A Novel De Novo Mutation in the Serine-Threonine Kinase STK11 Gene in a Korean patient with Peutz-Jeghers Syndrome

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Abstract

Background: The Peutz-Jeghers syndrome (PJS) is an unusual autosomal dominant disorder characterized by mucocutaneous pigmentation and multiple gastrointestinal hamartomatous polyps. Patient with PJS are at an increase risk of developing multi-organ cancer, most frequently gastrointestinal tracts. Germline mutation of the \textit{STK11} gene, encoding serine threonine kinase are responsible for the PJS.

Case presentation: We sequenced 9 exons and their flanking intron regions of \textit{STK11} gene using polymerase chain reaction (PCR) and direct sequencing in four family members including PJS proband. Sequencing of the \textit{STK11} gene in the proband of the family revealed a novel 1 base pair deletion of guanine (G) in exon 6 (c.826delG; Gly276AlafsX11). This mutation resulted in a premature termination at codon 286, predicting a partial loss of kinase domain and complete loss of C-terminal domain. We did not observe this mutation in both parents of the PJS patient, which is therefore a novel \textit{de novo} mutation.

Conclusion: These results enlarged the spectrum of mutations of the \textit{STK11} gene by identifying a novel \textit{de novo} mutation in a PJS patient and further support the hypothesis that \textit{STK11} mutations are disease causing mutations for PJS with or without a positive family history.

Background

Peutz-Jeghers syndrome (PJS; OMIM 175200) is a rare, autosomal dominant disorder characterized by melanocytic macules of the lips, buccal mucosa, and digits and multiple gastrointestinal hamartomatous polyps, most frequently in the small intestine [1, 2]. Patient with PJS are at an increased risk of developing gastrointestinal cancer and extraintestinal neoplasms involving such organs as the ovaries, testes, breasts, pancreas, lung or uterine cervix [3-5]. The relative cancer risk is 15.2 and no significant difference in overall cancer risk between genders [3].

Currently only mutations in the gene \textit{STK11} (also known as \textit{LKB1}; OMIM 602216) at chromosome 19p13.3 have been identified as a cause for PJS [6, 7]. Human \textit{STK11} gene encodes a 433 amino acid serine-threonine kinase. \textit{STK11} is known to be located both in the nucleus and the cytoplasm of all human tissues [8, 9], and orthologs include mouse \textit{Lkb1} [10], \textit{XEEK1} (\textit{Xenopus} egg and embryo kinase 1) [11], \textit{Caenorhabditis elegans} partitioning defective gene 4 (\textit{par-4}) [12], and \textit{drosophila Lkb1} [13].

Loss of the normal allele has been observed in polyps form patients with PJS, and loss of heterozygosity (LOH) has been occurred in some tumor tissues, suggests that \textit{STK11} is a tumor suppressor gene [14-16]. \textit{STK11} is shown to cause apoptosis in intestinal epithelial cells, and physically associated with p53 and regulates specific p53 dependent apoptosis pathways [17]. \textit{STK11} is also known to have effects on G1 cell cycle arrest [18], TGF-\beta signaling [19], polarity [20], and phosphorylating and activating the AMP activated protein kinase (AMPK) [21].

Screening for point mutations and large deletions by direct sequencing or MLPA increased the mutation detection rate in the \textit{STK11} gene up to 94% [22]. To date, more than 200 different mutations in the \textit{STK11} gene have been reported at the Human Gene Mutation database (HGMD) website (http://www.hgmd.cf.ac.uk/ac/all.php) and most of them are small
We report on a Korean PJS patient with novel \textit{STK11} mutation. During molecular genetic testing for \textit{STK11} mutation, we detected a novel small deletion in exon 6, causing a premature stop codon. This mutation was absent in both parents of the patient and was thus a \textit{de novo} mutation.

**Case Presentation**

**Subjects**
The proband was a 13-year-old Korean boy. He was diagnosed clinically with PJS at the age of 6 years based on the presence of characteristic mucocutaneous pigmentation of the lips and buccal mucosa and gastrointestinal hamartomatous polyps after polypectomy (Figure 1). The patient had one sibling and neither of his parents was affected. After obtaining informed consent, blood samples were collected from the patient and family members (Figure 2).

**Mutation studies**

Four family members including the proband were included in this study after obtaining informed consent and included in the molecular genentic study. The genomic DNA was isolated from the peripheral blood leukocytes using a Wizard Genomic DNA Purification kit according to the manufacturer’s instructions (Promega, Madison, WI, USA). The \textit{STK11} gene was amplified by polymerase chain reaction (PCR) by using the appropriate primers that had been designed by the authors (available upon request) and a thermal cycler (Model 9700; Applied Biosystems, Foster City, CA, USA). Direct sequencing of all nine coding exons along with the flanking intron regions of the \textit{STK11} gene was performed with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) in conjunction with an ABI Prism 3100 automated genetic analyzer (Applied Biosystems).

**Identification of mutation**

Direct sequencing analysis of the proband demonstrated a heterozygous 1-bp deletion of guanine (G) (c.826delG; Gly276AlafsX11) in exon 6 of the \textit{STK11} gene, which resulted in a frameshift leading to premature termination of the 433 amino acid protein at 286\textsuperscript{th} codon, disruption of kinase domain and complete loss of carboxyterminal non-catalytic region. This mutation was absent in his family members and 100 control chromosomes (Figure 3).

**Discussion**

Germline mutations in the \textit{STK11} gene on chromosome 19p13.3 have been shown the cause of PJS [6, 7]. Recent study suggests that \textit{STK11} mutation detection rate was above 90% [22]. However, some families with PJS have shown linkage to chromosomal region 19q13.4 [23] and 6 [24]. Human \textit{STK11} consists of nine coding exons with a 433 amino acid coding sequence and on non-coding exon 10 spanning 23 kb [9, 10, 19, 20, 25]. \textit{STK11} protein mainly comprises of three major domains, the N-terminal non-catalytic domain containing the nuclear localization signal, the catalytic kinase domain important for ATP binding and the carboxy-terminal non-catalytic regulatory domain containing prenylation motif (CAAX-box). Codons 49-309 encode the catalytic kinase domain. The C-terminal non-catalytic region of the \textit{STK11} protein is encoded by exon 8 and 9 and encompasses amino acids 309-433.

The patient recruited in this study fulfilled the well-established clinical diagnostic criteria for PJS [4]. These included histopathologically hamartoma together with classical mucocutaneous hyperpigmentation and small-bowel polyposis. Therefore, the possibility that
this patient is affected with hamartomatous polyposis syndromes other than PJS is highly unlikely. Such syndromes include juvenile polyposis syndrome, PTEN hamartoma tumor syndrome and Carney complex. In our study, we sequenced STK11 gene in this patient with PJS. We identified novel heterozygous 1-bp deletion (c.826delG; Gly276AlafsX11) in exon 6 of the STK11 gene, which resulted in a frameshift leading to premature termination of the 433 amino acid protein at 286th codon, which presumably encodes a truncating protein. This mutation was not detected in the sequencing analysis of STK11 of his family members, indicating that Gly276AlafsX11 is a novel de novo mutation.

The Gly276AlafsX11 mutation is located in the catalytic kinase domain of STK11 protein, so we hypothesize that this mutation may lead to partial loss of the kinase domain and complete loss of the C-terminal domain. Loss of STK11 protein kinase activity associated with loss of growth suppression function was reported in some mutations in STK11 associated with PJS [6, 7, 25, 26]. Thus, the development of the PJS phenotypes is believed to be due to the elimination of the kinase activity of STK11 [26, 27]. C-terminal domain of STK11 is important for the control of both the AMPK pathway and cell polarity [20]. Mutation leading to loss of C-terminal domain of STK11, as observed in this case, lead to loss of cell polarity, resulting development of malignancies. Taken together, these data suggests that STK11 the mutation in exon 6 of STK11 gene in this study may contribute to polyp formation and tumorigenesis through various mechanisms such as loss of growth arrest, apoptosis, and loss of cell polarity. Further studies will be needed to address these questions.

Although an increased cancer risk in PJS is well established [3-5], data on genotype-phenotype correlation is lacking. Schumacher et al. reported in 146 PJS patients that inframe deletions, splice site mutations, and missense mutations in the part of the gene encoding protein domain important for ATP binding and the site of catalysis (I-VIA) were rarely associated with cancer, but missense mutations in the C-terminal domain and in the part of the gene encoding protein domains important for substrate recognition (VIB-VIII), were more frequently associated with malignancies[25]. However, recently Hearle et al. reported in 419 PJS patients that the type or site of STK11 mutation did not significantly influence cancer risk[28]. Restricted by the few published papers on this topic, the genotype-phenotype correlation remains to be further investigated.

**Conclusion**

We enlarged the spectrum of mutations of the STK11 gene by identifying a novel mutation in a Korean patient with PJS. Because of the increased risk of PJS patients having multi-organ cancers, molecular diagnosis will be an important factor for genetic counseling, clinical management of patients, and tumor screening.

**Abbreviations**

PJS: Peutz-Jeghers syndrome

STK11: serine-threonine kinase 11

XEEK1: *Xenopus* egg and embryo kinase 1

LOH: loss of heterozygosity
AMPK: AMP activated protein kinase
MLPA: multiplex ligation probe-dependent amplification
PCR: polymerase chain reaction
DNA: deoxyribonucleic acid

Competing interests
The author(s) declare that they have no competing interests.

Authors’ contributions
JY recruited all the subjects investigated, carried out the molecular genetic studies and drafted the manuscript. YS and CK helped with the experiments. JY, YC and JK diagnosed the patient and participated in the editing of the manuscript. JC designed and supervised the study. All authors read and approved the final manuscript.

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References


**Figures**

**Figure 1**
The colonic polyp shows hyperplastic mucosal epithelium and arborizing pattern of smooth muscle, consistent with hamartomatous polyp (Hematoxylin-eosin stain, x200).

**Figure 2**
Pedigree of the family with PJS. *Circle* female; *square* male; *black symbol* affected. * marks indicate the family member who was available for genetic analysis.

**Figure 3**
Identification of the *STK11* gene mutation. Direct sequencing of the proband demonstrated a 1-bp deletion (c.826delG) in exon 6 of the *STK11* gene, resulting in frameshift deletion mutation (p.Gly276AlafsX11). The proband’s family members didn’t have the mutation. The localization of the deletion is indicated by arrow.
Figure 3

Father (I-1)

Mother (I-2)

Brother (II-1)

Proband (II-2)