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A new mutation of LKB1 gene in a Japanese patient with Peutz-Jeghers syndrome.

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Abstract

Germline mutations of the LKB1 gene are associated with Peutz-Jeghers syndrome (PJS), which is characterized by mucocutaneous pigmentation and gastrointestinal hamartoma with an increased risk of cancer development. In this study, we have employed polymerase chain reaction and DNA sequencing analysis to characterize the LKB1 gene in a 25-year-old Japanese PJS patient. Direct sequence analyses revealed a novel single base deletion at nucleotide 844 in exon 6 (844delC) in one LKB1 allele, resulting in a frame shift and in the introduction of a premature termination codon in this mutated allele.

KEYWORDS: Peutz-Jeghers syndrome(PJS), LKB1, deletion, frame shift

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Short Communication

A New Mutation of *LKB1* Gene in a Japanese Patient with Peutz-Jeghers Syndrome

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Germline mutations of the *LKB1* gene are associated with Peutz-Jeghers syndrome (PJS), which is characterized by mucocutaneous pigmentation and gastrointestinal hamartoma with an increased risk of cancer development. In this study, we have employed polymerase chain reaction and DNA sequencing analysis to characterize the *LKB1* gene in a 25-year-old Japanese PJS patient. Direct sequence analyses revealed a novel single base deletion at nucleotide 844 in exon 6 (844delC) in one *LKB1* allele, resulting in a frame shift and in the introduction of a premature termination codon in this mutated allele.

Key words: Peutz-Jeghers syndrome (PJS), *LKB1*, deletion, frame shift

Peutz-Jeghers syndrome (PJS, MIM#175200) is a rare autosomal dominant disorder characterized by hamartomatous polyposis of the gastrointestinal tract, melanin pigmentation of the mucous membranes and skin, and an apparently increased risk of developing cancer. In 1997 the PJS gene was mapped to chromosome 19 p13.3 [1] and found to encode a serine threonine kinase named *LKB1* or *STK11* [2, 3]. *LKB1/STK11* consists of 9 exons that span 23 kb and encode a 433-amino acid protein. *LKB1* is involved in not only carcinogenesis but also vasculogenesis. The overexpression of *LKB1* in a number of tumor cell lines that do not express this protein kinase has been shown to suppress cell growth by inducing G1 cell cycle arrest [4]. Many of the mutations that have been mapped to *LKB1* would be expected to impair its activity, suggesting that *LKB1* functions as a tumor suppressor. Knockout mice have also indicated that the *Lkb1* gene is a tumor suppressor gene. Nakau *et al.* reported that hepatocellular carcinoma was caused by a

loss of heterozygosity in the *Lkb1* gene knockout mice [5]. Mice homozygous for a targeted disruption of *Lkb1* undergo embryonic lethality at midgestation as a result of defective vasculogenesis associated with a tissue-specific deregulation of vascular endothelial growth factor [6].

Mutation screening of the *LKB1/STK11* gene in PJS patients has identified different mutations to date [2, 3, 7-12]. About three quarters of PJS cases occur in families, with the remainder resulting from new mutations or low penetrance variants.

Here, we report a new *LKB1* mutation in a PJS patient. The patient was a 25-year-old Japanese male with melanin pigmentation of the mucous membranes. Upper and lower gastrointestinal endoscopic examinations revealed polyposis not only in the stomach but also in the ileum, colon, and rectum, with the polyps being approximately 5 mm in size. As shown in Fig. 1A, histological diagnosis of the colon polyp showed a hamartomatous polyp. Therefore he was diagnosed as PJS. His mother is healthy and his father had died of esophageal cancer. Information regarding gastrointestinal polyposis of his father was uncertain and his parents were not consanguineous. We have attempted to identify the mutations of

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the *LKB1* gene from a colonic biopsy specimen. Informed consent for genetic analysis was obtained from his mother, not from the patient, because the patient was on a lengthy stay abroad when the analysis was carried out.

Genomic DNA was prepared from a formalin-fixed and paraffin-embedded tissue by standard methods using phenol/chloroform extraction and ethanol precipitation. The exons and the intron/exon boundaries in the *LKB1* gene were analyzed by polymerase-chain-reaction (PCR) and direct sequencing of the PCR products [13]. The primers used for the amplification of the genomic DNA were as follows: PJ, ex6F, 5'-TTG ACT GAC CAC GCC TTT CTT C-3', PJ, ex6R, 5'-CGC CTC CCT GGG GCT GCG GGC AG-3'. Amplified DNA fragments were recovered from a low melting temperature agarose gel and used for the sequencing analysis. We sequenced the PCR products directly by using a DNA sequencing system (model 377; Applied Biosystems). The direct sequencing was performed in both directions and the mutation analysis was repeated independently. Nucleotide numbering is based on Genbank sequence AF032984 (for exon 1) and AF032985 (for exons 2-8).

We successfully screened this patient with PJS for *LKB1* mutation. As shown in Fig. 1B, mutational analysis of the *LKB1* gene using a pair of primers, ex6F and ex6R, revealed a heterozygous single nucleotide deletion at nucleotide 844 in exon 6 which corresponds to amino acid position 282. Nucleotide position 1 is the A in the ATG translation initiation codon in the *LKB1* cDNA sequence. Because of this mutation, the frame shift and the introduction of a premature termination codon at codon position 286 are expected in this mutated allele. The genomic DNA used in this study originated from the whole biopsy specimen which included not only epithelia but also normal lymphocytes, plasma cells, endothelial cells, fibroblasts so on, therefore the mutation of 844delC is supposed to be the germline mutation.

To date, about 80 kinds of mutations of the *LKB1* gene have been reported [2, 3, 7-12], but the 844delC mutation has never been reported. Table 1 shows the mutations of exon 6 of the *LKB1* gene in PJS. Five of nine kinds of mutations were found in Japanese patients. The reported mutations of *LKB1* gene have been found from exon 1 to exon 9 and there are no hot spots, although Westerman *et al.* reported that exon 1 of the *LKB1* gene is a hot spot in PJS [7]. Nakagawa *et al.* reported that there was one kind of insertion mutation in

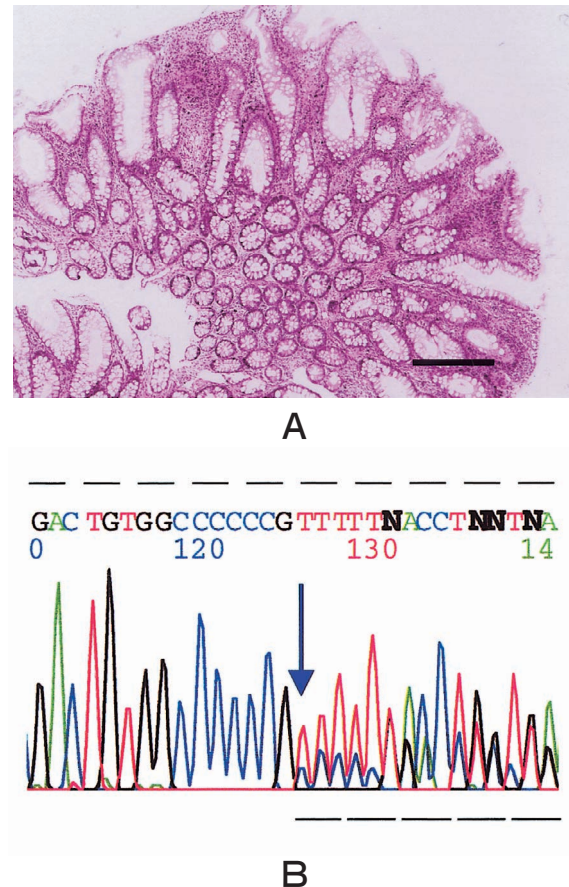


Fig. 1 **A**, Histopathology of the colon polyp in the PJS patient. Hamartomatous epithelia and thick lamina propria are shown (Hematoxylin-Eosin; HE stain). A bar indicates 500 μm . **B**, A single base deletion in exon 6 of the *LKB1* gene in a patient with PJS. Direct sequence analysis to amplify the genomic DNA obtained from the patient reveals a heterozygous C deletion (844delC) in exon 6 of the *LKB1* gene. The first nucleotide C of the 282nd codon in the wild-type allele is deleted in the mutant allele. Numbers from 277 to 286 indicate amino acid numbers. The nucleotide sequence shown on the top of Fig. 1B indicates the normal allele, while the mutated allele is shown on the bottom of Fig. 1B. The nucleotide deletion of C (844delC) in the mutated allele is shown by an arrow. The 286th codon of the mutated allele is a premature termination codon.

exon 1, one deletion in exon 3, one deletion in exon 5, and 4 kinds of mutations in exon 6 among Japanese patients [9]; therefore, exon 6 of the *LKB1* gene may be a hot spot in Japanese PJS. Mehenni *et al.* reported another responsible gene for PJS is located on 19q13.4 [11]. Their report, taken together with the report that shows no mutations in the *LKB1* gene in PJS, indicates that identification of the responsible gene is important to

Table 1 Mutations of exon 6 of *LKB1* gene in PJS

Type of mutation	Codon	Nucleotide change	References
2 bp substitution	246-247	CA- > AG	Nakagawa <i>et al.</i> , 1998
3 bp deletion	247	delAAC	Nakagawa <i>et al.</i> , 1998
1 bp substitution	251	G- > A, G251S	Resta <i>et al.</i> , 1998
4 bp deletion	263		Resta <i>et al.</i> , 1998
2 bp deletion	277-278		Hemminki <i>et al.</i> , 1998
1 bp deletion	281	delC	Nakagawa <i>et al.</i> , 1998
1 bp insertion	281	insC	Nakagawa <i>et al.</i> , 1998
1 bp deletion	281	delC	Wang <i>et al.</i> , 1999
1 bp deletion	282	delC	Present case

our understanding of the molecular basis of PJS. As well, somatic mutations of the *LKB1* gene have been demonstrated in lung adenocarcinomas [14]; therefore, the molecular mechanism of carcinogenesis involved in the *LKB1* gene is of importance in understanding the relationship between gastrointestinal polyposis and cancer.

Rossi DJ *et al.* demonstrated intriguing data in which mice heterozygous for a targeted inactivating allele of *Lkb1* developed severe gastrointestinal polyposis, and that histological features of polyps arising in the *Lkb1* ± mice were found to be hamartomas, similar to the polyps resected from PJS patients [15]. They also observed that COX-2 was highly up-regulated in 75% of the polyps examined, indicating that COX-2 induction was a common feature of hamartoma formation in *Lkb1* ± mice. As well, elevated COX-2 expression was observed in 70% of the polyps of PJS patients. Marignani *et al.* reported that *Lkb1* is associated with Brahma-related gene 1 (*Brg1*) and is necessary for *Brg1*-induced growth arrest [16]. *Brg1* is an essential component of chromatin remodeling complexes, therefore it is possible that the mutations of the *Lkb1* gene disturb *Brg1*-dependent growth arrest and cause the hyperplasia of gastrointestinal epithelia.

Since correlations between mutation sites of the *LKB1* gene and cancer development have not been reported, it is important to examine whether to determine the existence of any genotype-phenotype correlations.

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