Mutations in the STK11 gene in Czech Peutz-Jeghers families

P. Vasovčák1*, A. Puchmajerová1, J. Roubalík2 and A. Křepelová1

1Institute of Biology and Genetics, Charles University, Medical School, Prague, Czech Republic
2Bata Hospital, Digestive endoscopy Centre, Zlin, Czech Republic

Abstract

Background: Peutz-Jeghers syndrome (PJS) is an autosomal dominant hereditary disease characterized by mucocutaneous pigmentation and gastrointestinal hamartomatous polyposis. The germline mutations in the serine/threonine kinase 11 (STK11) gene have been shown to be associated with the disease. Individuals with PJS are at increased risk for various neoplasms. Molecular analysis could be helpful in disease management of PJS probands.

Methods: We investigated the promotor and the entire coding region including the splice-site boundaries of the STK11 gene in genomic DNA of 8 individuals from five Czech families by sequencing analysis and multiplex ligation probe-dependent amplification (MLPA) assay.

Results: One frameshift and two large deletions were found in 6 individuals from three families. One individual developed gastric and lung cancer. No other proband has developed carcinoma so far. Two patients with sporadic disease were not found to carry
any germline variation of the \textit{STK11} gene. A familial case with very aggressive gastric carcinoma is described.

**Conclusion:** We found germline mutations of the \textit{STK11} gene in probands fulfilling PJS diagnostic criteria. A sporadic case with hyperpigmentation and only one adenomatous polyp was also found to have a deletion of the whole \textit{STK11} gene.

*Corresponding author: Peter Vasovčák, Institute of Biology and Genetics, Charles University, Medical School, V Úvalu 84, Prague 5, 150 06, Czech Republic.

E-mail: pevas78@centrum.cz

**Introduction**

Peutz-Jeghers syndrome (PJS; OMIM 175200) is an autosomal dominant disorder characterized by mucocutaneous pigmentation and gastrointestinal hamartomatous polyposis with an increased risk of cancer [1-4]. Histologically, polyps in PJS are characterized as hamartomas; however, adenomatous changes may occur in polyps and they become malignant. In addition to an elevated risk of gastrointestinal cancers, an increased risk of cancers at other sites has been described, mainly: breast, ovary, uterus, cervix, pancreas, lung and testicular cancers [3, 5-8]. Testicular sex cord and Sertoli cell tumors, leading to sexual precocity and gynecomastia [9-11], sex cord tumors with annular tubules and cervical adenoma malignum have also been reported [12].

The gene responsible for PJS, serine/threonine kinase \textit{STK11}, was mapped to chromosome 19p13.3 and acts as a tumor suppressor [4, 13, 14]. It plays a role in the
p53-dependent apoptosis pathway, in the vascular endothelial growth factor (VEGF) signaling pathway and in the polarization of epithelial cells [15-17].

About one-third of patients with PJS are diagnosed before the age of 10 years and up to 60% cases develop their first clinical manifestations until the third decade of life [18]. In most cases, initial symptoms are abdominal pain due to intussusceptions, obstruction and gastrointestinal bleeding with anemia [19, 20]. A working definition of PJS has been suggested by Giardiello [3], where for individuals with a histopathologically confirmed hamartoma, a definite diagnosis of PJS requires two of the following three findings: family history consistent with autosomal dominant inheritance, mucocutaneous hyperpigmentation, or small-bowel polyposis. Tomlinson and Houlston [21] have modified the criteria for PJS for individuals without a family history of PJS, in which the diagnosis depends upon the presence of two or more histologically verified Peutz-Jeghers-type hamartomatous polyps.

We report here a clinical manifestation and mutational analysis of the PJS gene in eight individuals from five unrelated Czech families.

**Patients**

Eight patients from five unrelated families were included in the study (tab.1). Four probands from two families fulfilled and three sporadic cases did not fulfill criteria to establish the diagnosis of definite PJS [3, 21]. In one individual, a presumptive diagnosis of PJS was made due to a first-degree relative with PJS and the presence of mucocutaneous hyperpigmentation.
Family A includes mother (case A-1) and her daughter (case A-2).

Case A-1 was a 29-year-old female with negative family history. The diagnosis of PJS was made at her 10 years due to hyperpigmentation of the lips, buccal mucosa, and perinasal region. X-ray of abdomen did not reveal any polyp. The patient was free from any abdominal symptoms. At her 24 years she underwent gastroscopy because of dyspepsia lasting for a few months. A rigid mucosa of the stomach was noted with negative histology. Sixteen months later and seven months after giving birth, two hamartomatous polyps 4.5 cm and 1.5 cm in diameter and multiple small polyps 1-3 mm in diameter were found in the stomach. Colonoscopy revealed tubulous adenoma, 3 cm in diameter in caecum. Enteroclysis did not show any pathology of the small intestine. Since then she has been followed up every six months. Continually, tubulovillous adenoma from sigmoid colon and hamartomatous polyp from the transverse colon were removed, and at her 27, well differentiated mucinous adenocarcinoma in left inferior lung lobe was surgically removed. One year later, during second gestation, adenocarcinoma of the stomach was found. Patient refused termination of pregnancy; therefore operation was performed without previous neoadjuvant therapy. Unfortunately, because of deterioration of her state premature birth was induced at the 30-ith week of the gestation. Three months later patient died of gastric cancer.

Case A-2, a 7-year-old girl, presented with pale brown patches on the lower lip, which have been noted since her 2 years of age. Examination of the gastrointestinal tract (GIT) was not performed.

The younger daughter, 4 years old, was not included in the study. She was free of any symptoms typical for PJS.
Family B comprises mother (case B-1) and her two sons (case B-2 and B-3).

Case B-1, a 46-year-old female has presented with perioral and buccal pigmentation since childhood. She was found to have colonic and small intestinal hamartomatous polyps at 36 years of age. Since then repeated colonoscopy and enteroscopy with polypectomy has been performed. Total colectomy was made due to excessive polyposis and recurrent GI problems. Histology of the polyps did not reveal any malignancy. Family history is missing.

Case B-2 represented a 17-year-old boy with perioral brown pigmentation mostly on the lips. At his 10, hamartomatous polyps in small intestine were detected. Since then frequent gastroduodenoscopies and colonoscopies with polypectomies have been performed.

Case B-3, a 13-year-old boy, manifested with mucocutaneous brown to dark blue pigmentations on the lip, mostly on the lower one. At his 6, rectal bleeding due to polyp in rectum was noted. Since then he has undergone frequent gastroduodenoscopies and colonoscopies with polypectomies.

The remaining cases (C-1, D-1 and E-1) were sporadic.

Case C-1, a 20-year-old female has presented brown to dark blue pigmentations from her 2 years. At her 14, enteroclysis of small intestine showed one adenomatous polyp. Frequent colonoscopies and enteroclysies with negative results have been performed. Family history was negative.

Case D-1, a 50-year-old male with perioral and buccal pigmentation was found to have two hyperplastic polyps, one in sigmoideum and the other in colon ascendens. Tubulovilous adenoma with low-grade dysplasia was excised from the ascending colon.
Mother of the patient had colon cancer in 72 and father, a smoker, had lung cancer in 76. They were without hyperpigmentation.

Case E-1, a 10-year-old boy, was referred because of perioral and buccal pigmentation. Examination of the GIT did not reveal any polyp. Parents and step-siblings are without any PJS symptoms.

**Material and methods**

After an informed consent genomic DNA of the patients was isolated from blood leukocytes using Genomic DNA Purification Kit (Gentra). Genomic DNA was amplified using intronic primers [22, 23] flanking the nine exons and promoter region of the \textit{STK11} gene. PCR products were purified using the SureClean PCR purification kit (Bioline). Cycle sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and the Applied Biosystem 3130 Genetic Analyzer. Patients with negative results of sequencing analysis were examined by means of the multiplex ligation-dependent probe amplification (MLPA) method for identification of large exonic deletions or duplications with the P101-STK11 MLPA kit (MRC Holland).

**Results and discussion**

All eight patients except one (A-2) underwent endoscopic procedures to examine the whole GIT. Excised polyps from cases A-1, B-1, B-2, and B-3 were histologically
classified as hamartomatous, adenomatous from case C-1, and hyperplastic from case D-1. Case E-1 was free of any polyps (tab.1). All individuals had pigmentation of the lips and buccal mucosa, the most visible in children. None of the probands had pigmentation of extremities. The positive family history of cancer was only noted in case D-1. Mutation analysis revealed three different germline mutations. In family A, germline mutation (c.350dupT) in exon 2 (fig.1) was detected. The mutation is predicted to introduce a frameshift at codon Leu117, 46 novel amino acids, and premature termination codon (p.Leu117PhefsX46). It was found in heterozygosity in both patients (A-1 and A-2).

Individuals from family B (case B-1, B-2, and B-3) harboured deletion of the part of the promoter region and exon 1 (fig. 2). Case C-1 was a carrier of deletion of the whole STK11 gene (fig.3). In case D-1 and E-1, we have not revealed any variation of the STK11 gene by aforementioned methods.

PJS is a relatively very well characterized disorder with clear cut phenotype [21]. However, in sporadic cases, the diagnosis of PJS may be uncertain. Although multiple hamartomatous polyps of the GIT are pathognomonic of the PJS, hyperplastic and adenomatous polyps are commonly present [16]. Recently, it has been reported that STK11 deletions are not a rare cause of Peutz-Jeghers syndrome and account for up to 30% of patients with PJS [24, 25]. There was no difference in the clinical phenotype between patients with point mutations or with large genomic deletions [24]. However, detailed phenotype in patients with different types of mutations was not given.

Members of family B (case B-1, B-2, and B-3) had almost identical clinical symptoms with decreasing age of onset of the first symptoms and detection of polyps. They are
carriers of a germline mutation (deletion of the part of the promoter region and exon 1) of the *STK11*. All three affected individuals had mucocutaneous hyperpigmentation predominantly on the lips and on the buccal mucosa, the most prominent in the youngest patient (B-3) and very pale in mother (B-1). Sons of the former patient, B-2 and B-3, were initially classified as PJS suspected because of striking hyperpigmentation of the lips. Both of them are under careful surveillance. A few endoscopic examination of the whole GIT have been performed so far with polypectomy of hamartomatous polyps. This confirmed the diagnosis of definite PJS. Polyps were localized in small intestine and colon. No polyps were detected in the stomach.

Deletion of the promoter region and exon 1 was reported in three independent studies in 12 families overall [24-26]. It could be a recurrent mutation, probably a consequence of unequal recombination mediated by repetitive Alu (SINE elements) sequences. In accord with UCSC Genome Browser, region of chr19:1,129,999-1,196,665, where the *STK11* gene is located, is rich for these repetitive sequences which can be involved in large chromosome rearrangements. The implication of Alu repetitive elements in unequal genomic recombinations was described for another tumor suppressor gene, *MSH2*, implicated in Lynch syndrome (HNPCC) [27].

Sporadic case C-1 with pigmentation and adenomatous polyp was found to harbour a germline deletion of the entire *STK11* gene (fig.1). Although this mutation is expected to have the same effect on phenotype as the mutation (deletion of the part of the promoter region and exon 1) in family B, case C-1 has presented with hyperpigmentation and one adenomatous polyp in the small intestine so far. We cannot exclude developing another
polyps or tumour later in life. Patient is carefully followed up and without any changes related to PJS at present.

We failed to find any variation of the STK11 gene in sporadic case D-1 and E-1, which would explain their phenotype. On the other hand, they did not fulfill criteria for the diagnosis of definite PJS [3, 21]. We included these cases to the study on the basis of the result from the case C-1. Especially, case E-1 where PJS polyps could develop later on. Studies with more individuals not fulfilling PJS diagnostic criteria were reported. None of the patients harboured a germline mutation of the STK11 gene [24, 28]. Some studies suggested there could be another locus responsible for PJS phenotype [29, 30]. Other authors stated according to their results that another locus is unlikely and the causative variation could be in regulatory regions such as promoter, enhancers, or splicing sites deep in introns, which are not detectable by conventional methods [24, 25, 31].

The risk of developing various types of GI cancers (esophagus, stomach, small bowel and colon) was determined in several studies [3, 6, 7, 32, 33]. The cumulative risk for stomach cancer was 29% [7]. Amos et al. noted that gastric polyps are very common among individuals with PJS [28]. They did not specify proportion of patients with a detectable PJS germline mutation and gastric polyps/cancer. There are several case reports and reviews reporting gastric cancer in PJS patients [3, 19, 34-43]. In our group of probands, case A-1 developed gastric cancer at 28 years and died one year later. No genotype-phenotype correlations were published in PJS patients with gastric cancer [6, 28, 31]. Konishi et al. reviewed 103 literature PJS patients with malignancy and found out that mean age of 8 cases with gastric cancer was 31,2 years compared to duodenal carcinoma 39,7 years (9 cases), and colorectal carcinoma 48 years (13 cases). According
to literature and our results we suppose that gastric cancer has very aggressive course in some individuals with PJS and usually despite very frequent endoscopic examinations with relevant treatment the next course is poor. It would be particularly interesting to find out if there is a correlation between genotype and phenotype in relation to the development of gastric cancer. There have been only two reports dealing with gastric cancer in PJS patients and mutational analysis of the STK11 gene so far [22, 42]. Shinmura et al. described two PJS females (sisters) with gastric cancer in which a STK11 germline mutation (c.890delG) was identified [22]. Takahashi et al. reported a 14-year-old girl with sporadic PJS and early-onset gastric cancer harbouring a frameshift (c.757_758insT) STK11 mutation [42]. Similarly, as in our family A, the mutations led to truncated protein lacking a kinase domain. These results suggest that the truncation mutations leading to loss of STK11 kinase domain could act in a dominant negative fashion and be responsible of tumour development. Schumacher et al. summarized clinical and mutational data from 132 PJS cases (83 without and 49 with cancer) to find correlation between type/site of mutation and cancer. They proposed two different mechanisms for tumour development. One is based on loss of STK11 functions due to truncation mutations and subsequent LOH as a second hit. This hypothesis is not in accord with findings of other authors, where second hit was not a requisite for tumour development [22, 42].

In summary, we found germline mutations of the STK11 gene in three families. One patient (C-1) with germline mutation did not fulfill criteria for establishing the diagnosis of PJS. Therefore variability in time of onset of symptoms should be always born in mind when establishing the diagnosis and in the disease management.
Authors' contributions

PV carried out molecular genetic studies including sequencing, MLPA analysis for all the families, and drafted the manuscript. AP identified and diagnosed the patients. AK designed study and revised manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank to all the patients and their families for agreeing to participate in the study. We are grateful to Iveta Nimsova for the DNA isolation.

Grant support: VZ MZO 00064203

Competing interest

The authors declare they have no competing financial interests.


Legend to figures

Fig. 1 Sequencing chromatogram from the analysis of the STK11 gene in family A

Fig. 2 Representative chromatogram from MLPA analysis of STK11 in family B

Fig. 3 Representative chromatogram from MLPA analysis of STK11 in family C
<table>
<thead>
<tr>
<th>Family</th>
<th>Case no</th>
<th>Sex</th>
<th>Age at onset/admission</th>
<th>Initial symptoms/signs</th>
<th>Histology of polyps</th>
<th>Location</th>
<th>Cancer</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A-1</td>
<td>F</td>
<td>10</td>
<td>pigmentation</td>
<td>hamartomatous</td>
<td>throughout GIT</td>
<td>lung, stomach</td>
<td>+</td>
</tr>
<tr>
<td>A</td>
<td>A-2</td>
<td>F</td>
<td>2</td>
<td>pigmentation</td>
<td>NA</td>
<td>NA</td>
<td>NO</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>B-1</td>
<td>F</td>
<td>36</td>
<td>pigmentation</td>
<td>hamartomatous</td>
<td>throughout GIT</td>
<td>NO</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>B-2</td>
<td>M</td>
<td>10</td>
<td>pigmentation</td>
<td>hamartomatous</td>
<td>throughout GIT</td>
<td>NO</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>B-3</td>
<td>M</td>
<td>6</td>
<td>pigmentation</td>
<td>hamartomatous</td>
<td>throughout GIT</td>
<td>NO</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>C-1</td>
<td>F</td>
<td>2</td>
<td>pigmentation</td>
<td>adenomatous</td>
<td>small intestine</td>
<td>NO</td>
<td>+</td>
</tr>
<tr>
<td>D</td>
<td>D-1</td>
<td>M</td>
<td>50</td>
<td>pigmentation</td>
<td>hyperplastic</td>
<td>colon</td>
<td>NO</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>E-1</td>
<td>M</td>
<td>10</td>
<td>pigmentation</td>
<td>no polyps</td>
<td>no polyps</td>
<td>NO</td>
<td>-</td>
</tr>
</tbody>
</table>

NA - not analyzed  
GIT - gastrointestinal tract