3 Patient I/3 59

3.4 Patient I/2 61

3.5 Patient II/4 66

3.6 Patient II/3 67

3.7 Patient II/2 68

3.8 Patient III/4 69

3.9 Summary of Results 69

4 DISCUSSION AND CONCLUSIONS 73

4.1 SMAD4 Mutation and its Implications 73

4.2 Hypothesis (1) 74

4.3 Hypothesis (2) 77

4.4 HP Eradication 79

4.5 JPS as Differential Diagnosis 79

4.6 Implications for Surveillance 80

5 REFERENCES 81

Abbreviations

ACF = aberrant crypt foci
ACVRL1 or ALK1 = activin receptor-like kinase 1
APC = adenomatous polyposis coli
AVM = arteriovenous malformation
BMPR1A = bone morphogenetic protein receptor, type 1A (alternative titles: ACVRLK3, ALK3)
BRRS = Bannayan-Ruvalcaba-Riley syndrome
CAVM = cerebral arteriovenous malformation
CCS = Cronkhite-Canada syndrome
CS = Cowden syndrome
DCC = deleted in colorectal carcinoma
DPC4 = deleted in pancreatic carcinoma 4
EMR = endoscopic mucosal resection
ENG = endoglin
ESD = endoscopic submucosal resection
FAP = familial adenomatous polyposis
FISH = fluorescence in situ hybridization
FOBT = faecal occult blood test
GJP = generalized juvenile polyposis
HAVM = hepatic arteriovenous malformation
HGIEN = high-grade intraepithelial neoplasia
HHT = hereditary haemorrhagic telangiectasia
HMPS = hereditary mixed polyposis syndrome
HNPCC = hereditary non-polyposis colon cancer
HP = Helicobacter pylori
IEN = intraepithelial neoplasia
IRA = ileorectal anastomosis
JPC = juvenile polyposis coli
JPI = juvenile polyposis of infancy
JPS = juvenile polyposis syndrome
LGIEN = low-grade intraepithelial neoplasia
LOD = log. of the odds
LOH = loss of heterozygosity
MLPA = multiplex ligation-dependent probe amplification
N.e. = not examined
PAVM = pulmonary arteriovenous malformation
PJS = Peutz-Jeghers syndrome
PTEN = phosphatase and tensin homolog gene
SMAD4 = mothers against decapentaplegic, Drosophila, homolog 4 (alternative titles: MADH4, DPC4, SMA- and MAD-related protein 4)
SSCP = single-strand conformational polymorphism analysis
STRP = simple tandem repeat polymorphism
TGF-ß = tumor growth factor ß
TSG = tumour suppressor gene
UTR = untranslated region

Index of Figures

Figure 1: 4-bp deletion through slipped mispairing [41] 23
Figure 2: Germline mutations associated with JPS in the TGF-β and BMP pathways [47] 25
Figure 3: TGF-β and BMP pathways [6] 26
Figure 4: Hexameric complex formation [54] 28
Figure 5: The structure of SMAD4 [58] 29
Figure 6: Knudson's two-hit hypothesis for tumourigenesis [64] 31
Figure 7: Histology of a juvenile polyp 35
Figure 8: Histology of a juvenile polyp with adenomatous dysplasia and of an adenoma . 36
Figure 9: Characteristic histological findings in juvenile polyps and other polyposis syndromes [8] 37
Figure 10: EMR techniques [115] 46
Figure 11: Ileo-anal pouch anatomy [116] 47
Figure 12: Pedigree with affected family members 51
Figure 13: Colectomy specimen of patient III/2 with multiple pedunculated juvenile polyps 55
Figure 14: Upper endoscopy in patient II/6 57
Figure 15: Histology of early gastric cancer of the intestinal type G2, mucosa type pT1A in patient II/6 58
Figure 16: Massive gastric polyposis in patient I/3 60
Figure 17: Sonography of the gallbladder in patient I/2 63
Figure 18: Macroscopic image of the gallbladder with cholesterol polyps after cholecystectomy in patient I/2 63
Figure 19: Histology of cholesterol polyps found in the gallbladder in patient I/2 64
Figure 20: Findings during initial coloscopy in patient I/2 64
Figure 21: Upper endoscopy in patient I/2 65

Index of Tables

Table 1: Hamartomatous polyposis syndromes [3-7] 12
Table 2: Reported SMAD4 and BMPR1A mutations in the literature 22
Table 3: Mutations by sequencing and MLPA [43] 24
Table 4: The best-characterised TGF-ß subfamilies with their members, receptors and signalling molecules [50] 27
Table 5: Upper and lower gastrointestinal surveillance strategies [111] 45
Table 6: Patient III/2 53
Table 7: Patient II/6 56
Table 8: Patient I/3 59
Table 9: Patient I/2 62
Table 10: Patient II/4 66
Table 11: Patient II/3 67
Table 12: Patient II/2 68
Table 13: Patient III/4 69
Table 14: Overview of all results 72

1 INTRODUCTION

1.1 Colorectal Cancer

Colorectal cancer, besides lung cancer in men and breast cancer in women, the most common malignancy in adults, develops either sporadically or on the basis of inherited predispositions. Risk factors supporting its formation and progression are low-fibre nutrition rich in red meat and fat, chronic inflammatory diseases (especially ulcerative colitis), a positive family history of colorectal cancer, age over 40 years, smoking, and alcohol. Most colorectal carcinomas develop from adenomas in the colon or rectum [1].

The two most common inherited syndromes leading to the development of colorectal cancer are familial adenomatous polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC). Together, they account for about 6% of the total number of colorectal cancers. FAP is caused by germline mutations of the APC (adenomatous polyposis coli) gene located on chromosome 5 (5q21), HNPCC by germline mutations in six different mismatch repair genes including MLH1, MSH2, MSH3, MSH6, PMS1 and PMS2, located on chromosomes 2, 3 and 7 [1, 2].

Other inherited predispositions are the hamartomatous polyposis syndromes characterized by the development of hamartomas – meaning an overgrowth of cells native to the area – instead of adenomas. They are responsible for less than 1% of colorectal carcinomas [3].

1.2 Hereditary Hamartomatous Polyposis Syndromes

Seven syndromes belong to the group of hereditary hamartomatous polyposis syndromes leading to non-neoplastic malformations of the epithelium and connective tissue of the gut. They include Cowden syndrome (CS), Bannayan-Ruvalcaba-Riley syndrome (BRRS), Peutz-Jeghers syndrome (PJS), juvenile polyposis syndrome (JPS), basal cell nevus syndrome (BCNS), neurofibromatosis 1 (NF1) and multiple endocrine neoplasia syndrome 2B (MEN 2B) [4]. An eighth syndrome termed hereditary mixed polyposis syndrome (HMPS) is a variant of juvenile polyposis syndrome characterized by the development of hamartomas as well as adenomas in the gastrointestinal tract [4]. The other seven syndromes share the occurrence of hamartomatous polyps in the gastrointestinal tract. Another similarity is the autosomal dominant inheritance even though sporadic cases arising from de novo mutations are also known for each syndrome. The hamartomatous

polyposis syndromes can be distinguished in terms of their extraintestinal manifestations. Possibly, a ninth syndrome should be listed in this context: Cronkhite-Canada syndrome (CCS). All nine syndromes are summarized in Table 1.

Syndrome	Mutation	Main location of polyps	Extragastrointestinal manifestations
Cowden syndrome (CS)	PTEN	Throughout the GI tract	- Mucocutaneous manifestations : trichilemmomas, acral keratosis, papillomatous papules - Macrocephaly, high arched palate, hypoplastic mandible and maxilla, microstomia - Genitourinary abnormalities : ovarian cysts, multiple uterine leiomyomas and/or a bicornuate uterus - Breast: fibrocystic breast disease and adenocarcinoma - Thyroid: thyroid goiter, thyroid adenomas and follicular carcinoma - Esophageal glycogenic acanthosis
Bannayan-Ruvalcaba-Riley syndrome (BRRS)	PTEN	Distal ileum and colon	- Macrocephaly - Delayed psychomotor development - Lipomatosis - Haemangiomas - Pseudopapilledemas - Pigmentation on the glans penis
Peutz-Jeghers syndrome (PJS)	STK11	Small intestine	- Mucocutaneous pigmentation - Hamartomatous polyps in the upper and lower respiratory tract, pelvis and bladder - Malignancies in the pancreas, breast, ovary, testis and cervix
Basal cell nevus syndrome or Gorlin syndrome (BCNS or GS)	PTCH	Stomach	- Basal cell carcinomas - Medulloblastomas - Skeletal abnormalities: bifid ribs and bone cysts (especially in the mandibule) - Macrocephaly, frontal bossing, hypertelorism and intracranial calcification
Neurofibromatosis 1 (NF1)	NF1	Throughout the GI tract	- Dermal neurofibromas - Café au lait spots - Axillary and inguinal freckling - iIis Lisch nodules - Association with neuroendocrine tumours (somatostatinoma)
Multiple endocrine neoplasia syndrome 2B (MEN 2B)	RET	Throughout the GI tract	- Mucosal neuromas on the lips and tongue - Typical facies with enlarged lips - Marfanoid habitus - Thyroid carcinoma - Pheochromocytoma
Hereditary mixed polyposis syndrome (HMPS)	Chromosome 15q13-q14	Colon and rectum	None
Cronkhite-Canada syndrome	Unknown	Throughout the GI tract	- Onychodystrophy - Alopecia - Diffuse pigmentation
Juvenile polyposis syndrome (JPS)	SMAD4, BMPR1A, ENG	Throughout the GI tract	- Possible association with hereditary haemorrhagic telangiectasias (SMAD4 mutation carriers)

Table 1: Hamartomatous polyposis syndromes [3-7]

Differential diagnoses of hamartomatous polyposis syndromes in terms of causative mutations, locations of polyps and extragastrointestinal manifestations.

Cowden syndrome. CS is rare – even rarer than JPS – with a prevalence of 1 in 200 000. Apart from hamartomatous polyps found throughout the gastrointestinal tract this

syndrome is characterized by multiple extraintestinal manifestations – in these terms differing greatly from JPS. Its manifestations may include mucocutaneous lesions occurring in about 80% of CS patients: facial trichilemmomas (benign tumours of the hair shaft), acral keratosis, subcutaneous lipomas, papillomatous papules (e.g. oral cobblestoning – intraoral papilloma-like fibromas). Affected patients may additionally show macrocephaly, high arched palate, hypoplastic mandible and maxilla as well as microstomia. As for the patients' chests, supernumerary nipples and a pectus excavatum (caved-in chest) can be found. Genitourinary abnormalities such as ovarian cysts, multiple uterine leiomyomas and/or a bicornuate uterus occur. Importantly, patients afflicted with CS should be screened for abnormalities of the thyroid and breast as they may develop multinodular thyroid goiter, thyroid adenomas and follicular carcinomas as well as fibrocystic breast disease and adenocarcinoma of the breast. Another gastrointestinal manifestation apart from polyposis is oesophageal glycogenic acanthosis. All of these extraintestinal manifestations may also be subtle – thorough clinical examination is necessary to identify these characteristics in order to distinguish CS from JPS, especially in the rare case where mucocutaneous findings are not exhibited by the patients [3, 4].

Bannayan-Ruvalcaba-Riley syndrome. Bannayan-Ruvalcaba-Riley syndrome is the combination of three previously described syndromes: Bannayan-Zonana syndrome, Riley-Smith syndrome and Ruvalcaba-Myhre-Smith syndrome. BRRS is characterised by macrocephaly, delayed psychomotor development, lipomatosis, haemangiomas, pseudopapilledemas and pigmentation on the glans penis. About 45% of the afflicted patients are affected by intestinal polyposis – located mostly in the distal ileum and colon, though possible throughout the gastrointestinal tract [3, 4].

Both CS and BRRS are caused by germline mutations in the phosphatase and tensin homolog (PTEN) gene located on chromosome 10q23.31 – thus they have been summarized under the term PTEN hamartoma syndrome. It is discussed whether CS and BRRS may even represent different manifestations of the same syndrome [4].

Peutz-Jeghers syndrome. Typical of Peutz-Jeghers syndrome are mucocutaneus pigmentations located around the mouth, on the lips, in the perianal area, on the buccal mucosa and fingers. Freckling usually fades in the course of life (less so on buccal mucosa). [8] As the penetrance is variable some patients do not have to manifest with gastrointestinal polyposis – if they do, however, the most common location is the small intestine. Hamartomatous polyps can also manifest in the nares, lungs, bladder and pelvis. Extraintestinal tumours can develop in the pancreas, breast, ovary (sex cord tumours),

testis (Sertoli cell tumours) and cervix (adenoma malignum – histologically benign but biologically malignant). [3-5, 8] In 90% PJS is caused by germline mutations of STK11 (Serine/threonine-protein kinase 11), a tumour suppressor gene located on chromosome 19p13.3 [8].

Basal cell nevus syndrome. This syndrome, also referred to as Gorlin syndrome, encompasses the occurrence of multiple basal cell carcinomas, other tumours such as medulloblastomas, skeletal abnormalities such as bifid ribs (the sternal ends of which are cleaved into two) and bone cysts (especially in the mandibule), macrocephaly, frontal bossing (prominent forehead), hypertelorism and intracranial calcification [4]. Gastric hamartomatous polyps may occur. However, most patients afflicted with the syndrome do not show gastrointestinal manifestations. Gorlin syndrome is caused by a germline mutation in the PTCH gene (homologue of Drosophila patched) on chromosome 9q22.1 [4].

Neurofibromatosis 1. In this syndrome, even though not a typical hamartomatous polyposis syndrome, neurofibromas can involve the gastrointestinal tract causing abdominal pain and bleeding. Apart from that, patients manifest with multiple café au lait spots, axillary and inguinal freckling, iris Lisch nodules and multiple dermal neurofibromas [3, 4]. Neurofibromatosis 1 is associated with the development of neuroendocrine tumors, especially somatostatinomas, and mixed endocrine tumors (double neoplasms with glandular and endocrine components) [9, 10]. Mutations or deletions of the NF1 gene on chromosome 17q11 cause the development of this disease [3, 4].

Multiple endocrine neoplasia syndrome 2B. Characteristics of this syndrome are mucosal neuromas located on the lips and tongue, a typical face with enlarged lips and a marfanoid habitus. Patients can also develop gastrointestinal ganglioneuromatosis in about 40%. Besides, patients carry an increased risk of developing thyroid carcinoma and pheochromocytoma. Causative of MEN 2B are mutations in the RET proto-oncogene located on chromosome 10q11 [3, 4].

Hereditary mixed polyposis syndrome. The macroscopic and histological features of the polyps observed in this syndrome may be consistent with both adenomas as well as hamartomas, varying within affected families and even within individuals. Polyps occur mostly in the colon and rectum. As no extraintestinal features have been described, it has been confounded with JPS in the past [5]. Pedigrees showed linkage to a chromosomal region on 15q13-q21 and 15q14-q22. These regions contain the CRAC1 gene, a

susceptibility gene for colorectal adenomas and carcinomas. HMPS has also been mapped to chromosome 10q23 and mutations in BMPR1A have been discovered [11].

Cronkhite-Canada syndrome. Patients affected with CCS present with polyps in the gastrointestinal tract that resemble juvenile polyps together with onychodystrophy, alopecia and diffuse pigmentation. However, this syndrome is not familial and patients show their first manifestations when they are adults. [6] According to a case report, polyps are found throughout the gastrointestinal tract. [7] The same case report, however, leaves doubts as to whether CCS can truly be regarded as a hamartomatous polyposis syndrome. Okamoto et al. observed a remission of the polyps in the duodenum and colorectum after Helicobacter pylori eradication in one patient [7].

1.3 Juvenile Polyposis Syndrome

Even though JPS is the most common of all hamartomatous polyposis syndromes it is still a rare disease. Its incidence is approximately 1 per 100 000 births. Hamartomatous juvenile polyps in JPS manifest throughout the gastrointestinal tract – stomach, small intestine, colon and rectum and bear the risk of malignant transformation. Extraintestinal manifestations may be associated with JPS – none, however, is so common, typical or predominant that it could aid in diagnosis. Sporadic juvenile polyps on the other hand occur in about 2% of the paediatric population in the absence of JPS , in much smaller numbers and without the risk of developing colorectal cancer [4]. This is attributed to the fact that these polyps tend to outgrow their blood supply, become ischemic and autoamputate [12].

1.3.1 History

Diamond was the first to describe the histology of a juvenile polyp in 1939, the term ‘juvenile polyp’ being first coined by Horrilleno et al. in 1957. Five years later, Morson et al. differentiated them from adenomas in postulating them to be hamartomas. The term juvenile polyposis coli was first defined by McColl et al. in 1964 describing the presence of multiple juvenile polyps in the gastrointestinal tract and thus distinguishing this situation from solitary juvenile polyps on the one hand and adenomatous polyposis on the other. Smilow et al. first proposed an autosomal dominant inheritance pattern for juvenile polyposis syndrome [6, 13].

1.3.2 Diagnosis

First of all, the occurrence of solitary juvenile polyps needs to be distinguished from the manifestation of juvenile polyposis syndrome, as the latter carries an increased risk of malignant transformation whereas the first does not. Sachatello et al. [14] suggested in 1974, that at least one of the following conditions needs to be fulfilled in order to secure the diagnosis of juvenile polyposis syndrome: (1) more than ten colonic juvenile polyps; (2) juvenile polyps throughout the gastrointestinal tract; or (3) any number of juvenile polyps in an individual with a family history of juvenile polyposis. These diagnostic criteria have been revised by Jass et al. [15] in 1988 suggesting that more than five juvenile polyps of the colorectum are sufficient to diagnose JPS. In 1991, Giardiello et al. [16] came up with yet another definition for JPS. They recommended that patients with three or more juvenile polyps or with a family history of juvenile polyps should undergo colonoscopic surveillance.

1.3.3 Pathogenesis and Genetic Conditions

1.3.3.1 JP1

Up to 1998, the genetic source underlying JPS was unknown. The first to explore mutations corresponding to the syndrome were Leggett et al. in 1993 [17]. A linkage study for APC markers on chromosome 5 was performed and concluded that APC genes were not altered in JPS in contrast to FAP. This discovery together with the distinct histological characteristics of juvenile polyps suggested the existence of a separate genetic syndrome.

The first report of a novel tumour suppressor gene causing juvenile polyposis was provided by Jacoby et al. in 1997 [18]. A child with multiple congenital abnormalities and juvenile polyposis was cytogenetically analyzed and a de novo interstitial deletion of chromosome 10, del(10) (q22.3q24.1), was found.

Later, using polymerase chain reaction amplification of microsatellite markers and fluorescent in situ hybridization (FISH) Jacoby et al. [19] analyzed 47 juvenile polyps from 16 unrelated patients with either hereditary or sporadic juvenile polyps. The aim was to show that in both groups loss of function of a tumour suppressor gene is the basis for polyps to develop. Of the 47 sporadic as well as hereditary juvenile polyps 39 (83%) were caused by a somatic deletion mutation on the long arm of chromosome 10. This deletion of chromosomal areas is referred to as loss of heterozygosity (LOH) [20] and was found exclusively in stromal cells of the juvenile polyps and not in the epithelium. Progression

from hamartoma to adenoma was explained by interactions between the stromal clonal cells and the epithelium, inducing epithelial cell growth and neoplastic progression. This explanation of tumour development in juvenile polyposis was referred to as 'landscaper' hypothesis (see 1.3.3.9) [21].

Juvenile polyposis syndrome seemed to be linked with a congenital deletion of this locus referred to as JP1 [19]. Unclear remained, which of the genes mapped to the 10q22-24 region was responsible for actually causing the polyps, the most probable being the apoptosis-signalling receptor gene FAS. Loss of function of this locus might lead to the growth of juvenile polyps implicating it to be a novel tumour suppressor gene. Various reasons support this assumption: First, LOH could be found in 83% of the examined polyps. Second, a proliferation of lamina propria cells could be observed in those polyps where the deletion in chromosome 10 was detected. Third, the risk for malignancy in juvenile polyposis patients is heightened [19].

The region on chromosome 10 also encompasses a gene called PTEN, mapped to 10q23.3 and causative of both Cowden as well as Bannayan-Ruvalcaba-Riley Syndrome. PTEN encodes a dual specificity phosphatase [22].

1.3.3.2 PTEN

Considering the similar clinical features in terms of polyposis, the fact that CS and BRRS are caused by PTEN mutations and JPS by presumed mutations in JP1 located in close vicinity to PTEN, gave rise to the aim to determine whether JPS was allelic to CS and BRRS. Linkage analysis was carried out by Marsh et al. [22] for eight JPS families using microsatellite markers for the PTEN locus. LOD (log. of the odds) scores[1] of < -2.0 were calculated, thus excluding PTEN and any genes within a 20-cM[2] interval as possible loci for JPS. Two explanations are possible for these differing results found by Jacoby et al. on the one hand and Marsh et al. on the other [22]: First, more than one gene might be responsible for causing JPS – also referred to as genetic heterogeneity. In this case, PTEN would account for a small part of JPS cases. Second, as for the child with LOH on chromosome 10 described by Jacoby et al., it might be possible that this mutation causes the congenital abnormalities of the extremities, head and abdomen, but not the juvenile polyposis. In that case, a second independent mutation must exist which could not be found by the cytogenetic analysis used.

[1] Log. of the odds scores are used in linkage analysis. Linkage is significant if LOD > 3.0.
[2] 1 cM (Centi-Morgan) equals a distance of 1000 kilobases on the DNA.

Olschwang et al.'s [23] observations of 14 patients, three of which carry PTEN mutations, seem to substantiate the findings that PTEN mutations account for at least a part of JPS cases. As mutations in the same gene are also causative of CS and BRRS, this variation in expressivity may depend on the type of PTEN mutation. According to Olschwang et al., for a clear genotype-phenotype correlation the number of mutations identified up until now is insufficient [23]. Lynch et al. [24] found germline PTEN mutations in one family affected by JPS. These results raise doubts as to whether clinical diagnosis of JPS based on exclusion of the extraintestinal manifestations of CS and BRRS was far-reaching enough in these studies. The possibility remains that patients with PTEN mutations were in truth affected by CS or BRRS rather than JPS [25].

Rendering more precisely, critics argue that of the three affected patients described by Olschwang et al., one had laryngeal cancer and a thyroid nodule and thus possibly manifestations of Cowden syndrome. The other two were 14 and 7 years of age at the time of investigation. At that point, no other manifestations were reported in these patients apart from polyposis. At this age however, penetrance of CS is low and typical manifestations of the syndrome develop at a later age [26]. This age-related penetrance in CS is 90% by the age of 20 years and under 10% in patients younger than 15 years [27]. Eng et al. further criticize that the patients investigated by Lynch et al. also showed criteria consistent with a diagnosis of CS [26].

1.3.3.3 BMPR1A

Yet, in 2001 Howe et al. [28] again performed linkage analysis for chromosome 10q for four families affected by JPS without PTEN mutations or SMAD4 mutations that had been discovered before (see 1.3.3.4). They found a region corresponding to 10q22-23 with PTEN and the putative JPS gene JP1 in its direct vicinity. Since PTEN has been accepted as causing CS and BRRS but has been excluded as causative of JPS, other possible genes were screened. In all four kindreds analyzed BMPR1A/ALK3/SKR5 (bone morphogenetic protein receptor type 1A) mutations were found causing prematurely truncated proteins. This was the first report of BMPs playing an important part in controlling epithelial neoplasia. BMPR1A is a type I receptor on the cell membrane which upon mutation, loses its serine-threonine kinase domain and thus its ability to start intracellular signalling. Zhou et al. [29] analyzed 18 unrelated families and seven isolated cases of SMAD4 negative clinically ascertained JPS cases. Of these 25 patients 10 (6 familial and 4 isolated cases) revealed germline BMPR1A mutations. Of the described 10 mutations 8 were either

nonsense, frameshift of splice-site mutations leading to truncated receptors lacking all or part of their kinase domain. Binding of the ligands still takes place but intracellular signalling is disrupted. 8 of the 14 mutations described until then were located in the N-terminal domain, while no mutations could be found in the C-terminal domain of the gene [29].

The loss of function of the BMPR1A gene is traced back not only to the germline mutation itself, but also to its combination with the so-called somatic second hit, a somatic mutation in the second allele. In the tumours investigated, loss of heterozygosity (LOH) was found in the BMPR1A region together with the germline mutation in the other allele. These findings correspond to Knudson's two-hit hypothesis (see 1.3.3.9) and implicate that BMPR1A is a tumour suppressor gene [29].

Friedl et al. found 5 out of 29 patients to carry a BMPR1A mutation (17%) [30]. In the analysis of 77 JPS patients carried out by Howe et al. in 2004 [31], 16 BMPR1A mutations were reported, corresponding to 20.8% of the tested cases. One of four Korean JPS patients was identified as a BMPR1A mutation carrier by Kim et al. [32] and Rozen et al. [33] found two out of 10 Jewish pedigrees positive for mutations in this gene. In summary, 31 different mutations in the BMPR1A gene have been reported in JPS [31]. Howe et al. combined their results with those of Friedl et al. indicating a BMPR1A mutation rate of 19.8% (21 of 106) in JPS patients [31].

No germline mutations were found in the other components of the TGF-ß pathway SMAD1, SMAD2, SMAD3 and SMAD5 when screening 30 SMAD4 negative patients fulfilling the clinical JPS criteria [34]. Four JPS patients negative for SMAD4 mutations examined by Roth et al. [35] also did not have mutations of either SMAD2, SMAD3 or SMAD7.

1.3.3.4 SMAD4

Before linkage to BMPR1A was demonstrated, Howe et al. [25] were able to identify the genetic locus responsible for JPS in a large family. Thus linkage to JPS was revealed for a region on chromosome 18q21.1, between the markers D18S1118 and D18S487. The tumour suppressor genes DCC (deleted in colorectal carcinoma) and DPC4 (deleted in pancreatic carcinoma 4) can be mapped to this region thus being potentially causative of JPS. DCC is a tumour suppressor gene deleted in many sporadic colorectal carcinomas. [21] DPC4 – also known as MADH4 or SMAD4 (SMAD4 = small mothers against

decapentaplegic, Drosophila, homolog 4) – belongs to the Mad gene family and is important for signal transduction in the TGF-ß pathway.

The same research group that had discovered linkage to chromosome 18q21.1 started sequencing PCR products of an affected individual to determine the tumour suppressor gene causing JPS. After having sequenced 14 of 29 exons of DCC and all 11 exons of SMAD4, they found a 4-bp (base pair) deletion in exon 9 of the SMAD4 gene. Consequently another eight unrelated patients were analyzed using SSCP (single-strand conformational polymorphism analysis) and genomic sequencing. In four of those eight, SMAD4 mutations were found: two 4-bp deletions in exon 9, one 2-bp deletion in exon 8 and one 1-bp insertion in exon 5. All of these were frameshift mutations and led to the creation of a new stop codon [36].

Genome sequencing by Friedl et al. in 1999 [37] of another 11 unrelated JPS patients revealed SMAD4 mutations in three cases. Two of the patients showed a 4-bp deletion in exon 9, while for the third a novel mutation was discovered in exon 6. Summing up the results of both Howe et al. and Friedl et al., SMAD4 germline mutations could be identified in 8 out of 20 JPS patients. 5 of these mutations were 4-bp deletions in exon 9, thus accounting for 25% of JPS cases [37]. Another work published by Houlston et al. [38] showed that of 21 analyzed patients only one had a SMAD4 mutation on exon 8, leading to a missense mutation.

The discrepancy in results between this group and the two others described previously, may be traced back ‘to the nonuniform selection criteria for patients’ with JPS [37]. As supposed by Houlston et al. [38], neither the study by Howe et al. nor his own had used methods capable of detecting large deletions in DPC4 or had analyzed UTRs (untranslated regions), introns or promoter regions. The greater part of the mutations responsible for JPS still remains unclear, supporting the theory of genetic heterogeneity in this syndrome.

Roth et al. analyzed four JPS kindreds and three sporadic cases in 1999 and found three different mutations in the SMAD4 gene. Mutation analysis was also performed for SMAD2, 3 and 7. However, no mutations were found in these genes [35]. As the two patient groups analyzed by Howe et al. and Roth et al. partly overlap, they can be added up, revealing SMAD4 germline mutations in 8 out of 12 cases.

Woodford-Richens et al. [39] analyzed 56 patients, 47 of which belonged to 15 different families and nine of which were sporadic patients. Five of these 24 showed germline mutations in the SMAD4 gene, accounting for 21% of the cases. Failure to find SMAD4

mutations in the other patients is attested to the same problem as mentioned by Houlston et al.

Screening of five Korean JPS patients in 2000 by Kim et al. [40] revealed SMAD4 mutations in three of the cases – for two patients the mutation was located in exon 9, for one in exon 8. PCR-SSCP analysis and bi-directional sequencing were used for the screening. In 2002, Friedl et al. [30] again analyzed 29 patients and identified SMAD4 germline mutations in seven cases. Six of the seven mutations are causative of a truncated protein. The patients analyzed in 1999 were included in this study. In an analysis by Howe et al. in 2004 [31] of a large group of 77 JPS patients, SMAD4 mutations were revealed for 14 cases (18,2%).

The results of these six studies by Friedl et al. (2002), Howe et al. (two studies), Woodford-Richens et al., Kim et al. and Roth et al. were combined not including the results of Houston et al. [38]. Identical cases in the studies were identified resulting in a total of 141 patients investigated. Of the 141 JPS patients in these studies 32 had 26 different SMAD4 mutations, resulting in a SMAD4 mutation prevalence of 22.7% [31]. An overview of the results is provided in Table 2.

Of these 26 mutations of the SMAD4 gene, 1244delACAG in exon 9 was identified in six different families [30, 31]. This raises the question if these families had common ancestors or whether this mutation leading to a stop at codon 435-6 is a mutational hotspot. 15 simple tandem repeat polymorphism (STRP) markers flanking the SMAD4 gene were genotyped by Howe et al. [41] using the DNA from four families with JPS (from Iowa, Mississippi, Texas and Finland). Haplotype analysis did not reveal common haplotypes for these four families thus implicating that the members of these four families did not have common ancestors. It was shown, however, that the region flanking the SMAD4 gene (a 14-bp region containing the deletion) contained four direct repeats and one inverted repeat, predisposing to microdeletions and thus possibly representing a mutational hotspot. Krawczak and Cooper [42] studied the causative mechanisms leading to deletions in 60 cases and found that in all but one the deletion had direct repeats between 2 bp and 8 bp in the adjacent sequences. In the 4-bp deletion of exon 9 in the SMAD4 gene various AG repeats can be found: CTT**AGAC**AGAGAAG. The reasons leading to deletion can be either slippage of DNA polymerase, slipped mispairing or palindromic sequences. Slipped mispairing is explained in Figure 1.

Chromo-some	Gene	No. of patients	Mutation	Result	Author
18	SMAD4	8/12[3] = 67%	3x 4-bp deletion in exon 9, codon 414-416	Frameshift mutations→ stop at codon 434	Howe et al. 1998 [36] and Roth et al. 1999 [35]
			1x 2-bp deletion in exon 8	Frameshift → stop at codon 350	
			1x 1-bp insertion in exon 5	Frameshift → stop at codon 235	
			1x 4-bp deletion in exon 9, nucleotides 1372-1374	Frameshift → stop at codon 434	
			A → C transition in exon 8	Tyr → Ser	
			C → G transversion in exon 4	Ser → Stop	
		5/7[4] = 71%			
18	SMAD4	3/11[5]	2x 4-bp deletion in exon 9	Frameshift	Friedl et al. 1999 [37]
			Novel mutation in exon 6	Frameshift	
18	SMAD4	7/29 = 24%	**2x 1244_1247delACAG in exon 9**	Frameshift	Friedl et al. 2002 [30]
			1157G→A in exon 9	G386D	
			831_832delAC in exon 6	Frameshift	
			1342C→T in exon 10	Q448X	
			1554delG in exon 11	Frameshift	
			1550_1551insAGAG in exon 11	Frameshift	
10	BMPR1A	5/29 = 17%			
18	SMAD4	1/21[6]	Mutation in exon 8	Missense mutation	Houlston et al. 1998 [38]
18	SMAD4	**5/24** = 21%	11-bp deletion in exon 4	Frameshift → stop codon	Woodford-Richens et al. 2000 [39]
			CGC to TGC in exon 8	Arg to Cys	
			2-bp deletion in exon 11	Frameshift → stop codon	
			9-bp deletion in exon 1	Deletion of amino acids	
			CGA to TGA in exon 10	Substitution → stop codon	
18	SMAD4	**3/5** = 60%	GAA → AAA in exon 9	Glu → Lys (missense)	Kim et al. 2000 [40]
			CAG → TAG in exon 9	Gln → Stop (nonsense)	
			CGC → CAC in exon 8	Arg → His (missense)	
18	SMAD4	14/77 = 18,2%	608delC in exon 4	Stop 240-1	Howe et al. 2004 [31]
			989A>G in exon 8	E330G	
			1037delC in exon 8	Stop 383-4	
			1054G>A in exon 8	G352R	
			1081C>G in exon 8	R361G	
			1081C>A in exon 8	R361S	
			1162C>T in exon 9	E388X	
			1244delACAG in exon 9	Stop 435-6	
			1244delACAG in exon 9	Stop 435-6	
			1244delACAG in exon 9	Stop 435-6	
			1244delACAG in exon 9	Stop 435-6	
			del1343-1365 in exon 10	Stop	
			1529G>T in exon 11	G510V	
			1588delC in exon 11	Stop 536-7	
10	BMPR1A	16/77 = 21%			
Total amount of SMAD4 mutations: 32/141 = 22.7% These 32 patients had 26 different SMAD4 mutations.					

Table 2: Reported SMAD4 and BMPR1A mutations in the literature

SMAD4 and BMPR1A mutations with their corresponding results found by Howe et al., Roth et al., Friedl et al., Houlston et al., Woodford-Richens et al. and Kim et al. as well as the number of patients investigated in their studies. Mutations in bold indicate a possible mutational hotspot [30, 31, 35-40].

[3] Results of both studies were combined and double counts eliminated.
[4] Both studies were compared to the results by Howe et al. 2004 and identical cases were excluded.
[5] Cases were included in the results by Friedl et al. 2002.
[6] Not included in the total count.

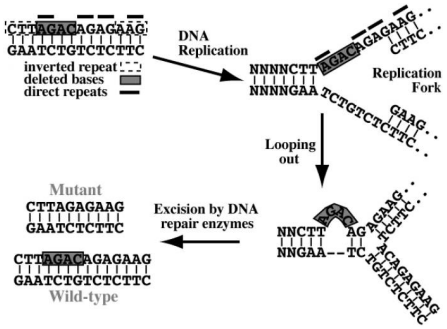

Figure 1: 4-bp deletion through slipped mispairing [41]

Slipped mispairing implicates that the second repeat pairs with the complement of the first causing the sequence that is left out to form a loop which is excised by repair enzymes. The upper-strand copies will include the 4-bp deletion, the lower-strand copies will correspond to the wild-type sequence.

1.3.3.5 Large Genomic Deletions

Considering the point mutation rates of BMPR1A (19.8%) and SMAD4 (22.7%) described above, about 60% of the JPS cases have unresolved genetic conditions. This suggests either that the corresponding susceptibility genes still have not been detected or that alternative mutations of the already known genes account for these cases. An alternative mechanism inactivating the two known genes could be large genomic deletions, which cannot be identified by sequencing [43]. Instead, multiplex ligation-dependent probe amplification (MLPA) needs to be used for the identification.

In 2007, Aretz et al. [44] analyzed 65 patients with typical JPS (80 patients when including 15 presumed JPS cases). In six patients large SMAD4 deletions were found and large BMPR1A deletions were detected in three patients. Adding up these results with the point mutations of SMAD4 and BMPR1A identified in these 65 cases, there were 23 SMAD4 and 16 BMPR1A mutations in total, corresponding to 35% and 25% of the cases respectively. In total, mutations of these two genes account for 60% (39/65) of JPS cases, or 49% when including the presumed JPS cases in the analysis (39/80). One of the patients analyzed had a deletion of the entire BMPR1A and PTEN gene.

In a study by van Hattem et al. [45] germline mutations of SMAD4 and BMPR1A were found in 13 of 27 JPS cases (48.1%), nine of which (33.3%) were point mutations and four of which (14.8%) were large genomic deletions. Of these four, two patients had a hemizygous deletion of both BMPR1A and PTEN.

Calva-Cerqueira et al. [43] sequenced 102 patients for point mutations in SMAD4 and BMPR1A and found 20/102 (19.6%) and 22/102 (21.6%) respectively. The 60 probands without point mutations were screened for large genomic deletions by MLPA. One patient

had a heterozygous deletion of the SMAD4 gene, another had a heterozygous deletion of the promoter and two non-coding exons of SMAD4. The third patient had a deletion of the promoter and the first non-coding exon of BMPR1A and in one case a contiguous deletion of both entire BMPR1A and PTEN genes was found. The results of the three studies are summarized in Table 3.

Comparing the results of all studies discussed above, four patients were found in total that had a deletion of both BMPR1A and PTEN. Deletions of this region have been described in 11 patients by different authors, with PTEN deletions in all, and additional definite BMPR1A deletions in six cases. BMPR1A deletions were supposed in another three patients but remained unclear [45]. The majority of these patients had juvenile polyposis of infancy – an early manifestation of JPS originally described by Sachatello et al. [46] (see 1.3.5.2).

Using sequencing as well as MLPA to detect large genomic deletions, the rate of identified mutations causative of JPS ranges from 45.1% [43] to 48,1% [45] and 49% to 60% [44] (depending on the cases included). For the other half of the patients no mutations have been found so far. Mutations in the promotor regions or in intronic sequences as well as yet undiscovered genes could be responsible for some cases.

	SMAD4 mutations		**BMPR1A mutations**	
Study	**Sequencing**	**MLPA**	**Sequencing**	**MLPA**
Calva-Cerqueira et al. 2008 [43]	20/102	2/60	22/102	2/60
Van Hattem et al. 2008 [45]	6/27	1/18	3/27	3/18
Aretz et al. 2007 [44]	17/65	6/50	13/65	3/50
Sub total		9/128 (7%)		8/128 (6.3%)
Total	52/194 (26.8%)		46/194 (23.7%)	

Table 3: Mutations by sequencing and MLPA [43]

Summary of results of three studies and shows the amount of point mutations detected by sequencing in SMAD4 and BMPR1A. The patients without point mutations were screened for large genomic deletions by means of MLPA. The amount of large genomic deletions found in the patients without point mutations is indicated as sub total. The amount of point mutations of all patients analyzed is indicated as total.

1.3.3.6 ENG

In 2005, Sweet et al. [47] tested 14 JPS patients who were mutation-negative for SMAD4 and BMPR1A and found germline mutations in ENG in two patients. ENG encodes endoglin, an accessory protein and coreceptor in the TGF-ß signalling pathway (see Figure 2). Endoglin is mostly expressed in vascular endothelial cells and thus might play a role in polyp development and carcinogenesis. This gene had previously been associated with hereditary hemorrhagic telangiectasia (HHT) – an autosomal dominantly inherited disease

causing vascular dysplasia in many organs. These two patients, however, showed no signs of HHT, but an unusually early onset of juvenile polyposis (aged 3 and 5 years). There are possible explanations why these two patients with ENG mutations develop JPS but not HHT. (1) There might be a subset of patients with ENG mutations developing JPS but not HHT, perhaps depending on the type and location of the mutation. Most of HHT type 1 associated ENG mutations map to exons 1 and 9, while JPS-related ENG mutations are located in exons 11 and 12. (2) HHT may have age-related penetrance and these patients might develop its manifestations later in life.

Howe et al. [48] also found ENG mutations in JPS patients negative for SMAD4 or BMPR1A mutations. These patients also did not show any signs of HHT nor gastric polyposis. Their mean age was 7.4 years at diagnosis compared to 14.4 years for patients without ENG mutations. Despite these findings, the role of ENG as predisposing to JPS still needs to be confirmed.

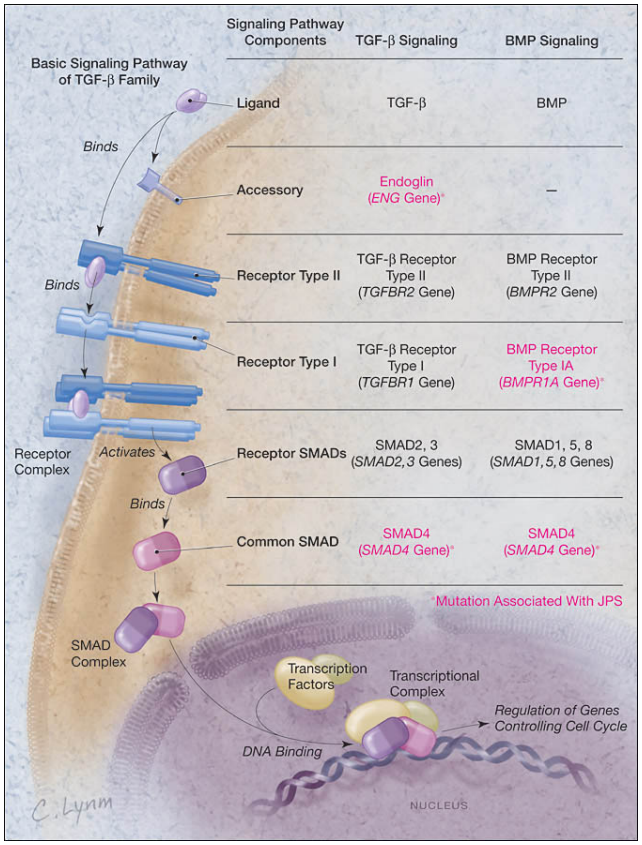

Figure 2: Germline mutations associated with JPS in the TGF-β and BMP pathways [47]

TGF-β is one of the most potent inhibitors of cell growth. Mutations in SMAD4, a common signalling element in the TGF-β signalling pathway and the BMP pathway, and in BMPR1A are responsible for JPS. A third mutation has been detected in ENG, encoding endoglin, which is an accessory component of the TGF-β receptor complex.

1.3.3.7 Summary of Mutations

The first chromosomal area associated with JPS was on chromosome 10q22-24 and named JP1. Further analysis of this region revealed the gene PTEN located on chromosome 10q23.31 as causative of CS and BRRS but not JPS. 10q22.3 in JP1 was finally found to be one responsible gene for JPS – it was termed BMPR1A. Another gene located on chromosome 18q21.1, named SMAD4, was detected as causative of the syndrome. Considering point mutations as well as large chromosomal deletions, these two genes account for about 60% of all JPS cases. A third gene, ENG, has been identified in some cases of JPS.

1.3.3.8 The TGF-ß Pathway

The TGF-ß (transforming growth factor ß) superfamily includes various cytokines and plays an important role in the regulation of cell proliferation and differentiation, in matrix production and apoptosis. A wide range of biological processes is regulated by these polypeptide growth factors. In adults, these cytokines regulate tissue repair and are involved in the modulation of the immune system. Besides, they are crucial for embryonal development, especially in terms of pattern formation and tissue specification [49, 50].

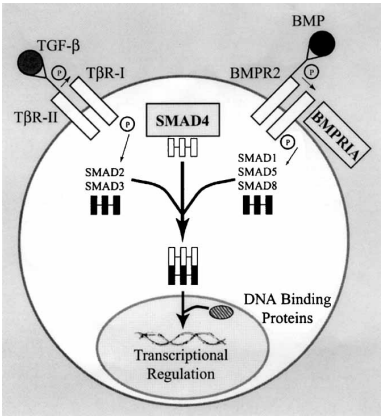

Figure 3: TGF-β and BMP pathways [6]

Signal transduction in the TGF-ß pathway is initiated once a ligand of the TGF-ß superfamily binds to the TßR-II receptor. This receptor phosphorylates and thus activates the TßR-I receptor. For signal transduction from the cell membrane to the nucleus the activated TßR-I receptor phosphorylates SMAD2 or 3 at their carboxy-terminal. As for BMPs, SMAD1 or SMAD5 is phosphorylated. The phosphorylated SMAD forms a heteromeric complex with SMAD4. This complex is transported to the nucleus where it interacts with DNA-binding proteins to activate transcription [50].

This large family consists of TGF-ßs, activins, inhibins and bone morphogenetic proteins (BMPs) (see Table 4) [50]. Signal transduction – as shown in Figure 3 – is initiated once a ligand of the TGF-ß superfamily binds to its receptor. TGF-ß1, for example, is the ligand for the TßR-II receptor, a serine/threonine kinase occurring in the cell membrane. [50]

Once bound to the TßR-II receptor, the latter phosphorylates and thus activates the TßR-I receptor. TGF-ß1 binds to the type II receptor with high affinity. The type I receptor is not needed for binding, but for starting the signal transduction [51]. Both receptors belong to the same family and resemble each other in structure. However, type I receptors have a region made up of glycine and serine residues, a so-called GS-domain, which is lacked by type-II receptors. This is the region where phosphorylation takes place [50]. Each ligand of the TGF-ß family requires its own receptor or its individual combination of receptors. For high-affinity binding TGF-ß2 is in need of TßR-I or TßR-III in addition to TßR-II, to which it binds only with low affinity [51]. For high-affinity binding BMPs require both BMP receptors at the same time [50].

Subfamily	**TGF-ß**	**Activin**	**BMP**
Examples of ligands	TGF-ß1 TGF-ß2 TGF-ß3	Activin A	BMP-2 BMP-4 BMP-7/OP-1
Type II receptors	TßR-II	ActR-II ActR-IIB	BMPR-II ActR-II ActR-IIB
Type I receptors	TßR-I	ActR-I? ActR-IB	BMPR-IA BMPR-IB ActR-I
Pathway-restricted SMADs	Smad2 Smad3	Smad2 Smad3	Smad1 Smad5 Smad9?
Common-partner SMAD	Smad4	Smad4	Smad4
Inhibitory SMADs	Smad6 Smad7	Smad6 Smad7	Smad6 Smad7
Responses	Inhibition of mitogenicity and induction of extracellular matrix	Induction of dorsal mesoderm, of erythroid differentiation and of follicle-stimulating hormone release	Induction of ventral mesoderm, of cartilage and bone and of apoptosis

Table 4: The best-characterised TGF-ß subfamilies with their members, receptors and signalling molecules [50]

For signal transduction from the cell membrane, where TGF-ßs, activins or BMPs bind to their receptors, to the nucleus, SMAD proteins have been identified as crucial. At least nine different proteins have been found so far and named SMAD as a vertebrate homologue for the Mad and Sma genes found in Drosophila and Caenorhabditis elegans [50]. In the TGF-ß pathway, the activated TßR-I receptor phosphorylates SMAD2 or 3 at their carboxy-terminal. The phosphorylated SMAD2 or 3 in turn forms a heteromeric complex with SMAD4 (see Figure 4). This complex is transported to the nucleus where it interacts with DNA-binding proteins to activate transcription [52, 53]. As for BMPs, SMAD1 or SMAD5 is phosphorylated and also forms a complex with SMAD4, which is

therefore referred to as a common-partner SMAD, as it plays an important role in signal transduction in both subfamilies. SMAD1, 2, 3 and 5 are thus receptor-dependent and pathway-restricted as they function either for TGF-ß or BMP [54].

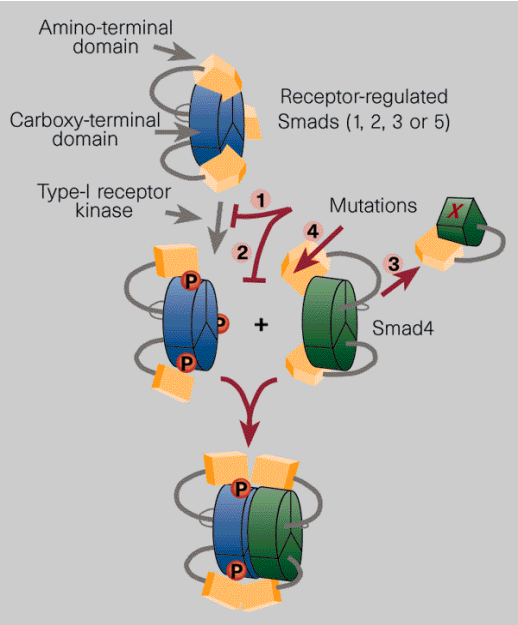

Figure 4: Hexameric complex formation [54]

The amino-terminal domain autoinhibits the carboxy-terminal domain. The phosphorylation of receptor-regulated SMADs stops this inhibition. Consequently, formation of a hexameric complex with SMAD4 can take place. This complex interacts with DNA-binding proteins to activate transcription.

SMAD4 is a tumour suppressor gene that is inactivated in pancreatic and other carcinomas [55]. Analysis of SMAD2 in sporadic colorectal carcinoma by Eppert et al. [56] identified four missense mutations in this gene. Three of these four mutations were found to be inactivating, thus implicating SMAD2 to be also a tumour suppressor gene.

SMAD structure. SMAD proteins are made up of an amino-terminal (N-terminal or MH1) and a carboxy-terminal (C-terminal or MH2) domain connected by a proline-rich linker region [49]. SMAD4 differs from the pathway-restricted SMADs in the carboxy-terminal, as it does not contain serine residues. These serine residues are needed by SMAD1, 2, 3, and 5 for phosphorylation [52, 53, 57]. The C-domain of all SMADs functions as effector domain, whereas the N-domain has an inhibitory effect on the C-domain and thus prevents the interaction between SMAD2 and SMAD4 for instance [54]. Shi et al. [58] have described the structure of SMAD4 (see Figure 5). This monomer consists of a ß-sandwich, one end is capped by three α-helices, the other is capped by three loops and one α-helix. The latter end together with a part of the ß-sandwich is also referred to as the loop/helix region. The loop/helix region of one subunit interacts with the triple helix of the other

subunit to form a trimeric structure resembling a disk. The amino termini stick out from one face of the disk, the carboxy termini emerge from the side. Essential for forming the heteromeric complex between the trimers of pathway-restricted SMADs and SMAD4 trimers is the third loop from the loop/helix region exposed on the face opposite the amino-termini.

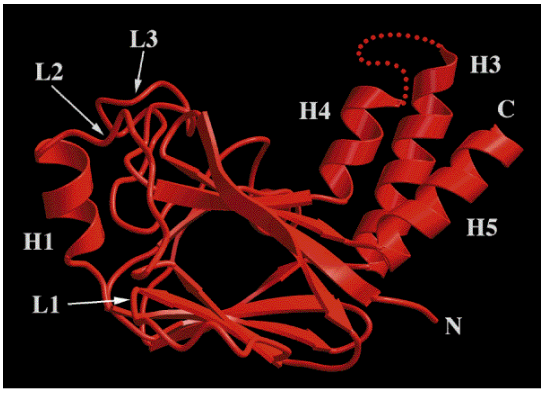

Figure 5: The structure of SMAD4 [58]

The three loops are labelled L1, L2 and L3 and the helix H1 on the one end, the three helices on the other end are labelled H3, H4 and H5.

Mutations. Of the 26 different SMAD4 mutations described so far [31], one mapped to the MH1 region, five to the linker region and the remaining 20 mutations to the MH2 domain. Mutations of the C-domain are thus the most common SMAD4 mutations. Three types of substitutions that cause failure in SMAD function have been identified by Shi et al. [58]. The first mutation affects the hydrophobic core of SMAD and leads to destabilization of the protein. The second affects the third loop and thus the formation of heteromeric complexes and the third maps to the interface region between the SMAD4 monomers. It hinders homotrimer formation. All of these mutations cause a stop in the signal transmission in the TGF-ß pathway. Mutations of the N-domain lead to an increase in the inhibitory function, to a greater affinity of the N-terminal to the C-terminal, thus resulting in a prevention of signalling. This so-called 'gain of autoinhibitory function' has been discovered by Hata et al. [59] and is an uncommon way of inactivating tumour suppressors. Mutations occurring in pathway-restricted SMADs interfere with their phosphorylation [56, 60].

Inhibitory SMADs. It was discovered that SMADs 6 and 7, also members of the TGF-ß superfamily, have C-domains relatively similar to those of the other SMADs but differ in terms of their N-domains from the other SMADs known so far [61, 62]. Imamura et al. [61] discovered that SMAD6 inhibits phosphorylation of SMAD2 and thus interrupts the signalling pathway. Interestingly, even though SMAD2 and 3 are closely related, SMAD6

acts differently on the latter, enhancing instead of suppressing its phosphorylation. Phosphorylation of SMAD1, induced by BMPR-IB, is also inhibited. Nakao et al. [62] showed that SMAD7, on the other hand, binds to TßR-I without being phosphorylated and thus inhibits phosphorylation of SMAD2 as well as SMAD3. It also associates with BMPR-IA and B and suppresses the phosphorylation of SMAD1. SMAD7 might play a role in the regulation of the TGF-ß signal in terms of intensity and duration and thus be responsible for autoregulatory negative feedback.

1.3.3.9 Tumourigenesis

In 1971, Knudson [63] postulated his two-hit hypothesis originally developed for retinoblastomas (see Figure 6). This hypothesis is equally valid for other tumours that are caused by mutations in tumour suppressor genes, consequently also for the development of colorectal carcinomas. Underlying this hypothesis is the fact that each autosomal gene exists in two copies, referred to as alleles. Tumour suppressor genes lose their function either through point mutations causing the formation of a new stop codon thus leading to a worthless protein or by the loss of larger chromosomal regions containing the respective gene. This process is referred to as loss of heterozygosity (LOH). As long as the other allele is not mutated, enough tumour suppressor gene products are produced to ensure a normal cell function. Inactivation of the second allele in the same cell, however, leads to a complete loss of function of the respective gene and to uncontrolled tumour cell growth. The probability that two somatic mutations might occur in the same cell is relatively small. In the case of inherited tumour predispositions with germline mutations in a tumour suppressor gene, the likelihood is much higher, as each cell carries the same mutation from the birth of the patient onwards. In such a case, one somatic mutation suffices for a complete loss of the tumour suppressor function [20].

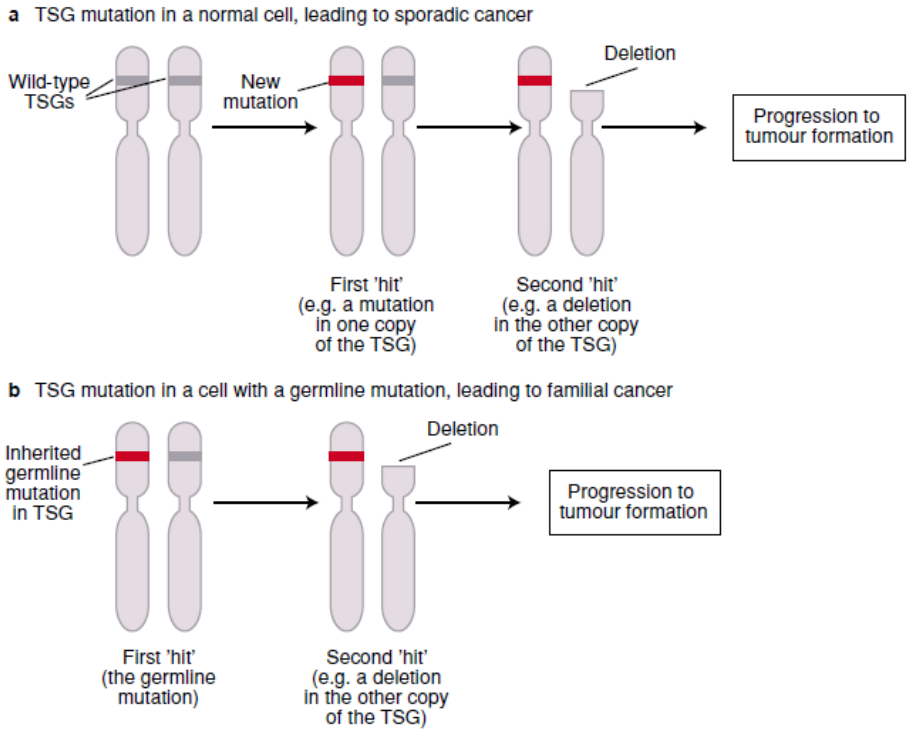

Figure 6: Knudson's two-hit hypothesis for tumourigenesis [64]

Part a) shows a mutation of a tumour suppressor gene (TSG) in a normal cell, leading to the development of sporadic cancer. Part b) shows a mutation of a TSG in a cell with a germline mutation. This leads to familial cancer. Normal genes are grey, mutated genes are red and deletions are indicated by the absence of a part of the gene.

Familial adenomatous polyposis is an example of this hypothesis. FAP is caused by germline mutations of APC, a gatekeeper of colonic epithelial cell proliferation, presumably regulating cell death. The result of complete APC inactivation is the development of numerous aberrant crypt foci (ACF), precursors of adenomas. For their progression to malignancy, however, further mutations in RAS, p53, etc. are required. Since for these tumour suppressor genes also both alleles need to be inactivated, at least seven mutations are necessary for the process of malignant transformation to take place. Germline mutations in six DNA repair genes (MLH1, MSH2, MSH3, MSH6, PMS1, and PMS2) were identified as causative of HNPCC, the two most important ones being MLH1 and MSH2, accounting for 30% and 50% of all cases. These proteins are referred to as 'caretakers' of the genome, as the inactivation of both of their alleles leads to genome instability, mismatch repair deficiency and to an increase in mutation rates [65].

Hereditary cancer syndromes have an important genetic component, but there is a striking difference to other genetic diseases such as cystic fibrosis or diabetes (type II). In cystic fibrosis the genetic alterations lead to the manifestation of the disease, while for diabetes diet and other environmental factors play a crucial role. Germline mutations such as the

ones occurring in JPS predispose to cancer, but do not automatically implicate the manifestation of the disease. For polyps to develop, further mutations are needed, which are influenced by environmental factors [65].

As juvenile polyps were considered stromal lesions with a normal epithelium in the past, the 'landscaper' hypothesis [21] (see 1.3.3.1) was developed to explain their progression to epithelial malignancy. This hypothesis postulated that the stromal environment influences the development of the adjacent epithelium. The resulting regeneration and growth of the epithelium would thus enhance the risk of cancer. A study by Woodford-Richens et al., however, analyzed juvenile polyps from JPS patients with germline SMAD4 mutations for loss of the second allele by fluorescence in situ hybridization (FISH). They found allelic loss of SMAD4 in the epithelial cells, in stromal fibroblasts and pericryptal myofibroblasts. No deletion of SMAD4 was found in stromal lymphocytes. These findings suggest a clonal origin of the epithelial cells and of those stromal cells showing the loss of the second SMAD4 allele – a contrast to the 'landscaper' hypothesis. As the regions lost around the locus vary, it might be concluded that different mechanisms could lead to the inactivation of the second copy of SMAD4. These results implicate that SMAD4 is a tumour suppressor gene. The somatic loss of its wild-type allele initiates hamartoma growth [66].

A study of a mouse model by Kim et al. aimed at reconciling these two opinions [67]. It had been shown before that the normal-appearing non-polypoid mucosa of patients affected by JPS contains a dense infiltrate of inflammatory cells [68]. It was concluded that disruption of the TGF-ß pathway – inter alia responsible for immune homeostasis – in the cells of the epithelial microenvironment would support tumour development in JPS. In the study by Kim et al. it was shown that disruption of SMAD4-dependent signalling in T cells leads to tumourigenesis in the gastrointestinal tract of the respective mice. Deletion of SMAD4 in the epithelial compartment alone did not result in this effect. This does not contradict the observation of SMAD4-LOH in tumour epithelia and the fact that until now SMAD4-LOH has not been detected in lymphoid lineages. It is, after all, possible, that haploinsufficiency might exist for SMAD4 in the T cells – this in combination with the loss of SMAD4 expression in the epithelial cells could lead to tumour development in the gastrointestinal tract [67].

Somatic loss of chromosome 18q also plays a role in sporadic colorectal cancer in over 60% of the cases [69], explicit loss of the SMAD4 gene, however, was found in 16,7% (5 of 31) of colorectal cancers in a study by Takagi et al. [70]. Somatic loss of SMAD4 is also frequently found in pancreatic carcinoma [55]. In an analysis by Schutte et al. [71] both

alleles of SMAD4 were found inactivated in 48% of sporadic pancreatic carcinoma. SMAD4 mutations are also found in other tumours, however to a lesser extent [71].
Knudson's second hit hypothesis also applies for JPS patients with mutations in BMPR1A – likewise a tumour suppressor gene. For the growth of hamartomatous juvenile polyps both a BMPR1A germline mutation and a somatic inactivation of the second allele are needed [29].

1.3.3.10 Hamartoma – Adenoma – Carcinoma Sequence

In the past, juvenile polyposis syndrome was not considered a premalignant condition, supposing that hamartomatous polyps did not lead to cancer (see 1.3.6). The simultaneous discovery of hamartomatous polyps, adenomatous polyps and carcinoma in the same patients affected by JPS, however, gave rise to the question whether this incident was due to a de novo adenoma formation or whether JPS might indeed be a precancerous condition leading to adenomas without juvenile residues. In 1979, Goodman et al. [72] reported of a JPS patient who had multiple juvenile polyps in the colon and stomach and had to undergo proctocolectomy and antrectomy. The patient had developed adenocarcinoma in the rectum. Goodman et al. analyzed the polyps microscopically and differentiated them according to their histological appearance. Five categories were distinguished: 1) hyperplastic epithelial foci and small hyperplastic polyps, 2) typical juvenile polyps, 3) juvenile polyps with focal adenomatous epithelium, 4) adenomas, and 5) adenocarcinoma [72]. These categories suggest a possible progression, a pathogenetic sequence, from hamartoma to carcinoma and thus might implicate malignant potential in JPS. Other authors [73, 74] have reported similar observations of a successive pattern implicating transformation from hamartomatous to adenomatous polyps to carcinoma. In contrast to these findings, cases of JPS patients with co-existing sporadic adenocarcinomas have been published [75, 76]. In summary, up to 50% of the untypical or multilobulated juvenile polyps reveal foci of low-grade intraepithelial neoplasia (IEN) upon histological evaluation [15], while simultaneous sporadic adenomas in JPS patients are rare.

1.3.3.11 Anticipation

The first report of the possible existence of the phenomenon of anticipation in juvenile polyposis came from Smilow, Pryor and Swinton in 1966 [77]. Anticipation describes the fact that the age of onset of an inherited disease as well as its severity increase from one generation to the next and that therefore the youngest generation is the most seriously

affected. In this report from 1966, the age of onset became younger with each generation. In 1998, Howe et al. [13] again discussed the existence of this phenomenon in a kindred with four generations affected by JPS. They discovered that the age at diagnosis decreased with each generation. However, the severity of juvenile polyposis with respect to the incidence of cancer did too. This leads to the conclusion that these results might be the effect of earlier diagnosis, surveillance and therapy rather than anticipation [13].

Anticipation is a phenomenon described in neurological diseases such as Chorea Huntington, Friedreich's ataxia, myotonic dystrophy or others. In these cases, however, a known genetic mechanism is responsible for anticipation. In Chorea Huntington the number of trinucleotide CAG repeats determines the penetrance of the disease – the more, the earlier the onset and the more severe the manifestation. In juvenile polyposis, caused by point mutations or large genomic deletions, a corresponding genetic mechanism has not been identified. Subsequently, either the mechanism leading to anticipation in these mutations has still not been found or the phenomenon of anticipation in JPS is a false conclusion and does not exist.

1.3.4 Histology

As already described by Veale et al. in 1966 [78] the first step towards differentiation between adenomatous and juvenile polyps is their macroscopic appearance. Juvenile polyps are known to be hamartomas, meaning a non-neoplastic malformation of the connective tissue and the epithelium of the gut. In contrast, adenomas are benign neoplasms.

Typically, the surface of adenomatous polyps has a lobulated appearance, the lobules separated by cracks and crevices. Juvenile polyps tend to have a smooth surface. Small juvenile polyps show minor inflammation and overgrowth of the superficial lamina propria while larger juvenile polyps consist of cystically dilated, elongated and branched mucinous glands (see Figure 7) [15, 79]. Problems in the differentiation by inspection alone arise when juvenile polyps deviate from their usual appearance, which is the case for about 20% of juvenile polyps. These so-called atypical juvenile polyps have a lobular appearance, as they are made up of several polyps attached to one common stalk. In addition to their altered macroscopic appearance, their histological appearance is different too. Atypical juvenile polyps consist of more epithelium and less lamina propria than typical juvenile polyps [15].

Histologically, adenomatous polyps (see Figure 8) also consist of epithelium and connective tissue – just like hamartomatous polyps. While adenomas are partly made up of smooth muscle fibres however, juvenile polyps completely lack them. This is the reason for their friable consistency and for a phenomenon called 'autoamputation', or the polyp's spontaneous falling off the stalk. Characteristics of neoplasms seen in adenomas also cannot be found in juvenile polyps: an increased number of mitoses, hyperchromatism of the nuclei, diminution of the goblet cells [78]. According to Jass et al., however, IEN can be found in typical as well as atypical juvenile polyps, more frequently so in the latter though. Almost 50% of all atypical juvenile polyps reveal some foci of adenomatous change [15]. Adenomatous change (see Figure 8) – usually seen in the polyp's periphery – implies a higher number of mitoses, a loss of the basal location of the nuclei and a decreased content of mucin in the cells. In these areas of IEN, glands are denser and more numerous. In contrast to usual adenomas, however, juvenile polyps with adenomatous change keep their original juvenile characteristics at the base of the polyp with oedematous and inflamed stroma, dilated glands and diffuse border with normal epithelium [73].

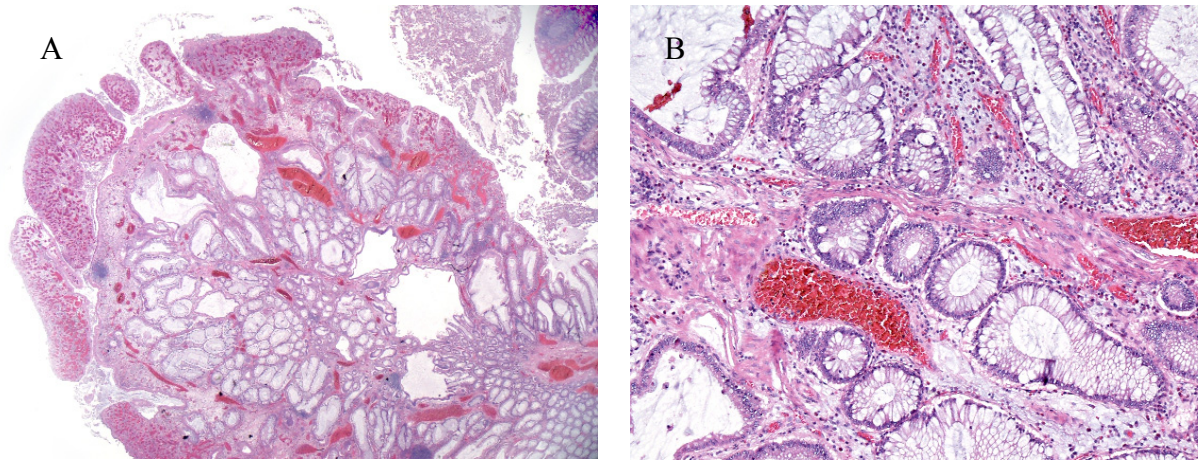

Figure 7: Histology of a juvenile polyp

Panel A shows a juvenile polyp located in the colon, panel B is an amplified section of the same polyp. The typical cystically dilated, elongated and branched mucinous glands as well as the oedematous and inflamed stroma can be observed. The pictures were provided by Sen. Scientist Dr. med. univ. Ekkehard Spuller.

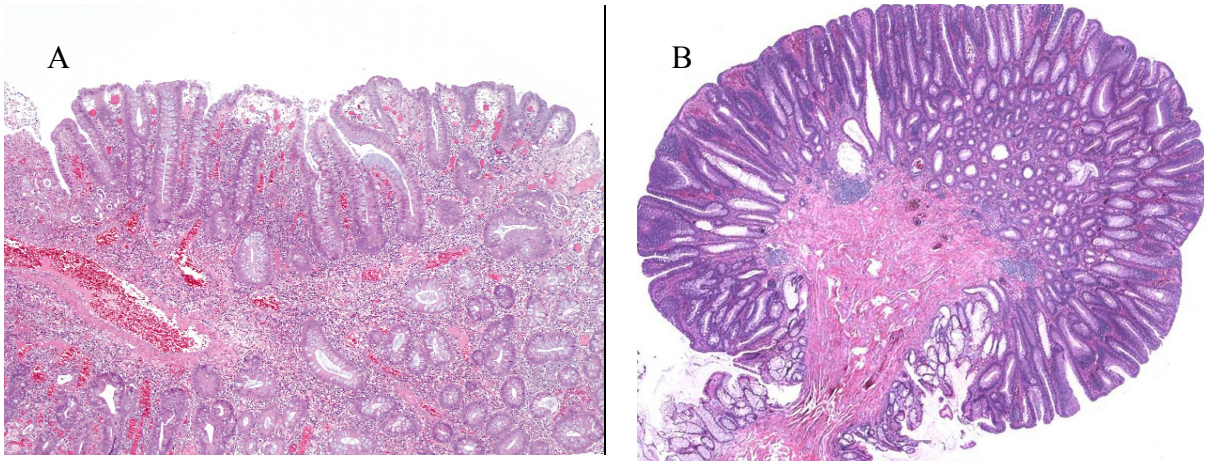

Figure 8: Histology of a juvenile polyp with adenomatous dysplasia and of an adenoma

Panel A: Adenomatous change can be seen in the juvenile polyp's periphery due to the higher number of mitoses, the loss of the basal location of the nuclei and the decreased content of mucin in the cells. The glands are denser in the area of IEN and more numerous. However, the original juvenile characteristics are still present at the base of the polyp with an oedematous and inflamed stroma and dilated glands. Panel B: Adenomas show crowded crypts. They consist of columnar, pseudostratified epithelium and show an increased amount of mitoses, hyperchromatism of the nuclei, diminution of the goblet cells as well as smooth muscle fibres. Picture A was provided by Sen. Scientist Dr. med. univ. Ekkehard Spuller, picture B by Sen. Scientist Univ.-Doz. Dr. med. Cord Langner.

As the differential diagnoses of juvenile polyposis include several other polyposis syndromes, which might not always be distinguishable according to clinical presentation alone, differences regarding histology are crucial for correct diagnosis. JPS polyps show only a minor amount of smooth muscle fibres. In contrast, Peutz-Jeghers polyps consist of a thick, tree-like branched muscularis mucosae covered by a lamina propria normal in composition and cellularity. Typically no signs of inflammation are evident. Cowden's disease polyps are characterized by non-neoplastic, inflamed or slightly fibrotic mucosa and also contain excessive smooth muscle. There is a slight architectural distorsion of the crypts. The epithelium is nondysplastic and contains the usual composition of goblet cells and absorptive cells found in the colon. In most cases they are indistinguishable from juvenile polyps. The majority of polyps, mostly sessile, occurring in the context of Cronkhite-Canada syndrome closely resembles juvenile polyps, but the intervening non-polypoid intestinal mucosa shows pathological changes such as oedema, cystically dilated glands and increased inflammatory cells, whereas the intervening mucosa in JPS is normal. Within the lamina propria dilated irregular foveolar glands can be found [79].

Other important differential diagnoses are hyperplastic polyps and adenomas. Hyperplastic polyps often occur in the stomach and thus make up 28-75% of all gastric polyps. They are characterized by elongated, branched and cystically dilated foveolae, eroded surfaces or central umbilication and regenerating epithelium often mistaken for IEN. Their

macroscopic appearance is shiny and soft, which discerns these polyps from others. Adenomas can have tubular, tubulovillous and villous appearances and consist of columnar, pseudostratified epithelium. Mitotic activity is increased and atypical nuclei can be found [79]. The characteristic histological findings of juvenile polyps and its differential diagnoses are displayed in Figure 9 below.

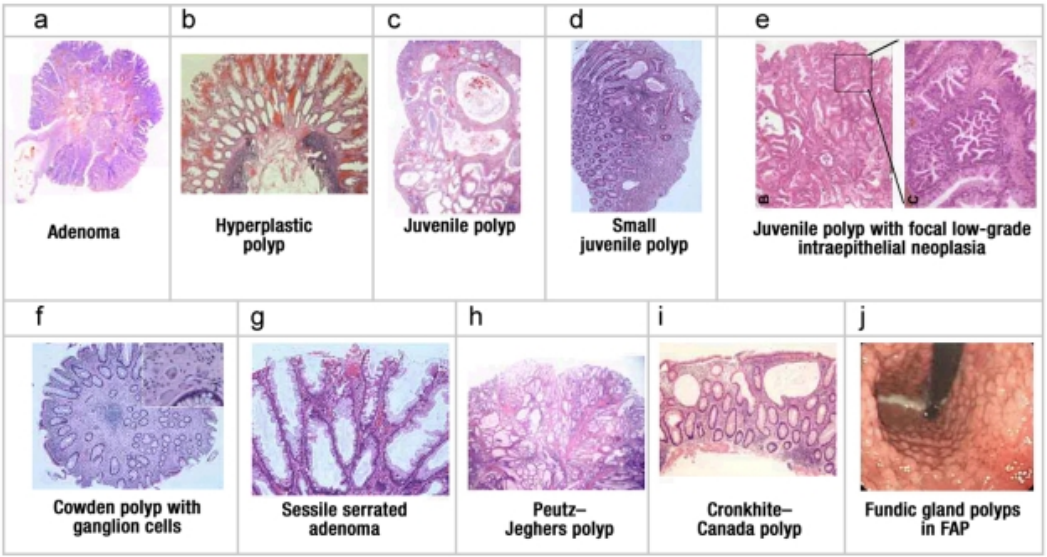

Figure 9: Characteristic histological findings in juvenile polyps and other polyposis syndromes [8]

Adenomas can have tubular, tubulovillous and villous appearances and consist of columnar, pseudostratified epithelium, have increased mitotic activity and atypical nuclei. Hyperplastic polyps in the colon have maintained architecture of the mucosa with elongated crypts which are serrated but covered by a normal appearing epithelium. Cowden's disease polyps are characterized by non-neoplastic, inflamed or fibrotic mucosa and excessive smooth muscle. Peutz-Jeghers polyps consist of normal mucosa and a tree-like branched muscularis mucosae. Cronkhite-Canada polyps, mostly sessile, have smooth muscle in the oedematous lamina propria and an inflammatory infiltrate. Within their lamina propria dilated irregular foveolar glands can be found.

1.3.5 Manifestations and Symptoms

In 1975, Sachatello et al. [46] described the variable clinical spectrum of the juvenile polyposis syndrome and thus divided it into three major subtypes: (1) a form that is limited to the colon (juvenile polyposis coli), (2) a form involving the stomach, small intestine and colon (generalized juvenile polyposis) and (3) a form causing extensive intestinal involvement (juvenile polyposis of infancy).

1.3.5.1 Generalized Juvenile Polyposis and Juvenile Polyposis Coli

Generalized juvenile polyposis (GJP) and juvenile polyposis coli (JPC) are regarded as two different expressions of the same disease [80]. They usually manifest in the first two

decades of life, but not before the age of six for the generalized form. This was shown in a review of 218 patients affected with juvenile polyposis [81]. 15%, however, present with symptoms later in life [82]. JPC patients show mostly distal colonic and rectal involvement, whereas in GJP patients a variable number of polyps ranging from 50 to 200 [15, 82] is found throughout the gastrointestinal tract, mostly in the stomach, distal colon and rectum. The predominant symptoms in JPC include blood per rectum, mild anaemia and rectal prolapse. In GJP patients present with symptoms varying from mild rectal bleeding and prolapse to recurrent gastrointestinal bleeding, protein-losing enteropathy, intussusception and severe malnutrition [81]. 20-50% of the patients have a family history of juvenile polyposis [80, 81]. 15% of the patients analyzed by Coburn et al. had congenital anomalies consisting mainly of cardiac, central nervous, gastrointestinal, genitourinary or soft tissue deformities [81].

1.3.5.2 Juvenile Polyposis of Infancy

Patients with juvenile polyposis of infancy (JPI) show a very early manifestation of disease within the first two years of life [80]. They show a severe course of disease and present with diarrhoea, recurrent gastrointestinal bleeding, rectal prolapse, intussusception, protein-losing enteropathy and failure to thrive [80-82]. The very early onset of disease and rectal bleeding were described as hallmarks of juvenile polyposis of infancy by Sachatello et al. [46] As a consequence of the severity of the symptoms, mortality is high in JPI and patients die at a young age. Thus the mutation responsible for the syndrome is usually not inherited. This inference is consistent with the finding that all patients described and analyzed so far do not present with a family history and are affected by de novo mutations [80]. As already mentioned above (see 1.3.3.5) the mutations leading to juvenile polyposis of infancy are usually deletions of both BMPR1A and PTEN. In reviewing the literature, van Hattem counted 11 patients with PTEN mutations, six of which had secured BMPR1A deletions in addition. Another three presumably also had both deletions [45].

PTEN protein functions as a lipoprotein phosphatase that downregulates the PI3K/AKT pathway which is important for survival, growth and proliferation of cells [83]. BMPR1A is the type I receptor in the BMP pathway. This pathway is responsible for the downregulation of the proliferation of cells, especially in the gastrointestinal tract. By inactivating BMPR1A in mice, He et al. [84] found that intestinal epithelial regeneration homeostasis is disturbed leading to an expansion of stem and progenitor cell populations. Consequently, the deletion of both genes leads to an increase in the proliferation of

gastrointestinal cells. Given the severity of this contiguous-gene syndrome, a cooperative rather than an additive effect between these two genes is suggested [80]. Indeed, Waite et al. have shown that stimulation with BMP2, a ligand on the BMPR1A receptor, in an MCF-7 breast cancer cell line inhibits PTEN protein degradation. This increases PTEN protein levels [85].

As for the malignant potential in JPI, opinions seem to differ. Coburn et al. [81] state that development of malignancy is rare in JP of infancy, as patients usually die of progressive malnutrition and small bowel obstruction before its onset. Delnatte et al. [80], however, found grade 3 dyplasia foci in one of their patients aged 3 years and an adenocarcinoma in a 14-year-old patient. They concluded that cancer risk is highly increased in patients with both PTEN and BMPR1A deletions compared to other JPS patients.

1.3.5.3 Gastric Polyposis

As already described above, Sachatello et al. [46] subdivided juvenile polyposis into three different entities: JPS restricted to the colon, JPS of infancy and JPS affecting the stomach, small intestine and colon. In 1979, however, Watanabe et al. [86] reported three members of a family which had exclusively gastric polyposis lacking polyps of the colon. The index patient of the family and her brother had massive gastric polyposis requiring subtotal gastrectomy. Their mother died of gastric cancer. Watanabe et al. proposed therefore the existence of a fourth entity – familial juvenile polyposis of the stomach. Although several other cases have been described [87-90], this stomach-restricted form is rare [89]. Due to this rarity, it remains unclear until now whether this form actually represents an independent entity distinct from generalized juvenile polyposis [88]. Possibly, a case identified as gastric JPS may only be the initial presentation of a generalized manifestation. In a study by Hizawa et al. [88] however, three patients affected by juvenile polyposis of the stomach showed no extragastric manifestations throughout a period of thirty years.

Patients affected by juvenile polyposis restricted to the stomach may present with protein-losing enteropathy, intermittent pyloric obstruction due to antral polyps prolapsing into the duodenum [86, 88, 91] or gastric haemorrhage [89].

Endoscopic results have shown that juvenile polyps are usually located in the gastric antrum. When the whole stomach is affected, the greater part of the polyps will be located in the antrum. In familial adenomatous polyposis, however, polyps occur mostly in the gastric fundus and body [91]. As a consequence of their gastric localisation, juvenile polyps may prolapse into the duodenum and cause an obstruction of the pylorus.

Some patients with colonic juvenile polyps can also show gastric involvement – thus subdividing juvenile polyposis into the colon-restricted and the generalized form. The genetic background determines the type of manifestation. The first report of a genotype-phenotype correlation was made by Friedl et al. in 2002 [30]. They had analyzed 29 patients and found 12 mutations causative of JPS, seven mutations in the SMAD4 and five in the BMPR1A gene. When comparing the clinical manifestations of these two groups, it could be shown that four out of the seven unrelated patients with SMAD4 mutations had massive gastric polyposis. Partial or total gastrectomy was necessary for those four patients. It is not mentioned in the study whether these four patients were H. pylori positive or not. Noticeable is that the patients with gastric polyposis were older than those without, suggesting age-related penetrance for stomach manifestation of JPS. In support of a genotype-phenotype correlation is the fact that BMPR1A mutation positive patients or patients for whom no mutation could be identified did not have such severe forms of gastric polyposis [30].

In 2007, the proposed higher frequency of gastric polyposis in SMAD4 mutation positive patients was confirmed by Aretz et al. [44]. 73% (22 of 30) of SMAD4 mutation positive patients had gastric polyposis whereas this was only the case for 1 of 13 with BMPR1A mutations. Consistent with these results is the fact that all seven gastric cancers reported in the analyzed families were found in SMAD4 mutation positive patients. Again, age-related penetrance is suggested for the stomach manifestation of JPS. In the study by Aretz et al. gastric polyposis was diagnosed later in life (median age at diagnosis: 41 years) than colorectal polyposis (median age at diagnosis: 12 years). H. pylori status was not commented on in any of the patients of the study [44].

1.3.5.4 Hereditary Haemorrhagic Telangiectasia (HHT)

In 1980, two patients were described with pulmonary AV-malformations and juvenile polyposis. This was the first report of an association between hereditary haemorrhagic telangiectasia and JPS, as a consequence of which a common syndrome was proposed [92]. Reports followed of patients with both disorders or of patients who had JPS and additionally symptoms of hereditary haemorrhagic telangiectasia [93-95].

HHT, or Osler-Weber-Rendu disease, is an autosomal dominant vascular dysplasia that affects many organs. Typically, patients show telangiectasias of the skin (face, lips, fingers, etc.), oral and nasal mucosa, epistaxis (from nasal mucosal telangiectasias) as well as pulmonary (PAVMs) and cerebral (CAVMs) – more common in younger patients –,

hepatic (HAVMs) and gastrointestinal arteriovenous malformations – more common in older patients – that can lead to haemorrhages [96-99]. Frequently associated with PAVMs is digital clubbing. Patients with JP-HHT show AVMs in 87% of the cases, epistaxis in 78% and telangiectasias in 72%. The latter two are considered hallmark features of HHT [98]. Gastrointestinal mucosal telangiectasias can be found in approximately half of the HHT patients. Nasal and GI bleeding can lead to chronic iron-deficiency anaemia [100]. Pulmonary arteriovenous malformations, connecting the pulmonary artery and the pulmonary vein thus bypassing the capillary bed, occur in about 20% of the patients. They may lead to paradoxical embolic stroke or brain abscess and can cause chronic hypoxemia and cyanosis. Complications of the cerebral manifestation include migraine headache and aneurysm [100, 101]. Intrahepatic AVMs can cause hepatic shunting, consequently leading to high-output heart failure, portal hypertension and cirrhosis [100]. The age at manifestation of these symptoms is variable, by the fourth decade, however, they are usually developed. Also variable is the phenotype, even within the same family. Genetic as well as environmental factors may play a role in the variable presentation of symptoms [98].

Shovlin et al. [102] have developed four diagnostic criteria, three of which need to be fulfilled in order for diagnosis of HHT to be definite. These criteria are recurrent spontaneous epistaxis, mucocutaneous telangiectasias, visceral AVMs and a positive family history. If only two of the symptoms are present no definite diagnosis can be made, but HHT is regarded as possible. If case of fewer than two criteria, HHT is considered unlikely. With regard to children of affected patients with less than two criteria, age-related penetrance should be taken into account.

Two genes have been identified as responsible for up to 90% of the cases: Endoglin (ENG) and activin receptor-like kinase 1 (ACVRL1 or ALK1). Endoglin, a member of the TGF-ß receptor complex, was discovered in 1994 as causative of HHT type 1 and mapped to chromosome 9q34.1. HHT was thus the first syndrome discovered to be caused by a mutation in the TGF-ß pathway [101]. The second gene, activin receptor-like kinase 1, which is responsible for HHT type 2, is a member of the serine-threonine kinase receptor family and is mapped to chromosome 12q11-q14 [103]. HHT type 1 is linked to a higher prevalence of PAVMs and CAVMs, while HHT type 2 is associated with a higher frequency of HAVMs and a reduced penetrance [103, 104]. A third possible gene, HHT3, has recently been mapped to chromosome 5 [105]. A fourth gene, HHT4, is located on chromosome 7 [100].

Knowledge of mutations causing either HHT or JPS (SMAD4, BMPR1A) did not suffice to resolve the genetic condition responsible for the combination of both which was thus unknown until 2004. In an analysis of a subset of 7 JP-HHT patients, who were all ENG and ACVRL1 negative, SMAD4 mutations were found in all cases [96]. Of 30 unrelated HHT patients who were all negative for mutations in ENG and ALK1, three harboured mutations in SMAD4. All three patients had no family history of HHT, as most other HHT patients with SMAD4 mutations. Thus, a high rate of de-novo cases can be proposed in this syndrome [97]. SMAD4 mutations found in JP-HHT patients strongly resemble those found in JPS patients in terms of type and distribution. Consequently, no diagnostic hints can be derived from the genotype alone, implying that any HHT patient with a SMAD4 mutation is at risk for colorectal cancer, and any SMAD4 mutation positive JPS patient is at risk for HHT [98]. JP-HHT patients show an earlier onset of HHT symptoms and a higher severity compared to individuals suffering from HHT1 or HHT2 [100]. Aretz et al. [44] found out that of 23 JPS patients with SMAD4 mutations, five had HHT, corresponding to a frequency of 22%.

An explanation why ENG and ALK1 mutations cause vascular dysplasia but not colonic polyps, whereas SMAD4 mutations cause both, lies in the location of their expression. ENG and ALK1 are expressed only in vascular endothelium. SMAD4 is expressed both in vascular endothelium as well as in colonic mesenchymal and epithelial cells. A SMAD4 mutation can consequently cause both vascular dysplasia and juvenile polyps. As one single defect causes disorders in both the gastrointestinal epithelium as well as the vascular endothelium, the dual occurrence of JP and HHT may be described as a genetic syndrome [96].

What consequences can be derived from these findings? Molecular diagnosis is essential in patients with either HHT or JPS. An example: Is the genotype unknown in an HHT patient, GI bleeding may be solely attributed to gastrointestinal AVMs. In SMAD4 mutation positive patients, however, the cause of bleeding could also be juvenile polyps. Patients with HHT and SMAD4 mutations are at high risk for developing juvenile polyposis and thus colorectal cancer. More intensive colorectal cancer screening is thus required for these patients than for the normal population. Likewise, JPS patients with SMAD4 mutations should be screened for visceral manifestations of HHT, as they can lead to serious complications [96-98].

1.3.6 Malignant Potential

In the past, juvenile polyposis syndrome was not considered a precancerous condition. Later studies have shown that risk for malignancies is indeed elevated in JPS but not in sporadic juvenile polyps. In 1984, Järvinen and Franssila [73] estimated the risk for colorectal cancer to be 9%, as nine out of 102 patients with hereditary and sporadic juvenile polyps had developed colorectal cancer. However, 33 additional cases with gastrointestinal cancer seen in these families were not included in this analysis as these individuals were not regarded as affected. Thus, 9% may be too low and might not reflect the actual cancer risk.

Jass et al. [15] reviewed 87 patients and found colorectal cancer in 18 cases, corresponding to an incidence of 20.7%. The average age at diagnosis was 34 years (15 to 59 years). In contrast to the two studies described above, Howe et al. [13] included family members with GI cancer who died before being evaluated for juvenile polyps. Strictly seen, these patients cannot be considered as affected by juvenile polyposis. However, it is more likely that they developed GI cancer on the basis of juvenile polyps than in a sporadic way. Not including them would induce a considerable bias against an association of juvenile polyposis with cancer, including them might lead to a slight increase in the presumed association. Of the 29 family members (revealing upper or lower gastrointestinal polyps or cancer or both) included in the analysis, 16 had gastrointestinal cancer (55%). 11 (38%) had developed colon cancer and 6 upper GI cancer (16%). Brosens et al. [106] calculated a life-time risk of 38.7% for developing colorectal cancer while Lynch et al. described in their review that colorectal carcinoma risk in JPS is considered to be 22% by the fourth decade in life and 68% by the age of 60 [2].

A literature review of 51 reports including 271 juvenile polyposis patients revealed an overall adenoma incidence of 18.5% (50/271) and an overall carcinoma incidence of 17.3% (47/271). Of the 50 patients with adenomatous changes, 48 had colorectal adenomas, one had a gastric and one a duodenal adenoma. Of the patients developing a carcinoma, two had gastric cancers, two duodenal and pancreatic (= perivaterian) cancers and two jejunal cancers. The rest had developed colorectal carcinoma [107].

1.3.7 Surveillance Scheme

Definite diagnosis on a clinical level for patients with hamartomatous polyps is often difficult due to phenotypic overlap between the different hamartomatous polyposis

syndromes. Mutation analysis is therefore important. Direct sequence analysis of coding regions and exon-intron boundaries in SMAD4 as well as BMPR1A should be carried out in patients suspected of being affected with the syndrome. Direct genetic analysis can identify mutations in about 30% of unrelated JPS cases [108]. If the gene-carrier status is confirmed, family members should also undergo genetic testing. Autosomal dominant inheritance and the fact that about 20-50% of JPS patients have a family history of polyposis [15] emphasize its importance. Direct sequence analysis is a straight-forward and cost-effective method. The advantages of having to examine only these two genes are a limited number of exons and a low frequency of polymorphisms. However, the fact that there is a high number of unique mutations and a limited number of mutational hotspots implies that all coding regions and exon-intron boundaries need to be examined. If no mutations are found MLPA analysis should be done to exclude intragenic deletions.

Before detection of the genetic locus responsible for JPS, annual upper and lower endoscopy was recommended for patients known to be affected with polyps. Family members without polyps or those cleared of polyps, were to be screened every three years. With the detection of a germline mutation causative of JPS, presymptomatic diagnosis is possible to identify other family members carrying the mutation. Subsequently, only family members known to have inherited the causative mutation need to undergo regular endoscopic surveillance and noncarriers can be spared the procedure. Endoscopic polypectomy should be carried out not only to reduce malignant transformation but also to reduce risk of bleeding and anaemia [13].

Surveillance schemes for the upper as well as the lower gastrointestinal tract have been defined by Howe et al. [109] and Dunlop et al. [110]. Howe et al. suggest full blood examination and endoscopy of the lower gastrointestinal tract from 15 years of age onwards, in symptomatic patients even earlier. If polyps are found, they should be removed and the patient should be screened annually until free of polyps. Endoscopy can then be carried out every three years [109].

Dunlop et al. suggest screening intervals from one to two years starting from the age of 15-18 onwards or earlier in the case of symptomatic patients. This surveillance strategy should be continued until the mutation carriers or affected patients reach the age of 70 years [110]. Upper gastrointestinal surveillance is recommended by Howe et al. to be carried out contemporaneously with coloscopy, providing no exact age when to start gastric screening. However, biliary and/or pancreatic duct brushings are suggested in case of elevated amylase or abnormal liver function tests [109]. Dunlop et al.'s advice is to perform

gastroscopy from the age of 25 years onwards with a frequency of every one to two years. It ought to be done contemporaneously with lower gastrointestinal endoscopy [110]. These recommendations are summarized in Table 5.

	Recommendation by Howe et al. [109]	**Recommendation by Dunlop et al. [110]**
Upper gastrointestinal surveillance	Contemporaneously with coloscopy In case of elevated amylase or abnormal liver function tests, biliary and/or pancreatic duct brushings are advised;	From age 25 Intervals: 1-2 years Contemporaneously with coloscopy
Lower gastrointestinal surveillance	From age 15, earlier in case of symptoms: full blood examination and endoscopy If normal → screening every 3 years In case of polyps → removal and annual screening until free of polyps; then screening every 3 years;	From age 15-18, earlier in the case of symptoms Intervals 1-2 years Mutation carriers or affected patients should continue surveillance until age 70.

Table 5: Upper and lower gastrointestinal surveillance strategies [111]

In addition to upper and lower gastrointestinal endoscopy, small bowel screening is carried out in various institutions. However, most experts do not recommend small bowel screening in spite of the assumption that the amount of small bowel polyps is probably underestimated [112]. Reasons for underestimation may be reporting bias, categorization problems and inaccuracy of imaging techniques used in the past. The only study until now that examined the small bowel of JPS patients by means of capsule endoscopy gives an estimation of the real number of patients affected with polyps in the small intestine. 3 of 10 patients had been identified as carrying duodenal juvenile polyps by gastroscopy. Another 3 patients showed polyps of the small intestine upon screening with capsule endoscopy. These numbers are one reason to justify capsule endoscopy as a baseline investigation in JPS. Another is that small-bowel manifestation lacks correlation to either a certain mutation or to another predominant manifestation that could help in its diagnosis. The authors of the study suggest carrying out capsule endoscopy – a safe and well-tolerated technique – for JPS patients to identify those with large or dense small-bowel polyps. Only these patients ought to be screened again [112].

1.3.8 Therapy

Polypectomy. Single polyps detected by endoscopic screening ought to be endoscopically removed. Small polyps – less than 6 mm in size – can be removed by biopsy forceps. Larger, sessile polyps up to 10 mm as well as pedunculated polyps can be removed by snare polypectomy. Sessile polyps larger than 15 mm or big pedunculated polyps require

piecemeal polypectomy – a step-by-step-resection – and repeated submucosal injections to lift the polyp off the submucosa to obtain as much neoplastic tissue as possible. A new technique called endoscopic submucosal resection (ESD) can be applied for very large sessile polyps located in the distal colon – it implies an en-bloc removal of the neoplastic tissue requiring great experience. Limitations to polypectomy are shape, size and localization of polyps. Polyps using more than half of the luminal circumference in the caecum (thinner wall), polyps between or beyond two folds, ulcerated polyps anywhere in the colon or villous polyps in the caecum should imply a surgical removal. Complications of endoscopic removal of polyps may be bleeding and perforation of the colon [113].

EMR. Endoscopic mucosal resection (see Figure 10) is an appropriate technique for the removal of early gastrointestinal cancer located to the mucosa, or high-grade intraepithelial neoplasia, in cases with minimal risk of regional lymph node metastasis. Indications for the stomach are well and/or moderately differentiated adenocarcinoma restricted to the mucosa. Lesions should be not larger than 1 to 2 cm [114]. Lymphatic vascular involvement, evidence of ulcer or ulcer scar and tumour size above 3 cm are risk factors for the development of lymph node metastasis in early gastric cancer [114]. The evidence of one of these risk factors excludes endoscopic mucosal resection as the treatment of choice. Before EMR is carried out, histologic confirmation of early gastric cancer is needed and size and depth need to be analyzed by endoscopy. Then, one of four techniques is applied for resection: (1) the inject and cut technique, (2) the inject, lift and cut technique, (3) EMR using a cap and (4) EMR with ligation [115].

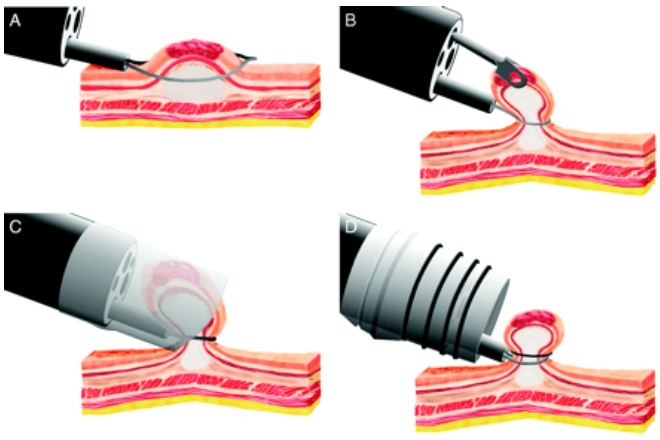

Figure 10: EMR techniques [115]

(A) The inject and cut technique. After injection of the submucosa to increase the distance of the mucosa from the muscularis propria, the diseased mucosa is resected using a snare loop. (B) The inject, lift, and cut technique. A pair of forceps is applied to lift the diseased mucosa from the muscularis propria. (C) The EMR with cap technique. After the mucosa is suctioned into the cap it is cut by a snare loop. (D) The EMR with ligation technique. Before resection, the mucosal lesion is ligated.

Surgery. In case of a high number of polyps difficult to treat endoscopically, impossibility of endoscopic removal, persistent severe diarrhoea and bleeding, high-grade IEN or invasive adenocarcinoma, surgery should be taken into consideration for treatment of JPS manifestations [82]. The two options for treating colonic JPS surgically are colectomy with ileorectal anastomosis (IRA) and proctocolectomy with pouch creation, also referred to as restorative proctocolectomy [82]. In this procedure the colon and rectum are removed in total and a pouch is constructed from the last 30-40 cm of ileum functioning as a reservoir. Subsequently, ileoanal anastomosis is carried out. Two different types of pouches are common: the technically easier J or two-loop pouch (see Figure 11) and the more difficult W or four-loop pouch that reduces the frequency of defecation. If the anastomosis is stapled, 1-2 cm of rectum remain and with it potential polyp development. The hand sewn technique includes anorectal mucosectomy. Here, the anastomosis is located just above the dentate line. Both mucosectomy and the fact that no residual is left of the rectum minimize the neoplastic risk. A disadvantage as compared to the stapled technique may be the increased risk of incontinence [116].

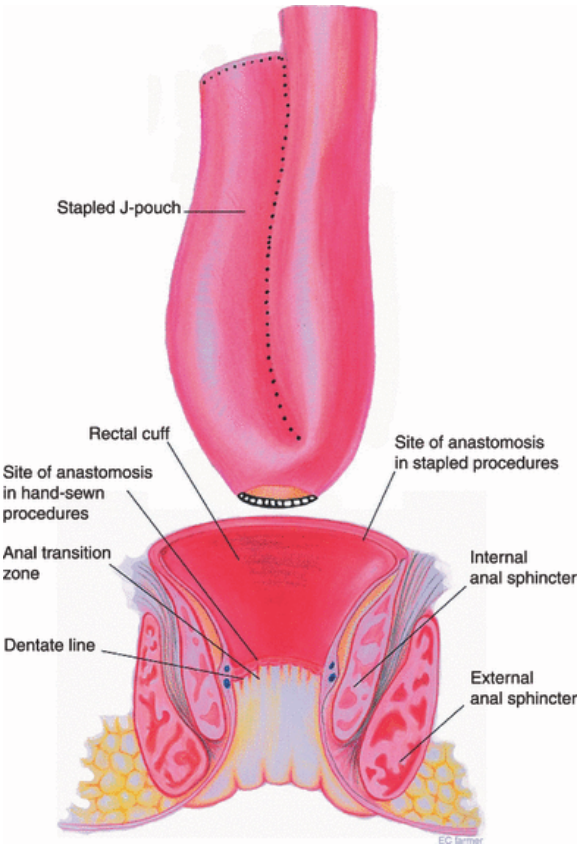

Figure 11: Ileo-anal pouch anatomy [116]

The best procedure remains unclear as long-term outcomes to compare restorative proctocolectomy and colectomy with IRA, requiring endoscopic removal of polyps occurring in the rectal remnant, do not exist [117]. In a retrospective review by Scott-Conner et al., however, rapid recurrence of polyps was found in patients treated with subtotal colectomy, implying that restorative proctocolectomy may be the better choice of surgical treatment [117]. Decision whether to include proctectomy in the surgical procedure or whether to create an ileorectal anastomosis in patients with FAP has been based on the number of polyps in the rectum. As for JPS, no study exists so far that would allow a similar inference [118]. In their study, Oncel et al. show that five of ten JPS patients treated by means of subtotal colectomy with IRA or partial colectomy developed symptomatic polyps, in situ carcinoma or both in the rectal remnant in the nine years following the operation. Subsequently, these patients had to undergo proctectomy. Of the other patients, four needed recurrent endoscopic polypectomies. No connection between the amount of polyps found in colon or rectum and the need for proctectomy could be derived from this study. For both procedures, endoscopic surveillance after the operation is necessary as recurrence rates of polyps in the rectum as well as the pouch are high.

2 MATERIAL AND METHODS

2.1 Aims

The aim of the study was to describe the manifestations of JPS in a kindred of SMAD4 mutation carriers. The number of polyps in the stomach, small intestine, colon and gallbladder was to be determined, as well as the patients' symptoms and the severity of the disease.

2.2 Hypotheses

In this prospective study of a kindred affected by juvenile polyposis the following hypotheses were postulated: (1) Juvenile polyps occur not only in the stomach, small intestine and colon, but also in the gallbladder and biliary tract – a manifestation not yet described in the literature. (2) For the family at hand, three generations of which are afflicted by JPS, the phenomenon of anticipation exists – meaning an earlier age of onset and an increased severity of disease in the younger generation.

By examining the symptoms and the severity of the manifestations of JPS in the eight family members affected by it, these two hypotheses will be discussed.

2.3 Methods

The study was approved of by the Ethics committee of the Medical University of Graz and informed consent was obtained from the patients.

The index patient, an 8-year old girl, was admitted to hospital in August 2006. Six months passed until the correct diagnosis was assumed. Genetic testing followed to confirm the diagnosis and detect the causal mutation. After the mutation responsible for causing JPS had been found, relatives of the index patient were summoned to undergo genetic testing as well. Of those relatives who agreed to undergo genetic testing at the Institute of Human Genetics at the Medical University of Graz the ones identified as mutation carriers were invited for clinical evaluation. This examination was either carried out at the Department of Internal Medicine, Division of Gastroenterology and Hepatology and at the Department of Paediatrics and Adolescence Medicine (for patients under 18 years of age) at the Medical University of Graz or at the hospital of 'Barmherzige Brüder' Graz .

After the clinical evaluation, medical records were reviewed for symptoms and manifestations of JPS. Review included upper endoscopy, coloscopy, capsule endoscopic and radiographic reports as well as operative and pathology reports.

In addition, discharge letters were reviewed for patient's symptoms. The results were then included in databases created for each patient. They include all the clinical, endoscopic and pathologic information obtained.

3 RESULTS

The genetic analysis for the index patient was carried out at the Department of Medical Genetics Basel, Switzerland. By means of PCR-amplification and bi-directional sequencing the heterocygote SMAD4 germline mutation c.543delC was found in the index patient – a mutation so far not described in the literature. This frameshift mutation leads to the formation of a new stop codon at position 201 (p.Leu201X).

In total, 19 relatives that agreed with genetic testing were analyzed. 9 were identified as mutation carriers and were subsequently clinically examined by means of upper, lower as well as capsule endoscopy and sonography of the abdomen. The clinical manifestations of JPS in the index patient and in seven of her family members also identified as mutation carriers are described below. For the eighth patient (patient II/8 in the pedigree) no information was available as clinical examination was carried out in another hospital. An overview of the affected family members is provided in the pedigree in Figure 12.

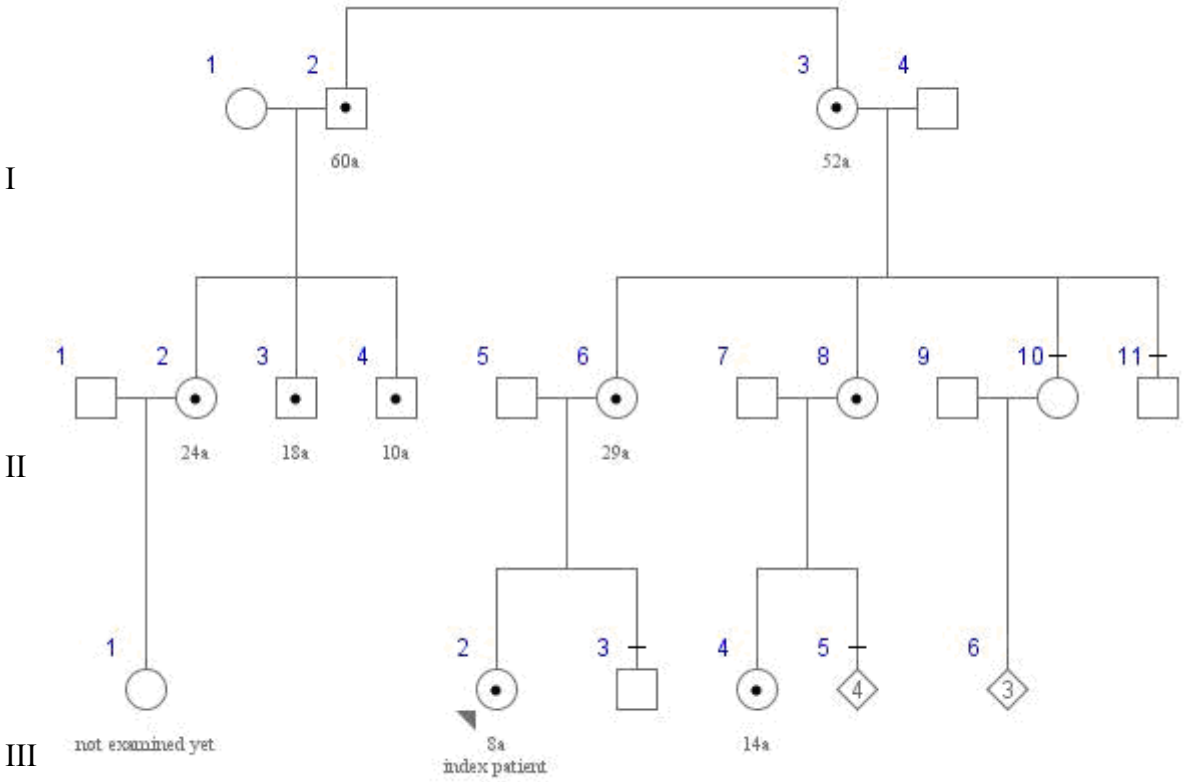

Figure 12: Pedigree with affected family members

Dots indicate a SMAD4 mutation carrier status. Family members with a horizontal line do not carry the SMAD4 mutation. I/2 and I/3 are brother and sister. III/1 is only a few months old and has thus not been examined yet. II/8 is known to be affected but no information on her clinical presentation was available. In generation III unaffected siblings were grouped and the number written inside the symbol.

Not shown in the pedigree are the parents and grandparents of generation I. The first refrained from genetic testing. They were both 83 years old and asymptomatic at the time

of screening of the other family members and had never had a diagnosis of a carcinoma. The grandparents of generation I were both dead at the time of screening – the grandmother had died of gastric cancer.

3.1 Index Patient (III/2)

Index patient (III/2)			
		Macroscopic results	**Histologic results**
Age at first diagnosis	8a		
Symptoms	Tiredness Lack of strength Diarrhoea Rectal bleeding		
FOBT	Positive		
Complications	Hypoproteinaemia Chronic anaemia 2 intestinal invaginations		
Gastroscopy	10/2006	Normal	
	03/2007	Normal	HP-associated, moderate chronic gastritis;
	08/2008	Normal	
	02/2009	Normal	Low-grade, chronic inactive gastritis;
HP status	+		
Eradication	03/2007		
Capsule endoscopy	03/2007: stuck in the stomach	No information on small bowel	
Coloscopy	09/2006	Multiple polyps in the rectum and sigmoid;	Suspected infectious colitis;
	10/2006	Multiple polyps in the entire colon and terminal ileum;	Hamartomatous polyps, possibly inflammatory pseudopolyps;
	03/2007	No polyps in the terminal ileum, hypertrophy of the Peyer's plaques; massive polyposis (sessile and pedunculated) of the entire colon;	Hamartomatous juvenile polyps, lymphatic hyperplasia in the terminal ileum;
	08/2008	Normal large bowel	
	02/2009	Normal large bowel	Lymphatic hyperplasia in the ileum;
Sonography gallbladder and biopsy of biliary tract	Sonography 06/2006, 12/2006,	Normal	
First diagnosis	6 months after first admission 03/2007		
Concomitant disorders	Cyst of the choroid plexus		
Therapy	Proctocolectomy with J-pouch (anojejunal anastomosis) 07/2007		Juvenile polyps, no intraepithelial neoplasia;

Table 6: Patient III/2

FOBT = faecal occult blood test, HP = Helicobacter pylori;

At the end of August 2006 the index patient, an 8-year old girl, was admitted to hospital. She had had rectal bleeding and diarrhoea during the last months. Besides, the school

doctor had noticed the girl's paleness, had diagnosed microcytic, hypochromic anaemia and had started an iron substitution therapy. In addition, the mother had reported of the patient's unusual tiredness and lack of strength. Lab results done in the hospital again showed microcytic, hypochromic anaemia (Hb 9,9g/dl, Hkt 30,9%, MCV 70,9fl, MCH 22,7pg), hypoproteinaemia and hypalbuminaemia. The faecal occult blood test was positive. Crohn's disease, ulcerative colitis or possibly even celiac disease were taken into consideration as differential diagnoses.

Ultrasound and an x-ray of the abdomen showed signs of an ileocolic invagination – an incidental finding, particularly since the patient did not have abdominal pain. Pneumatic disinvagination followed. Ileocolic invagination recurred about one month later and as a consequence laparoscopy with disinvagination and ileoascendopexia had to be carried out. Due to the two invaginations the possibility of the existence of a polyp, a tumour or possibly even a foreign body in this region became more likely. Coloscopy performed in October revealed multiple polyps throughout the colon histologically classified as hamartomatous polyps.

In the course of the following months anaemia aggravated. The patient had to be operated on due to an adhesive strangulation of intestines. Gastroscopy and coloscopy repeatedly showed multiple colonic hamartomatous polyps and a complete lack of polyps in the stomach. A Helicobacter pylori-associated chronic gastritis was diagnosed and an eradication therapy started. Since the clinical condition did not improve, proctocolectomy with an anojejunal anastomosis was carried out in July 2007. Macroscopic results showed multiple, mostly pedunculated juvenile polyps (see Figure 13) without signs of intraepithelial neoplasia. However, at that time, definite diagnosis had not yet been established. After proctocolectomy, regular bouginages were necessary, diarrhoea decreased, no incontinence was reported and the patient started gaining weight.

Figure 13: Colectomy specimen of patient III/2 with multiple pedunculated juvenile polyps

Macroscopic aspect of the index patient's colon after proctocolectomy with multiple, pedunculated juvenile polyps. The pictures were provided by Sen. Scientist Dr. med. univ. Ekkehard Spuller.

In June 2008 the result of genetic testing showed the heterozygote germline mutation c.543delC in the SMAD4 gene. Subsequently genetic counselling and predictive testing of the family was started. A surveillance scheme was agreed upon for the index patient. Controls of the pouch should be carried out every year, of the stomach and duodenum every 1 to 3 years. A chest x-ray was done once and the existence of an AV-malformation associated with this syndrome excluded. Gastroscopy and coloscopy carried out during the last two years showed no pathologic results.

3.2 Patient II/6

Mother of index patient (II/6)			
		Macroscopic results	**Histologic results**
Age at screening	29a		
Symptoms	No		
FOBT	N.e.		
Complications	No		
Gastroscopy	08/2008	1 sessile polyp in the antrum 2 cm in size, EMR carried out; 2 small polyps in the corpus; several other small polyps;	Tubular adenocarcinoma (early gastric cancer, mucosa type); Small juvenile polyps; HP-associated low-grade chronic gastritis;
	10/2008	St.p. EMR (scar), 2nd EMR, no pathological findings;	Without pathological findings, HP negative;
	01/2009	No pathological findings;	Without pathological findings, HP negative;
	04/2009	Small polyp-like lesions around the cardia;	No pathological findings;
	07/2009	Small polyp in the antrum, small polyp in the duodenum proximal of the papilla;	Hamartomatous juvenile polyp in the duodenum;
	09/2009	No pathological findings;	No pathological findings;
HP status	+ (08/2008)		
Eradication	08/2008		
Capsule endoscopy	No evidence of small bowel polyps		
Coloscopy	08/2008	1 pedunculated polyp in the descending colon 1,5 cm in size; 1 small polyp in the sigmoid; 1 small polyp in the caecum; Lymphatic hyperplasia in the ileum;	Tubular adenoma in the descending colon (LGIEN); Mucosal hyperplasia in the sigmoid; Mucosal hyperplasia in the caecum; No pathologic results;
	09/2009	Normal	
Sonography gallbladder and biopsy of biliary tract	Sonography 08/2008 Sonography 04/2009	2 polyps about 4 mm in size 1 polyp about 1 mm in size	
First diagnosis	08/2008		
Concomitant disorders	None		
Therapy	Polypectomy in the colon EMR in the stomach		

Table 7: Patient II/6

FOBT = faecal occult blood test, HP = Helicobacter pylori, N.e. = not examined, EMR = endoscopic mucosal resection, LGIEN = low-grade intraepithelial neoplasia;

In August 2008 the mother of the index patient came for clinical evaluation after testing positive for the pathogenic SMAD4 mutation. Gastroscopy showed a 2 cm big sessile

polyp in the antrum and two small polyps in the gastric corpus. Mucosectomy of the polyp was performed. Histological results revealed an early gastric cancer (tubular adenocarcinoma with moderate differentiation, intestinal type G2, mucosa type pT1A). Endoscopic and histological results are shown in Figure 14 and Figure 15.

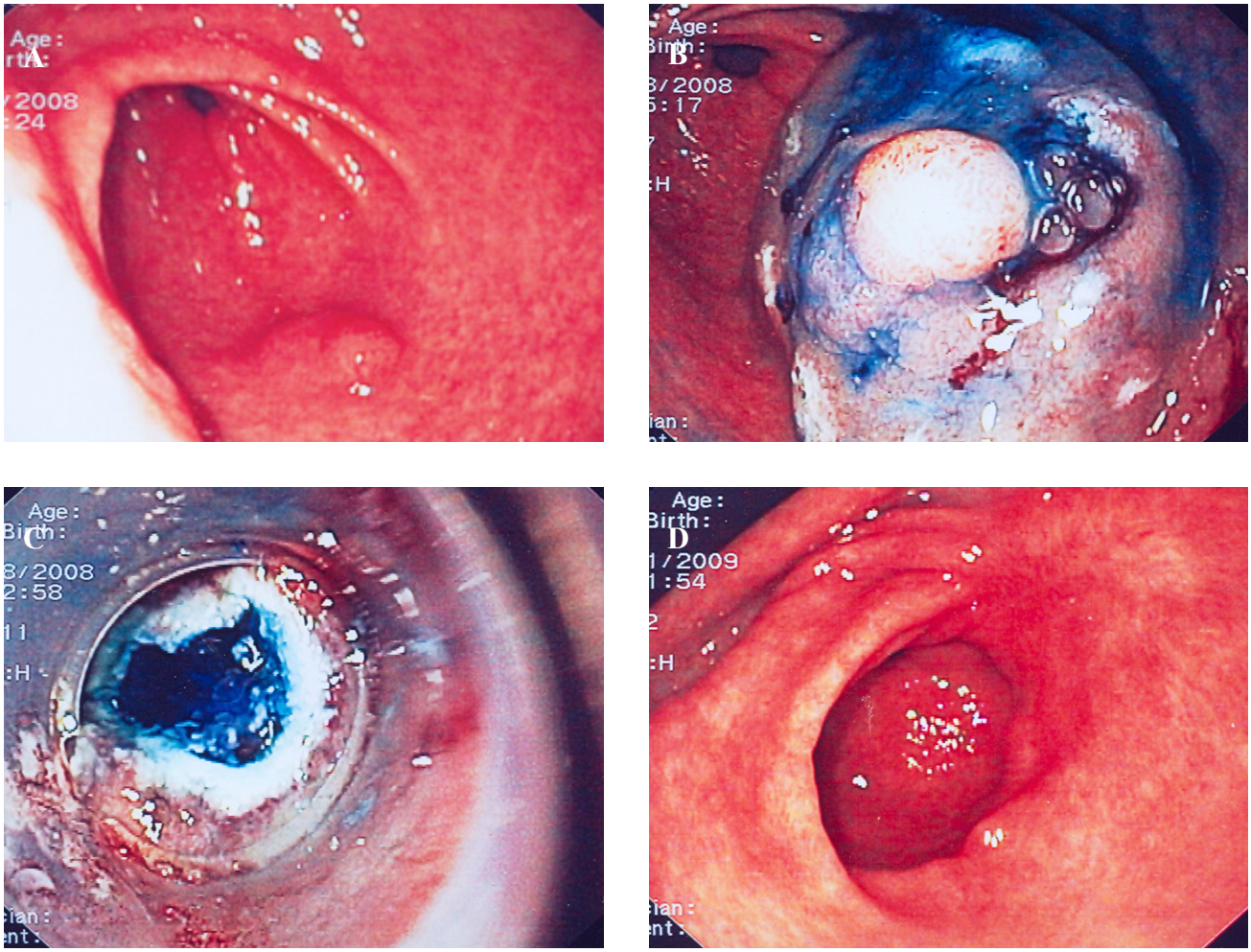

Figure 14: Upper endoscopy in patient II/6

EMR of the early gastric cancer in patient II/6. Early gastric cancer before EMR (A), the resection area with injected Methylene blue (B and C), upper endoscopy three months after EMR (D). Pictures provided by a.o. Univ. Prof. Dr. Christoph Högenauer.

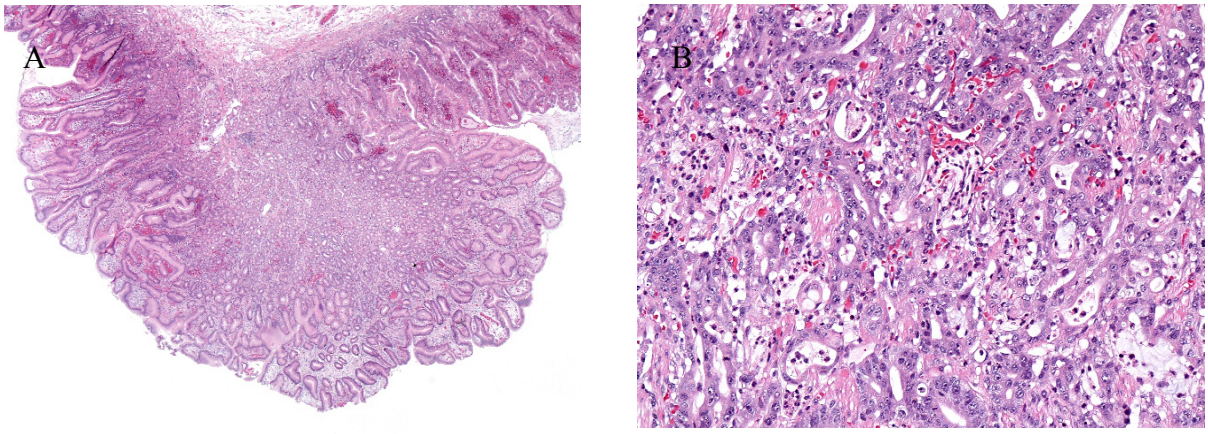

Figure 15: Histology of early gastric cancer of the intestinal type G2, mucosa type pT1A in patient II/6

Panel A shows a juvenile polyp with an early gastric cancer (tubular adenocarcinoma with moderate differentiation) of the intestinal type G2, limited to the mucosa (mucosa type pT1A). Panel B is an enlarged section showing the early gastric cancer. Characteristics are irregular tubular structures with secondary lumina, pluristratification, cellular pleomorphism, hyperchromatic nuclei and an increased number of mitoses. These pictures were provided by Sen. Scientist Dr. med. univ. Ekkehard Spuller.

As the patient was positive for Helicobacter pylori an eradication therapy was performed. Coloscopy revealed lymphatic hyperplasia of the ileum, two small polyps – one in the sigmoid and one in the caecum – as well as one pedunculated polyp in the descending colon. Polypectomy was carried out. Histology showed a tubular adenoma of the colon with low-grade intraepithelial neoplasia and two small polyps interpreted as mucosal hyperplasia. Sonography of the abdomen showed two polyps of the gallbladder about 4 mm in size.

In October 2008 the patient was again admitted to hospital to repeat mucosectomy in the area of the early gastric cancer. Histological findings of the resected mucosa showed no signs of malignancy or other neoplastic tissue. A prophylactic total gastrectomy was discussed, which was rejected by the patient. The following surveillance scheme for the patient was initiated: Gastroscopy was to be repeated every three months at the beginning. If no residues of the early gastric cancer were to be found after one year, gastroscopic controls could be carried out every six months. Coloscopy should be repeated once a year, capsule endoscopy of the small intestine every three years.

Subsequent gastroscopies showed small hamartomatous polyps of the duodenum. Sonography carried out in April 2009 showed only one polyp 1 mm in size in the gallbladder.

3.3 Patient I/3

Grandmother of index patient (I/3)			
		Macroscopic results	**Histologic results**
Age at screening	52a		
Symptoms	Recurrent tiredness since 2004 Recurrent lack of strength since 2004		
FOBT	Negative		
Complications	Recurrent chronic anaemia since 2004 Hypoproteinaemia diagnosed in 2005		
Gastroscopy	04/2005	Several polyps up to 1 cm in size in the corpus, polypoid mucosa in the fundus;	Hyperplastic polyp (probably juvenile polyp), no malignancy, low-grade chronic active gastritis, HP negative;
	09/2008	Pronounced polyposis of the corpus and fundus, partly friable with traces of haematin;	HP-associated high-grade chronic gastritis; hyperplastic polyp (probably juvenile polyp);
HP status	+ (09/2008)		
Eradication	(Gastrectomy 10/2008)		
Capsule endoscopy	09/2008	1 suspect small polyp in the small intestine;	
Coloscopy	04/2005	Small polyp in the caecum;	Small hyperplastic polyp (probably juvenile polyp);
	09/2008	1 polyp in the ascending colon;	Inflammatory polyp (probably juvenile polyp);
MRI abdomen	Several haemangiomas in the liver, one lesion of unknown origin in the liver;		
Sonography gallbladder and biopsy of biliary tract	Biopsy of the lesion of unknown origin in the liver 10/2008		Bile duct hamartoma (von Meyenburg complex);
First diagnosis	09/2008 (genetic screening)		
Concomitant disorders	Epilepsy in childhood Hypothyreosis, st.p. hemithyroidectomy Haemangiomas in the liver Obesity		
Therapy	Gastrectomy 10/2008	Massive polyposis	Massive juvenile polyps, no intraepithelial neoplasia;

Table 8: Patient I/3

FOBT = faecal occult blood test, HP = Helicobacter pylori;

The patient was admitted to hospital in 2005 at the age of 49 years due to tiredness and lack of strength caused by a chronic anaemia diagnosed by her general practitioner (Hb 5.2g/dl). A year before she had complained about a similar condition. Back then no source of bleeding could be detected in gastroscopy carried out by a doctor in private practice. In 2005 again no sources of bleeding could be determined either in gastroscopy or in

coloscopy that revealed several polyps up to 1 cm in size in the stomach and one small polyp in the caecum. Iron uptake showed no disorders. After the transfusion of two units of packed red cells and parenteral iron substitution, haemoglobin increased and clinical symptoms ameliorated. The patient was dismissed without a correct diagnosis of her gastric polyposis.

In September 2008, after positive testing for the pathogenic SMAD4 mutation, the patient was again admitted to hospital for gastrointestinal evaluation and clarification of her chronic anaemia and hypoproteinaemia. Massive gastric polyposis was again diagnosed (see Figure 16) and gastrectomy planned subsequently. The patient was also HP positive. Sonography of the abdomen revealed several lesions in the liver. An MRI of the abdomen showed multiple haemangiomas in the liver as well as one lesion of unknown origin which was biopsied during gastrectomy. Biopsy revealed a bile duct hamartoma.

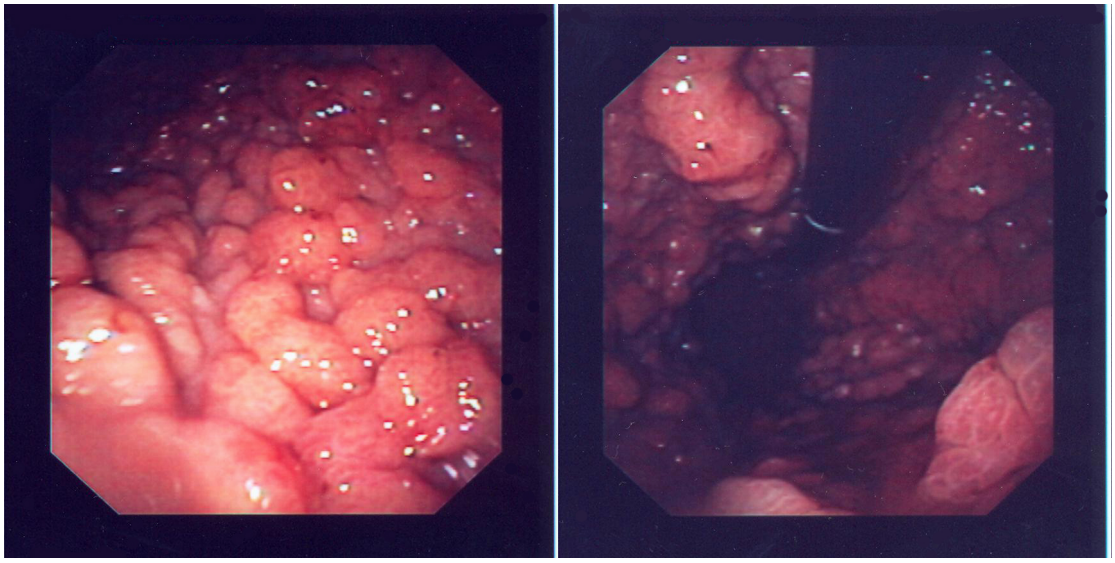

Figure 16: Massive gastric polyposis in patient I/3

Due to this massive gastric polyposis and severe chronic anaemia because of bleeding gastrectomy was performed. These pictures were provided by a.o. Univ. Prof. Dr. Christoph Högenauer.

3.4 Patient I/2

Brother of grandmother (I/2)			
		Macroscopic results	**Histologic results**
Age at screening	60a		
Symptoms	No		
FOBT	Positive		
Complications	No		
Gastroscopy	01/2009	Several sessile polyps in the antrum about 1 cm in size (EMR),	HP-associated low-grade chronic gastritis, incipient hyperplastic polyps;
		Various small polyps in the duodenal bulb;	N.e.
	01/2009 + EMR	3 sessile polyps in the antrum;	Hamartomatous juvenile polyps;
HP status	+ (01/2009)		
Eradication	01/2009		
Capsule endoscopy	01/2009	Multiple haemorrhages in the jejunum, possible intramural lesion in the distal jejunum (DD: GIST), polypoid lesion in the distal ileum;	
MRI duodenum		GIST could not be verified;	
Coloscopy	01/2009 + polypectomy	1 small polyp in the rectum,	N.e.
		1 small polyp in the caecum,	N.e.
		1 pedunculated polyp in the sigmoid about 2 cm in size,	Tubulovillous adenoma with LGIEN
		in the ascending colon (near the right colic flexure) 1 small polyp and 1 sessile polyp 5 cm in size;	Hamartomatous polyp Villous adenoma with HGIEN
	06/2009 + polypectomy	1 small polyp in the caecum,	Hamartomatous polyp
		1 small polyp in the ascending colon,	Hamartomatous polyps
		in the transverse colon (near the right colic flexure) a 6-7 cm big sessile polyp covering ¾ of the circumference,	Hamartomatous polyp with LGIEN
		in the sigmoid a 1 cm big polyp,	Vascular malformation
		two small polyps in the rectum;	Hamartomatous polyps
	10/2009	Near the right colic flexure the remains of the big sessile polyp removed before,	Hamartomatous polyp with LGIEN
		1 small polyp in the transverse colon,	Hamartomatous polyp
		1 polyp in the sigmoid about 1 cm in size;	Hamartomatous polyp
Sonography gallbladder and biopsy of biliary tract	Sonography 01/2009	Several polyps in the gallbladder a few mm in size;	
	Sonography 01/2009	Multiple polyps in the	

		gallbladder, the biggest 15 mm;	
	Sonography 06/2009	Several polyps up to 1 cm in size;	
First diagnosis	01/2009		
Concomitant disorders	Rheumatoid arthritis Arterial hypertension		
Therapy	Polypectomy EMR Resection of transverse colon, ascendodescendostomia, cholecystectomy;		Tubulovillous adenoma with low-grade intraepithelial neoplasia and incipient juvenile polyps; Gallbladder: hyperplastic polyps (first diagnosis); re-evaluation: cholesterol polyps;

Table 9: Patient I/2

FOBT = faecal occult blood test, HP = Helicobacter pylori, EMR = endoscopic mucosal resection, LGIEN = low-grade intraepithelial neoplasia, HGIEN = high-grade intraepithelial neoplasia, GIST = gastrointestinal stromal tumour, n.e. = not evaluated;

This patient, the brother of the index patient's grandmother, was identified by molecular genetic screening and was asymptomatic at initiation of screening. Gastroscopy revealed various small polyps in the duodenal bulb and several sessile polyps in the antrum about 1 cm in size. The latter were removed by means of EMR (see Figure 21). Since the patient was positive for Helicobacter pylori an eradication therapy was started. Coloscopy showed small polyps in the caecum, the ascending colon and the rectum. In the ascending colon a bigger, sessile polyp was also found about 5 cm in size and located near the right colic flexure. In the sigmoid a pedunculated 2 cm big polyp was found (see Figure 20). Polypectomies were carried out.

Five months later, coloscopy was repeated and again showed several smaller polyps, one pedunculated one in the sigmoid and the remaining big polyp in the right colic flexure – 6-7 cm in size covering ¾ of the circumference. Polypectomies were again performed.

Histologic results confirmed the polyps' hamartomatous origin. One polyp turned out to be a tubulovillous adenoma with low-grade intraepithelial neoplasia (LGIEN), another turned out to be a villous adenoma with high-grade intraepithelial neoplasia (HGIEN). In the subsequent coloscopy in October 2009 the remnants of the villous adenoma with HGIEN located in the right colic flexure were marked with ink to facilitate the planned resection.

In addition to the juvenile polyps found in the stomach and the colon, polyps were also found in the gallbladder by means of sonography (see Figure 17). On three separate occasions several polyps up to 1 cm (the biggest one even up to 15 mm) in size were detected. In January 2010 the patient underwent a planned resection of the transverse colon for the villous adenoma with an ascendodescendostomia and a cholecystectomy for the gallbladder polyps (see Figure 18) which turned out to be cholesterol polyps (see Figure

19). Another interesting finding was revealed in the histologic results of the colonic polyps – a vascular malformation in the sigmoid.

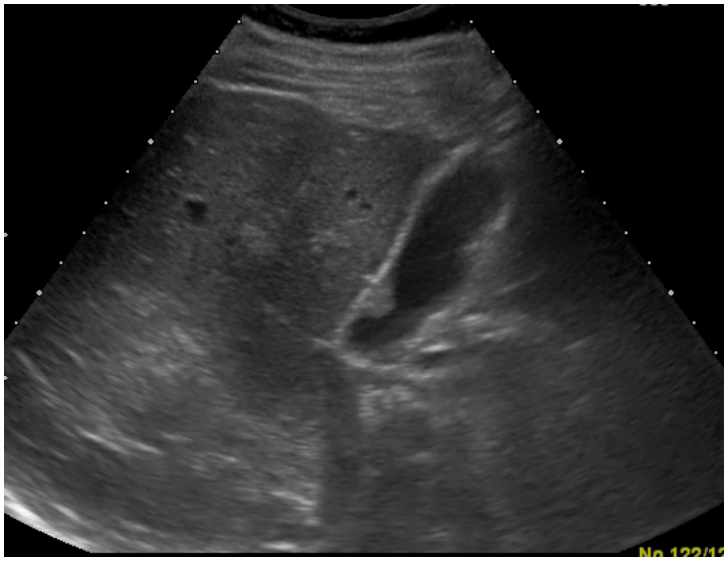

Figure 17: Sonography of the gallbladder in patient I/2

In the gallbladder multiple small polyps were found. In this sonographic image one polyp detected in patient I/2 can be seen measuring 10 mm in size. This picture was provided by a.o. Univ. Prof. Dr. Christoph Högenauer.

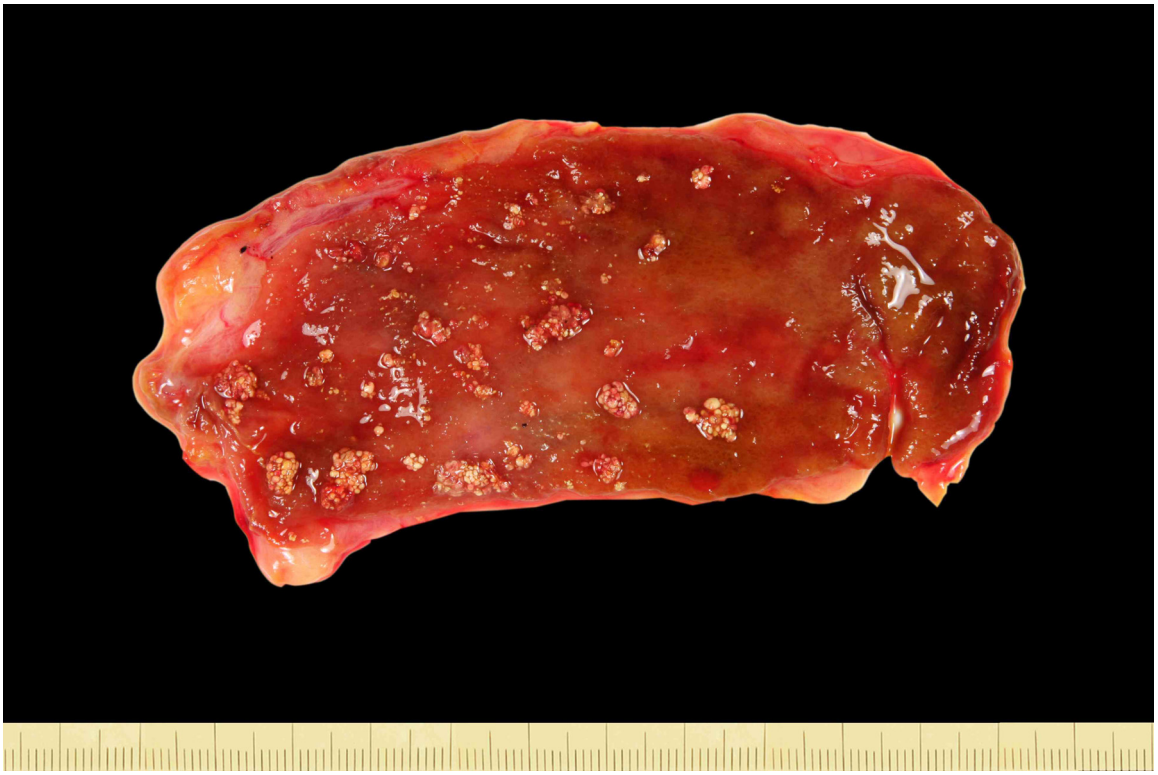

Figure 18: Macroscopic image of the gallbladder with cholesterol polyps after cholecystectomy in patient I/2

This picture was provided by Sen. Scientist Dr. med. univ. Ekkehard Spuller

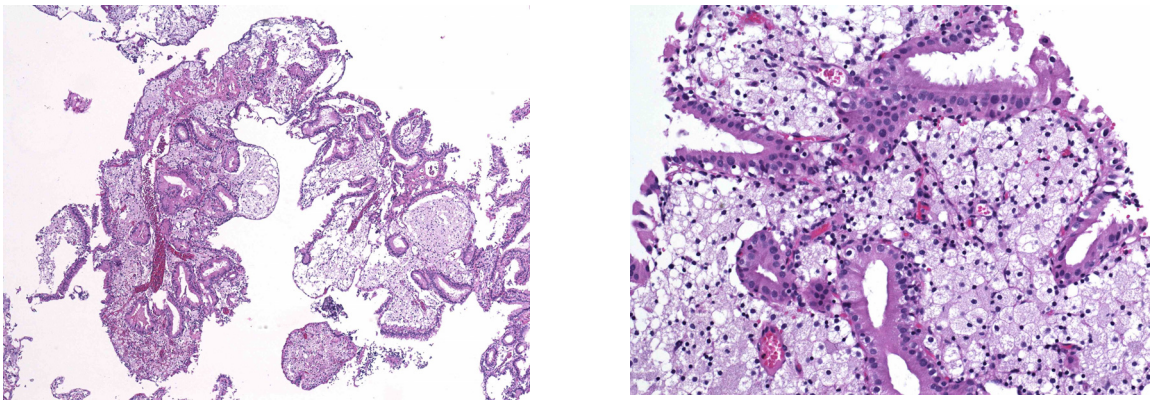

Figure 19: Histology of cholesterol polyps found in the gallbladder in patient I/2

The panels show the polypoid mucosa of the gallbladder with foam cells (cholesterol-storing macrophages). The epithelium shows no neoplastic changes. Inflammatory cells can be seen. These panels were provided by Sen. Scientist Dr. med. univ. Ekkehard Spuller.

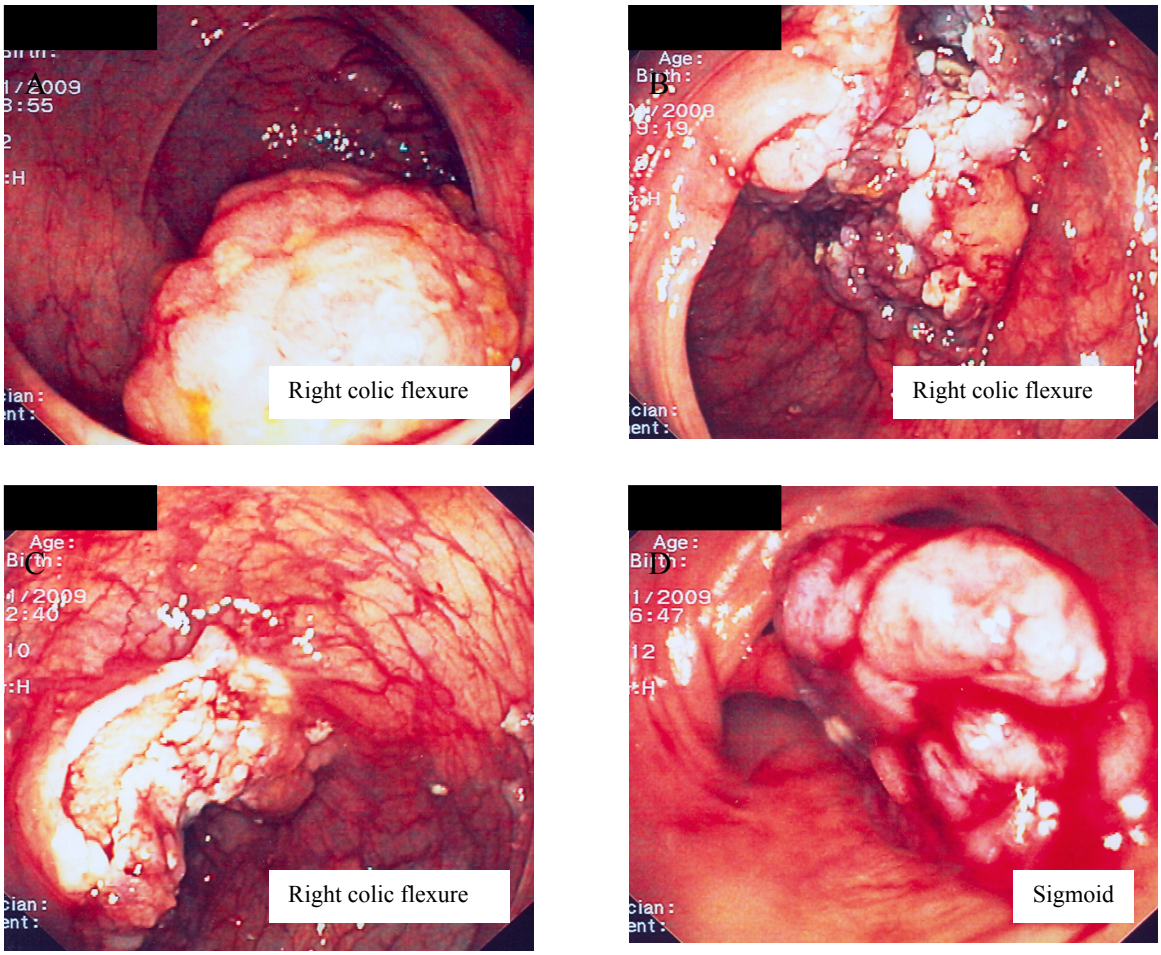

Figure 20: Findings during initial coloscopy in patient I/2

In coloscopy, a villous adenoma with HGIEN was revealed in the right colic flexure (panel A and B) and a tubulovillous adenoma with LGIEN in the sigmoid (panel D). Polypectomies were performed. The polyp in the right colonic flexure could not be removed completely by endoscopic polypectomy (panel C). Pictures provided by a.o. Univ. Prof. Dr. Christoph Högenauer.

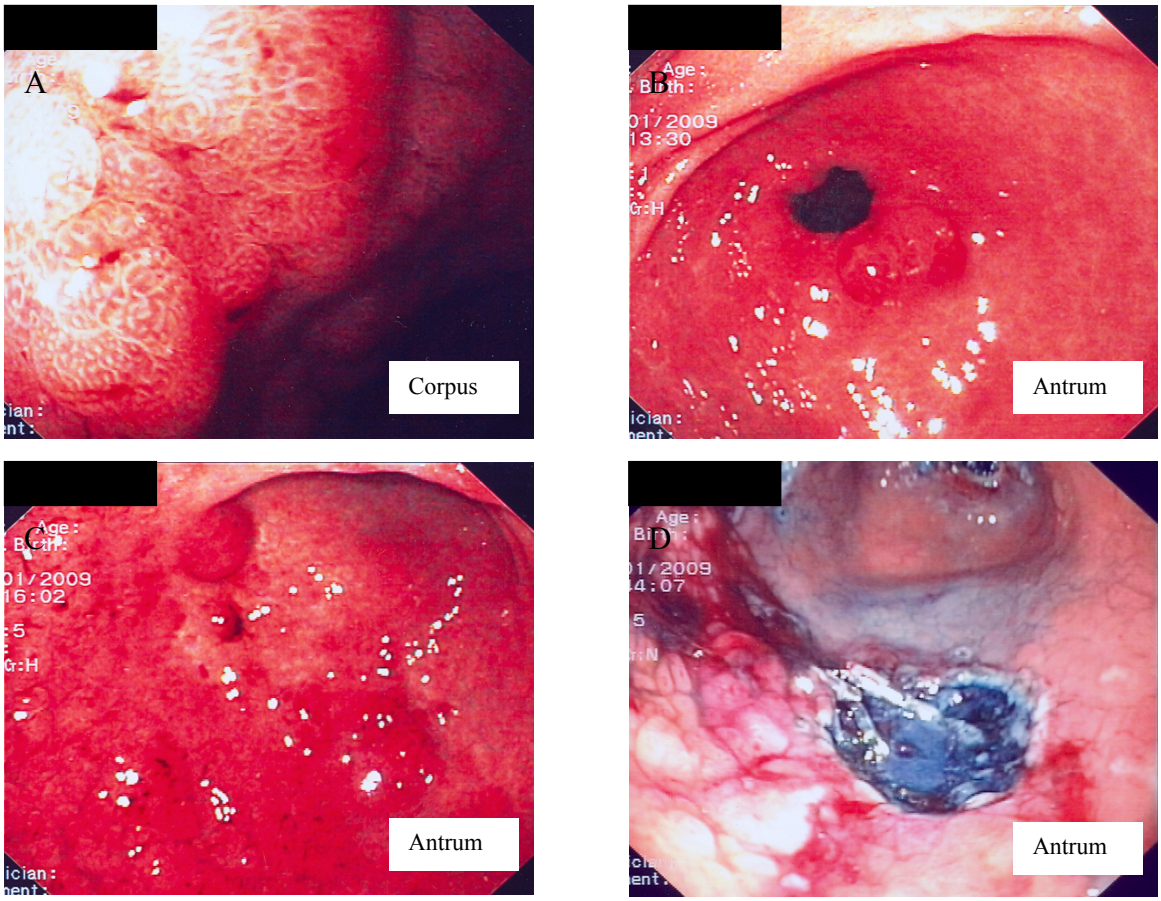

Figure 21: Upper endoscopy in patient I/2

Upper endoscopy in patient I/2 showed several small polyps in the corpus (panel A) and 2 sessile polyps in the antrum (panels B and C). After EMR the resection area appeared blue due to injected Methylene blue with saline solution before EMR (panel D: after EMR). Pictures provided by a.o. Univ. Prof. Dr. Christoph Högenauer.

3.5 Patient II/4

Son of patient I/2 (II/4)			
		Macroscopic results	**Histologic results**
Age at screening	10a		
Symptoms	No		
FOBT	Negative		
Complications	No		
Gastroscopy	08/2009	Suspect small polyp in the duodenal bulb;	HP-associated low-grade chronic gastritis; normal duodenal mucosa;
HP status	+		
Eradication	No		
Capsule endoscopy	N.e.		
Coloscopy	08/2009	Multiple polyps (5 to 10) from the caecum to the rectum, the biggest one located in the caecum/ascending colon;	Juvenile polyps;
Sonography gallbladder and biopsy of biliary tract	N.e.		
First diagnosis	05/2008		
Concomitant disorders	Psychomotor retardation, hypertelorism;		
Therapy	No therapy so far		

Table 10: Patient II/4

FOBT = faecal occult blood test, HP = Helicobacter pylori, N.e. = not examined;

The son of patient I/2 was first screened at the age of 10 years. He had always been asymptomatic. Gastroscopy revealed suspect small polyps in the duodenal bulb which, however, showed normal histology and a Helicobacter pylori positive gastritis. In the colon 5 to 10 juvenile hamartomatous polyps were found. No information concerning a possible manifestation of the gallbladder is available as sonography of the abdomen was not carried out.

3.6 Patient II/3

Son of patient I/2 (II/3)			
		Macroscopic results	**Histologic results**
Age at screening	18a		
Symptoms	No		
FOBT	N.e.		
Complications	No		
Gastroscopy	06/2009	No polyps found;	HP-associated low-grade chronic gastritis;
HP status	+		
Eradication	07/2009		
Capsule endoscopy	06/2009	Healed ulcer in the jejunum;	
Coloscopy	06/2009	Lymphatic hyperplasia in the ileum, 2 small polyps in the caecum, in the sigmoid 1 pedunculated polyp 1 cm in size, 1 small sessile polyp, in the rectum 2 small polyps;	Hamartomatous polyps, no malignancy;
Sonography gallbladder and biopsy of biliary tract	Sonography 06/2009	-	
First diagnosis	06/2009		
Concomitant disorders	No		
Therapy	Polypectomy		

Table 11: Patient II/3

FOBT = faecal occult blood test, HP = Helicobacter pylori, N.e. = not examined;

The asymptomatic elder son of patient I/2 was first screened at the age of 18. He had no polyps in the stomach or small intestine, but presented with 6 hamartomatous juvenile polyps located throughout the colon which were removed by polypectomy. No polyps were seen in the gallbladder.

3.7 Patient II/2

Daughter of patient I/2 (II/2)			
		Macroscopic results	**Histologic results**
Age at screening	24a		
Symptoms	No		
FOBT	N.e.		
Complications	No		
Gastroscopy	03/2010	2 incipient small polyps in the duodenal bulb;	Incipient juvenile polyps, HP-associated moderate chronic gastritis;
HP status	+		
Eradication	03/2010		
Capsule endoscopy	03/2010	-	
Coloscopy	03/2010	In the sigmoid 1 sessile polyp 3 mm in size;	Incipient juvenile polyp;
Sonography gallbladder and biopsy of biliary tract	Sonography 03/2010	-	
First diagnosis	03/2010		
Concomitant disorders	Epilepsy in childhood		
Therapy	Polypectomy		

Table 12: Patient II/2

FOBT = faecal occult blood test, HP = Helicobacter pylori, N.e. = not examined;

The daughter of patient I/2 was first screened at the age of 24 years. She had no symptoms and only two very small incipient juvenile polyps in the duodenal bulb and one incipient sessile polyp 3 mm in size in the sigmoid. Apart from that, she had no other manifestations of JPS. Helicobacter pylori-associated gastritis was diagnosed and an eradication therapy initiated.

3.8 Patient III/4

Cousin of index patient (III/4)			
		Macroscopic results	**Histologic results**
Age at screening	14a		
Symptoms	No		
FOBT	N.e.		
Complications	No		
Gastroscopy	04/2009	In the fundus 1 polyp, multiple smaller ones;	HP negative, no pathological findings;
	11/2009	Small polyps in the fundus;	Hamartomatous polyp;
HP status	Negative		
Capsule endoscopy	N.e.		
Coloscopy	04/2009	From the caecum to the rectum about 20-50 bigger and smaller polyps;	Hamartomatous polyps;
	11/2009	About 20 bigger and smaller polyps throughout the colon;	Hamartomatous polyps;
Sonography gallbladder and biopsy of biliary tract	Sonography 04/2009	-	
First diagnosis	04/2009		
Concomitant disorders	Mild mental retardation, signs of dysmorphia, hypertelorism; no causal chromosomal aberration was found;		
Therapy	Polypectomy (2 juvenile polyps) planned for 07/2010		

Table 13: Patient III/4

FOBT = faecal occult blood test, HP = Helicobacter pylori, N.e. = not examined;

The cousin of the index patient was refereed for gastrointestinal screening when she was 14 years of age after testing positive for the SMAD4 mutation. In the stomach she had multiple very small juvenile polyps and a slightly bigger one, while in the colon 20-50 bigger and smaller juvenile polyps were found. She had no manifestation of JPS in the gallbladder.

3.9 Summary of Results

Summarizing the results (see Table 14), it can be concluded that all SMAD4 mutation carriers of the kindred analyzed had manifestations of JPS – with varying expression. There was a high variation in number and localisation of polyps, neoplastic progression of polyps and severity of symptoms – independent of the age of the patients.

Only 2 of the 8 patients – the index patient and her grandmother – were symptomatic at the time of diagnosis. The index patient presented with tiredness, lack of strength, diarrhoea and rectal bleeding. Hypoproteinaemia and chronic anaemia were detected and during the process of diagnosis two intestinal invaginations were detected. Her grandmother also reported of recurrent tiredness and lack of strength due to chronic anaemia. A source of bleeding was not found at initial evaluation. Additionally, hypoproteinaemia was diagnosed. The other 6 patients never showed any symptoms or complications associated with JPS.

Until the correct diagnosis was made for the index patient six months passed. With the diagnosis of JPS and subsequent genetic testing for all family members her grandmother's year-long clinical condition was explained and the diagnosis of JPS established after three years.

All of the eight patients showed colonic manifestations of JPS – as mentioned, the number of polyps varied greatly within the family. The index patient's mother had a tubular adenoma with LGIEN, the grandmother's brother a tubulovillous adenoma with LGIEN and a villous adenoma with HGIEN in the colon. Five of the eight patients had juvenile polyps in the stomach. The mother of the index patient presented with early gastric cancer of the mucosa type treated by endoscopic mucosal resection, the grandmother of the index patient with massive polyposis necessitating gastrectomy due to chronic blood loss. The two patients showing advanced neoplastic lesions were asymptomatic at the time of diagnosis.

Four patients had polyps in the small intestine – diagnosed either by means of gastroscopy or capsule endoscopy. For the two patients with polyps located in the duodenum histological verification was obtained revealing a hamartomatous origin. In the other two patients the existence of polyps in the small intestine was diagnosed by means of capsule endoscopy – thus no histological evaluation exists.

Three of the eight patients had manifestations of JPS in the gallbladder (two patients) or the biliary tract (one patient). Polyps in the gallbladder in the mother and the grandmother's brother were detected sonographically. When the resection of the transverse colon was carried out in the grandmother's brother, cholecystectomy was also done due to several polyps seen in sonography. Histologic results showed that they were most likely cholesterol polyps. As for the grandmother, the lesion of unknown origin detected in sonography and the MRI was biopsied when gastrectomy was carried out. Histologic

evaluation revealed a bile duct hamartoma. Thus, for two of the three patients with JPS manifestations in the gallbladder and biliary tract histologic verification exists.

Another interesting finding is that the grandmother's brother had a histologically detected vascular malformation in the sigmoid.

Furthermore, of the eight examined family members, seven had a Helicobacter pylori-positive gastritis. Five had eradication therapy, in the grandmother gastrectomy was performed after diagnosis and for the seventh patient eradication therapy has not been recommended so far.

As for the concomitant diseases, the index patient has a cyst of the choroid plexus, two had epilepsy in their childhood and two patients have developmental delay. For one patient the reason for his psychomotor retardation is probably hypoxia at birth. For the other patient showing mild developmental delay and signs of dysmorphia, no causal chromosomal aberrations were found.

Patient	**Index patient (III/2)**	**Mother of index patient (II/6)**	**Grandmother of index patient (I/3)**	**Brother of grandmother (I/2)**	**Son of patient I/2 (II/4)**	**Son of patient I/2 (II/3)**	**Daughter of patient I/2 (II/2)**	**Cousin of index patient (III/4)**
Age at diagnosis (index pat)/screening	**8a**	**29a**	**52a**	**60a**	**10a**	**18a**	**24a**	**14a**
Stomach **HP status** **Eradication**	Normal + 03/2007	2 jP + early gastric cancer mucosa type + 08/2008	Massive jP + (2008) Gastrectomy	Multiple jP (3 jP, multiple smaller ones) + 01/2009	1 jP + No	Normal + 07/2009	Normal + 03/2010	1 jP, multiple smaller jP -
Duodenum	N.ev.	1 jP	Normal	Normal	N.e.	Normal	2 small jP	N.e.
Jejunum, ileum	N.ev.	Normal	1 small P (capsule endoscopy)	Multiple haemorrhages	N.e.	Normal Jejunum: healed ulcer	Normal	N.e.
Terminal ileum	Normal	Normal	Normal	1 P (capsule endoscopy)	Normal	Normal	Normal	Normal
Colon	Multiple jP	2 P (no biopsy) + tubular A (LGIEN)	1-2 jP	4 jP, 1 tubulovillous A with LGIEN, 1 villous A HGIEN; Vascular malformation in the sigmoid;	5-10 jP	6 jP	1 jP	≈20 jP
Gallbladder (sonography) **Biliary tract (biopsy)**	Normal	1-2 P	 1 hamartoma of the biliary tract	Multiple P	N.e.	Normal	Normal	Normal
First diagnosis	6 months after first admission 03/2007	08/2008	09/2008	01/2009	05/2008	06/2009	03/2010	04/2009
Concomitant disorders	Cyst of the choroid plexus	None	St. p. epilepsy Hypothyreosis	Rheumatoid arthritis Arterial hypertension	Psychomotor retardation, hypertelorism;	None	Epilepsy in childhood	Mild mental retardation, hypertelorism, signs of dysmorphia;
Complications	Hypoproteinaemia Chronic anaemia 2 invaginations	None	Chronic anaemia	None	None	None	None	None
Therapy	Proctocolectomy	Mucosectomy in the stomach Polypectomy	Gastrectomy	Polypectomy; Cholecystectomy and resection of the transverse colon, ascendo-descendotomia;	No therapy so far	Polypectomy	Polypectomy	Polypectomy (2 jP) planned for 07/2010

Table 14: Overview of all results

jP = juvenile polyps
P = polyp without histologic results
+/- = HP positive/negative
A = adenoma
C = carcinoma

LGIEN = low-grade intraepithelial neoplasia
HGIEN = high-grade intraepithelial neoplasia
N.e. = not examined
N.ev. = could not be evalutated

4 DISCUSSION AND CONCLUSIONS

4.1 SMAD4 Mutation and its Implications

JPS in the family at hand is caused by a novel SMAD4 mutation that has not been reported so far in the literature [30, 31, 35-40]. All affected patients of the family are heterozygote carriers of the SMAD4 germline mutation c.543delC leading to a frameshift and the creation of a new stop codon at position 201 (p.Leu201X). This in turn causes a preterm derogation of SMAD4 protein synthesis.

Friedl et al. [30] who proposed the first genotype-phenotype correlation in JPS reported seven SMAD4 mutation carriers four of which presented with massive gastric polyposis. Aretz et al. [44] showed stomach manifestations of JPS in 22 of 30 SMAD4 mutation carriers, corresponding to 73% of the tested patients. In analogy to these findings, five of the eight patients of the family in this study showed gastric juvenile polyps – two of them revealing a severe manifestation. The mother of the index patient had early gastric cancer treated by means of EMR and the grandmother who had been symptomatic for years had massive gastric polyposis requiring gastrectomy. She was 52 years of age when diagnosis was established – this could be consistent with Aretz et al.'s suggestion of age-related penetrance in gastric manifestation as mentioned in chapter 1.3.5.3 [44] Five of eight patients, 63%, in the kindred presented with gastric manifestation. It needs to be taken into account when comparing these numbers that the family of this study represents affected individuals all carrying the same mutation. The patients analyzed by Friedl et al. [30] and Aretz et al. [44] were unrelated and all showed different SMAD4 germline mutations. From the results of the present study alone surveillance implications cannot be derived due to this reason. In combination with the results described in the literature however they emphasize the importance of regular upper gastrointestinal screening in SMAD4 mutation carriers.

Another association with SMAD4 mutations apart from gastric manifestation is the occurrence of hereditary haemorrhagic telangiectasias. For establishing the diagnosis of HHT three of four diagnostic criteria need to be fulfilled [102]. Of the eight patients examined no one seemed to have spontaneous epistaxis, mucocutaneous telangiectasias, visceral AVMs or a positive family history of HHT. Only one patient revealed a vascular malformation in the colon – possibly a manifestation of HHT or perhaps only a coincidence. One criterium does not suffice for the diagnosis of HHT. Yet this finding

places emphasis on the necessity of thorough screening of all SMAD4 mutation positive patients for AVMs and telangiectasias.

4.2 Hypothesis (1)

In hypothesis (1) it was postulated that juvenile polyps could occur not only in the stomach, small intestine and colon, but also in the gallbladder and biliary tract – a manifestation not yet described in the literature. Of the eight SMAD4 mutation positive patients examined clinically two showed polyps in the gallbladder. In one of these, polyps were classified histologically as cholecystectomy was performed together with the resection of the transverse colon due to a large adenoma. A confirmation of their hamartomatous composition could not be achieved, as histological results were ambiguous – indicating the possibility of the existence of cholesterol rather than juvenile polyps in the gallbladder. The second patient did not undergo cholecystectomy since polyps in the gallbladder were very small and thus no histological sample of the polyps was available. A third patient was biopsied due to a lesion of unknown origin in the liver and revealed a bile duct hamartoma. Thus, three related patients possibly might have had a manifestation of JPS in the biliary tract that has not been described before.

Assuming that the family at hand does indeed show manifestations of JPS in the gallbladder, it raises the question what causes the difference between this family and other JPS kindreds without gallbladder manifestations. The mutation in this family has not been detected before – it is thus a new SMAD4 mutation in the group of many different described before. It leads to a frameshift, a new stop codon and consequently to a truncated protein – as most other SMAD4 point mutations. Nevertheless, it remains possible that this mutation is different from the others in terms of the JPS manifestations it causes – evoking a broader spectrum of manifestations involving not only the stomach, small intestine and colon but the gallbladder as well.

Yet, why do only three of eight patients have gallbladder or biliary tract manifestations even though they all have the same mutation? These three patients are the eldest examined SMAD4 mutation carriers in this kindred. The patient with the multiple polyps in the gallbladder was 60 years of age at the time of first screening. The grandmother with the bile duct hamartoma was 52 at the time of biopsy. Her daughter, the second patient with polyps in the gallbladder – not confirmed histologically – was 29 years old when the first screening was performed. The other five patients, of which all but one were examined by

means of sonography, were between 8 and 24 years old when screening was started or – as for the index patient – when diagnosis was made. This observation gives rise to the suspicion that polyps in this location might manifest later in life than those in the colon or stomach, a phenomenon referred to as age-related penetrance. If this were true, it would implicate and emphasize the necessity of regular surveillance of the gallbladder by means of sonography.

Could the polyps in the gallbladder and the bile duct hamartoma have been coincidental? Approximately 1.5-9.5% of the general adult population are affected by gallbladder polyps of different types [119]. The benign polypoid lesions of the gallbladder include pseudotumors such as cholesterol polyps, adenomyomatosis and inflammatory polyps and true tumors such as adenomas, leiomyomas and lipomas [120]. When comparing various publications, the highest prevalence of gallbladder polyps is always found in patients between their third and fifth decade of life [121-123]. Opinions diverge on age-dependent prevalence in the literature. Chen et al. [121] and Jorgensen at al. [122] could not show an effect of age on the development of polypoid lesions in the gallbladder. Segawa et al. [123], on the other hand, showed that in the Japanese population the prevalence of gallbladder polyps considered cholesterol polyps was highest among middle-aged (40- and 50-year old) males.

Bile duct hamartomas are rare hepatic lesions first described by Von Meyenburg in 1918 [124] with an incidence at autopsy of 5-6% [125]. They consist of epithelium-lined dilated small bile ducts or cystically dilated bile ducts within a fibrous stroma. They develop at a late embryogenic stage during which peripheral bile ducts grow and are thus a ductal plate malformation [126].

These findings suggest that the discovery of gallbladder polyps in two of the JPS patients, as well as the bile duct hamartoma might also be coincidental and not associated with JPS. As for the bile duct hamartoma, a coincidental finding seems highly likely as von Meyenburg complexes are described as incidental findings in asymptomatic patients without inherited gastrointestinal diseases in the literature and also due to their incidence [124-126].

The fact that the polyps in the gallbladder of the grandmother's brother were histologically classified as cholesterol polyps seems to support the idea of coincidence in these two patients. The findings by Segawa et al. [123] might help to understand why only two of eight patients are actually concerned with polyps in the gallbladder, whether hamartomatous or cholesterol. He found out that the prevalence of gallbladder polyps was

highest among 40 to 50 year-olds. As the two patients with gallbladder polyps were 60 and 29 years of age at screening, they are, apart from the 52 year-old grandmother of the index patient with the bile duct hamartoma, the oldest patients. The other five patients are all younger. Therefore, age-related penetrance may play a role and polyps in the gallbladder might develop later in life.

Reports by Wada et al. [127] and Vogel et al. [128] could support the newly found JPS manifestations in the family examined. In 1987, Wada et al. documented a case of a patient with Peutz-Jeghers syndrome (PJS) who had carcinoma and two polyps characterized as hamartomatous polyps in the gallbladder [127]. In 2000 Vogel et al. reported of three unrelated patients with PJS who presented with polyps in the gallbladder. They recommend no operative therapy as long as polyps are small and asymptomatic, but regular sonographic controls [128]. Thus, as the gallbladders of these patients were not removed, no histological evaluation is available in this report – it remains unclear whether histological evaluation would have revealed cholesterol, hamartomatous or even other polyps. In any case, both studies document gallbladder manifestations in patients with a hamartomatous polyposis syndrome.

Cases of gallbladder polyps have also been reported in the literature for familial adenomatous polyposis– 10 adenomas and 6 adenocarcinomas until 2007– and for Gardner's syndrome in a publication by Brevet et al. [129]. The adenomatous gallbladder lesions are usually discovered in patients older than 40 years of age [129] – consistent with the data of Segawa et al. and also comparable with the findings in the kindred analyzed in this work.

Regular sonographic controls for any polypoid lesions of the gallbladder are not only recommended by Vogel et al. [128] as mentioned above but are also advised by Lee et al. [130]. They suggest three- to six-monthly ultrasonography examination in the initial follow-up period. If polyps are small and static and thus appear benign, surveillance can be loosened after 1 or 2 years. They regard age over 50 years and size of polyp over 1 cm as the most important risk factors. These predictors of malignancy in polypoid lesions of the gallbladder are considered an indication for laparoscopic cholecystectomy [130].

In the grandmother's brother (patient I/2) laparoscopic cholecystectomy was carried out simultaneously with the resection of the transverse colon – the patient being 60 years of age and the biggest polyp being 15 mm in size and several others up to 1 cm. Thus, the two most important risk factors for malignancy as proposed by Lee et al. were fulfilled in this patient, histology, however, showed no signs of neoplasia. The index patient's mother

(patient II/6) will undergo regular sonographic surveillance to observe the polyps' behaviour in terms of number and size.

Ambiguity as to the origin of the polyps in the grandmother's brother, the occurrence of bile duct hamartomas in other patients without gastrointestinal polyposis and the lack of histological evaluation in the third patient raise doubts if manifestations of JPS involve the gallbladder and the biliary tract.

On the other hand, three of eight SMAD4 mutation carriers had polypoid or hamartomatous lesions in the biliary tract. This rate is higher than would be expected in the normal population. Furthermore, there is no investigation on the biliary tract in JPS patients so far. Finally, in other polyposis syndromes such as FAP, Gardner's syndrome and Peutz-Jeghers syndrome biliary polyps are present however seem to be rarer than other manifestations. In any case, a heightened awareness and thorough sonographic screening in JPS patients is warranted for biliary manifestations.

4.3 Hypothesis (2)

Hypothesis (2) postulated that for the family at hand with three generations afflicted by JPS, the phenomenon of anticipation exists – meaning an earlier age of onset and an increased severity of disease in the younger generation. In neurodegenerative or developmental neuropsychiatric diseases expansion of trinucleotide repeat sequences in successive generations is the molecular basis for anticipation.

The phenomenon of anticipation has also been discussed for inherited cancer syndromes such as Lynch syndrome which is caused by point mutations in mismatch repair genes (MMR). Authors such as Nilbert et al. [131] have observed cancer development taking place at a significantly earlier age in children as compared to their parents. They had analyzed 290 parent-child pairs [131]. The molecular mechanisms underlying anticipation in tumour syndromes are still unknown. Suggested are repeated sequences other than trinucleotide sequences, telomere shortening, and epigenetic factors such as methylation and/or chromatin formation changes, imprinting, recombination, replication and DNA repair [131].

As less people are affected by JPS than by Lynch syndrome, studies as the one presented by Nilbert et al. do not exist for JPS. The comparison of the age at cancer development between child and parent is also more difficult as the rate of malignant transformation is much lower in JPS. No statistical analysis on the phenomenon of anticipation in JPS has

thus been carried out so far. Only hypotheses based on clinical observations were set up and discussed as done by Smilow, Pryor and Swinton [77] and Howe et al [13].

JPS in the family presented here is also caused by a point mutation – again, no genetic correlate is known to explain the hypothesis that the younger generations have an earlier onset of disease and a more severe phenotype than the older ones.

Comparing the age and the clinical manifestations of the patients examined reveals the following. The most symptomatic patient was also the youngest – only eight years of age. She had massive polyposis of the colon, and despite the absence of cancer development, required proctocolectomy. Her mother developed early gastric cancer and a tubular adenoma with LGIEN but on the basis of a small number of juvenile polyps. As additional risk factors she was Helicobacter pylori positive and had a history of smoking. At the time when early gastric cancer was diagnosed in the course of surveillance she was 29 years old and asymptomatic. It is very likely she would have become symptomatic and more severe in phenotype had gastroscopy and coloscopy not been performed and diagnosis not made. The grandmother who presented with massive gastric polyposis and who had been symptomatic for years had a severe phenotype, in spite of a small number of colonic polyps (1 small polyp in the caecum diagnosed in 2005, 1 polyp in the ascending colon diagnosed in 2008). At the time of diagnosis, however, she was 52 years of age already. The findings in these three generations could be consistent with anticipation: the patient of the first generation symptomatic, with massive gastric polyposis but already 52 years old, the patient in the second generation with early gastric cancer at the age of 29, but asymptomatic and the highly symptomatic patient in the third generation, with massive colonic polyposis, but no cancer development (yet).

The grandmother's brother with the same amount of colonic polyps or less than his two sons has the most severe phenotype in this part of the family in terms of neoplastic transformation (1 tubulovillous adenoma with LGIEN, 1 villous adenoma with HGIEN, 4 additional juvenile polyps). However, it can be presumed that both of these adenomas developed in the more recent past, as an earlier development in life would have entailed symptoms or complications by the age of 60. His two sons had 5-10 and 6 juvenile polyps at the age of 10 and 18 years respectively. It seems likely that, without treatment, the number of polyps in the two sons will increase with age and neoplastic transformation will take place – presumably leading to a more severe phenotype at the age of 60 than seen in their father now. Only the sister of the two boys does not support the idea of anticipation.

At the age of 24 years she is hardly affected – revealing only two very small juvenile polyps in the duodenal bulb and one in the colon.

The index patient's cousin presented asymptomatically with two gastric and 20 colonic juvenile polyps – a high amount, especially for the age of 14 years. She is part of the third generation – without treatment, at the age of 52, the age her grandmother is now, or earlier she will very likely have a severe phenotype in terms of malignancy, symptoms and complications.

With only eight patients to analyze and without causal molecular mechanisms anticipation cannot be proven – it might exist, but equally likely, it might only be the consequence of coincidence and misinterpretation. As the phenomenon of anticipation in JPS can neither be proven nor refuted at the moment, the possibility of its existence must suffice for surveillance implications in JPS. If JPS is diagnosed in a family, it is especially important for the younger generations to carry out genetic testing and clinical examination as soon as possible as these patients might be more severely affected by the disease than the older generation.

4.4 HP Eradication

In seven of eight patients examined in this kindred, Helicobacter pylori-associated gastritis was diagnosed. As Helicobacter pylori is known to have an association with gastric cancer [132, 133] this is a second risk factor in JPS patients for developing gastric malignancy and could thus represent the second hit leading to cancer according to the two-hit hypothesis. Eradication is thus especially recommended for patients affected by both JPS and Helicobacter pylori. In JPS families in the literature, Helicobacter pylori status has not been commented on.

4.5 JPS as Differential Diagnosis

Only two of the eight patients were symptomatic – the index patient and her grandmother. Time to diagnosis took six months and three years respectively. In the latter case, the correct diagnosis was only established due to the genetic screening carried out in all relatives of the index patient. This shows that JPS, although rare, should be an important differential diagnosis in patients with chronic diarrhoea, rectal bleeding, chronic anaemia and hypoproteinaemia.

4.6 Implications for Surveillance

Though of hamartomatous origin, the malignant potential of juvenile polyps must not be underestimated and regular surveillance should be performed thoroughly, especially since both patients with advanced neoplastic lesions in the kindred at hand were asymptomatic. The findings in the family at hand are not sufficient and unambiguous enough to definitely interpret a manifestation of JPS in the gallbladder – the possibility remains nevertheless. For this reason, surveillance should include not only upper and lower gastrointestinal endoscopy but also sonographic evaluations of the gallbladder. As the phenomenon of anticipation might exist, the genetic and endoscopic screening of children should be performed especially thoroughly.

5 REFERENCES

1. Herold, G., *Innere Medizin*. 2007, Köln. 441-442.
2. Lynch, H.T., *Hereditary Colorectal Cancer: An Updated Review* Gastroenterology and Hepatology, 2005. **1**(1): p. 39-48.
3. Zbuk, K.M. and C. Eng, *Hamartomatous polyposis syndromes.* Nat Clin Pract Gastroenterol Hepatol, 2007. **4**(9): p. 492-502.
4. Schreibman, I.R., et al., *The hamartomatous polyposis syndromes: a clinical and molecular review.* Am J Gastroenterol, 2005. **100**(2): p. 476-90.
5. Jass, J.R., *Colorectal polyposes: from phenotype to diagnosis.* Pathol Res Pract, 2008. **204**(7): p. 431-47.
6. Merg, A. and J.R. Howe, *Genetic conditions associated with intestinal juvenile polyps.* Am J Med Genet C Semin Med Genet, 2004. **129C**(1): p. 44-55.
7. Okamoto, K., et al., *A case of Cronkhite-Canada syndrome: remission after treatment with anti-Helicobacter pylori regimen.* Digestion, 2008. **78**(2-3): p. 82-7.
8. Aretz, S., *The differential diagnosis and surveillance of hereditary gastrointestinal polyposis syndromes.* Dtsch Arztebl Int. **107**(10): p. 163-73.
9. Deschamps, L., et al., *Mixed endocrine somatostatinoma of the ampulla of vater associated with a neurofibromatosis type 1: a case report and review of the literature.* Jop. **11**(1): p. 64-8.
10. Reichardt, M., W. Rexroth, and C. Hasslacher, *[Von Recklinghausen type I neurofibromatosis and neuroendocrine tumor (somatostatinoma) in a 50-year-old woman].* Med Klin (Munich), 1998. **93**(9): p. 550-3.
11. Chen, H.M. and J.Y. Fang, *Genetics of the hamartomatous polyposis syndromes: a molecular review.* Int J Colorectal Dis, 2009. **24**(8): p. 865-74.
12. Adolph, V.R. and K. Bernabe, *Polyps in children.* Clin Colon Rectal Surg, 2008. **21**(4): p. 280-5.
13. Howe, J.R., F.A. Mitros, and R.W. Summers, *The risk of gastrointestinal carcinoma in familial juvenile polyposis.* Ann Surg Oncol, 1998. **5**(8): p. 751-6.
14. Sachatello, C.R., I.S. Hahn, and C.B. Carrington, *Juvenile gastrointestinal polyposis in a female infant: report of a case and review of the literature of a recently recognized syndrome.* Surgery, 1974. **75**(1): p. 107-14.
15. Jass, J.R., et al., *Juvenile polyposis--a precancerous condition.* Histopathology, 1988. **13**(6): p. 619-30.
16. Giardiello, F.M., et al., *Colorectal neoplasia in juvenile polyposis or juvenile polyps.* Arch Dis Child, 1991. **66**(8): p. 971-5.
17. Leggett, B.A., et al., *Exclusion of APC and MCC as the gene defect in one family with familial juvenile polyposis.* Gastroenterology, 1993. **105**(5): p. 1313-6.
18. Jacoby, R.F., et al., *Del(10)(q22.3q24.1) associated with juvenile polyposis.* Am J Med Genet, 1997. **70**(4): p. 361-4.
19. Jacoby, R.F., et al., *A juvenile polyposis tumor suppressor locus at 10q22 is deleted from nonepithelial cells in the lamina propria.* Gastroenterology, 1997. **112**(4): p. 1398-403.
20. Jungck, M., W. Friedl, and P. Propping, *[Hereditary gastrointestinal tumors].* Internist (Berl), 1999. **40**(5): p. 502-12.
21. Kinzler, K.W. and B. Vogelstein, *Landscaping the cancer terrain.* Science, 1998. **280**(5366): p. 1036-7.
22. Marsh, D.J., et al., *Exclusion of PTEN and 10q22-24 as the susceptibility locus for juvenile polyposis syndrome.* Cancer Res, 1997. **57**(22): p. 5017-21.

23. Olschwang, S., et al., *PTEN germ-line mutations in juvenile polyposis coli.* Nat Genet, 1998. **18**(1): p. 12-4.
24. Lynch, E.D., et al., *Inherited mutations in PTEN that are associated with breast cancer, cowden disease, and juvenile polyposis.* Am J Hum Genet, 1997. **61**(6): p. 1254-60.
25. Howe, J.R., et al., *A gene for familial juvenile polyposis maps to chromosome 18q21.1.* Am J Hum Genet, 1998. **62**(5): p. 1129-36.
26. Eng, C. and H. Ji, *Molecular classification of the inherited hamartoma polyposis syndromes: clearing the muddied waters.* Am J Hum Genet, 1998. **62**(5): p. 1020-2.
27. Nelen, M.R., et al., *Localization of the gene for Cowden disease to chromosome 10q22-23.* Nat Genet, 1996. **13**(1): p. 114-6.
28. Howe, J.R., et al., *Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis.* Nat Genet, 2001. **28**(2): p. 184-7.
29. Zhou, X.P., et al., *Germline mutations in BMPR1A/ALK3 cause a subset of cases of juvenile polyposis syndrome and of Cowden and Bannayan-Riley-Ruvalcaba syndromes.* Am J Hum Genet, 2001. **69**(4): p. 704-11.
30. Friedl, W., et al., *Juvenile polyposis: massive gastric polyposis is more common in MADH4 mutation carriers than in BMPR1A mutation carriers.* Hum Genet, 2002. **111**(1): p. 108-11.
31. Howe, J.R., et al., *The prevalence of MADH4 and BMPR1A mutations in juvenile polyposis and absence of BMPR2, BMPR1B, and ACVR1 mutations.* J Med Genet, 2004. **41**(7): p. 484-91.
32. Kim, I.J., et al., *Identification of a novel BMPR1A germline mutation in a Korean juvenile polyposis patient without SMAD4 mutation.* Clin Genet, 2003. **63**(2): p. 126-30.
33. Rozen, P., et al., *An audit of familial juvenile polyposis at the Tel Aviv Medical Center: demographic, genetic and clinical features.* Fam Cancer, 2003. **2**(1): p. 1-7.
34. Bevan, S., et al., *Screening SMAD1, SMAD2, SMAD3, and SMAD5 for germline mutations in juvenile polyposis syndrome.* Gut, 1999. **45**(3): p. 406-8.
35. Roth, S., et al., *SMAD genes in juvenile polyposis.* Genes Chromosomes Cancer, 1999. **26**(1): p. 54-61.
36. Howe, J.R., et al., *Mutations in the SMAD4/DPC4 gene in juvenile polyposis.* Science, 1998. **280**(5366): p. 1086-8.
37. Friedl, W., et al., *Frequent 4-bp deletion in exon 9 of the SMAD4/MADH4 gene in familial juvenile polyposis patients.* Genes Chromosomes Cancer, 1999. **25**(4): p. 403-6.
38. Houlston, R., et al., *Mutations in DPC4 (SMAD4) cause juvenile polyposis syndrome, but only account for a minority of cases.* Hum Mol Genet, 1998. **7**(12): p. 1907-12.
39. Woodford-Richens, K., et al., *Analysis of genetic and phenotypic heterogeneity in juvenile polyposis.* Gut, 2000. **46**(5): p. 656-60.
40. Kim, I.J., et al., *Germline mutations of the dpc4 gene in Korean juvenile polyposis patients.* Int J Cancer, 2000. **86**(4): p. 529-32.
41. Howe, J.R., et al., *Common deletion of SMAD4 in juvenile polyposis is a mutational hotspot.* Am J Hum Genet, 2002. **70**(5): p. 1357-62.
42. Krawczak, M. and D.N. Cooper, *Gene deletions causing human genetic disease: mechanisms of mutagenesis and the role of the local DNA sequence environment.* Hum Genet, 1991. **86**(5): p. 425-41.
43. Calva-Cerqueira, D., et al., *The rate of germline mutations and large deletions of SMAD4 and BMPR1A in juvenile polyposis.* Clin Genet, 2009. **75**(1): p. 79-85.

44. Aretz, S., et al., *High proportion of large genomic deletions and a genotype phenotype update in 80 unrelated families with juvenile polyposis syndrome.* J Med Genet, 2007. **44**(11): p. 702-9.
45. van Hattem, W.A., et al., *Large genomic deletions of SMAD4, BMPR1A and PTEN in juvenile polyposis.* Gut, 2008. **57**(5): p. 623-7.
46. Sachatello, C.R. and W.O. Griffen, Jr., *Hereditary polypoid diseases of the gastrointestinal tract: a working classification.* Am J Surg, 1975. **129**(2): p. 198-203.
47. Sweet, K., et al., *Molecular classification of patients with unexplained hamartomatous and hyperplastic polyposis.* Jama, 2005. **294**(19): p. 2465-73.
48. Howe, J.R., et al., *ENG mutations in MADH4/BMPR1A mutation negative patients with juvenile polyposis.* Clin Genet, 2007. **71**(1): p. 91-2.
49. Wrana, J.L. and L. Attisano, *MAD-related proteins in TGF-beta signalling.* Trends Genet, 1996. **12**(12): p. 493-6.
50. Heldin, C.H., K. Miyazono, and P. ten Dijke, *TGF-beta signalling from cell membrane to nucleus through SMAD proteins.* Nature, 1997. **390**(6659): p. 465-71.
51. Rodriguez, C., et al., *Cooperative binding of transforming growth factor (TGF)-beta 2 to the types I and II TGF-beta receptors.* J Biol Chem, 1995. **270**(27): p. 15919-22.
52. Lagna, G., et al., *Partnership between DPC4 and SMAD proteins in TGF-beta signalling pathways.* Nature, 1996. **383**(6603): p. 832-6.
53. Macias-Silva, M., et al., *MADR2 is a substrate of the TGFbeta receptor and its phosphorylation is required for nuclear accumulation and signaling.* Cell, 1996. **87**(7): p. 1215-24.
54. Wrana, J. and T. Pawson, *Signal transduction. Mad about SMADs.* Nature, 1997. **388**(6637): p. 28-9.
55. Hahn, S.A., et al., *DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1.* Science, 1996. **271**(5247): p. 350-3.
56. Eppert, K., et al., *MADR2 maps to 18q21 and encodes a TGFbeta-regulated MAD-related protein that is functionally mutated in colorectal carcinoma.* Cell, 1996. **86**(4): p. 543-52.
57. Nakao, A., et al., *TGF-beta receptor-mediated signalling through Smad2, Smad3 and Smad4.* Embo J, 1997. **16**(17): p. 5353-62.
58. Shi, Y., et al., *A structural basis for mutational inactivation of the tumour suppressor Smad4.* Nature, 1997. **388**(6637): p. 87-93.
59. Hata, A., et al., *Mutations increasing autoinhibition inactivate tumour suppressors Smad2 and Smad4.* Nature, 1997. **388**(6637): p. 82-7.
60. Hoodless, P.A., et al., *MADR1, a MAD-related protein that functions in BMP2 signaling pathways.* Cell, 1996. **85**(4): p. 489-500.
61. Imamura, T., et al., *Smad6 inhibits signalling by the TGF-beta superfamily.* Nature, 1997. **389**(6651): p. 622-6.
62. Nakao, A., et al., *Identification of Smad7, a TGFbeta-inducible antagonist of TGF-beta signalling.* Nature, 1997. **389**(6651): p. 631-5.
63. Knudson, A.G., Jr., *Mutation and cancer: statistical study of retinoblastoma.* Proc Natl Acad Sci U S A, 1971. **68**(4): p. 820-3.
64. 2001 [cited 2010 April 24]; Available from: http://journals.cambridge.org/fulltext_content/ERM/ERM3_08/S1462399401002654sup003.pdf.
65. Kinzler, K.W. and B. Vogelstein, *Lessons from hereditary colorectal cancer.* Cell, 1996. **87**(2): p. 159-70.

66. Woodford-Richens, K., et al., *Allelic loss at SMAD4 in polyps from juvenile polyposis patients and use of fluorescence in situ hybridization to demonstrate clonal origin of the epithelium.* Cancer Res, 2000. **60**(9): p. 2477-82.
67. Kim, B.G., et al., *Smad4 signalling in T cells is required for suppression of gastrointestinal cancer.* Nature, 2006. **441**(7096): p. 1015-9.
68. Subramony, C., et al., *Familial juvenile polyposis. Study of a kindred: evolution of polyps and relationship to gastrointestinal carcinoma.* Am J Clin Pathol, 1994. **102**(1): p. 91-7.
69. Thiagalingam, S., et al., *Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers.* Nat Genet, 1996. **13**(3): p. 343-6.
70. Takagi, Y., et al., *Somatic alterations of the DPC4 gene in human colorectal cancers in vivo.* Gastroenterology, 1996. **111**(5): p. 1369-72.
71. Schutte, M., et al., *DPC4 gene in various tumor types.* Cancer Res, 1996. **56**(11): p. 2527-30.
72. Goodman, Z.D., J.H. Yardley, and F.D. Milligan, *Pathogenesis of colonic polyps in multiple juvenile polyposis: report of a case associated with gastric polyps and carcinoma of the rectum.* Cancer, 1979. **43**(5): p. 1906-13.
73. Jarvinen, H. and K.O. Franssila, *Familial juvenile polyposis coli; increased risk of colorectal cancer.* Gut, 1984. **25**(7): p. 792-800.
74. Grigioni, W.F., et al., *Atypical juvenile polyposis.* Histopathology, 1981. **5**(4): p. 361-76.
75. Bentley, E., et al., *Generalized juvenile polyposis with carcinoma.* Am J Gastroenterol, 1989. **84**(11): p. 1456-9.
76. Rozen, P. and M. Baratz, *Familial juvenile colonic polyposis with associated colon cancer.* Cancer, 1982. **49**(7): p. 1500-3.
77. Smilow, P.C., C.A. Pryor, Jr., and N.W. Swinton, *Juvenile polyposis coli. A report of three patients in three generations of one family.* Dis Colon Rectum, 1966. **9**(4): p. 248-54.
78. Veale, A.M., et al., *Juvenile polyposis coli.* J Med Genet, 1966. **3**(1): p. 5-16.
79. Oberhuber, G. and M. Stolte, *Gastric polyps: an update of their pathology and biological significance.* Virchows Arch, 2000. **437**(6): p. 581-90.
80. Delnatte, C., et al., *Contiguous gene deletion within chromosome arm 10q is associated with juvenile polyposis of infancy, reflecting cooperation between the BMPR1A and PTEN tumor-suppressor genes.* Am J Hum Genet, 2006. **78**(6): p. 1066-74.
81. Coburn, M.C., et al., *Malignant potential in intestinal juvenile polyposis syndromes.* Ann Surg Oncol, 1995. **2**(5): p. 386-91.
82. Desai, D.C., et al., *Juvenile polyposis.* Br J Surg, 1995. **82**(1): p. 14-7.
83. Waite, K.A. and C. Eng, *Protean PTEN: form and function.* Am J Hum Genet, 2002. **70**(4): p. 829-44.
84. He, X.C., et al., *BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling.* Nat Genet, 2004. **36**(10): p. 1117-21.
85. Waite, K.A. and C. Eng, *BMP2 exposure results in decreased PTEN protein degradation and increased PTEN levels.* Hum Mol Genet, 2003. **12**(6): p. 679-84.
86. Watanabe, A., et al., *Familial juvenile polyposis of the stomach.* Gastroenterology, 1979. **77**(1): p. 148-51.
87. Covarrubias, D.J. and J.E. Huprich, *Best cases from the AFIP. Juvenile polyposis of the stomach. Armed Forces Institute of Pathology.* Radiographics, 2002. **22**(2): p. 415-20.

88. Hizawa, K., et al., *Juvenile polyposis of the stomach: clinicopathological features and its malignant potential.* J Clin Pathol, 1997. **50**(9): p. 771-4.
89. Winkler, A., et al., *Juvenile polyposis of the stomach causing recurrent upper gastrointestinal bleeding.* Eur J Gastroenterol Hepatol, 2007. **19**(1): p. 87-90.
90. Hofting, I., et al., *[Familial juvenile polyposis with predominant stomach involvement].* Z Gastroenterol, 1993. **31**(9): p. 480-3.
91. Jarvinen, H.J. and P. Sipponen, *Gastroduodenal polyps in familial adenomatous and juvenile polyposis.* Endoscopy, 1986. **18**(6): p. 230-4.
92. Cox, K.L., et al., *Hereditary generalized juvenile polyposis associated with pulmonary arteriovenous malformation.* Gastroenterology, 1980. **78**(6): p. 1566-70.
93. Baert, A.L., et al., *Generalized juvenile polyposis with pulmonary arteriovenous malformations and hypertrophic osteoarthropathy.* AJR Am J Roentgenol, 1983. **141**(4): p. 661-2.
94. Desai, D.C., et al., *A survey of phenotypic features in juvenile polyposis.* J Med Genet, 1998. **35**(6): p. 476-81.
95. Inoue, S., et al., *Juvenile polyposis occurring in hereditary hemorrhagic telangiectasia.* Am J Med Sci, 1999. **317**(1): p. 59-62.
96. Gallione, C.J., et al., *A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4).* Lancet, 2004. **363**(9412): p. 852-9.
97. Gallione, C.J., et al., *SMAD4 mutations found in unselected HHT patients.* J Med Genet, 2006. **43**(10): p. 793-7.
98. Gallione, C., et al., *Overlapping spectra of SMAD4 mutations in juvenile polyposis (JP) and JP-HHT syndrome.* Am J Med Genet A. **152A**(2): p. 333-9.
99. Plauchu, H., et al., *Age-related clinical profile of hereditary hemorrhagic telangiectasia in an epidemiologically recruited population.* Am J Med Genet, 1989. **32**(3): p. 291-7.
100. Poletto, E.D., et al., *Hereditary hemorrhagic telangiectasia and juvenile polyposis: an overlap of syndromes.* Pediatr Radiol, 2009.
101. McAllister, K.A., et al., *Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1.* Nat Genet, 1994. **8**(4): p. 345-51.
102. Shovlin, C.L., et al., *Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome).* Am J Med Genet, 2000. **91**(1): p. 66-7.
103. Johnson, D.W., et al., *Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2.* Nat Genet, 1996. **13**(2): p. 189-95.
104. Letteboer, T.G., et al., *Genotype-phenotype relationship in hereditary haemorrhagic telangiectasia.* J Med Genet, 2006. **43**(4): p. 371-7.
105. Cole, S.G., et al., *A new locus for hereditary haemorrhagic telangiectasia (HHT3) maps to chromosome 5.* J Med Genet, 2005. **42**(7): p. 577-82.
106. Brosens, L.A., et al., *Risk of colorectal cancer in juvenile polyposis.* Gut, 2007. **56**(7): p. 965-7.
107. Agnifili, A., et al., *Juvenile polyposis: case report and assessment of the neoplastic risk in 271 patients reported in the literature.* Dig Surg, 1999. **16**(2): p. 161-6.
108. Pyatt, R.E., R. Pilarski, and T.W. Prior, *Mutation screening in juvenile polyposis syndrome.* J Mol Diagn, 2006. **8**(1): p. 84-8.
109. Howe, J.R., et al., *Direct genetic testing for Smad4 mutations in patients at risk for juvenile polyposis.* Surgery, 1999. **126**(2): p. 162-70.

110. Dunlop, M.G., *Guidance on gastrointestinal surveillance for hereditary non-polyposis colorectal cancer, familial adenomatous polypolis, juvenile polyposis, and Peutz-Jeghers syndrome.* Gut, 2002. **51 Suppl 5**: p. V21-7.
111. Chow, E. and F. Macrae, *A review of juvenile polyposis syndrome.* J Gastroenterol Hepatol, 2005. **20**(11): p. 1634-40.
112. Postgate, A.J., et al., *Capsule endoscopy for the small bowel in juvenile polyposis syndrome: a case series.* Endoscopy, 2009. **41**(11): p. 1001-4.
113. Monkemuller, K., et al., *Polypectomy techniques for difficult colon polyps.* Dig Dis, 2008. **26**(4): p. 342-6.
114. Gotoda, T., et al., *Incidence of lymph node metastasis from early gastric cancer: estimation with a large number of cases at two large centers.* Gastric Cancer, 2000. **3**(4): p. 219-225.
115. Soetikno, R., et al., *Endoscopic mucosal resection for early cancers of the upper gastrointestinal tract.* J Clin Oncol, 2005. **23**(20): p. 4490-8.
116. McLaughlin, S.D., et al., *Review article: restorative proctocolectomy, indications, management of complications and follow-up--a guide for gastroenterologists.* Aliment Pharmacol Ther, 2008. **27**(10): p. 895-909.
117. Scott-Conner, C.E., et al., *Familial juvenile polyposis: patterns of recurrence and implications for surgical management.* J Am Coll Surg, 1995. **181**(5): p. 407-13.
118. Oncel, M., et al., *Colonic surgery in patients with juvenile polyposis syndrome: a case series.* Dis Colon Rectum, 2005. **48**(1): p. 49-55; discussion 55-6.
119. Ben Schalom-Dejordy, L., et al., *[Gallbladder polyps].* Harefuah, 1992. **122**(11): p. 707-9, 751.
120. Myers, R.P., E.A. Shaffer, and P.L. Beck, *Gallbladder polyps: epidemiology, natural history and management.* Can J Gastroenterol, 2002. **16**(3): p. 187-94.
121. Chen, C.Y., et al., *Risk factors for gallbladder polyps in the Chinese population.* Am J Gastroenterol, 1997. **92**(11): p. 2066-8.
122. Jorgensen, T. and K.H. Jensen, *Polyps in the gallbladder. A prevalence study.* Scand J Gastroenterol, 1990. **25**(3): p. 281-6.
123. Segawa, K., et al., *Prevalence of gallbladder polyps among apparently healthy Japanese: ultrasonographic study.* Am J Gastroenterol, 1992. **87**(5): p. 630-3.
124. Duran-Vega, H.C., et al., *[Hamartoma of the bile ducts. Report of a case and review of the literature].* Rev Gastroenterol Mex, 2000. **65**(3): p. 124-8.
125. Mimatsu, K., et al., *Preoperatively undetected solitary bile duct hamartoma (von Meyenburg complex) associated with esophageal carcinoma.* Int J Clin Oncol, 2008. **13**(4): p. 365-8.
126. Bailador Andres, M.C., S. Vivas Alegre, and R. Rueda Castanon, *Multiple bile-duct hamartomas (Von Meyemburg complexes).* Rev Esp Enferm Dig, 2006. **98**(4): p. 306-7.
127. Wada, K., et al., *Carcinoma and polyps of the gallbladder associated with Peutz-Jeghers syndrome.* Dig Dis Sci, 1987. **32**(8): p. 943-6.
128. Vogel, T., et al., *Extraintestinal polyps in Peutz-Jeghers syndrome: presentation of four cases and review of the literature. Deutsche Peutz-Jeghers-Studiengruppe.* Int J Colorectal Dis, 2000. **15**(2): p. 118-23.
129. Brevet, M., et al., *[Adenomatous polyposis of the gallbladder and Gardner's syndrome. A rare association].* Gastroenterol Clin Biol, 2007. **31**(4): p. 425-7.
130. Lee, K.F., et al., *Polypoid lesions of the gallbladder.* Am J Surg, 2004. **188**(2): p. 186-90.
131. Nilbert, M., et al., *Role for genetic anticipation in Lynch syndrome.* J Clin Oncol, 2009. **27**(3): p. 360-4.

132. Parsonnet, J., et al., *Helicobacter pylori infection and the risk of gastric carcinoma.* N Engl J Med, 1991. **325**(16): p. 1127-31.
133. *Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts.* Gut, 2001. **49**(3): p. 347-53.