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CASE REPORT

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A novel stop codon mutation in STK11 gene is associated with Peutz-Jeghers Syndrome and elevated cancer risk: a case study

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ABSTRACT

Based on the analysis of patients with Peutz-Jeghers syndrome (PJS), Serine threonine kinase11 (STK11) is known as a tumor suppressor gene, which is involved in cell polarization, regulation of apoptosis, and DNA damage response. In this case report study, we examined STK11 gene sequencing in a 42-year-old woman with mucocuta neous pigmentation and positive family history. Endoscopy and colonoscopy showed >1000 polyps throughout the stomach/colon (PJ-type hamartomas). The larger polyp in the stomach was resected and the small bowel imaging detected multiple jejunum/ileum small polyps. The data released from the sequencing results revealed five alterations in exons 1 to 5. The major mutation in stop codon was reported as converted to the amino acid tryptophan (TRP) to tyrosine (TER). The TGG codon was converted to TAG by mutation. Finally, another novel mutation in STK11 stop codon as a 'de novo' variant was seen. It is predicted that stop codon mutations make the affected person susceptible to developing colorectal cancer.

Keywords: Peutz-Jeghers syndrome (PJS), STK11gene, Novel mutation, Sequencing, Genetic analysis.

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Introduction

Peutz-Jeghers syndrome (PJS, OMIM 175200) is a rare uncommon autosomal dominant disorder characterized by skin pigmentation of the mucosa around the mouth and inside the lips with intestinal polyposis (1). The affected individuals have melanotic pigmentation in the skin and mucous membranes, especially around the lips and gums (2). In this syndrome, as with other autosomal dominant hereditary disorders, the affected person is prone to the formation of hamartomatous polyps of the gastrointestinal tract, especially in the colon and rectum. Indeed, the presence of these polyps is one of the main clinical features of this disease (3). The malignancy potential of hamartomas before tube formation is low (4).

In addition to complications such as anemia, bleeding, abdominal pain, and intussusception due to hamartoma at an early age in this rare syndrome, there is growing evidence that PJS also increases the risk of cancers, including gastrointestinal cancers and extra-gastrointestinal cancers such as breast cancer (5-7).

Germline mutations in STK11 (Liver kinase 1, LKB1) gene are believed to be the causative agent of this syndrome (6) and plays an important role in regulating cellular energy metabolism, chromatin regeneration, DNA damage response, cell cycle arrest, p53-mediated apoptosis as well as cellular polarization (8). Although

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PJS is a rare genetic disease, the presence of hamartoma polyps can cause severe clinical damage and marked heterogeneity of clinical phenotypes (9). In addition to the above, the STK11 gene is believed to be involved in the regulation of somatic endothelial growth factor (VEGF) and Wnt signal transduction (10). The STK11 tumor suppressor gene, located on chromosome 19p13.3, is responsible for PJS (11). STK11 appears to be inactivated or mutated in diffuse cancers whose spectrum of tumor types cooperates with exposure to environmental carcinogens (7, 12). STK11 is a tumor suppressor gene and is a mutation in the loss of oncogenic function, at least in part due to a loss of regulation of AMP-activated protein kinase (AMPK) mammalian target of rapamycin (Mtor) (12).

STK11 functions as an AMP-kinase kinase in cells. The activity of STK11 is limited in cells with adequate ATP. AMPK also phosphorylates Tuberous sclerosis ½ (TSC1/2), and when mutations occur in STK11, the TSC1/2 complex inhibits mTOR function (13) (Figure 1).

STK11 has a dual role as a tumor suppressor and metabolic regulator by antagonizing the phosphatidylinositol 3-kinase-AKT pathway, which propagates anabolic signals from insulin and proliferation signals from growth factors/oncogenes (14).

Although the genetic heterogeneity of disease has been recognized in over 500 mutations such as serine/threonine-protein kinase 11 in STK11, this genetic heterogeneity is so widespread that many mutations are still unknown (15).

Note that in this case study, in addition to reporting a new mutation in the STK11 gene, as a 'de novo' type, the authors aimed to describe the genotype-phenotype relationship as well as the clinical and molecular

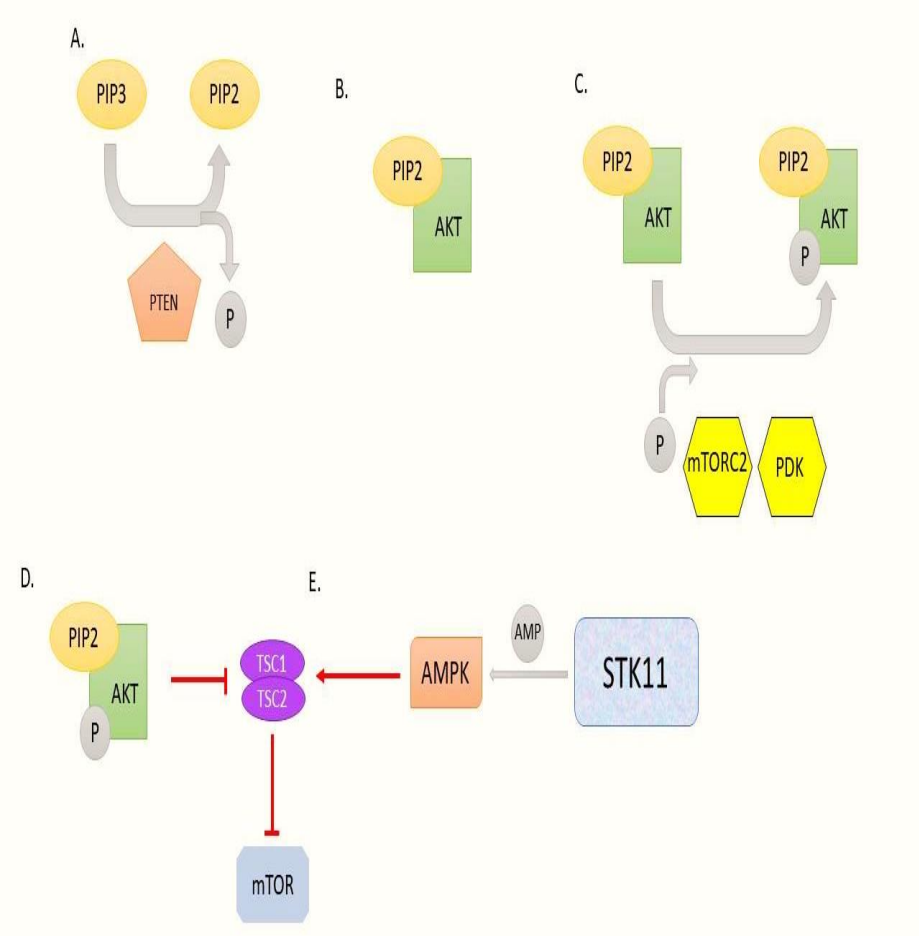

Figure 1. The STK11 /AMPK/mTORC1-dependent regulation of protein translation. STK11 is a master kinase controlling 14 substrates, including AMPK. Following AMP binding to AMPK, this substrate is activated. AMPK activation directly phosphorylates TSC2 to inhibit mTORC1 activity.

characteristics of an image sample. PJS case has been chosen as there is a distinct phenotypic variation between and within the family in PJS. Thus, the availability of predictive genetic testing may be of particular value, but it cannot determine the potential severity of the phenotype.

Both PTEN, phosphatase and tensin homolog and STK11, serine– threonine protein kinase 11 potentially inhibit the mTOR pathway through modulation of TSC1-TSC2, and this dysregulation contributes to the pathogenesis of PJS. Cells sense changes in their AMP/ATP ratio and use the STK11- AMPK kinase cascade to maintain proper anabolic-catabolic homeostasis.

Case report

Clinical information

A 42-year-old Iranian woman with a positive family history of PJS and mucosal nevus pigmentation referred to Taleghani Hospital in Tehran two years ago with abdominal pain and underwent colonoscopy.

This woman's brother died due to PJS at the age of 15, and since this syndrome is an autosomal dominant disease, one of the parents must have been affected by this disease. However, when the patient returned for follow-up and treatment, the father and his mother were not alive, and it was not clear to us which of the parents was the carrier of this disease. Also, the 5-year-old son of this patient also based on evidence and clinical

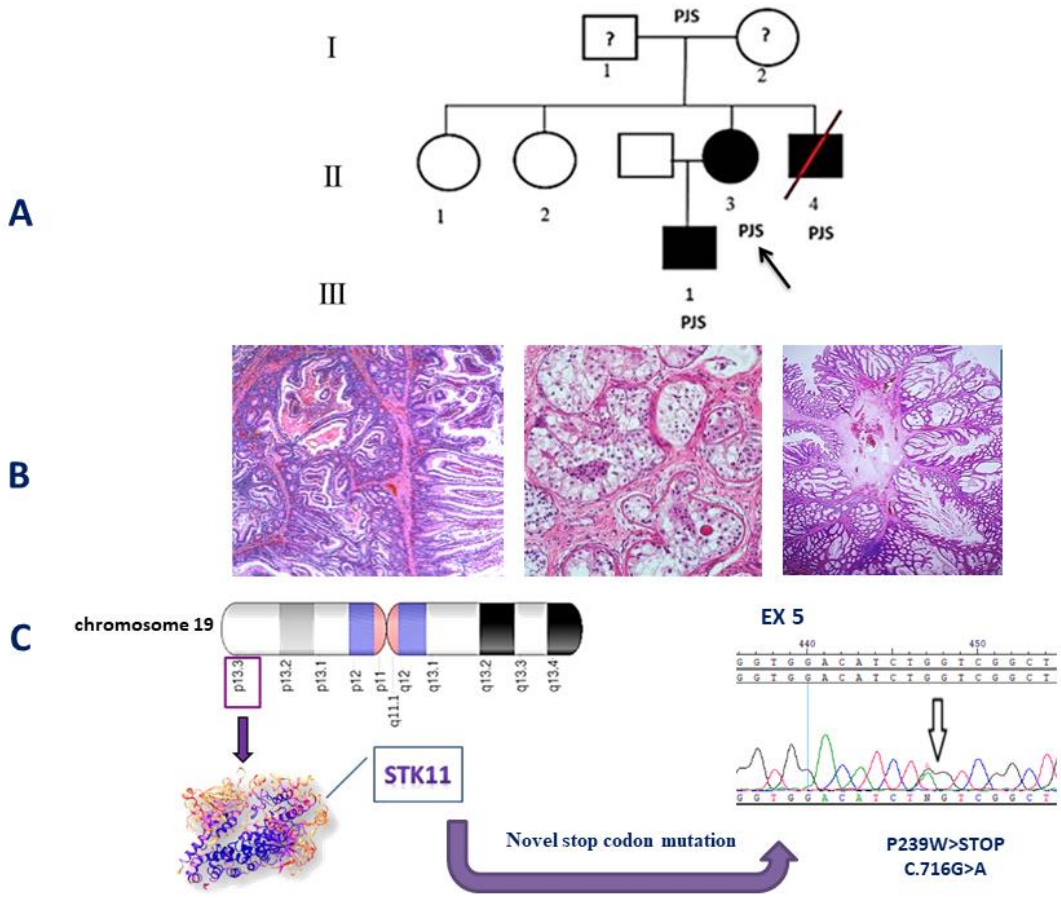

Figure 2. Genogram, pathology, and electropherogram of the examined patient. (A) The genogram showing an isolated PJS patient. Roman numerals indicate generations and Arabic numerals reveal individuals. Squares = males, circles = females. The affected individuals are denoted by solid symbols and unaffected individuals are represented by open symbols. The index patient is indicated by an arrow. (B) View of tissue sections stained with hematoxylin-eosin of gastrointestinal polyp specimens, confirming hamartomatous polyps. (C) Sanger sequencing revealing a heterozygous substitution new mutation, in 5 exon structure of STK11 gene.

symptoms, such as the presence of pigment in both palms as well as in the sole of the right foot, is going through the early stages of this syndrome (Figure 2A).

The report of this colonoscopy indicated the presence of two polyps with dimensions of 2 and 4 cm in the transverse colon. After diagnosis, primary colonoscopy and subsequent annual endoscopic evaluations revealed the presence of multiple diffuse small polyps as well as several large polyps throughout the stomach, colon, and rectum.

The patient referred to the relevant doctor after 2 years with more severe clinical complications as well as the presence of plaque on the eyes and lips, for whom clinical measures such as: endoscopy, colonoscopy, enteroscopy, and CT scan of the abdomen and pelvis were performed.

Endoscopy and colonoscopy showed >1000 polyps throughout the stomach/colon (PJ-type hamartomas); the larger polyp in stomach was resected and the small bowel imaging detected multiple small jejunum/ileum polyps. Also, due to the enlargement of intestinal polyps, the patient had intestinal obstruction and had undergone surgery. The cut tissue samples were sent to the pathology department by the surgeon and the samples were stained with hematoxylin-eosin, and finally confirmed by three expert pathologists (Figure 2B).

Note that all procedures involving human participants were in accordance with the ethical standards of the Medical Ethics Committee, Taleghani Hospital and with the 1964 Helsinki Declaration along with its later amendments or comparable ethical standards.

Mutation analysis

Peripheral blood of the index patient was collected in EDTA anticoagulant tubes. In the laboratory, the genomic DNA of peripheral blood was extracted routinely by Isolation Kit (Roche kit, Germany). According to the manufacturer's instructions, all nine coding exons of the STK11 gene were amplified using the primers listed in Table 1. PCR of STK11 exons was performed in a 50 ul reaction containing 0.4 μM of each primer, 50 ng of genomic DNA, and 25 μl of 2× premix. Ex Taq DNA polymerase (Takara Bio, Japan). PCRs were performed under the following conditions: denaturation at 95°C for 4 min, followed by 35 thermal cycles, each consisting of 95°C for 30 s, 58°C for 30 s, and 72°C for 45 s. Is. The PCR products underwent gel electrophoresis and purification treated with ExoSAP-IT (USP Corporation, Cleveland, OH). The treated PCR fragments were then sequenced using BigDye Terminator (Applied Biosystems, Foster City, CA, USA). The data released from the Sanger sequencing results were analyzed and evaluated using SeqMan from DNASTAR-Lasergene v6 package.

Results

Investigations of the results of Sanger sequencing finally showed five mutations in exons 1 to 5. Further, we performed T vector assay and identified the exact haplotype, which is a two-nucleotide deletion. The major heterozygous germline mutation in stop codon, exon 5 is reported as converted to the amino acid TRP to TER; The TGG codon is converted to TAG by mutation (p.239W>stop, c.716G>A). (Figure 2C).

Table 1. Primers for 9 exon-specific sequencing of STK11 gene

Exon	Forward primer (5′-3′)	Reverse primer (5′-3′)
1	CCGTTGGCACCCGTGACCTA	ACCATCAGCACCGTGACTGG
2	GGGCGGATCACAAGGTCA	AGGAGACGGGAAGAGGAGC
3	TGTGCCCAGAGCAAGAGC	GCAGAAGAATGGCGTGAACC
4 & 5	AGGAGACGGGAAGAGGAGC	TGAACCACCATCTGCCGTAT
6	TGACTGACCACGCCTTTCTT	TGAGGGACCTGGCAAACC
7	CAGGGTCTGTCAGGGTTGTCC	CCGTCCGCTGCTCTGTCTT
8	ACTGCTTCTGGGCGTTTGC	AGGTGGGCTGGAGGCTTT
9	GGTTCTGTGCTGGCATTTCG	GGCTCTGACGCTGGTGGAT

The mutation found in similar studies as well as related databases such as the Human Gene Mutation Database (HGMD), DisGeNET, Human Genome Variation (HGV) and Online Mendelian Inheritance in Man (OMIM) were reviewed and evaluated. No similar items were found.

Discussion

In this case report study, we demonstrated a novel mutation in STK11 causing PJS in a 42-year-old Iranian woman with a positive family history. We identified a novel stop codon heterozygous mutation as a 'de novo' variant (p.239W>stop, c.716G>A) of STK11. This mutation was found only in the target patient. Since the detected mutation consonances with the disease phenotype in the family and the structure-function prediction shows its pathological effect, we conclude this mutation is disease-specific and not a polymorphic variant of the STK11 gene.

Dr Connor first reported this syndrome in the literature in 1895, and finally Dr Bruwer named it in 1954 after two researchers who contributed significantly to it (16).

The pathogenic gene in PJS was eventually identified as STK11 and cloned in 1997 (17).

Serine/threonine kinase STK11 located on chromosome 19p13.3 acts as a Tumor suppressor gene (TSG) by regulating cellular polarity, motility, differentiation, metastasis, and cellular metabolism (18). Serine/threonine-protein kinase is a tumor suppressor which controls the activity of members of the AMP-activated protein kinase (AMPK) family (15). It also phosphorylates non-AMPK family proteins such as STRADA, PTEN, and possibly p53/TP53. It acts as a key upstream regulator of AMPK by mediating phosphorylation (16) and activation of AMPK catalytic subunits PRKAA1 and PRKAA2 thereby regulating processes including inhibition of signaling pathways that promote cell growth and proliferation when energy levels are low, glucose homeostasis in the liver, activation of autophagy when cells undergo nutrient deprivation, and B-cell differentiation in the germinal center in response to DNA damage (17).

Mutations in the STK11 gene have been identified in people with the disease. Many of these mutations lead to the production of an abnormally short and dysfunctional version of the enzyme serine/threonine kinase 11 (18). Other mutations modify the building blocks of proteins (amino acids) used to make enzymes. Mutations in the STK11 gene disrupt the tumor suppressor function of the enzyme, allowing cells to grow and divide uncontrollably (16, 18). This uncontrolled cell growth can lead to the formation of hamartomatous polyps and cancerous tumors. This gene also provides instructions for making an enzyme called serine/threonine kinase 11 (19). This enzyme is a tumor suppressor, meaning that it helps prevent cells from growing and dividing too fast or in an uncontrolled manner (20).

In this regard, during a case series study Ladrian B et al presented in 2020 on three American patients with STK11 mutated tumors, they evaluated genetic changes in STK11 as well as carcinogenesis caused by these changes (21).

In another case study conducted in 2017 by Zhao ZY et al., they examined a patient with PJS, and stated that a new mutation in the STK11 gene led to the development of colorectal cancer in the patient (22). In 2018, the first comprehensive national study based on genetic and clinical analysis of patients with PJS in Greece was conducted by Fostira F et al and reports the spectrum of STK11 mutations in eight families with clinical diagnosis of PJS, briefed the clinical characteristics of sixteen mutation carriers and a National Registry for PJS launched (23).

A genotype-phenotype correlation has been sought in PJS, but the relationship between the type or locus of STK11 gene variants and cancer risk is unclear (24).

Forming a complete genotype-phenotype correlation map according to the findings presented by researchers around the world is very difficult (24, 25, 26). The cancer caused by PJS should be considered as a complex disease in which the interaction of genetic and epigenetic factors plays a significant role in its occurrence and development (25).

According to the mentioned studies conducted in the field of population genetics, many mutations identified in different populations are based on the lifestyle and specific genetics of that population and have been passed as a new mutation.

Conflict of interests

Authors have no potential conflicts of interest to disclose.

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