Gastrointestinal polyposis syndromes
Keller, J.J.

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Chapter 1

General Introduction

Molecular and phenotypic aspects of gastrointestinal polyposis syndromes

Josbert J. Keller, Francis M. Giardiello, G. Johan A. Offerhaus
Gastrointestinal polyposis syndromes are autosomal dominant disorders, caused by germline mutations in a variety of tumor-suppressor genes. These syndromes are characterized by the development of multiple gastrointestinal polyps, extra-intestinal manifestations, and an increased cancer risk. Cancer may occur in the gastrointestinal tract, but also at extra-intestinal sites. Polyposis syndromes are classified by the histological appearance of polyps; further subclassifications can be made by phenotypic expression, extra-intestinal manifestations, or underlying genetic cause. These disorders may present in patients with a negative family history, representing de novo mutations or masked existence of familial polyposis due to intra-family differences in phenotypic expression.

Classification:
The most important distinction is made by the presence of adenomas, which are defined as macroscopically visible lesions that carry dysplastic mucosa and are therefore, by definition, neoplastic. Adenomas are pre-cursors of colorectal carcinoma (CRC); lesions ≥ 10mm have a 24% risk for malignant transformation within 20 years. Adenomas are the phenotypic hallmark of familial adenomatous polyposis (FAP) (see below), which is characterized by a virtually 100% risk for the development of CRC. FAP is caused by a germline mutation in the APC (adenomatous polyposis coli) gene, considered the key tumor suppressor of the large bowel. Expression variants are attenuated FAP, Gardner syndrome (FAP with extra-intestinal manifestations), and Turcot syndrome. Gardner syndrome is the combination of polyposis with epidermoid cysts, osteomas, dental abnormalities or desmoids; Turcot syndrome is the combination of FAP with brain tumors, most often medulloblastoma. Of note, Turcot-pedigrees have also been linked to hereditary nonpolyposis colorectal cancer (HNPCC), caused by germline mutations in one of the mismatch repair genes.

Distinct from the adenomas in FAP are the hamartomatous polyps found in the Peutz-Jeghers syndrome (PJS) (see below), Juvenile Polyposis syndrome (JPS), and in Cowden's syndrome (CS) and its expression variant Bannayan-Riley-Ruvalcaba syndrome (BRRS). Hamartomatous polyps are non-neoplastic at the histological level, composed of tissue elements present in the normal mucosa at that particular site. However, dysplastic transformation has occasionally been reported in PJS hamartomas, and ~30% of JPS hamartomas harbor dysplastic foci. PJS is caused by a mutation in the STK11/LKB1 gene. JPS is caused by mutations in the SMAD4 or BMPRIA genes, which are components of the TGF-β-RII signaling pathway. CS and BRRS are caused by mutations in the PTEN gene. JPS patients with mutations in PTEN have also been described, however, these individuals may have been misdiagnosed, and are in fact affected by CS. Other syndromes with gastrointestinal (hamartomatous) polyps are Gorlin syndrome (naevoid basal cell carcinoma syndrome) and Cronkhite-Canada syndrome, which are not associated with an increased risk for gastrointestinal carcinoma. In addition to FAP and the hamartomatous polyposis syndromes, there are reports of less established conditions, such as familial hyperplastic polyposis syndrome and hereditary mixed polyposis syndrome. Whether these are distinct disorders or variations of the above syndromes remains to be seen.
**Phenotype:**

The intestinal and extra-intestinal phenotype, cancer risk and underlying genetic cause of the different syndromes are listed in Table 1. The phenotypes have been defined by detailed clinical descriptions. Also, a molecular genetic approach may help to define the phenotype, linking the pathogenesis of specific lesions to the underlying genetic defect of a syndrome \(^{22}\) (chapter 10, this thesis). There is wide inter- and intra-familial variation in the phenotypic expression of the different syndromes. The inter-familial variation can in part be explained by genotype-phenotype correlations, linking site and type of the germline mutations within a causative gene to phenotypic expression of the syndrome \(^{3,24,25}\). Intra-familial variation may in part be explained by polymorphisms in modifier loci \(^{26}\).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype, hallmark symptoms are listed in italics</th>
<th>Cancer risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GI polyposis</td>
<td>Extra-intestinal</td>
</tr>
<tr>
<td></td>
<td>Fundic gland polyp</td>
<td>CHRPE, nasopharyngeal angiofibroma, osteomas, jaw lesions, dental anomalies, lipomas, fibromas, epidermoid cysts, desmoides hepatoblastoma</td>
</tr>
<tr>
<td>FAP</td>
<td>Gastric adenomas</td>
<td>CRC ~100%</td>
</tr>
<tr>
<td>APC</td>
<td>Small intestinal adenomas</td>
<td>Pa 2%</td>
</tr>
<tr>
<td>APC</td>
<td>Colorectal adenomas</td>
<td></td>
</tr>
<tr>
<td>PJS</td>
<td>Gastric, small intestinal and colorectal hamartomas</td>
<td>mucocutaneous pigmentation, ovarian cysts, SCTAT, adenoma malignum, urinary tract polyps, nasal polyps, bronchus polyps, gallbladder polyps</td>
</tr>
<tr>
<td>STK11</td>
<td></td>
<td>Du/Je/Bl 13%</td>
</tr>
<tr>
<td>STK11</td>
<td></td>
<td>CRC 39%</td>
</tr>
<tr>
<td>STK11</td>
<td></td>
<td>Pa 36%</td>
</tr>
<tr>
<td>JPS</td>
<td>Gastric juvenile polyps</td>
<td>congenital anomalies, arteriovenous malformations</td>
</tr>
<tr>
<td>SMAD4</td>
<td>Small intestinal juvenile polyps</td>
<td></td>
</tr>
<tr>
<td>BMPRIA(^a)</td>
<td>Colorectal juvenile polyps</td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>Gastrointestinal juvenile polyps</td>
<td>mucocutaneous papules(^b), trichilemmoma(^c), breast fibroadenomas, thyroid adenomas, goitre, cerebellar gangliocytoma, macrocephaly(^d), lipomatosis(^e), speckled penis(^f)</td>
</tr>
<tr>
<td>&amp; BRRS</td>
<td></td>
<td></td>
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</tbody>
</table>

1. Recently, a **BMPRIA** mutation has also been described in a patient with Cowden/BRRS syndrome \(^{35}\).
2. **PTEN** mutations have also been linked to JPS, probably reflecting difficulties in the clinical distinction between JPS and CS in some cases \(^{17-19}\).
3. Hallmarks of CS, present in ~100 of patients.
4. Classic triad of BRRS: macrocephaly, lipomatosis and pigmented maculas of the giant penis (speckled penis, not reported in CS)
Chapter 1

Management:

There is wide variation in the clinical presentation, and therefore, patient care and surveillance guidelines differ between polyposis syndromes. Adequate risk assessment is required and includes complete diagnostic work up and genetic testing. Surveillance protocols should be based on formal cancer risk investigations within cohorts of affected individuals. Recommendations for FAP and PJS are presented in Table 2 and 3. Genotype-phenotype correlations and the identification of modifier loci may enable further differentiation of surveillance guidelines in the future. In addition to surveillance, prophylactic surgery is required in FAP, but is not recommended for the other polyposis disorders. Furthermore, patients with gastrointestinal polyposis syndromes may benefit from chemopreventive treatment against the development of intestinal and extra-intestinal cancer. To date, NSAIDs and selective COX-2 inhibitors appear promising chemopreventive agents, although clinical use is still restricted to patients with FAP (chapter 2, this thesis).

Familial adenomatous polyposis (FAP)

FAP is an autosomal dominantly disorder caused by germline mutations in the APC (adenomatous polyposis coli) tumor suppressor gene. FAP occurs between 1 per 7,000 and 1 per 30,000 newborns; CRC in FAP accounts for <1% of all diagnosed cases of colorectal cancer. The diagnosis of FAP is based on the presence of: (i) 100 or more colorectal adenomas, or (ii) germline mutation of the APC gene.

Phenotype

The hallmark of FAP is the development of innumerable colorectal adenomas, which become visible between the age of 10 and 20 years and inevitably will result in the development of CRC at a mean age of 39 years. Most adenomas are tubular, ranging in size from barely visible nodules up to 1 cm or more. Unique are the single crypt adenomas observed at microscopy, which can be considered pathognomonic for FAP. The number of adenomas may vary between families. If less than 100 adenomas are present, attenuated FAP (AFAP) should be considered. In addition to colorectal adenomas, adenomas of the small intestine have been described in up to 92% of patients, resulting in a 330 times increased risk for duodenal carcinoma compared to the general population. In the stomach, adenomas appear rare but fundic gland polyps are commonly found. However, there is no increased risk for gastric cancer in FAP in Western patients.

Extra-intestinal manifestations of FAP are listed in Table 1, and may serve as marker for FAP if the diagnosis is uncertain. Of clinical concern are extra-intestinal malignancies, including hepatoblastoma, medulloblastoma and thyroid carcinoma. In addition, desmoid tumors, which are typically found in retroperitoneal tissues or in the abdominal wall, can cause severe morbidity and may even become life threatening. Desmoids are composed of sheets of elongated fibroblasts, arranged in fascicles and whorls with a dense consistency and a variable amount of collagen, and follow an expansive growth pattern.

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Genetics and pathogenesis

A causative germline mutation in the APC gene may be found in ~95% of FAP families. The APC tumor suppressor gene is considered the gatekeeper of the colorectal mucosa and inactivation of the wild type allele by loss of heterozygosity (LOH) or somatic mutations can be found in the vast majority of FAP related tumors. Loss of APC appears mandatory for the initiation of the adenoma-carcinoma sequence in FAP, which describes the transformation of normal colorectal epithelium into invasive adenocarcinoma. Wild type APC binds β-catenin, enabling the phosphorylation and degradation of this proto-oncogene. Inactivation of APC results in nuclear accumulation of β-catenin, and β-catenin/TCF4 mediated transcription of target genes, amongst which is cyclinD1, Met, and CD44. As a consequence, apoptosis, proliferation and migration, which are key processes for the maintenance of colorectal epithelial homeostasis, are disturbed. In addition to a β-catenin regulating function, wild type APC is involved in the integrity of the genome, playing a role in mitotic spindle formation. The latter function may provide an explanation for the observed chromosomal instability found during colorectal carcinogenesis.

Over 300 different germline APC mutations have been identified, and phenotypic expression correlates to the mutation site. Classical FAP is caused by mutations between codon 167 and 1596, whereas mutations at the 5' or 3' end of the APC gene are associated with AFAP. Alterations between codon 1250 and 1464 can result in a severe phenotype. Furthermore, mutations between codons 1403 and 1578 may predispose to the development of extra-intestinal manifestations, including desmoids. Explaining those genotype-phenotype correlations may provide further insights in the role of APC in carcinogenesis. Interestingly, the site of a germline APC mutation may also predict whether inactivation of the wild type APC allele occurs through LOH or a truncating mutation.

After inactivation of APC, progression of the adenoma-carcinoma sequence is driven by an accumulation of activating and inactivating mutations in oncogenes, such as K-RAS, and tumor suppressor genes, e.g. TP53, respectively. As a result, the expression of growth-regulatory enzymes is altered, amongst others COX-2, which is involved in the conversion of arachidonic acid into prostaglandins, promoting tumor growth by inhibition of apoptosis, and increasing angiogenesis. Importantly, COX-2 is a target for chemopreventive treatment with NSAIDs or COX-2 inhibitors (chapter 2, this thesis).

Management

First-degree relatives of FAP patients and suspected FAP patients should undergo screening and genetic testing for the APC gene mutation. At the time of diagnosis, surgery is recommended. There are several surgical options including subtotal colectomy with ileorectal anastomosis (IRA), and total proctocolectomy with Brooke ileostomy or with mucosal proctectomy and ileoanal pull-through. Surveillance should be offered to those who are APC gene mutation tested positive, or to all first-degree relatives of FAP patients in whom no mutation has been identified. Guidelines are listed in Table 2.
Table 2. Screening and surveillance guidelines in FAP.

**At risk individuals**

<table>
<thead>
<tr>
<th>Genotyping:</th>
<th>APC mutation: sigmoidoscopy annually starting at age 12 years; children up to age 7 years: alpha-feto-protein levels and ultrasound imaging of the liver. No APC mutation: sigmoidoscopy at age 25 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>No genotype available:</td>
<td>Sigmoidoscopy annually starting at age 12 years, then every 2 years starting at age 35 years, then every 3 years starting at age 35 years, then per guidelines for average-risk individuals starting at age 50 years. Children of affected parents up to age 7 years: alpha-feto-protein levels and ultrasound imaging of the liver.</td>
</tr>
</tbody>
</table>

**Affected individuals**

Upper GI surveillance every 3 to 4 years, and annually if upper GI tract polyps are found. If retained rectum or J-pouch, sigmoidoscopy every 6 months or 1 to 2 years, respectively. Annual physical exam and routine blood testing.

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Chemoprevention may be considered in patients with FAP who have rectal adenomas after colectomy with IRA. The NSAID sulindac and the selective COX-2 inhibitor celecoxib have shown to reduce the number and size of adenomas \(^{66,67}\). Although sulindac appears more effective, celecoxib may be the initial treatment of choice considering the potential gastrototoxicity of NSAIDs \(^{37}\). NSAIDs based chemoprevention against duodenal adenomas appears of limited value \(^{66-68}\). Also, sulindac appeared ineffective as primary chemopreventive treatment against the development of adenomas in patients genotypically affected but phenotypically unaffected by FAP \(^{69}\). Although NSAIDs and selective COX-2 inhibitors are of use as chemopreventive agents, the effect is incomplete, and there is a need for biomarkers, which can predict treatment outcome. To date, the most promising biomarker appears mucosal prostaglandin levels, which predicted treatment response in FAP patients on sulindac \(^{70-72}\). Another potential biomarker is rectal epithelial apoptosis \(^{73}\) (chapter 3 and 4, this thesis).

**Peutz-Jeghers syndrome (PJS)**

In 1921, the Dutch physician Peutz reported a family with "a highly remarkable combination of polyposis of the mucosa of the intestinal tract and of the nasopharynx, together with typical mucocutaneous pigmentation", a rare syndrome which is now named after him \(^{74,75}\) (chapter 7, this thesis). PJS is an autosomal dominant gastrointestinal hamartomatous polyposis disorder with a very high cancer risk, caused by a germline mutation in the STK11 gene \(^{11,12,28,76}\). The incidence is estimated between 1 per 50,000 and 1 per 120,000 newborns \(^{77,78}\). The diagnosis is based on the presence of (i) 2 or more characteristic PJS hamartomas, or (ii) one hamartoma together with either classical PJS pigmentation or a family history of PJS, or (iii) a pathogenic STK11 germline mutation \(^{36}\). Melanin pigmentation may serve as a marker for disease, with a limited sensitivity and specificity (chapter 12, this thesis).
**Phenotype**

The phenotype of PJS includes distinctive mucocutaneous melanin pigmentation, gastrointestinal hamartomas and benign extra-intestinal tumors. Furthermore, there is a high intestinal and extra-intestinal cancer risk, as listed in Table 1.

The mucocutaneous pigmentation consists of dark blue through brown to black macula typically found on the lips and oral mucosa, and on the skin around the mouth, orbita and nose (chapter 12, this thesis). These lesions are usually noticed at infancy and may fade after puberty. The pigmentation are formed by a proliferation of melanoblastic cells, and are not of clinical concern. However, they may point to the diagnosis PJS.

Hamartomatous polyps can be found in the small intestine of ~80% of PJS patients, and in the colon and stomach of ~40% of those individuals. Hamartomas are pedunculated polyps with a central core of arborizing smooth muscle surrounded by disorganized and branching glandular crypts with many goblet-like cells. Dysplastic changes are generally absent. Notably, epithelial misplacement of intestinal mucosa, found in ~10% of small intestinal hamartomas, may mimic invasive malignancy (pseudo-invasion) and represents a diagnostic pitfall. Recurrent intussusception of hamartomatous polyps causes significant morbidity in PJS patients, resulting in a high laparotomy rate.

Polyps have also been reported at many extra-intestinal sites, including the gallbladder, bronchus, esophagus, urether, urinary bladder, renal pelvis, and nasal cavities. Rare extra-intestinal tumors in PJS were reported in a variety of organs, including sex cord tumors with annular tubules (SCTAT) of the ovaries, Sertoli cell tumors of the testis, and adenoma malignum of the uterine cervix. Of interest, SCTAT in PJS appears to be a rather benign condition, often bilateral and multi-focal, whereas sporadic SCTAT is characterized by a malignant clinical course.

**Genetics and pathogenesis**

PJS is caused by a germline mutation in the **STK11** (serine threonin kinase) tumor suppressor gene on 19p13.3. Mutations can be identified in ~70% of PJS patients. A second PJS gene may exist, possibly associated with a distinct spectrum of carcinomas. In hamartomas, carcinomas and extra-intestinal polyps from patients with a **STK11** germline mutation, LOH of the wild type allele has been reported. In addition, inactivation can occur by somatic mutations. Whether inactivation of **STK11** is required for initiation of the development of hamartomas is not known. Hamartomas from **Stk11**+/− mice may harbor wild type **Stk11**, suggesting that haploinsufficiency may suffice for a clinically manifest phenotype.

**STK11** is a kinase and most point mutations reported in PJS disrupt the kinase activity of **STK11**, suggesting that intact kinase activity is essential to its tumor-suppressive function. In vitro, **STK11** inhibits proliferation by causing G1 cell cycle arrest. This effect may be mediated by a p53 dependent up-regulation of p21WAF1/CIP. In addition, **STK11** appears to be involved in p53-mediated apoptosis, although p53 mediated growth arrest was not impaired in **Stk11**−/− mouse embryonic fibroblasts (MECs). The effects of **STK11** on proliferation and apoptosis are both dependent of cytoplasmic localization, which seems thus required for its tumor-suppressive activity. **Stk11**−/− mice die in utero and display many congenital defects, including vascular anomalies. The latter may be related to increased Vegf expression in **Stk11**−/− mice. However, a
role for VEGF in PJS-related tumorigenesis remains to be defined. Notably, the expression of other stromal signaling proteins, such as MMp2 and Pdgfra, was increased in Stk11−/− hamartomas and MECs 97, possibly explaining the prominent stromal proliferation in hamartomas.

Interestingly, Stk11−/− MECs not only showed immortalization in culture, but also resistance to activated RAS induced transformation 97. This investigation suggests that, under certain circumstances, loss of STK11 may protect cells against progression towards malignancy, providing a possible explanation for the limited malignant potential of hamartomas. Such a paradoxical effect of the STK11 gene places it in a distinct class of tumor suppressors.

To date, carcinogenesis in PJS is not well understood and the premalignant potential of hamartomas has not been established. Although rare 79, dysplastic transformation of hamartomas may occur, suggesting the existence of a hamartoma-adenoma-carcinoma sequence 79. At the molecular level, this PJS tumor-progression model differs from the adenoma-carcinoma sequence in FAP. PJS hamartomas and carcinomas show LOH at the STK11 locus 19q13.3, but lack LOH at 5q (APC locus) and 17p (TP53 locus) 90,91. In addition, mutations in APC and K-RAS are rare 92,99 (chapter 9, this thesis). One group reported β-catenin (CTNNB1) mutations in a subset of hamartomas, including one dysplastic lesion 92, suggesting a relationship to progression of the hamartoma-adenoma-carcinoma sequence. However, we were unable to find β-catenin mutations in either hamartomas or carcinomas. Also, we discovered only focal alterations in the expression or localization of β-catenin, cyclinD1, Ki-67 and p21/waf1/cip1, which are commonly observed in the adenoma-carcinoma sequence (chapter 9, this thesis). Taken together, carcinogenesis in PJS appears to be distinct from FAP. Importantly, COX-2 expression was found in hamartomas from Stk11−/− mice, and in nondysplastic and dysplastic hamartomas and carcinomas from PJS patients, providing a rationale for the investigation of chemoprevention with NSAIDs or COX-2 inhibitors in PJS 100 (chapter 9, this thesis).

Table 3. Screening and surveillance guidelines in PJS 34.

<table>
<thead>
<tr>
<th>Interval</th>
<th>Test</th>
</tr>
</thead>
</table>
| Yearly   | haemoglobin, bilirubin  
abdominal ultrasound (assessment of liver and pancreas)  
pelvic ultrasound (assessment of cystic ovarian lesions)  
cervical smears (endo and ecto-cervical) |
| Biennially | upper GI-endoscopy with polypectomy  
colonoscopy with polypectomy  
small bowel series (or enteroscopy with video capsules) |
| Variable Male | regular testicular self-examination  
scrotal ultrasound until puberty or in the presence of feminizing feature |
| Female | regular breast self-examination  
breast radiology every 5 years from 25 till 45 years, then biennially until 50, then yearly |
Management

Patients and their relatives should be investigated for the presence of hamartomas and a germline STK11 mutation. Counseling is required before genetic testing may be performed. Once the diagnosis is established, surveillance should be offered, although the benefit of surveillance has not been evaluated. Recommendations published by McGrath and Spigelman are shown in Table 3. To decrease the risk for intussusception of hamartomas, regular polypectomy is indicated. Intra-operative endoscopy can be applied for polyps >1.5 cm or polyps which are not accessible by standard endoscopic techniques, avoiding small bowel resections. The high cancer risk in PJS points to the need for chemopreventive strategies against the development of intestinal and extra-intestinal carcinomas. To date, no treatment is available. However, we found COX-2 expression in PJS carcinomas and dysplastic hamartomas suggesting that NSAIDs or COX-2 inhibitors may be beneficial (chapter 9, this thesis).

Outline of the thesis

Phenotypic and molecular studies are needed to improve patient care for patients affected with polyposis syndromes. Important targets are the further optimization of surveillance protocols, and the development of screening tools and chemopreventive strategies. Studies addressing pathogenesis, screening modalities and chemoprevention of polyposis syndromes may provide important clues to the development of preventive strategies against sporadic CRC, since these disorders represent unique models of carcinogenesis in the general population.

This thesis describes two lines of research. The focus of part one of this thesis is chemoprevention of FAP, addressing the molecular mechanism of the effect of sulindac against colorectal adenomas, and evaluating apoptosis as potential biomarkers. In chapter 2, recent advances in chemoprevention of colorectal cancer with NSAIDs or COX-2 inhibitors are reviewed. Chapters 3 and 4 address the value of rectal epithelial apoptosis as a biomarker for treatment outcome and adenoma development in phenotypically positive and negative FAP patients respectively, treated with sulindac. In chapter 5, molecular alterations potentially related to sulindac resistance are investigated in adenomas from sulindac resistant and sulindac responsive patients. Chapter 6 evaluates nuclear β-catenin and Wnt-signaling as targets for NSAIDs based chemoprevention in adenomas of FAP patients and in colorectal cancer cell lines.

Part two of this thesis describes studies investigating molecular and phenotypic aspects of PJS. Chapter 7 is a historical note about the initial descriptions of the syndrome by Peutz and Jeghers. Chapters 8 and 9 aim to further describe the molecular pathogenesis of PJS related hamartomas and carcinomas, to define a possible preneoplastic potential of hamartomas at the molecular level and to assess expression of COX-2, a target for chemopreventive therapy. Chapter 10 and 11 investigate the molecular genetic association between PJS and nasal polyposis and address the pathogenesis of PJS related nasal polyps. In chapter 12, the diagnostic value of mucocutaneous pigmentation in PJS is assessed. Chapter 13 describes the tumor spectrum and estimates the cancer risk in a cohort of well-documented Dutch PJS patients.
Chapter 1

References


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