Germline mutations of the LKB1 (STK11) gene in Peutz-Jeghers patients

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Abstract

Germline mutations of the LKB1 (STK11) serine/threonine kinase gene (chromosome 19p13.3) cause Peutz-Jeghers syndrome, which is characterised by hamartomas of the gastrointestinal tract and typical pigmentation. Peutz-Jeghers syndrome carries an overall risk of cancer that may be up to 20 times that of the general population. Here, we report the results of a screen for germline LKB1 mutations by DNA sequencing in 12 Peutz-Jeghers patients (three sporadic and nine familial cases). Mutations were found in seven (58%) cases, in exons 1, 2, 4, 6, and 9. Five of these mutations, two of which are identical, are predicted to lead to a truncated protein (three frameshifts, two nonsense changes). A further mutation is an in frame deletion of 6 bp, resulting in a deletion of lysine and asparagine; the second of these amino acids is conserved between species. The seventh mutation is a missense change in exon 2, converting lysine to arginine, affecting non-conserved amino acids and of uncertain functional significance. Despite the fact that Peutz-Jeghers syndrome is usually an early onset disease with characteristic clinical features, predictive and diagnostic testing for LKB1 mutations will be useful for selected patients in both familial and non-familial contexts.

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Peutz-Jeghers syndrome (PJS, MIM 175200) is characterised by a specific type of hamartomatous polyp of the gastrointestinal tract, and by freckling of the lips, buccal mucosa, and other sites.[1] PJS often presents in the first decade of life with pigmentation (usually in a familial context) or with complications of small bowel polyps, such as obstruction or intussusception. Older PJS patients have an increased risk of neoplasia of multiple sites, predominantly the colon, breast, stomach, ovary, uterus, and pancreas. This risk may approach a 20-fold increase over the general population if all organs are considered, although the increased risk for any particular site is necessarily more modest.[2] About three quarters of PJS cases occur in families, with the remainder resulting from new mutations or low penetrance variants.

The gene for PJS has recently been shown to be a serine/threonine kinase, known as LKB1 or STK11, which maps to chromosome subband 19p13.3.[3 4] This gene has a putative coding region of 1302 bp, divided into nine exons, and acts as a tumour suppressor in the hamartomatous polyps of PJS patients and in the other neoplasms which develop in PJS patients. It is probable that these neoplasms develop from hamartomas, but remains possible that the LKB1 locus plays a role in a different genetic pathway of tumour growth in the cancers of PJS patients.

Previous studies have found germline LKB1 mutations in 50-75% of Peutz-Jeghers patients using genomic DNA or cDNA sequencing as a primary screen.[4–6] Most of these mutations are frameshifts or nonsense changes and thus result in a truncated protein. In frame deletions or missense mutations occur less frequently, generally at conserved amino acids in the kinase core (codons 50-337). Although data are currently insufficient for a formal analysis, the germline mutations of Peutz-Jeghers patients appear to occur throughout the gene, but with a possible bias towards exons 1 and 6.[5 6] Most studies have reported few somatic LKB1 mutations in sporadic cancers,[7–10] despite screening tumours from most of the sites (colon, breast, testis, ovary, and pancreas) at which PJS patients have an excess of cancers. One study has, however, found a high frequency of missense LKB1 mutations in left sided sporadic colon cancers.[11] In addition, one somatic mutation with convincing pathogenic effects has been reported in a sporadic testicular cancer[5] and two mutations have been found in malignant melanoma.[12] There is some evidence of a second, minor PJS locus not on 19p13.3[13 14] and, although its existence remains unproven, it may explain why mutation screening does not detect a higher frequency of LKB1 mutations in PJS patients.

The ability to screen for LKB1 mutations has four important applications in clinical practice. First, the existence of a germline LKB1 mutation confirms a diagnosis of PJS (although the absence of a mutation does not, of course, rule out the diagnosis). Second, although many patients with PJS present in the first decade of life, predictive or confirmatory genetic testing may be useful for initiating screening for gastrointestinal polyps as early as practicable in young children who are at risk, but who do not have unequivocal clinical features of PJS. Third, some PJS patients in families do not present early in life (if, for example, their polyps cause no symptoms and if pigmentation is subtle or absent); predictive genetic testing may allow screening for PJS

Table 1 Clinical features of patients studied

Patient	*S/F*	*Pigmentation*	*PJS polyps*	*Cancer*	*Notes*
2	S	Classical	Multiple	No	
3	S	Classical	10	No	
4	F	Classical	Multiple	Ca endometrium (mother), 35 y	
5	F	Classical	5	No	
6	S	Classical	Multiple	Not known	
8	F	Classical	Multiple	Not known	
11	F	Classical	3	Ca ovary (aunt), 19 y	Other family members have larger numbers of polyps
12	F	Classical	Multiple	No	One affected family member has no pigment to age 33
16	F	Classical	Multiple	Not known	
20	F	Classical	Multiple	No	
21	F	Classical	Multiple	No	
22	F	Classical	Multiple	No	

S/F = sporadic/familial. Pigmentation refers only to circumoral and buccal mucosa. "Classical" is used to describe multiple lentigines of the lips and buccal mucosa. In familial cases, the clinical features of the proband are presented as regards polyps and pigmentation. Usually, the number of polyps could not be determined with precision from medical records, hence the use of the term "multiple" to describe cases in whom there was good evidence from patient histories, referring clinicians, and medical records that more than one PJS polyp had been found, but in whom the total number of polyps was uncertain; in cases with a reported number of polyps, the data probably underestimate the total number of polyps, owing to polyps left in situ or not visualised, or not recorded in notes, or not kept in archives. "Cancer" describes malignant tumours in any family member with PJS. Any noteworthy clinical features of family members other than the proband are given in "Notes" if they differ from those of the proband.

associated cancers later in life to be targeted to mutation carriers. Fourth, given that sporadic patients comprise a significant minority of PJS cases,[1] diagnostic testing for PJS may be useful for patients with PJS-like patterns of pigmentation or with small numbers of hamartomatous polyps. We have screened a set of 12 Peutz-Jeghers patients for germline mutations in LKB1 and report the results of this screening below.

Methods

Patients with PJS were identified from colorectal surgeons, dermatologists, and gastroenterologists. Five cases were from England, one from Wales, one from Scotland, three from China, and two from Germany. Using standard methods, DNA was extracted from peripheral blood samples from the 12 patients. Where possible, DNA was also derived from additional affected and unaffected members of the same family. Clinicopathological data (family history, presence and site of pigmentation, polyp number, site, and histology (wherever possible), and development of cancers) were obtained from hospital records or the referring clinician (table 1).

Published oligonucleotides and reaction conditions were used for exon by exon amplification of LKB1 exons and flanking intronic sequences in the PCR.[9] As an initial screen, purified PCR products were sequenced in forward and reverse orientation using the ABI Ready Reaction Dye Terminator Cycle Sequencing kit and the 377 Prism sequencer. Possible mutations were detected by inspection of the resulting electrophoretograms. For all samples with possible mutations, sequencing was repeated in forward and reverse orientation using an additional affected member of the same family (or using the original patient if no other affected subject from that family had been sampled) in order to confirm the presence of the mutation. All sequencing reactions were performed alongside samples with wild type genotypes.

Table 2 Mutation status of patients studied

Patient	*Nucleotide change*	*Base(s)*	*Codon(s)*	*Exon*	*Amino acid change*
2	delCAAAAA	321-326	107-109	2	HKN→H
3	AAA→AGA	323	108	2	K→R
4	AAG→TAG	1246	416	9	K→Stop
5	ND				
6	TAC→TAA	180	60	1	Y→Stop
8	ND				
11	ND				
12	ND				
16	delC	528	176	4	Stop codon 286
20	delC	842	281	6	Stop codon 283
21	delC	842	281	6	Stop codon 283
22	ND				

Positions given refer to LKB1 cDNA sequence (Genbank U63333). ND = none detected.

Results and discussion

Of the 12 PJS patients, three were sporadic and nine were familial cases. Each patient had a personal or family history of both pigmentation and polyps which were of a type characteristic of PJS. Germline LKB1 mutations were found in seven (58%) of the 12 cases (table 2), in exons 1, 2, 4, 6, and 9. No bias towards location of mutations in exons 1 or 6 was found. Examples of mutations are shown in fig 1. None has been reported previously. Five of the mutations are predicted to lead to a truncated protein (two nonsense changes and three frameshifts). Of these five mutations, two are identical frameshifts. A sixth mutation is an in frame deletion of 6 bp and a seventh mutation is a missense change (lysine→arginine), both of these occurring within the LKB1 kinase core. Linkage of disease to the D19S886 marker (less than 0.2 Mb from LKB1, http://www-bio.llnl.gov/) and cosegregation of mutations with disease were observed in those families (of patients 16, 20, and 21) from which suitable samples were available.

Five of the eight mutations are worthy of particular comment. The identical frameshift mutations found in two families (patients 20 and 21) produced a stop at codon 283 and occurred in a poly(C) tract at a site of potential slippage during DNA replication (table 2). These two families are not known to have any common ancestry. Nevertheless, both kindreds are from northern China and the disease is associated with the same D19S886 allele in both families (not shown). It is most likely, therefore, that these mutations have a common origin, although identical LKB1 mutations without evidence of a common origin have been reported previously.[5 6]

Another mutation (patient 2) occurred in a poly(A) tract, although this change results in a

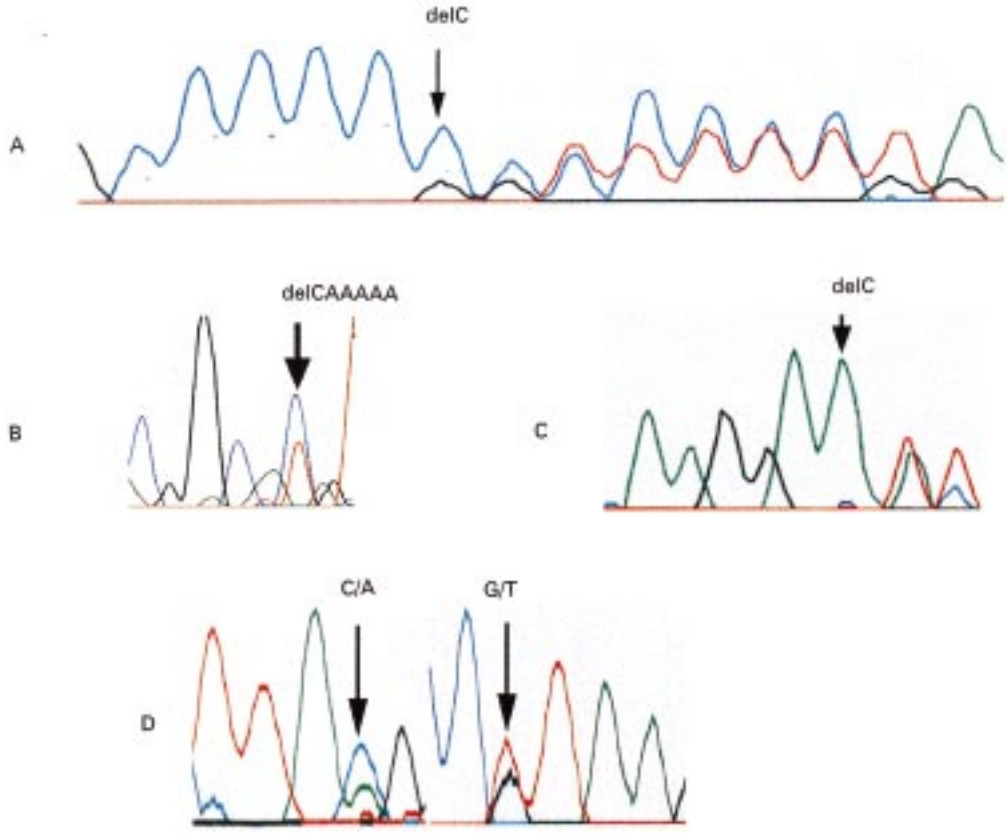

Figure 1 Examples of germline LKB1 mutations in Peutz-Jeghers patients. Mutations are arrowed. See table 2 for details of mutations shown. As per standard ABI sequencing analysis format, blue=C, black=G, red=T, green=A. (A) Patient 20 (forward sequence). CCCCCCGCTCTCTG (wt), CCCCCGCTCTCTGA (mutant). (B) Patient 2 (forward sequence). CGGCACAA (wt), CGGCATGT (mutant). (C) Patient 16 (forward sequence). Note the visible, but weak "wild type" C peak at the mutation site. AAGGACAT (wt), AAGGAATC (mutant). (D) Patient 6 (forward (left) and reverse (right) sequences are shown). TTACG (wt, forward), CGTAA (wt, reverse). TTAAG (mutant, forward), CTTAA (mutant, reverse).

deletion of six bases, rather than base slippage. The mechanism underlying the origin of this mutation is unclear. The mutation results in the removal of amino acids lysine and asparagine at codons 108 and 109 in the protein. The asparagine residue is conserved among the human, mouse, and *Xenopus* homologues of LKB1.

A further mutation (patient 4) produces a premature stop codon in exon 9, removing just 56 residues from the protein of 434 amino acids. Clearly, there is some doubt as to the pathogenic effect of a mutation which occurs so late in the gene and which lies outside the LKB1 protein kinase core.[5] This case is familial, but material from other family members is not available and cosegregation of the variant with disease cannot therefore be determined. The mutation has not, however, been observed in any of more than 65 other PJS and normal subjects sequenced for exon 9 to date. Other germline mutations in LKB1 have been found in exon 8,[4] although no exon 9 mutation has been reported previously.

The missense mutation in patient 3 which converts lysine to arginine substitutes one amino acid for another with a similar basic side chain. The cosegregation of the mutant with disease cannot be tested, since the case is sporadic. This amino acid is neither conserved among human, mouse, and *Xenopus*, nor between LKB1 and other human protein kinases. Thus, although this mutation has not been observed in more than 70 other PJS and normal subjects sequenced for exon 2 to date, and the mutation in patient 2 affects the same amino acid, the pathogenic effects of this sequence change must remain uncertain and we cannot exclude it as a very rare polymorphism.

Associations between the molecular data and the clinicopathological data (table 1) are difficult to assess formally for a rare disease such as PJS with a necessarily limited number of cases. In our sample, every case had clinical features typical of PJS, since these were the referral criteria specified to clinicians. The existence of intrafamilial variation in PJS was shown by the absence of pigmentation in a relative of case 12. As in previous studies,[4 5] there is no clear association in our sample between a detectable LKB1 mutation and a family history of PJS. Thus, using molecular criteria to define disease, the presence of typical PJS pigmentation and polyps is a much stronger indication of PJS than having a positive family history. It is not clear whether most classical PJS cases without a family history result from new mutations, from variable penetrance, or from under-reporting

of family history. To the date of study, subjects in two families had developed carcinomas at early ages and at sites which are at increased risk of cancer in PJS: one of these came from a family with a mutation in exon 9 and the other had no detectable mutation.

DNA sequencing is a viable method of genetic testing for LKB1 mutation in the diagnostic laboratory. In the few studies published previously,[4 5] and in our study, germline mutations can be detected using DNA sequencing in up to two thirds of patients with typical features of PJS, irrespective of whether or not these cases have a family history. Similar success rates are achieved using SSCP analysis.[6] The reason for the failure to detect LKB1 mutations in more than one third of all PJS patients is unclear. In our sample, we found no mutation in four families (of patients 8, 11, 12, and 22) which were suitable for linkage analysis, yet each family was compatible with linkage to LKB1.[14] We suspect that a combination of the imperfect (although high) sensitivity of DNA sequencing and SSCP analysis, some large scale mutations, promoter changes, and, perhaps, an uncharacterised minor PJS locus[13 14] combine to reduce the proportion of PJS patients in whom LKB1 mutations can be easily found. We suspect, however, that alternatives to sequencing or SSCP, such as Southern analysis, will not be of sufficient yield[5] to warrant their routine use in the diagnostic genetics laboratory, although about 5% of LKB1 variants are detectable by this method.[5 15]

Our results and those of other studies suggest the following characteristics for LKB1 mutations in PJS. Most germline mutations result in a truncated protein, but a minority of changes are missense or small in frame deletions; in sporadic tumours, most mutations are missense, even though they are generally accompanied by loss of the other allele. Germline mutations in LKB1 can occur throughout the gene, with a possible bias towards exons 1 and 6.[4–6] Predictive testing for PJS is likely to find a clinical application in selected relatives of PJS patients. To date, however, no study has examined the prevalence of LKB1 mutations in patients with atypical features of PJS (such as isolated hamartomas or pigmentation without family history) and it is unclear as to how many of these patients are truly variants of PJS. Diagnostic testing for LKB1 mutations in these patients may also be of importance, not least for the subject's risk of cancer and their relatives' risk of disease.

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