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


J. Med. Genet. 2006;43:18-
doi:10.1136/jmg.2005.039875

Updated information and services can be found at:
http://jmg.bmjjournals.com/cgi/content/full/43/5/e18

These include:

References
This article cites 31 articles, 10 of which can be accessed free at:
http://jmg.bmjjournals.com/cgi/content/full/43/5/e18#BIBL

Rapid responses
You can respond to this article at:
http://jmg.bmjjournals.com/cgi/eletter-submit/43/5/e18

Email alerting service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Topic collections
Articles on similar topics can be found in the following collections

Other Gastroenterology (738 articles)
Genetics (3845 articles)

Notes

To order reprints of this article go to:
http://www.bmjjournals.com/cgi/reprintform

To subscribe to Journal of Medical Genetics go to:
http://www.bmjjournals.com/subscriptions/
**ELECTRONIC LETTER**

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


*Correspondence:* e.volikos@uva.nl


---

**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 typical pigmented lesions, 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, 15 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

---

**Abbreviations:** MLPA, multiplex ligation dependent probe amplification; PJS, Peutz-Jeghers syndrome; SNP, single nucleotide polymorphism
carried out according to the manufacturer’s instructions and the products were analysed using the Applied Biosystems PRISM™ 3100 DNA sequencer. MLPA data were analysed using the Applied Biosystems Genotyper software (PE Applied Biosystems, Foster City, California, USA). Finally, the MLPA data were evaluated by a Microsoft Excel based program originally designed to detect APC copy number changes (www.ngrl.org.uk/ManchesterPages/Downloads/Dosage/) and adapted to evaluate copy number changes with the MLPA STKI1 test kit. Samples were analysed alongside 22 normal healthy individuals to exclude detection of false positives. All samples with possible MLPA changes were tested at least twice to confirm the change. Single nucleotide polymorphism (SNP) analysis was undertaken by sequencing to exclude heterozygosity within deletions reported by MLPA.

RESULTS AND COMMENT

Eleven of the 28 PJS patients tested (39%) had germline LKB1 mutations detected by MLPA (table 1). Five patients had whole gene deletions, two had the promoter region and exon 1 deleted, and in one patient exon 8 was deleted. The remaining detected deletions, each in a single patient, involved the following: loss of exons 2–10; deletion of the promoter region and exons 1–3; and loss of part of the promoter region. No duplications of any part of the gene were detected. Twenty two healthy individuals were screened by MLPA and no copy number changes detected.

For nine samples with deletions (75%), the appropriate region of the gene was sequenced in the patients concerned, so as to confirm the MLPA data by excluding heterozygosity at all reported SNPs. Two SNPs (rs3764640, rs3764641) lying near to exon 1 of LKB1 were found to be homozygous in the three patients with the promoter region and exon 1 deletions. Five SNPs (rs3795063, rs2075604, rs2075606, rs741764, and rs2075608), spread through LKB1 from intron one to intron eight, were homozygous in the five patients with loss of the entire copy of LKB1. Absence of polymorphic sites underlying the MLPA detection oligonucleotides was additionally confirmed using the latest version of dbsNP (BUILD 125, 29 September 2005). Given the original sample of 76 patients, we estimate the proportion of PJS cases with large deletions at 14% (11/76). These patients had no clinicopathological features which distinguished them from the other patients with or without LKB1 mutations in terms of family history, number of polyps, presence of pigmentation, or development of cancer (details not shown).

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 al40 found that phosphorylation of residue 431 was essential for cell growth suppression by LKB1 and Forcet et al41 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 al42 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.43 44 Another study7 used long range polymerase chain reaction to amplify 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 germ-line 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.4 5 7 9 10 16 20 23 30 In addition, we estimate that approximately 10% of cases appear to have mutations which are detectable using RNA based methods only.1 23 10 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 deletions45 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.

ACKNOWLEDGEMENTS

The study was supported by grants from the Academy of Finland (44870/Finnish Centre of Excellence Programme 2000–2005, 76227, 77547), the Sigrid Juselius Foundation, the Cancer Society of Finland, and Helsinki University Central Hospital. The support of Cancer Research UK is also acknowledged.

Authors’ affiliations
E Volikos, J Robinson, A Silver, Cancer Research UK Colorectal Cancer Unit, St Mark’s Hospital, Harrow, Middlesex, UK
K Aittomäki, Clinical Genetics, Haartman Institute, University of Helsinki, Helsinki, Finland

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>241/1</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, 52/1/1</td>
<td>Promoter and exon 1 deletion</td>
<td>Familial</td>
</tr>
<tr>
<td>59/1, 11181</td>
<td>Promoter and exon 1 deletion</td>
<td>Familial</td>
</tr>
<tr>
<td>13/1</td>
<td>Exon 8 deletion</td>
<td>Sporadic</td>
</tr>
<tr>
<td>7282</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>
Conflict of interest: none declared

Evolutionary models have been applied to determine relationships and divergences among species, where the likelihood of homologous segments can be evaluated by statistical methods. As a result, the potential evolution of homologous sequences can be inferred. By using these techniques, the evolutionary relationships between different species can be analyzed, allowing researchers to infer the evolutionary history of the species. This can provide insights into the diversity of species and the potential processes that have shaped the evolution of life on Earth. The study of homologous sequences is crucial for understanding the evolutionary processes that have occurred over millions of years.

**REFERENCES**


