Molecular genetic alterations in gastrointestinal polyposis syndromes: with emphasis on the Peutz-Jeghers syndrome
Entius, M.M.

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Chapter 6

Peutz-Jeghers Syndrome: 78-year Follow-Up of the Original Family

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Chapter 6

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Summary

**Background**
The association between heredity, gastrointestinal polyposis, and mucocutaneous pigmentation in Peutz-Jeghers syndrome (PJS) was first recognised in 1921 by Peutz in a Dutch family. This original family has now been followed-up for more than 78 years. We did mutation analysis in this family to test whether the recently identified LKB1 gene is indeed the PJS gene in this family.

**Methods**
The original family was retraced and the natural history of PJS was studied in six generations of this kindred by interview, physical examination, chart view, and histological review of tissue specimens. DNA-mutation analysis was done in all available descendants.

**Findings**
Clinical features in this family included gastrointestinal polyposis, mucocutaneous pigmentation, nasal polyposis, and rectal extrusion of polyps. Survival of affected family members was reduced by intestinal obstruction and by the development of malignant disease. A novel germline mutation in the LKB1 gene was found to cosegregate with the disease phenotype in the original family. The mutant LKB1 allele carried a T insertion at codon 66 in exon 1 resulting in frameshift and stop at codon 162 in exon 4.

**Interpretation**
The morbidity and mortality in this family suggest that PJS is not a benign disease. An inactivating germline mutation in the LKB1 gene is involved in the PJS phenotype in the original and oldest kindred known to be affected by PJS.

*Lancet* 1999; 353: 1211-15

Introduction

In 1921, the Dutch physician Peutz described the combination of gastrointestinal polyps and mucocutaneous melanin spots in three young siblings. Of the seven children in this family, five had numerous dark pigmented spots on the face, on the lips, and in the mouth; three of them, one girl and two boys, suffered from attacks of colicky abdominal pain and rectal blood loss. Intussusception of the small bowel leading to ileus developed in two patients, who were both found to have multiple polyps throughout the small and large intestine. The resected jejunal polyps in one patient showed malignant degeneration. Both patients reported severe nasal polyposis. Their father, who had no complaints, was found to have some pigmented spots on the mucous membranes of his mouth. He recalled having facial pigmented spots which apparently had disappeared. Two of his sisters with similar pigmentations had died, at the age of 11 years and 20 years, of intestinal obstruction.

The observations made by Peutz led to the definition of autosomal dominant syndrome characterised by gastrointestinal polyposis and mucocutaneous pigmentation, now known as Peutz-Jeghers syndrome (PJS). Jeghers published ten additional cases in 1942. The Dutch family originally described by Peutz was followed-up by van Wijk, who published his findings in a thesis in 1950 and included a new generation. We retraced the family 78 years after the initial description by Peutz and present further follow-up over six generations. The PJS gene has now been identified and found to encode the serine threonine kinase LKB1 or STK11. We therefore did mutation analysis to determine whether a defect in the LKB1 gene is responsible for the PJS phenotype in the original Peutz kindred.

**Methods**

**Family study**
We requested physicians in the Netherlands with an interest in gastroenterology to ask patients with PJS to participate in a clinical genetic study. Patients who agreed were visited by one of us. Two of these patients were found to be members of the original family described by Peutz. With their help, all other living descendants in this family were contacted, interviewed, and examined. Charts and tissue specimens were collected with their consent. Medical histories of deceased family members were reconstructed with the help of living family members and the previous descriptions by Peutz and van Wijk. The diagnosis of PJS was considered definite if all the following clinical criteria were present: gastrointestinal polyposis with histological verification of the polyp, characteristic mucocutaneous pigmentation, and positive family history. The age at which diagnosis could be regarded as conclusive was set at 25 years.

To check the pedigrees described by Peutz and van Wijk for completeness and accuracy, the exact dates of birth and death of every family member were obtained from municipal registries. The study was approved by the medical ethics committee of the Erasmus University and University Hospital Rotterdam, and informed consent was given by all participating individuals.
**Mutation detection**

Peripheral blood samples were taken from all available family members. Lymphoblastoid cell lines were established by Epstein-Barr virus transformation of lymphocytes. DNA was isolated by standard techniques and amplified by PCR. PCR products were submitted to denaturing gradient-gel electrophoresis (DGGE) for initial mutation screening in all nine exons of the LKB1 gene. The nucleotide sequences of PCR products that showed an abnormal DGGE pattern were determined by direct sequencing. Sequencing was done with a T7 sequenase kit (Amersham Life Science) according to the dideoxy-chain termination method. For DGGE analysis in exon 1 the following flanking intron primer pair was selected: 5'-ACCATCAGCACCGTGACTGG-3' (sense) and 5'-GGGAGGAGAGAAGGAAGGAA-3' (antisense). A GC clamp AGCCGCGCCGCAAGCGGGCC-3' (antisense primer was used for sequencing in combination with the antisense primer 5'-

**Results**

**Pedigree**

Figure 1 shows the updated pedigree of the original family. In total, 22 people (nine female, 13 male) were diagnosed as being affected and 31 as unaffected. The phenotypic status of 25 individuals is unknown because of lack of information, or (in three cases) because the symptom-free individual has not yet reached the age of 25 years. Members of the second and third generation

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**Table 1: Characteristics of the affected individual Peutz family members**

<table>
<thead>
<tr>
<th>Individual</th>
<th>Sex</th>
<th>Age at onset mucocutaneous pigmentations</th>
<th>Age at onset abdominal symptoms</th>
<th>Age*</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>II:3</td>
<td>F</td>
<td>?</td>
<td>20</td>
<td>20*</td>
<td>Closed colic</td>
</tr>
<tr>
<td>II:4</td>
<td>M</td>
<td>?</td>
<td>7</td>
<td>80*</td>
<td>Sigmoid carcinoma</td>
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<tr>
<td>II:11</td>
<td>F</td>
<td>&lt;12</td>
<td>7</td>
<td>10*</td>
<td>Closed colic</td>
</tr>
<tr>
<td>II:13</td>
<td>M</td>
<td>?</td>
<td>9</td>
<td>60*</td>
<td>Gastric carcinoma</td>
</tr>
<tr>
<td>III:9</td>
<td>F</td>
<td>First months</td>
<td>25</td>
<td>50*</td>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>III:11</td>
<td>F</td>
<td>&quot;Young age&quot;</td>
<td>17</td>
<td>40*</td>
<td>Colon carcinoma</td>
</tr>
<tr>
<td>III:13</td>
<td>M</td>
<td>First year</td>
<td>15</td>
<td>40*</td>
<td>Small-bowel ileus</td>
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<tr>
<td>III:15</td>
<td>M</td>
<td>2 years</td>
<td>8</td>
<td>70*</td>
<td>Peritonitis</td>
</tr>
<tr>
<td>III:17</td>
<td>M</td>
<td>First year</td>
<td>10</td>
<td>70*</td>
<td>Pneumothorax</td>
</tr>
<tr>
<td>III:19</td>
<td>M</td>
<td>3 months</td>
<td>6</td>
<td>30*</td>
<td>Adenocarcinoma of digestive tract</td>
</tr>
<tr>
<td>IV:9</td>
<td>M</td>
<td>First year</td>
<td>8</td>
<td>20*</td>
<td>Small-bowel ileus</td>
</tr>
<tr>
<td>IV:12</td>
<td>F</td>
<td>First year</td>
<td>24</td>
<td>50*</td>
<td>Squamous cell carcinoma of nasal cavity</td>
</tr>
<tr>
<td>IV:13</td>
<td>F</td>
<td>2 years</td>
<td>2</td>
<td>&lt;10*</td>
<td>Small-bowel ileus with peritonitis</td>
</tr>
<tr>
<td>IV:17</td>
<td>M</td>
<td>&quot;Young age&quot;</td>
<td>4</td>
<td>40*</td>
<td>Colon carcinoma</td>
</tr>
<tr>
<td>IV:19</td>
<td>F</td>
<td>First weeks</td>
<td>17</td>
<td>20*</td>
<td>Small-bowel ileus</td>
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<tr>
<td>IV:20</td>
<td>M</td>
<td>First year</td>
<td>2</td>
<td>60*</td>
<td>-</td>
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<td>V:9</td>
<td>F</td>
<td>First weeks</td>
<td>14</td>
<td>40*</td>
<td>-</td>
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<tr>
<td>V:13</td>
<td>F</td>
<td>First weeks</td>
<td>4</td>
<td>40*</td>
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<td>V:14</td>
<td>M</td>
<td>First year</td>
<td>20</td>
<td>30*</td>
<td>-</td>
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<tr>
<td>V:15</td>
<td>M</td>
<td>6 years</td>
<td>8</td>
<td>30*</td>
<td>-</td>
</tr>
<tr>
<td>V:16</td>
<td>M</td>
<td>9 years</td>
<td>19</td>
<td>30*</td>
<td>-</td>
</tr>
</tbody>
</table>

*At death, at the request of one of the family members, age now or at death is rounded off to the nearest decade.

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**Figure 1: Pedigree of the original Peutz family**

Roman numerals indicate generations, arabic numerals individuals. Squares=males, circles=females, diamonds=unknown sex. Affected individuals are denoted by solid symbols, unaffected individuals by open symbols, question marks denote unknown status. A slash means that the individual is deceased. The initial proband for this study is indicated by an arrow, participants in the DNA analysis are marked with an asterisk. Pedigree of the original Peutz-Jeghers family

**78-year Follow-up of the Original Peutz-Jeghers Family**

**Table:**

<table>
<thead>
<tr>
<th>Age at Onset</th>
<th>Abdominal Symptoms</th>
<th>Age</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;24</td>
<td>Colon carcinoma</td>
<td>80*</td>
<td>Closed colic</td>
</tr>
<tr>
<td>&lt;30</td>
<td>Sigmoid carcinoma</td>
<td>10*</td>
<td>Closed colic</td>
</tr>
<tr>
<td>&lt;24</td>
<td>Gastric carcinoma</td>
<td>50*</td>
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<td>&lt;24</td>
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<td>Small-bowel ileus</td>
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</tr>
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<td>Small-bowel ileus with peritonitis</td>
<td>&lt;10*</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1: Characteristics of the affected individual Peutz family members
Chapter 6

Acute rectal blood loss

Anaemia

Clinical symptoms

Total number of operations

Chronic rectal blood loss

Paralytic ileus

Nasal polyposis

Gastrointestinal polyposis

Mucocutaneous pigmentation

Clinical characteristics

Gastro-intestinal

For intussusception

For paralytic ileus

Number of patients operated

Abdominal surgery

Rectal prolapse by polyps

Abdominal symptoms had started in all patients in the characteristic pattern. At adolescence the pigmentation started to fade, but at that age the gastrointestinal symptoms had started in all patients (table 1).

Nasal polyps developed in six patients (table 2). Unfortunately, no tissue specimens of nasal polyps had been preserved for re-examination. In previous reports they had been diagnosed as "inflammatory polyps". In four patients the nasal polyposis was severe, obstructing the nasal cavity and sinuses, requiring repeated surgery. In one (IV:12), a 51-year-old woman who had had extremely severe nasal polyposis since childhood, a squamous-cell carcinoma of the nasal cavity developed. She died of this tumour 4 years later (table 1).

Six other cancers developed among the 22 affected individuals (table 2), five of which occurred in the gastrointestinal tract. Three of the five gastrointestinal cancers in this family were in the colon, one was in the stomach, and one was of unknown primary origin. All cancers had metastasised at the time of death, when patients were at a mean age of 50 years (SD 15). In another patient (V:13) a polyp was resected when the patient was aged 15 years and it was originally thought to contain a jejunal carcinoma in situ. Metastasis was not detected and the patient, who is now 36 years old, has remained in good health. Histological review of the resected polyp showed that it apparently malignant character was due to pseudo-invasion. Whether pseudo-invasion also occurred in the jejunal polyps from patient III:13 described by Peutz\(^1\) is not clear, because these specimens have been lost. However, this patient survived without metastasis for more than 23 years and it seems probable that a similar error of interpretation was made. None of 82 other polyps (nine stomach, seven duodenal, 50 small bowel, 14 colon, two rectal) resected from 20 patients had malignant degeneration. All polyps had the characteristic PJS hamartoma appearance on histological examination.

Breast cancer occurred in a female patient at the age of 47 (III:9, table 2). Premenopausal breast cancer was diagnosed in a sibling (III:1) at the age of 44. It is not known whether this patient was affected by PJS. No other cancers of the reproductive tract were found in this family.

**Mutation analysis**

For mutation analysis we were able to include six patients with continued PJS, six unaffected individuals, and three spouses of affected individuals (figure 1).
characteristic mucocutaneous melanin pigmentation and nasal polyposis has not been reported before, development of cancer of the nasal cavity in a PJS patient of the syndrome to include nasal polyposis. The development of cancer of the nasal cavity in a PJS patient with nasal polyposis has not been reported before, although De Faqu and colleagues showed adenomatous change in two nasal polyps in a PJS patient.

Affected individuals in this family had both the characteristic mucocutaneous melanin pigmentation and gastrointestinal polyposis. Interindividual variation was seen in the intensity of freckling and in the severity of gastrointestinal symptoms. The close association between pigmentation and polyps in this family is possibly the result of the close observation for the development of the disease, instigated by the family. The reported dissociation of symptoms in PJS could be due to age at diagnosis, because the occurrence of symptoms in PJS tends to be age specific. However, as in other genetic disorders, phenotypic variability has been reported in PJS both in and between families. One of the first presenting clinical symptoms of PJS in the family we studied was rectal extrusion of polyps (table 2). Prolapse of PJS polyps through the rectum has been reported by others. In the remainder of the affected family members the first symptomatic presentation of the polyps was colicky abdominal pain, caused by intussusception. Small-bowel intussusception progressing to paralytic ileus was the main cause of death in the earlier generations in this kindred (table 1), contributing to the decreased survival. Although PJS polyps are hamartomas, frequent association of this syndrome with both gastrointestinal and non-gastrointestinal tumours has led to reassessment of the cancer risk in this hereditary disorder. Seven out of the 22 (32%) affected family members developed cancer, five of which occurred in the gastrointestinal tract. This proportion does not necessarily reflect the risk of cancer in present generations, because there have been syndrome-associated competing causes of death in the earlier generations. The young age at which cancer death occurred in this family was striking, with a mean of 50 years (SD 15). All cancers had metastasised at the time of death, and therefore represented true cases of cancer. We also found one case of pseudo-invasion, a benign displacement of polyp epithelium mimicking invasive jejunal carcinoma. It is possible that the two "malignantly degenerated" jejunal polyps in one of the patients described by Peutz were not cancer but cases of pseudo-invasion. With three of the true gastrointestinal cancers arising in the colon and one in the stomach, and one of the extraintestinal cancers arising in the breast, the tumour spectrum in the family studied by Peutz correlates with our experience of other PJS families (unpublished data) and previous reports. None of the rare gonadal tumours that have been associated with PJS was encountered in this family. Looking for the genetic defect underlying the disease phenotype in this family, we founded a novel mutation in the recently identified PJS gene to be germline inherited among the affected individuals in this family. The mutation consisted of a T insertion in exon 1 (codon 66) of the LKB1 gene resulting in a stopcodon (162) in exon 4. This mutation is predicted to cause truncation of the encoded protein, a serine threonine kinase, and therefore to inactivate it. The germline inheritance of the familial susceptibility for developing aberrant growths, both within and outside the gastrointestinal tract, suggests that the PJS gene is a tumour-suppressor gene. Inherited loss of one allele confers susceptibility, whereas a second mutation in the other allele may be required to produce the phenotype at a cellular level. The loss of heterozygosity for the 19p-locus seen in PJS polyps by Hemminki and colleagues supports this hypothesis. The natural course of PJS in the original Peutz kindred shows clearly that this is not a benign disease. Decreased...
survival was encountered in the affected individuals due to gastrointestinal complications, especially in the (untreated) earlier generations, and early development of malignancies. Identification of gene carriers at an early age and the development of suitable screening programmes will possibly reduce morbidity and mortality in PJS families.

Contributors
Anne Marie Westerman, Paul Wilson, and Dick Lindhout designed the study. Anne Marie Westerman traced the family, collected blood samples and clinical data, and did the genealogical study. Mark Enius, Loes van Velthuysen, and Johan Offerhaus collected and reviewed the histology of the tissue specimens. Mutation analysis was done by Ellen de Baar, Patrick Boor, Rita Koole, and Felix de Rooij. Paul Wilson was the coordinating investigator. Anne Marie Westerman and Paul Wilson wrote the article, and all investigators helped to revise it.

Acknowledgments
We thank Th W van Wijk and D Overbosch for their help in tracing the original family.

References
1 Peutz JLA. Over een zeer merkwaardige, gecombineerde familiaire polypsies van de slimmvlies van den tractus intestinalis met die van de neus- en keelholte en gepaard met eigenaardige pigmentaties van huid-en sulmvlies. Ned Maandschr v Gen (Netherlands (1950)).