Further observations on LKB1/STK11 status and cancer risk in Peutz–Jeghers syndrome

Germline mutations in the LKB1/STK11 tumour suppressor gene cause Peutz–Jeghers syndrome (PJS), a rare dominant disorder. In addition to typical hamartomatous gastrointestinal polyps and pigmented perioral lesions, PJS is associated with an increased risk of tumours at multiple sites. Follow-up information on carriers is limited and genetic heterogeneity makes counselling and management in PJS difficult. Here we report the analysis of the LKB1/STK11 locus in a series of 33 PJS families, and estimation of cancer risks in carriers and noncarriers. Germline mutations of LKB1/STK11 were identified in 52% of cases. This observation reinforces the hypothesis of a second PJS locus. In carriers of LKB1/STK11 mutations, the risk of cancer was markedly elevated. The risk of developing any cancer in carriers by age 65 years was 47% (95% CI: 27–73%) with elevated risks of both gastrointestinal and breast cancer. PJS with germline mutations in LKB1/STK11 are at a very high relative and absolute risk of multiple gastrointestinal and nongastrointestinal cancers. To obtain precise estimates of risk associated with PJS requires further studies of genotype–phenotype especially with respect to LKB1/STK11 negative cases, as this group is likely to be heterogeneous.

Keywords: Peutz–Jeghers syndrome; LKB1/STK11; mutation; cancer risk

Peutz–Jeghers syndrome (PJS; MIM 175200) is an autosomal dominant disorder characterised by a specific form of hamartomatous polyposis of the gastrointestinal tract, and by melanin pigmentation of the lips, perioral region and buccal mucosa, fingers and toes, and other sites (Tomlinson and Houlston, 1997). Approximately three-quarters of PJS are familial, the remainder resulting from new mutations or low-penetration variants. PJS typically presents in early childhood with pigmentation or with complications of small bowel polyps—intussusception, obstruction or bleeding.

Although PJS polyps are seen most commonly in the small bowel, they can occur throughout the gastrointestinal tract (Tomlinson and Houlston, 1997) and at other extra-intestinal sites such as the kidney, ureter, gall bladder, bronchus and nasal passage (Westerman et al, 1999; Sommerhaug et al, 1970; Wada et al, 1987). The polyps seen in PJS have a muscular core and are generally classified as being hamartomas. Nevertheless, adenomatous change may occur in polyps and they may become malignant, and an increased risk of jejunal and other small bowel tumours is recognised (Gruber et al, 1998).

In addition to an elevated risk of gastrointestinal malignancies, an increased risk of cancers at other sites is recognized; in particular, breast, pancreas, ovary, uterus, cervix, lung and testicular cancers have been reported (Giardello et al, 1987, 1999; Spigelman et al, 1989). Testicular sex cord and Sertoli-cell tumours may occur in prepubertal boys affected with PJS leading to sexual precocity and gynaecomastia (Wilson et al, 1986; Coen et al, 1991; Young et al, 1995). The production of oestrogen in ovarian tumours in girls with PJS has also been reported causing isosexual precocity (Sohl et al, 1983).

Germline mutations in the serine/threonine kinase gene (LKB1/STK11) on chromosome 19p13.3 have been shown to cause PJS (Hemminki et al, 1997; Hemminki et al, 1998; Jenne et al, 1998).
This gene has a putative coding region of ~1.3 kb, composed of nine exons, and functions as a tumour suppressor.

Previous studies have shown that between 30 and 82% of patients have no detectable germline mutations in LKB1/STK11 (Mehenni et al, 1998; Nakagawa et al, 1998; Jiang et al, 1999; Wang et al., 1999; Westerman et al, 1999b; Ylikorkala et al, 1999; Boardman et al, 2000; Yoon et al, 2000; Olschwang et al, 2001). Families with PJS unlinked to 19p13.3 have also been reported, suggesting that the disease is heterogeneous (Jiang et al, 1999; Westerman et al, 1999b; Yoon et al, 2000). Furthermore, a second PJS locus on chromosome 19q13.4 has been proposed on the basis of linkage in one family (Mehenni et al, 1998).

The clinical features of PJS are variable especially with respect to cancer risks. It is likely that inter- and intrafamilial differences in disease expression reflect in part the influence of different germline mutations.

To further our knowledge about the relation between genotype and cancer risk in PJS, we have related disease expression to LKB1/STK11 status in 33 families.

**PATIENTS AND METHODS**

**Patients**

Thirty-three index patients with PJS were ascertained through colorectal surgeons, gastroenterologists and geneticists within the UK. Clinical information was collected on all patients using a standard proforma and through access to patients’ medical records. PJS was defined according to published diagnostic criteria (Giardello et al, 1987) – histopathologically verified hamartomaticous polyps with at least two of the following: small bowel polyposis, mucocutaneous melanotic pigmentation and family history of the disease. Patients were asked to provide details of any cancer in first- and second-degree relatives. There was no selection of cases for a family history of cancer. Clinical information and samples were obtained with informed consent and Local Ethical Review Board approval in accordance with the tenets of the Declaration of Helsinki.

**Mutational analysis of LKB1/STK11**

Genomic DNA from PJS patients was isolated from EDTA venous blood samples using a standard sucrose lysis protocol. The search for germline mutations in LKB1/STK11 was performed using conformational sensitive gel electrophoresis (CSGE) as described by Ganguly et al (1993). Published oligonucleotide primer sequences were used to amplify each of the nine exons of LKB1/STK11 (Bignell et al, 1998). Any fragments showing migration shifts were reamplified and sequenced directly using the ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, UK). Sequencing was performed using age-, sex- and calendar period-specific mortality rates for England and Wales referenced to the International Classification of Diseases, ninth revision (ICD-9) – all cancers 140–208, cancers of the digestive organs and peritoneum 150–159 and breast carcinoma 174. Two-sided 95% CIs for relative risk estimates are based on the Poisson distribution. A P-value of 0.05 was considered statistically significant.

**RESULTS**

Table 1 details the clinical characteristics and family histories of the 33 index patients analysed. Of these cases, 13 were familial and 20 sporadic. Germline LKB1/STK11 mutations were identified in 17 of the 33 (52%; 95% CI: 33–69%) patients (Table 1), in exons 1–8.

We cannot exclude the possibility that some mutations may have gone undetected; however, under test conditions, we have found that CSGE can detect all small insertions and deletions and ~90% of single-base substitutions. In addition, we have examined for the possibility that some cases might harbour large-scale deletions in LKB1/STK11. It is therefore unlikely that we have failed to detect coding mutations, and, allowing for 90% sensitivity, the results suggest that the mutations in LKB1/STK11 account for at best 75% of PJS cases (the upper 95% confidence limit). Two patients carried the same mutation in exon 6 (PJ42 and PJ51) and two carried the same mutation at the 5′ splice site of exon 8 (PJ33 and PJ61). These four patients were ascertained from different centres and were not known to have any common ancestry. Nevertheless, as all are from the UK, it is probable that these mutations have a common origin, although identical LKB1/STK11 mutations without evidence of common ancestry have been reported (Hemminki et al, 1998; Resta et al, 1998; Wang et al, 1999; Westerman et al, 1999b; Ylikorkala et al, 1999). None of the patients studied were shown to harbour large-scale deletions of LKB1/STK11.

No significant bias towards mutations in exons 1 or 6 was observed, but no exon 9 mutations were identified. Seven of the 15 different mutations identified have not been reported previously – 336delG (Q112fsX17), 369delG (Q123fsX6), 427delG (V143fsX14), 718_719insA (S240fsX26), G725A (G242E), 815_816insA (Y272X), IVS8-2A>G (altered splicing). In all, 11 of the mutations are predicted to lead to a truncated protein (four nonsense mutations, four frameshift deletions, one frameshift insertion and two splice site mutations). The other mutations identified were missense.
mutations, three of which have previously been reported to be pathogenic (Resta et al, 1998; Westerman et al, 1999b; Ylikorkala et al, 1999). All are nonconservative amino-acid changes that are highly conserved among human, mouse and Xenopus homologues of \( \text{LKB1/STK11} \) and reside within the protein kinase core of \( \text{LKB1/STK11} \) (Collins et al, 2000).

Table 2 shows the positions of the mutations observed in our study and in previously published reports (Hemminki et al, 1998; Gruber et al, 1989; Jenne et al, 1998; Mehmendi et al, 1998; Nakagawa et al, 1998; Resta et al, 1998; Jiang et al, 1999; Kruse et al, 1999; Wang et al, 1999; Westerman et al, 1999b; Ylikorkala et al, 1999; Boardman et al, 2000; Miyaki et al, 2000; Yoon et al, 2000; Olschwang et al, 2001; Abed et al, 2001). Overall, most mutations reported to date have been frameshift or nonsense mutations and thus result in a truncated protein. In-frame deletions or missense mutations appear to occur less frequently generally at conserved amino acids in the kinase core.

Very few cases of PJS appear to be the consequence of large-scale deletions of \( \text{LKB1/STK11} \); however, not all studies have systematically searched for such genetic changes (Table 2).

Disease expression in PJS is well documented to display inter- and intrafamilial variation (Burdick et al, 1982; Foley et al, 1998). Establishing a relationship between a number of the features of the disease and genotype is, however, inherently problematic because features typical of the disease are criteria for ascertainment. Nevertheless, there was no evidence that the ages at diagnosis are significantly different in carriers and noncarriers—mean ages of index cases, 13.9 y and 13.6 years, respectively. Furthermore, the distribution of polyps and rates of laparotomy were not significantly different between the groups.

Some previously reported studies have reported no association between detectable \( \text{LKB1/STK11} \) mutation and family history (Hemminki et al, 1998; Wang et al, 1999; Ylikorkala et al, 1999). In our study, 13 of the 33 index cases had a family history of PJS (39%). Of these 10 were carriers of mutations in \( \text{LKB1/STK11} \) (77%), but only seven (35%) patients with sporadic disease had mutations in \( \text{LKB1/STK11} \). The higher prevalence of \( \text{LKB1/STK11} \) mutations in PJS patients with a family history of the disease compared with sporadic cases is statistically significant \( (P = 0.03) \).

Extra-gastrointestinal polyps are a recognised feature of PJS. Four of the patients in our study had extra-intestinal polyps: one of these harboured an \( \text{LKB1/STK11} \) mutation and three did not.

Two patients had developed breast cancer since the diagnosis of PJS had been made—at ages 52 and 35 years. Both are carriers of an \( \text{LKB1/STK11} \) mutation. In addition, one patient had presented at age 6 with a Sertoli–Leydig cell stromal tumour. He did not
| Reference | Exon 1 | Exon 2 | Exon 3 | Exon 4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 | Exon 9 |
|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| This study | E70X| E123fs | Q152X | F157S | 240fs | 287fs | K296X | E291V | IVS7-1 |
| t | E57X | 36fs | E70X | L67X | Y60X | 55fs | K84X | 156_307 | invdel |
| ii | 36fs | I65fs | Q100X | D162N | 170fs | 281fs | K108R | K416X | |
| vi | L67R | Y118X | M136R | IVS3-2 | IVS4-2 | 263fs | 383fs | 108del2 | |
| xi | Y160X | K108R | 176fs | 281fs | |
| xii | 41fs | ex2-7del | 172fs | Q220X | |
| xxiii | 37fs | ex2 del | 188fs | 213fs | 262fs |
| xvi | IVS1-2 | A>G | Q170X | |

*a search for large-scale deletions made.

Mutation changes are described at the protein level.

Key to cancers: c = colon; p = pancreas; pr = prostate; b = breast; cx = cervix; ut = uterus; ov = ovary; k = kidney; d = duodenum; te = testis.

References: i, Hemminki et al (1998); ii, Jenne et al (1998); iii, Gruber et al (1998); iv, Nakagawa et al (1998); v, Ylikorkala et al (1999); vi, Resta et al (1998); vii, Mehenni et al (1998); viii, Boardman et al (2000); xi, Westerman et al (1999); x, Olschwang et al (2001); xi, Wang et al (1999); xii, Jiang et al (1999); xiii, Yoon et al (2000); xiv, Kruse et al (1999); xv, Miyake et al (2000); xvi, Abed et al (2001).
harbour an, LKB1/STK11 mutation. A high frequency of cancer was seen in the relatives of the familial cases – stomach (n = 2, ages 32, 33 years), breast (n = 2, ages 39, 51 years), colorectal (n = 2, ages 43, 67 years), pancreas (n = 1; age 50 years) and adenoma malignum of the cervix (n = 1, age 43 years). All but the one case of stomach cancer was associated with LKB1/STK11 mutations. Excluding the case presenting with a Sertoli–Leydig cell tumour, the index cases and their relatives provided a total of 70 individuals with PJS from which cancer risks could be estimated. These individuals provided a total of 2120 years at risk.

The probability of developing cancer by age 65 years in all PJS patients was 37% (95% CI: 21–61%). The observation of seven cancer deaths, four from gastrointestinal disease, between ages 5 and 65 years, equates to the SMR for all cancer of 9.9 (95% CI: 0.4–20.4; P < 0.001) and for gastrointestinal cancer of 24.8 (95% CI: 0.7–63.6; P < 0.001). Confining the analysis to LKB1/STK11 mutation carriers, the probability of developing cancer by age 65 is 47% (95% CI: 27–73%), SMR of all and gastrointestinal cancers of 13.2 (95% CI: 0.5–27.1, P < 0.001) and 32.0 (95% CI: 0.5–81.8, P < 0.001), respectively. The risk of breast cancer in carriers was markedly increased, 29% by age 65 (95% CI: 12–62%); SMR, 13.9 (95% CI: 0.2–50.3, P < 0.001).

DISCUSSION

It is now well recognised that cancer risks are markedly elevated in PJS (Giardello et al., 1987, 2000; Spigelman et al., 1989). Diagnosing PJS in the absence of mutation data, especially in those without a prior family history of the disease, can however be difficult as pigmentation may not always be present or can fade and polyposis is not always an invariable feature. Moreover, there is substantial phenotypic overlap with other syndromes such as Carney complex (Stratakis et al., 1998).

Over 75% of LKB1/STK11 mutations reported have been frameshift or nonsense mutations and thus result in a truncated protein (Hemminoki et al., 1998; Jenne et al., 1998; Mehenni et al., 1998; Nakagawa et al., 1998; Resta et al., 1998; Jiang et al., 1999; Kruse et al., 1999; Wang et al., 1999; Westerman et al., 1999b; Ylikorkala et al., 1999; Boardman et al., 2000; Miyaki et al., 2000; Yoon et al., 2000; Olschwang et al., 2001). In-frame deletions or missense mutations appear to occur less commonly at conserved amino acids within the kinase core of the expressed protein. Mutations reported to date have been scattered across exons 1–8. The distribution of mutations within the protein kinase core encoding region of LKB1/STK11 does not appear to be random (P < 0.05) and exons 1 and 6 appear to be preferentially involved accounting for ~38% of all reported mutations. Only one mutation has been described in exon 9 (Wang et al., 1999) – a nonsense mutation removing 56 residues from the protein of 434 amino acids and as such resides outside the protein kinase core. Although the case was familial, other members of the family were not evaluated and hence the pathologic significance of this mutation is questionable.

Our study showed that the risk for cancer, gastrointestinal and breast, associated with germline LKB1/STK11 mutations is high and supports recent implementation of screening protocols suggested for patients (Wirtzfeld et al., 2001). In contrast to a number of other inherited cancer syndromes, cancer risks associated with germline LKB1/STK11 mutations cancer risks are not so site specific. LKB1/STK11 functions as a tumour suppressor in hamartomatic polyps and in neoplasms. Some neoplasms develop from hamartomas; however, as LKB1/STK11 has a role in a number of pathways involved in control of cell growth, it is likely that some mutations may confer an increased cancer risk through alternative mechanisms.

In our study, cancers were found in association with mutations in most exons. From studies published so far, there does not seem to be a specifically higher prevalence of any cancer associated with mutations in specific exons (Figure 1). However, one of the mutations we detected, R304W, appeared to be associated with a high risk of breast cancer. It is highly conceivable that certain mutations may be associated with higher risks of cancer at certain sites, as seen with BRCA2 (The Breast Cancer Linkage Consortium 1999; Murphy et al., 2002). To formally assess such relationships will require a large number of observations.

Since Hemminki et al. (1998) first showed that germline mutations in LKB1/STK11 cause PJS, a number of studies have examined the prevalence of mutations in the syndrome. In our study, we identified the LKB1/STK11 mutation in 52% of our patients, implying that approximately half of the cases are not caused by mutations in this gene, reinforcing the suggestion that the disease is genetically heterogeneous. Other studies have reported similar estimates for the prevalence of germline LKB1/STK11 mutations in PJS patients (Wang et al., 1999; Westerman et al., 1999b; Yoon et al., 2000; Olschwang et al., 2001). Some mutations may have been undetected such as those in regulatory elements which may be undetectable in some PCR-based assays; however, families with PJS unlinked to 19p13.3 have been reported confirming that the disease is heterogeneous (Mehenni et al., 1998; Jiang et al., 1999; Westerman et al., 1999b; Yoon et al., 2000). Studies that have formally estimated cancer risks in PJS have not computed separate estimates according to LKB1/STK11 status. Olschwang et al. (2001) recently reported a high frequency of proximal bile duct adenocarcinomas in PJS who did not carry LKB1/STK11 mutations. Similarly, Boardman et al. (2000) reported a high frequency of cancer in this group of patients, although no cases of bile duct cancers were observed. In our study, we had few familial cases not caused by LKB1/STK11 mutations to enable us to compute a separate estimate of risk for noncarriers.

In conclusion, our results confirm that there is significant genetic heterogeneity in PJS. Future studies characterising the mutational status and disease manifestation in large numbers of PJS patients will allow better genotype–phenotype correlation to be made, which should assist clinicians in formulating cancer surveillance and individual predictive genetic testing.

ACKNOWLEDGEMENTS

Funding for this work was undertaken with support from Cancer Research UK. W Lim was in receipt of a grant from the Epsom Hospital NHS Trust Gastroenterology R & D fund. We are grateful to the patients who participated in this study.

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British Journal of Cancer (2003) 89 (2), 308–313 © 2003 Cancer Research UK
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