**Immunocytochemistry.** Oocyte pairs were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) and embedded in acrylamide. Cryostat sections (14–20 μm) were labelled with Shab-B antisera (1:400 in blocking solution; 50 mM Tris-HCl, 4% normal goat serum, 0.2% Triton X-100 in PBS) followed by an FITC-conjugated secondary antibody (Pierce Warriner, 1:100 in blocking solution) and mounted in Citifluor (Agar Scientific). Sections were imaged with a confocal microscope (MRC 600, Bio-Rad).

**Electrophoresis and western blotting.** Oocyte membrane proteins and Drosophila nervous system proteins were solubilized in SDS gel-loading buffer, containing 8 M urea, at room temperature. Proteins were electrophoretically separated on 12.5% SDS-polyacrylamide gels and transferred, in CAPSO buffer (25 mM CAPSO (Sigma), 20% methanol, pH 10), to nitrocellulose (Hybond-C, Amersham). Blots were labelled with Shab-B antisera (1:500 in blocking solution: 5% Marvel, 2% bovine serum albumin, 0.1% Tween-20 in PBS) followed by a peroxidase-conjugated secondary antibody (Sigma, 1:1000 in blocking solution) and developed with ECL (Amersham). Gels were scanned with a laser densitometer (Sharp) using ImageMaster ID (Pharmacia).

**Electrophysiology.** Individual oocytes of pairs were impaled with two borosilicate glass microelectrodes (1–4 MΩ) filled with a solution: 5% Marvel, 2% bovine serum albumin, 0.1% Tween-20 in PBS) and 10 mM HEPES, 10 mM EGTA, pH 7.5, and recorded using the double voltage-clamp procedure. Protocols to determine junctional conductance (gj) and its sensitivity to transjunctional voltage (Vj) and inside-outside voltage (Vio) were as described. Received 18 August; accepted 16 September 1997.

were exclusively on the chromosome inherited from the unaffected parent, raising the possibility that the target of the deletions was a tumour-suppressor gene underlying PJS\(^2\). Linkage analysis in PJS families using chromosome 19p markers confirmed the presence of a high-penetrance susceptibility locus telomeric to marker D19S565.

A search for the predisposing gene was made by using different approaches; efforts were facilitated by a complete cosmid contig across the putative PJS gene region, created as part of the human chromosome-19 mapping project (ref. 9, and http://www-bio.llnl.gov/). The PJS region was first narrowed down to 800 kilobases (kb) around markers D19S886 and D19S883 by meiotic recombination mapping in PJS families using microsatellite and biallelic polymorphisms developed for this purpose. Transcripts in this interval were identified by database searches and by solution hybridization of complementary DNA derived from human testis or colon RNA to genomic DNA from cosmids mapping to the PJS gene region (direct cDNA selection\(^10,11\)). Twenty-seven transcripts were screened for mutations, typically by sequencing of products obtained by reverse transcription followed by polymerase chain reaction (RT-PCR) from lymphoblastoid cell lines derived from PJS patients. In some cases, Southern blot analysis of genomic DNA was also done. Among the transcripts detected by cDNA selection was \(LKB1\), a gene predicted to encode a 433-amino-acid protein showing strong sequence similarity to the serine/threonine protein kinases (GenBank accession number U63333; ref. 4). The genomic location of \(LKB1\) has not previously been reported, but, using genomic cosmid sequence data, we confirmed that \(LKB1\) is located between the genes CIRP and GPX4, in accordance with the cDNA selection results (a map of genes in this area is available at http://www-bio.llnl.gov/).

Primer were designed to analyse mutations in the \(LKB1\) cDNA. The sequence available in the database covered only the translated region, and hence it was not possible to amplify the whole coding region for mutational screening purposes. However, the primers chosen amplified most of the coding sequence (87%), and this could be screened for mutations by RT-PCR and sequencing in 12 familial PJS patients. Each of the 12 families was compatible with linkage of the PJS phenotype to chromosome 19p. Evidence of a putative mutation in this gene was first obtained from the case (SL8) whose polyps were originally used in the localization of PJS. RT-PCR products from this individual gave a smaller cDNA fragment in addition to the normally sized band. Sequence analysis revealed a heterozygous 188-bp deletion (nucleotides 921–1,108) in the cDNA which is predicted to generate a translational frameshift (Table 1). Subsequently, eight further heterozygous mutations predicted to cause truncation of the encoded protein were identified among the patients. Genomic sequences (see Methods) are presented in the 5' to 3' direction and the location of the mutation is marked by an arrow. a, Patient SL32: a nonsense mutation in codon 84 (lysine \(\rightarrow\) stop). b, Patient SL31: a 2-bp deletion in codons 277–278. c, Patient SL25: a missense mutation in codon 67 (leucine \(\rightarrow\) proline). d, Patient SL26: a 9-bp deletion in codons 303–306. e, Patient SL12: a nonsense mutation in codon 57 (glutamic acid \(\rightarrow\) stop).

![Figure 1](image1.png)

**Figure 1** Examples of heterozygous mutations identified in \(LKB1\) gene in PJS patients. Genomic sequences (see Methods) are presented in the 5' to 3' direction and the location of the mutation is marked by an arrow. a, Patient SL32: a nonsense mutation in codon 84 (lysine \(\rightarrow\) stop). b, Patient SL31: a 2-bp deletion in codons 277–278. c, Patient SL25: a missense mutation in codon 67 (leucine \(\rightarrow\) proline). d, Patient SL26: a 9-bp deletion in codons 303–306. e, Patient SL12: a nonsense mutation in codon 57 (glutamic acid \(\rightarrow\) stop).

![Figure 2](image2.png)

**Figure 2** Segregation of a nonsense mutation in a PJS family including patient SL20 (lane 3). The heterozygous mutation is detected by Accl digestion of a 450-bp PCR product. The mutant allele (displayed as two fragments of sizes 286 and 164bp) segregates with the disease phenotype. The numbers above the lines correspond with those in the pedigree. A molecular size marker (\(\Phi X174\)) is shown on the left.
the other a deletion of 9 bp. Although these were not detected in 98 and 49 control individuals respectively (Table 1), at present we cannot exclude the possibility that they represent rare polymorphisms. Owing to an ongoing large-scale genomic sequencing effort in the Lawrence Livermore National Laboratory (http://www-bio.llnl.gov), we were able to design genomic primers for detection of these two changes, as well as for seven of the other changes, enabling us to confirm the genomic presence of these nine sequence variants and to study segregation of seven of the variants in the respective PJS families. All seven changes segregated with the PJS phenotype (Table 1 and Fig. 2). One of the 12 PJS cases screened does not currently show sequence variants in LKB1. The absence of mutations in this case may be due to incomplete screening of the gene, insensitivity of our mutation-detection methods, transcript instability as a result of the presence of a mutation, large genomic deletions that are undetectable by the PCR-based approach used, or genetic heterogeneity, with mutations present at another distinct locus. Northern blotting and RT-PCR analysis indicated that LKB1 is expressed in all tissues examined: liver, skeletal muscle, kidney, pancreas, heart, brain, placenta, lung, spleen, thymus, testis, peripheral blood leukocyte, colon, ovary, small intestine and prostate.

LKB1 was originally identified as a serine/threonine protein kinase expressed in human fetal liver and shows weak sequence homology to many protein kinases over the conserved catalytic core of the kinase domain (between codons 50–337) that is common to both serine/threonine and tyrosine protein kinase family members. The kinase domain (between codons 50–337) is located between codons 50 and 337 in the human LKB1 sequence and which was identified through a systematic search of genes encoded kinase activity. Although activation of kinase activity may be responsible for cancer susceptibility in multiple endocrine neoplasia type 2 (RET)13, familial renal papillary cancer (MET)14 and in familial melanoma (CDK4)15, to our knowledge this is the first gene that predisposes to cancer as a result of disabling its encoded kinase activity.

### Table 1 LKB1 sequencing results in the 12 PJS patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Codon</th>
<th>Nucleotide change</th>
<th>Predicted effect</th>
<th>Control individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLB</td>
<td>307–370</td>
<td>188-bp deletion</td>
<td>Frameshift, stop at codon 404</td>
<td>0/86</td>
</tr>
<tr>
<td>SL12*</td>
<td>57</td>
<td>G → T</td>
<td>Glutamic acid → stop</td>
<td>0/98</td>
</tr>
<tr>
<td>SL14*</td>
<td>66–75</td>
<td>29-bp deletion</td>
<td>Frameshift, stop at codon 152</td>
<td>0/98</td>
</tr>
<tr>
<td>SL20*</td>
<td>70</td>
<td>G → T</td>
<td>Glutamic acid → stop</td>
<td>0/98</td>
</tr>
<tr>
<td>SL25*</td>
<td>67</td>
<td>T → C</td>
<td>Leucine → proline</td>
<td>0/98</td>
</tr>
<tr>
<td>SL26*</td>
<td>303–306</td>
<td>9-bp deletion</td>
<td>Tyrrosine → stop</td>
<td>0/98</td>
</tr>
<tr>
<td>SL27*</td>
<td>60</td>
<td>G → G</td>
<td></td>
<td>0/98</td>
</tr>
<tr>
<td>SL28*</td>
<td>55–57</td>
<td>1-bp insertion</td>
<td>Frameshift, stop at codon 162</td>
<td>0/98</td>
</tr>
<tr>
<td>SL29</td>
<td>98–165</td>
<td>174-bp deletion</td>
<td>Truncated (in-frame) product of 376 amino acids</td>
<td>0/59</td>
</tr>
<tr>
<td>SL30</td>
<td></td>
<td>No changes</td>
<td></td>
<td>0/72</td>
</tr>
<tr>
<td>SL31†</td>
<td>277–278</td>
<td>2-bp deletion</td>
<td>Frameshift, stop at codon 283</td>
<td>0/72</td>
</tr>
<tr>
<td>SL32†</td>
<td>84</td>
<td>A → T</td>
<td>Lysine → stop</td>
<td>0/66</td>
</tr>
</tbody>
</table>

*Variant shown to segregate with the PJS phenotype in the respective family.
†Confirmed by genomic sequencing.

Figure 3 Sequence alignment of LKB1 and XEEK1 proteins and a homologous mouse EST (mLKB1) predicted protein sequence created by using the ClustalW17 e-mail server. Numbers refer to amino acids in the respective sequences. The protein kinase domain presenting a strong consensus sequence locates between codons 50 and 250 in the human LKB1 sequence. The analysis of the mouse EST reading frames revealed a possible sequencing error at the nucleotide position 266 (ref. 13). A putative loss of a single nucleotide T in the GenBank sequence at this site has led to a frameshift and loss of homology for the rest of the predicted mouse amino-acid sequence. The homology between human and mouse protein sequence was retained (as shown here) by adding a nucleotide G at position 265 in the mouse sequence.

12 patients (Table 1, Fig. 1). The nature of the mutations is consistent with the idea that the gene is inactivated during oncogenesis and conforms with the model of a recessive tumoursuppressor gene suggested by allele loss in PJS polyps2. Two nontruncating changes were detected, one an amino-acid substitution,
No major disturbances of development are seen in PJS individuals heterozygous for the LKB1 defect. It is likely that initiation of hamartoma formation occurs after somatic inactivation of the wild-type LKB1 allele. The function of LKB1 in human development remains obscure, but may be revealed by model systems, such as mice homozygous for the defect.

It should now be possible to offer predictive genetic testing to members of PJS families. The relatively small size of the gene and the nature of the mutations should facilitate the implementation of such diagnostic procedures.

Methods

Patient samples and RNA. Cell lines were prepared from PJS patients’ blood samples using standard methods, and RNA was extracted using the RNAeasy kit (Qagen).

RT-PCR. 20 μl cDNA was created from 0.8 μg of each of the patients’ RNA samples using standard random priming methods with M-MLV reverse transcriptase (Promega) and RNase inhibitor (Promega). RT-PCR was usually performed in DNA Engine thermal cyclers (MJ Research) under the following conditions: 3 μl cDNA, 1 × buffer (with MgCl₂), 250 μM dNTPs, 0.8 μM reverse and forward primers, 2 units of AmpliTaq Gold polymerase (Perkin Elmer) and water in a final volume of 50 μl. Primers were designed for each transcript studied using primer 3 (http://www-genome.wi.mit.edu/). For LKB1, only the protein-coding sequence without surrounding untranslated regions was available. In most cases, the following primer pair was used to amplify 1,135 of the total 1,302 bp (87%) of the reading frame (each primer 5′ to 3′): LKB1F: GAGCTGATGTCGGTGGGTAT; LKB1R: GCCCTGGATTTGGACCTTG.

Selection of transcripts from the candidate region was made using solution hybrid capture according to ref. 11. Individual cosmid clones spanning the critical region were digested with restriction enzymes I. These digests were combined and ligated to cDNA, which was then amplified in the PCR using the primer AGCAAGTTCAGCCTGGTTAAG. After amplification, 1,135 of the total 1,302 bp (87%) of the reading frame (each primer 5′ to 3′): LKB1F: GAGCTGATGTCGGTGGGTAT; LKB1R: GCCCTGGATTTGGACCTTG.

Sequencing of PCR products. Sequencing was performed using either ABI PRISM Dye Terminator or ABI PRISM 4Dhodamine cycle sequencing kits (Perkin-Elmer) according to the manufacturer’s instructions, and reactions were run on an ABI 373 A or 377 sequencer (Perkin Elmer), respectively.

Genomic PCR. The following intronic primers were used for genomic LKB1 PCR amplifications: for nucleotides 1–290: GGAAGTCAGGAAACAAGAGGA and GGGAGGAGGAGGAGGAGGAA; for nucleotides 735–862: TACAAGATCCATGACA and ACCACCCCAAAACCCTAGATT; for nucleotides 863–920: GAGGATGGTCGGCCCTGTTG and CTTAACCCGGTCGCCAC.

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COPII–cargo interactions
direct protein sorting into ER-derived transport vesicles

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Vesicles coated with coat protein complex II (COPII) selectively transport molecules (cargo) and vesicle fusion proteins from the endoplasmic reticulum (ER) to the Golgi complex1–3. We have investigated the role of coat proteins in cargo selection and recruitment. We isolated integral membrane and soluble cargo proteins destined for transport from the ER in complexes formed in the presence of Sar1 and Sec23/24, a subset of the COPII components, and GTP or GMP-PNP. Vesicle fusion proteins of the vSNARE family and Emp24, a member of a putative cargo carrier family4, were also found in COPII complexes. The inclusion of amino-acid permease molecules into the complex depended on the presence of Sh3, a protein required for the permease to leave the ER-derived transport vesicles.