**LKB1 exonic and whole gene deletions are a common cause of Peutz-Jeghers syndrome**


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**Background:** LKB1/STK11 germline mutations cause Peutz-Jeghers syndrome (PJS). The existence of a second PJS locus is controversial, the evidence in its favour being families unlinked to LKB1 and the low frequency of LKB1 mutations found using conventional methods in several studies. Exonic and whole gene deletion or duplication events cannot be detected by routine mutation screening methods.

**Objective:** To seek evidence for LKB1 germline deletions or duplications by screening patients meeting clinical criteria for PJS but without detected mutations on conventional screening.

**Methods:** From an original cohort of 76 patients, 48 were found to have a germline mutation by direct sequencing; the remaining 28 were examined using multiplex ligation dependent probe amplification (MLPA) analysis to detect LKB1 copy number changes.

**Results:** Deletions were found in 11 of the 28 patients (39%)—that is, 14% of all PJS patients (11/76). Five patients had whole gene deletions, two had the promoter and exon 1 deleted, and in one patient exon 8 was deleted. Other deletions involved: loss of exons 2–10; deletion of the promoter and exons 1–3; and loss of part of the promoter. No duplications were detected. Nine samples with deletions were sequenced at reported single nucleotide polymorphisms to exclude heterozygosity; homozygosity was found in all cases. No MLPA copy number changes were detected in 22 healthy individuals.

**Conclusions:** These results lessen the possibility of a second PJS locus, as the detection rate of germline mutations in PJS patients was about 80% (59/76). It is suggested that MLPA, or a suitable alternative, should be used for routine genetic testing of PJS patients in clinical practice.

**Methods**

A diagnosis of PJS was made when the patient presented with two or more PJS polyps, or one polyp and a family history of PJS. A cohort of 76 patients meeting these criteria had been screened previously for germline LKB1 mutations using direct sequencing of each exon plus intron–exon boundaries. In 48 patients, small scale mutations had been found, including protein truncating changes, missense variants targeting phosphorylation sites or other highly conserved residues, and splice site variants. In all, 28 patients without detected mutations (13 familial, 13 isolated cases, two unknown) were identified for the present study. The multiplex ligation dependent probe amplification (MLPA) LKB1/STK11 kit, designed to search for LKB1 deletions or duplications at exon or whole gene level, was obtained from MRC-Holland (Amsterdam, Netherlands). MLPA reactions were carried out using the MLPA kit protocol.

**Conclusion**

The original report of LKB1 mutations in PJS demonstrated mutations in about 90% of cases (11/12) using direct sequencing of constitutional DNA and mRNA. Most subsequent screens for mutations in PJS have produced a similar, although slightly lower, frequency of patients with LKB1 mutations, although the methods used for mutation detection have varied. Two studies, however, found mutations in only 10% and 18% of PJS cases, although the proportion of familial cases in the latter study was low. These findings have led to claims that a single PJS locus cannot explain all cases of PJS. Support for this contention has been provided by linkage studies in PJS families, which have reported exclusion of the LKB1 locus (chromosome 19p13.3). Countervailing arguments have been that many tumour susceptibility genes harbour a large proportion of mutations which cannot be detected by routine screening methods, and that several conditions—even including normal variants—may mimic the pigmentation of PJS, leading to diagnostic misclassification.

Whole gene and exonic deletions and duplications cannot be detected using routine mutation screening methods. The high frequency of such changes in diseases such as von Hippel-Lindau syndrome, and the increasing realisation that conditions such as hereditary non-polyposis colon cancer (HNPCC) are often caused by such changes, we screened for gene and exon scale mutations in a set of PJS patients without detected LKB1 mutations. The results have implications for diagnostic genetic testing in PJS patients and for the hypothesised second PJS locus.

**Abbreviations:** MLPA, multiplex ligation dependent probe amplification; PJS, Peutz-Jeghers syndrome; SNP, single nucleotide polymorphism

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**P**eutz-Jeghers syndrome (PJS) is diagnosed by the presence of multiple hamartomatous polyps of characteristic morphology, usually accompanied by mucocutaneous “freckling”. PJS patients often present in their first decade of life with intussusception, obstruction, or volvulus caused by small bowel polyps. The main complication of PJS later in life is a greatly increased risk of colorectal and upper gastrointestinal cancers, and of tumours at multiple other sites. PJS is caused by germline mutations in the LKB1/STK11 gene. LKB1 acts as a tumour suppressor in humans, and loss of function may cause multiple defects, including those of cell polarity, activation of the hypoxia pathway through AMP kinase and hypoxia inducible factor 1α, and increased Wnt signalling.

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**Figure:** This article is available free on JMG online via the JMG Unlocked open access trial, funded by the Joint Information Systems Committee. For further information, see http://jmg.bmj.com/cgi/content/full/42/2/97

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**Abbreviations:** MLPA, multiplex ligation dependent probe amplification; PJS, Peutz-Jeghers syndrome; SNP, single nucleotide polymorphism
Table 1: MLPA results for patients with LKB1 changes

<table>
<thead>
<tr>
<th>Patient ID(s)</th>
<th>Result</th>
<th>Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>11200</td>
<td>Whole gene deletion</td>
<td>Sporadic</td>
</tr>
<tr>
<td>11199</td>
<td>Whole gene deletion</td>
<td>Sporadic</td>
</tr>
<tr>
<td>11668</td>
<td>Whole gene deletion</td>
<td>Sporadic</td>
</tr>
<tr>
<td>24/1/1</td>
<td>Whole gene deletion</td>
<td>Unknown</td>
</tr>
<tr>
<td>02-551</td>
<td>Whole gene deletion</td>
<td>Familial</td>
</tr>
<tr>
<td>000/1/1, 52/1/1</td>
<td>Promoter and exon 1 deletion</td>
<td>Familial</td>
</tr>
<tr>
<td>09/1/1, 11181</td>
<td>Promoter and exon 1 deletion</td>
<td>Familial</td>
</tr>
<tr>
<td>13/1/1</td>
<td>Exon 8 deletion</td>
<td>Familial</td>
</tr>
<tr>
<td>7282/2</td>
<td>Exon 2–10 deletion</td>
<td>Familial</td>
</tr>
<tr>
<td>AXY</td>
<td>Promoter deletion</td>
<td>Sporadic</td>
</tr>
<tr>
<td>LEH</td>
<td>Promoter and exon 1–3 deletion</td>
<td>Familial</td>
</tr>
</tbody>
</table>

LKB1 is a highly conserved protein, especially within the kinase domain (codons 48–309), and deletion of the promoter region or single or multiple exons are all predicted to have profound effects on kinase activity. Exon 8 encodes amino acids 308–369, distal to the kinase domain, but functional effects of such C-terminal mutations have been shown using in vitro assays. Sapkota et al.10 found that phosphorylation of residue 431 was essential for cell growth suppression by LKB1 and Forget et al11 showed that C-terminal LKB1 mutations reduced activation of AMPK and diminished the ability of LKB1 to induce and maintain cell polarity.

Large scale germline deletions have very rarely been reported in PJS, probably because methods suitable for their detection have not been used. Jiang et al.12 found one PJS patient with a deletion spanning exons 2–7 of LKB1. One whole gene deletion and one genomic rearrangement (a ~2 kb deletion) has been reported in two PJS patients.13 21 Another study7 used long range polymerase chain reaction to abrogate13 order from exon 3 to exon 8 of LKB1 in order to search for products of novel sizes, but found no changes in their PJS patients. However, this method would not have detected any of the cases in our study with whole gene deletions or deletion of the promoter region and exon 1.

The finding of relatively frequent, large scale germline LKB1 mutations in PJS patients strongly suggests that MLPA testing (or some suitable alternative) should be introduced into the diagnostic genetics laboratory. Our findings also have consequences for the likelihood that there exists a second uncharacterised PJS locus. Analysis of published LKB1 mutations shows that a mean of 59% of PJS cases (median = 58%, range = 10%–100%) have harboured a germline mutation, which was detectable using standard DNA based screening methods such as single stranded conformational polymorphism (SSCP) analysis, conformation specific gel electrophoresis (CSGE), DHPLC, and direct sequencing of coding regions and intron–exon boundaries.1 4 7 9 10 12 13 14 15 16 17 18 19 20 21 In addition, we estimate that approximately 10% of cases appear to have mutations which are detectable using RNA based methods only.12 13 14 Therefore, allowing for a mutation detection rate of about 40% by MLPA in the remaining 30% of PJS cases, the frequency of PJS patients with detectable mutations becomes about 80–85%. Given that the mutation detection rate in familial PJS cases has been consistently higher in families than in isolated cases, it is likely that a proportion of the latter have only had a presumptive or possible diagnosis of PJS.

In our view, therefore, the proportion of unambiguous PJS cases with LKB1 mutations is sufficiently high for there to be no good case for a second PJS locus based on these data alone. During the preparation of this paper, another study on germline LKB1 deletions10 reached identical conclusions. Seventeen of 34 PJS patients without small scale LKB1 mutations were found to have germline deletions by MLPA. The authors found that when only patients who met the clinical criteria for PJS were considered, the overall mutation detection rate was 94% (64% point mutations and 30% large deletions), very close to our estimate from this study. We therefore conclude that the remaining evidence for the existence of an uncharacterised minor PJS locus therefore comes solely from families whose disease is unlinked to LKB1.

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