ELECTRONIC LETTER

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 events 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.

Peutz-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.[1] *LKB1* acts as a tumour suppressor in humans, and loss of function may cause multiple defects, including those of cell polarity,[2] activation of the hypoxia pathway through AMP kinase and hypoxia inducible factor 1α,[3 4] and increased Wnt signalling.[5]

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.[1] 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%[6] and 18%[7] 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,[8–10] 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. Given the high frequency of such changes in diseases such as von Hippel-Lindau syndrome,[11] and the increasing realisation that conditions such as hereditary non-polyposis colon cancer (HNPCC) are often caused by such changes,[12–14] 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.

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.[15] 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.[1 16–19] 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

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

Table 1 MLPA results for patients with *LKB1* changes

Patient ID(s)	Result	Family history
11200	Whole gene deletion	Sporadic
11199	Whole gene deletion	Sporadic
11668	Whole gene deletion	Sporadic
24/1/1	Whole gene deletion	Unknown
02-551	Whole gene deletion	Familial
000/1/1; 52/1/1	Promoter and exon 1 deletion	Familial
59/1/1; 11181	Promoter and exon 1 deletion	Familial
13/1/1	Exon 8 deletion	Sporadic
7282	Exon 2–10 deletion	Familial
AXY	Promoter deletion	Sporadic
LEH	Promoter and exon 1–3 deletion	Familial

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 *STK11* 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 al*[20] found that phosphorylation of residue 431 was essential for cell growth suppression by LKB1 and Forcet *et al*[21] 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*[6] 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.[19 22] Another study[7] 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 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 6 7 9 10 18 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 30] 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* deletions[31] 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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