Molecular Genetics of Peutz-Jegher Syndrome

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Abstract. Peutz-Jeghers Syndrome (PJS) is one of the inherited syndromes associated with polyposis. It is characterized by mucocutaneous pigmentation, especially in the vermillion border of the lips and gastrointestinal tract. This is known as hamartomatous polyposis. The disease results from an autosomal dominant mutation localized in the LKB1 (liver kinase B1) or STK11 (serine/threonine kinase 11) gene on chromosome 19p13.3. The STK11 gene plays a role as a tumor suppressor gene. Mutation in STK11 results in an abnormally shortened or truncated protein, transcriptional splicing errors, and loss of kinase activity. Therefore, somatic inactivation of STK11 will cause formation of hamartomas and tumors in PJS. Yoon et al. identified several types of STK11 gene mutation, including nonsense, missense, splicing site, and ameshift mutations. The other mutation STK11 lead to complications such as cancers, surgical treatment, and increased numbers of polyps. Another mechanism for the inactivation of tumor suppressor genes is promoter hypermethylation of normally unmethylated CpG islands located in promoter regions of DNA repair and tumor suppressor genes. In conclusion, STK11/LKB1 gene mutation is the etiology of PJS.

Keywords: Peutz–Jegher Syndrome, STK11 gene, Genetics, Mutation

1 Introduction

Peutz–Jegher syndrome (PJS) is one of the inherited syndromes associated with polyposis. This syndrome presents with mucocutaneous pigmentation, especially in the vermillion border of the lips and gastrointestinal hamartomatous polyposis [1,2]. Polyps can be located in the gastrointestinal tract; however, the majority are found in the colon (50%–64%) and small bowel (60%–90%) [2]. This syndrome is usually diagnosed in early childhood and can exhibit skin pigmentation or complications such as intussusception, small intestinal bleeding, and obstruction caused by hamartomatous polyps [1].

This disease is caused by an autosomal dominant mutation localized in the LKB1 (liver kinase B1) or STK11 (serine/threonine kinase 11) gene on chromosome 19p13.3.[1,3,4]. The LKB1/STK11 gene is organized into nine exons encompassing nearly 1.3 kb¹ and encodes a 433-amino-acid protein [2,4,5]. Mutations in this gene cause abnormal truncation of the protein, transcriptional splicing errors, and loss of kinase activity [1,4]. Some authors suggest that the STK11 gene plays a role as a tumor suppressor [1,6]. Loss of STK11 function could result in several defects, such
as hypoxia inducible factor 1α, cell polarity, and AMP-kinase-mediated activation of the hypoxia pathway [7].

It is reported that individuals diagnosed with PJS are at a higher risk of developing malignant tumors located in the gastrointestinal and extraintestinal tracts [1,4] such as the reproductive organs, pancreas, and breast [1]. The risk of cancer in patients with PJS is approximately 9–18 times higher than that in unaffected people [4].

1.1 Definition
Peutz–Jeghers syndrome (PJS) is one of the inherited syndromes associated with polyposis and presents with numerous polyps found in the gastrointestinal tract. It commonly presents with mucocutaneous pigmentation, mainly in the vermilion border of the lips. The inheritance pattern of this syndrome is autosomal dominant [2].

![Autosomal dominant inheritance](http://www.daviddarling.info/encyclopedia/A/autosomal_dominant.html)

The main cause of PJS is a germline mutation in the STK11 or serine threonine kinase (formerly named liver kinase B1/LKB1) gene. This gene is located on human chromosome 19p13.3 [2,9]. This mutation has been found in more than 70% of familial PJS cases [9].

![STK11 gene on human chromosome 19p13.3](http://www.daviddarling.info/encyclopedia/A/autosomal_dominant.html)
The first reported discovery of PJS was by Bruwer et al. in 1954. The estimated incidence rate of this syndrome is approximately 1 in 50,000 to 1 in 200,000 births [2].

2 Clinical Features

The first manifestation of PJS is lesions of pigmented mucocutaneous cells (found in nearly 95% of cases). Lesions are usually found during infancy, occurring near the nostrils, mouth, dorsal, and volar aspects of the hands and feet, toes, fingers, and perianal area. Lesions can fade after puberty but tend to remain in the buccal mucosa. Polyps are identified throughout the colon (50%–64%) and gastrointestinal tract (60%–90%) [2]. The main clinical symptoms are small bowel obstruction, abdominal pain, intussusception, anemia, and rectal blood loss, which could cause an increasing rate of laparotomy [11]. Inflammatory block of melanin migration from melanocytes to keratinocytes showed higher melanin in basal cells of pigmented macules in the histology of PJS [2].

Differential diagnosis of PJS is the Carney complex. It is a syndrome that presents with lentigines and spotty skin pigmentation occurring on the face, mainly the oral mucosa, lips, eyelids, and conjunctiva [2].

Fig. 3. Clinical characteristics of PJS patients. Adapted from Hosogi H, Nagayama S, Kawamura J, et al. Molecular insights into Peutz-Jeghers syndrome: two probands with a germline mutation of LKB1. J Gastroenterol [1].

Patients with PJS syndrome are at an increased risk of cancer commonly located in areas of the gastrointestinal tract such as the stomach, pancreas, and upper small bowel. Other extra-gastrointestinal cancers can also occur, such as cancer of the breast, testis, ovary, and pancreas [11].

3 Pathogenesis

The potential pathogenesis of numerous hamartomas in PJS is a mutation located in the STK11 gene, which inhibits phosphorylation of AMP-activated protein kinase, a direct substrate of STK11. Authors confirmed that the loss of heterozygosity found in PJS polyps causes biallelic loss of STK11 expression or function [1].
Another study predicted that mutation in the \textit{STK11} gene could cause a shortened or truncated protein, thus resulting in activity loss, functional loss, and disturbance of the kinase domain. Loss of serine/threonine kinase catalytic activity can cause cancer predisposition syndrome in PJS patients. Another finding suggested that loss of the second functional allele in somatic cells will trigger several disease manifestations. Analysis of an experimental mouse with a heterozygous mutation in \textit{STK11} strongly demonstrated that haploinsufficiency caused polyph formation [12].

Most mutations in the \textit{STK11} gene in PJS cases showed complete loss of the protein product. Functional disruption has been identified in the kinase domain. Therefore, tumor and hamartoma formation is the consequence of somatic inactivation of the wild-type \textit{STK11} allele in PJS patients. Karuman et al. suggested that \textit{STK11} function loss found in PJS caused insufficiency in intestinal epithelial cell apoptosis. Therefore, this defect could be the main reason for benign hamartoma formation and high susceptibility to malignant transformation in PJS patients [13].

4 \textit{STK11} gene and its function

\textit{STK11} is expressed extensively in every tissue [13] The \textit{STK11} gene in humans consists of 10 exons of which nine encode a 433-amino-acid protein, while exon 10 is a noncoding exon [11].

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Fig.4. Coding exons in the \textit{STK11} gene. Adapted from V. Schumacher, T. Vogel, B. Leube et al. \textit{STK11} genotyping and cancer risk in Peutz-Jeghers syndrome. J Med Genet [14].

There are three major domains in the \textit{STK11} protein:
1. N-terminal noncatalytic domain (amino acids 1–49)
2. Catalytic kinase domain (amino acids 49–309)
3. C-terminal noncatalytic regulatory domain (amino acids 309–433) by exons 8 and 9 [4,15,16].

Fig.5. \textit{STK11} gene (Source: https://fr.wikipedia.org/wiki/LKB1) [17]

Sequence analysis showed that the \textit{STK11} kinase domain (codons 50–337) has a weak homology to the conserved catalytic core of the kinase domain common to both Ser/Thr and tyrosine protein kinase family members, whereas the C-terminal domain is not homologous to any known protein [13].
5 STK11 gene mutation

Mutations in PJS are mainly found in the catalytic domain and result in kinase activity dysfunction, which interrupts STK11 expression or function [16]. The STK11 gene acts as a tumor suppressor gene. Therefore, polyps in PJS have demonstrated loss of heterozygosity, which result in loss of STK11 biallelic function. This gene is also recognized to facilitate cellular functions via connections with a number of proteins, for instance, inducing cell cycle arrest through p21 and involvement in the pathway of p53-dependent programmed cell death (apoptosis) [11,18]. STK11 has important roles in energy homeostasis, cell polarity, and cell metabolism [2,18].

One of the key causes of cancer development is homeostatic imbalance between proliferation and cell death. Proliferation of tumor cells could occur without environmental clues and could avoid apoptosis. One of the most important roles of the tumor suppressor gene p53 is its potential capability to suppress cell proliferation and trigger programmed cell death (apoptosis). Therefore, functional loss of STK11 in PJS leads to programmed cell death of intestinal epithelial cells [13].

Yoon et al. determined numerous types of STK11 gene mutations, such as nonsense, missense, splice site, and frame shift mutations. Distinct types and locations of mutations in the STK11 gene correspond with distinct complications such as cancer, surgical treatment, and increasing numbers of polyps. Huang et al. reported that patients who underwent more surgical treatment and suffered from more gastrointestinal polyps were found to have frameshift mutations. Lim et al. proposed that mutations occurring in exon 3 were correlated with a greater risk of developing cancer. Thus, distinct exons and types of mutation may have distinct roles in the impact of PJS [16].


1. Missense
- A study by Mehenni et al. reported a nucleotide change (924G → T) in exon 8, which result in the substitution of Trp308 by Cys or W308C. This mutation causes a dramatic change in the three-dimensional structure of STK11 and elimination of its activity [5].
Fig. 7. Missense mutation with a nucleotide change (924G → T) in exon 8. Adapted from Mehenni H, Gehrig C, Nezu J, et al. Loss of LKB1 Kinase Activity in Peutz-Jeghers Syndrome and Evidence for Allelic and Locus Heterogeneity. Am J Hum Genet [5].

- Another case is a nucleotide change (526G → A) in exon 4, resulting in the substitution of Asp176 by Asn (D176N). Asp176 is an amino acid in the catalytic loop that is important for catalytic enzymatic activity. This amino acid accepts protons from the hydroxyl group of the attacking substrate during the phosphotransferase mechanism [5].


- A missense mutation takes place at c.1062C > C/G in exon 8 in the C-terminal noncatalytic regulatory domain. Loss of this domain results in improper overgrowth of differentiated cells, which leads to loss of cell polarity and the appearance of hamartomas [16].

2. Nonsense

- Transversion of nucleotide 904 in exon 7 from C to T changes the codon CAG (glutamine) to TAG (stop) at position 302. This mutation caused truncation of the \textit{STK11} protein. Structural prediction showed that the mutation caused complete loss of the C-terminal end of the α-helix and damaged half of the kinase domain [4].

![Wild type and codon 302, Glu](image1)

![Mutant type and codon 302, stop](image2)


- Nonsense mutation at codon 240 (exon 5), changing TCG (Ser) to TAG (STOP) and resulting in a truncated protein defective in kinase activity [1].
Mutation located in the catalytic kinase domain of the \textit{STK11} protein. Substitution of nucleotide C to T occurs at exon 5 and results in production of a truncated protein. This patient also developed more gastric polyps [16].

3. Frameshift
   - A single base (A) insertion between nucleotides 574 and 575 in exon 4 resulting in a frameshift at codon K191 produces 73 novel amino acids and premature termination of a 265-residue protein in contrast to normal 433-amino-acid protein [5].
Deletion of one nucleotide (903G) in exon 7 causes a frameshift after codon R301, resulting in 33 novel amino acids and premature termination of a 335-amino-acid protein [5].

4. Splicing site
   • Substitution of G to A at nucleotide +5 in intron 5 of the \( LKB1 \) gene at the donor site. This mutation results in abnormal splicing and a shortened protein because G is the preferred nucleotide at position +5 of the splice site. No cancer was reported in this patient [5].
Fig. 15. Substitution of G to A at nucleotide +5 in intron 5 causes a splicing site mutation. Adapted from Mehenni H, Gehrig C, Nezu J, et al. Loss of LKB1 Kinase Activity in Peutz-Jeghers Syndrome and Evidence for Allelic and Locus Heterogeneity. Am J Hum Genet [5].

- Huang et al. reported a heterozygous de novo mutation in which substitution of nucleotide G by A at intron 6 caused a splice site mutation [16].


- Deletion of 52 base pairs at the terminal of intron 6 and most of exon 7, which ends at nucleotide 904 in exon 7, results in abnormal splicing and a truncated protein. This type of deletion is compatible with the slipped-mispairing model [5].
6 Epigenetics

Epigenetics is the study of inherited alterations in gene expression that occur mitotically and meiotically without alteration in the genomic and chromatin nucleotide sequence [19]. This process is influenced by flexible and dynamic responses to intracellular and extracellular stimuli, physiology, or exposure to the environment, through cell–cell communication between adjacent cells. The environment could modulate alteration of hormone levels, growth factors, neurotropic factors, cytokines, and the stress response [20].

From Fig. 18, some factors might confer an advantage for both behavior and health, while others could be unfavorable and inhibit the human mind and body, resulting in homeostatic disturbance or imbalance that may lead to disease or psychological disorder. These factors are divided into
a. Beneficial: alternative medicine, microbiome (beneficial intestinal bacteria), and exercise
b. Harmful: drugs abuse and toxic chemical exposure
c. Possibly beneficial or harmful depending on the specific influences: disease exposure, diet, therapeutic drugs, psychological state, seasonal changes, social interactions, and financial status [20].

Several chemical modifications could create distinct epigenetic processes that affect DNA, RNA, and proteins. These modifications are DNA methylation, chromatin remodeling factors, histone modifications (phosphorylation, ubiquitylation, and SUMOylation), and noncoding RNAs [19,21].

1. DNA methylation is a process of covalent addition of a methyl group at position 5 on the pyrimidine ring of cytosine. Protein transcription occurs at the cytosine located beside the guanine nucleotide connected by phosphate, known as CpG. CpGs in short stretches form CpG islands, of which 60%–80% are methylated in the human genome [20]. DNA methyltransferases or DNMTs will create 5-methylcytosine (5mC) by catalyzing the transfer of a methyl group to location 5 of a cytosine. This process is achieved by one or more activities of DNMTs and requires a cofactor called S-adenosylmethionine (AdoMet) [19].

![DNA hypermethylation](image19)

Gene promoter regions that are hypermethylated have a role in the suppression of gene expression. This is the most general alteration known in human cancer and leads to broad or wide-spectrum abnormal gene expression [19]. DNA hypermethylation also causes chromatin condensation and silences tumor suppressor genes. DNA hypomethylation activates oncogenes and results in loss of chromosomal stability and activates transposons [22].

2. Histone modification
Histones are the central protein components of chromatin complexes. Histones provide a structural backbone around which DNA is wrapped at certain intervals to
produce chromatin [16]. Histones pack and order DNA into nucleosomes, the building blocks of chromatin. It is reported that each nucleosome has two subunits. The core histones are known as H2A, H3, H2B, and H4 [20]. Histones regulate DNA packaging, which influences transcriptional activity and silencing of transcriptional activity [20]. Histone modifications are posttranslational changes that take place at histone tails, which contain flexible stretches of either terminal residues of C or N and expand from the globular histone octamer [20]. This modification is divided into two main areas:

a. Histone acetylation

This action is executed by enzymes known as histone acetyltransferases (HATs). HATs are responsible for facilitating transcription and adding acetyl groups to lysine residues located on histone tails [20,23].

![Fig.20. Histone acetylation. Adapted from Rodenhisier D, Mann M. Epigenetics and human disease: translating basic biology into clinical applications. CMAJ [22].](image)

b. Histone deacetylation

The acting enzymes are histone deacetylases (HDACs), which eliminate acetyl groups from acetylated lysines, resulting in chromatin compaction and thus inactivation of transcription [20,23].

![Fig.21. Histone deacetylation. Adapted from Rodenhisier D, Mann M. Epigenetics and human disease: translating basic biology into clinical applications. CMAJ [22].](image)

c. Histone methylation

Histone methylation does not alter the charge of the modified residues or histone protein [24,25]. Therefore, it is less likely to directly alter nucleosomal interactions required for chromatin folding and could either repress or activate transcription depending on its location [25]. It mainly occurs on the side chains of lysines and arginines [24]. Arginine methylation of histone H3 and H4 promotes transcriptional activation, whereas lysine methylation of histone H3 and H4 could activate or repress transcription depending on the methylation site [25].
There are two main types of histone methyltransferases, namely lysine-specific and arginine-specific histone methyltransferases. Histone demethylases are classified based on the residue they modify, such as KDM1/LSD1 (lysine-specific demethylase 1) and JmjC (Jumonji domain-containing) [25].

d. Histone phosphorylation

This type of modification mainly occurs on tyrosines, serines, and threonines. However, phosphorylation not only takes place in the tails of the histone N-terminus. Phosphatases and kinases determine the modification level by adding and removing the modification. Transfer of a phosphate group originating from ATP to a hydroxyl group in the target chain amino acid is provided by histone kinases, influencing chromatin structure and adding a large negative charge to histones [24].

e. Histone Ubiquitylation

Ubiquitylation causes a much larger covalent modification than other types of histone modification. It is known that ubiquitin is a 76-amino-acid polypeptide attached to histone lysines by three enzymes, namely E1 activating, E2 conjugating, and E3 ligating enzymes. These enzymes determine substrate specificity and the degree of ubiquitylation. However, this modification can be dismissed via the action of isopeptidases known as de-ubiquitin enzymes. It is reported that this action is fundamental to gene silencing and activity [24].

f. Histone SUMOylation

SUMOylation is related to ubiquitylation and requires small modifier molecules similar to ubiquitin, which could engage a covalent attachment to histone lysines through E1, E2, and E3 enzyme mechanisms. Four core histones were predicted to function by antagonizing acetylation and ubiquitylation, which took place in the same lysine side chain. Thus, more research is needed to elucidate the molecular mechanism [24].

3. RNA silencing

This is a posttranscriptional gene alteration. In RNA silencing, the expression of one or more genes is contained or repressed by small stretches of noncoding RNA called small interfering RNAs (siRNA) and microRNAs (miRNA) [20]. Both miRNA and siRNA hinder translation but by different mechanisms; both are associated with a ribonucleoprotein complex called RNA-induced silencing complex (RISC) [26].

Proteins in the RISC incorporates siRNA and cause it to remain as a single antisense strand to bind with mRNA in a sequence-specific manner. Then, a protein component of the RISC known as slicer cuts the mRNA in the middle binding region. This mRNA cut is identified as abnormal by the cell and thus targets the mRNA for destruction [22].
The miRNA mechanism is a microRNA-induced silencing complex (miRISC) related to mature miRNA. This complex bind to mRNA and inhibits translation [22].

7 Epigenetics in PJS
Another inactivation mechanism acting on tumor suppressor gene in PJS is promoter hypermethylation. The main epigenetic modification in humans is methylation. Changes in methylation patterns have significant roles in the process of tumorigenesis. Particularly, hypermethylation of unmethylated CpG islands that takes place in promoter regions of several DNA repair genes and tumor suppressors,
namely p16, p15, and hMLH1, is associated with loss of expression in primary tumors and cancer lines [29].

Esteller et al. reported STK11 deactivation involving specific patterns of 5’ hypermethylation of CpG islands, thereby causing epigenetic inactivation of the STK11 tumor suppressor gene. This alternative pathway was found in sporadic tumors. In conclusion, the study of Esteller et al. showed the existence of inactivation correlated with hypermethylation of the STK11 gene in a subset of primary tumors and cancer cell lines, which was found in patients with PJS [23].

8 Conclusion
PJS is one of inherited syndromes associated with polyposis and presents with numerous polyps found in the gastrointestinal tract. The main cause is germline mutation of the STK11 or serine threonine kinase (formerly named liver kinase B1/LKB1) gene. This gene is located on human chromosome 19p13.3. Most mutations in the STK11 gene in PJS cases resulted in complete loss of the protein product. Disruption of kinase activity has been identified in the kinase domain. Therefore, tumor and hamartoma formation is the consequence of somatic inactivation of the wild-type STK11 allele in PJS patients. Another inactivation mechanism acting on tumor suppressor gene in PJS is promoter hypermethylation. STK11 deactivation involves a specific pattern of 5’ hypermethylation of CpG islands, thereby causing epigenetic inactivation of the STK11 tumor suppressor gene.

References