Relative Frequency and Morphology of Cancers in *STK11* Mutation Carriers

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Background & Aims: **There is limited data on the spectrum and risk for cancer associated with germline serine/threonine protein kinase 11 (*STK11*) mutations that cause Peutz–Jeghers syndrome (PJS).** ***Methods:*** **We analyzed the incidence of cancer in 240 individuals with PJS possessing germline mutations in *STK11*.** ***Results:*** **Fifty-four cancers were found among carriers. Overall, the risk for developing cancer at ages 20, 30, 40, 50, 60, and 70 years was 1%, 3%, 19%, 32%, 63%, and 81%, respectively. Kaplan–Meier analysis showed similar cancer risks between missense and truncating mutation carriers (log-rank $\chi^2 = 2.48$; $P = 0.12$). There was some evidence that mutations in exon 3 of *STK11* were associated with a higher cancer risk than mutations within other regions of the gene. We found no difference in overall cancer risk between male and female carriers (log-rank $\chi^2 = 1.31$; $P = 0.25$) or between familial and sporadic cases (log-rank $\chi^2 = 1.16$, with 1 df; $P = 0.28$). The most common cancers represented were gastrointestinal in origin—gastroesophageal, small bowel, colorectal, and pancreatic—and the risk for these cancers at ages 30, 40, 50, and 60 years was 1%, 10%, 18%, and 42%, respectively. In women, the risk for breast cancer was substantially increased, being 32% by age 60 years.** ***Conclusions:*** **These results quantitatively show the spectrum of cancer risk associated with *STK11* germline mutations in the context of PJS and provide a valuable reference for defining surveillance regimens.**

Peutz–Jeghers syndrome (PJS) is an autosomal-dominant disorder characterized by mucocutaneous pigmentation and gastrointestinal hamartomatous polyposis.[1,2] In addition, patients with PJS are known to be at an increased risk for gastrointestinal and extraintestinal malignancies.

A number of studies have sought to quantify cancer risks associated with PJS. Most have been based on small numbers and estimates of cancer risk vary markedly.[3–6] To derive better estimates of the risk for various cancers associated with PJS a meta-analysis of 6 published reports was conducted by Giardiello et al.,[7] unambiguously showing that the cumulative risk for all cancers was 93% by age 65. Importantly, a wide spectrum of malignancies has been described in association with PJS. In addition to an increased risk for gastrointestinal cancers, an increased risk for cancers at other sites such as breast, ovary, uterus, cervix, lung, and testis have been described.[3–5,7] Also, rare tumors have been attributed to PJS, such as testicular sex cord and Sertoli-cell tumors in prepubertal boys affected with PJS leading to sexual precocity and gynecomastia,[8–10] or adenoma malignum of the uterine cervix.[11,12]

Germline mutations in the serine/threonine kinase gene (*STK11*) on chromosome 19p13.3 have been shown to cause PJS.[13–15] However, between 30% and 82% of patients have no detectable mutation.[6,16–25] This may indicate the existence of another PJS gene or can be explained by detection failure owing to technical reasons. Families with PJS unlinked to 19p13.3 have been reported, however, reinforcing the suggestion that the

Abbreviations used in this paper: PJS, Peutz-Jeghers syndrome; *STK11*, serine/threonine protein kinase 11.

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disease is genetically heterogeneous.[26] Furthermore, support for the existence of a second PJS locus is provided by the finding of linkage of PJS to chromosome 19q13.4 in one large family.[16]

Some studies, albeit based on small numbers, suggest that the cancer spectrum associated with PJS is different in carriers and noncarriers of *STK11* mutations.[6,24] Robust estimates of cancer risk and detailed descriptions of the cancer spectrum associated with PJS are of immediate clinical relevance to define cancer screening algorithms and develop chemopreventative strategies for patients with the disease. Importantly, such information should incorporate mutation status to avoid bias owing to a potential distinct phenotype in *STK11*-mutation–negative PJS patients. To address the shortcomings of current information on cancer risks associated with PJS we have undertaken a collaborative study of a large series of patients in whom a germline *STK11* mutation has been documented.

Materials and Methods

Patients

A series of PJS patients were ascertained through specialist centers within Europe, Australia, and the United States: Institute of Cancer Research, United Kingdom; INSERM U343, Paris, France; Academic Medical Center, Amsterdam, The Netherlands; Erasmus MC University Medical Center, Rotterdam, The Netherlands; VU University Medical Center, Amsterdam, The Netherlands; John Hunter Hospital, New South Wales, Australia; Mayo Clinic, Rochester, Minnesota; and The Johns Hopkins University School of Medicine, Baltimore, Maryland. The study was performed with ethical review board approval from the relevant authority in each country in accordance with the tenets of the declaration of Helsinki.

All patients included in the study fulfilled the established criteria for a PJS diagnosis based on the presence of characteristic mucocutaneous pigmentation and histologically verified hamartomatous intestinal polyps. None of the index patients were selected for inclusion in this study preferentially because of a past history of cancer. For each patient the following data were obtained: sex, dates of birth, diagnosis of PJS, family history of PJS, and neoplasms with dates of diagnosis and dates of death. Cancers that were eligible for inclusion in the analysis were all primary tumors (excluding nonmelanotic skin cancer).

Detection of *STK11* Mutations

Several different techniques were used to identify germline *STK11* mutations—conformational sensitive gel electrophoresis, single-strand conformational polymorphism, denaturing high-performance liquid chromatography, denaturing gradient gel electrophoresis, and direct sequencing of exons. Two centers (Institute of Cancer Research and the Mayo Clinic) additionally made use of long-range polymerase chain reactions to screen for large-scale gene deletions. Patients were classified as carriers if they or a family member with PJS had a fully characterized germline *STK11* mutation. In addition, patients in whom no mutation could be shown but who were from a family linked to chromosome 19p13.4 also were considered to be carriers of a germline mutation in *STK11*.

Mutations in *STK11* identified in PJS patients were coded according to the published sequence of the gene (Genbank accession numbers: exon 1, AF032984; exons 2–8, AF032985; exon 9, AF032986) and referenced to the Human Gene Mutation Database[27] adhering to standard convention.[28] STK11 protein sequences of *Homo sapiens* (GenBank accession no. NP 000446), *Mus musculus* (NP 035622), and *XEEK1* (Q91604) were obtained from the National Center for Biotechnology Information protein database. Alignments were made by using the Clustal W (1.82) multiple sequence alignment program.[29]

Statistical Analyses

Cancer incidence in patients was truncated at age 75 years. A classification scheme for grouping cancers by diagnosis was devised primarily based on topography to subdivide carcinomas by primary site. Cancers were coded using the 10th revision of the International Classification of Diseases. We tested for differences in cancer risk according to both mutation status and the patient's sex by using the Kaplan–Meier product-limit method. Differences in age at cancer diagnosis between male and female carriers and between those with a truncating vs. a missense mutation were assessed by using the log-rank test. We further tested for risk differences by using Cox's proportional-hazards regression model to adjust for potential confounding factors such as site of the mutation in *STK11*. Because most patients were isolated cases or were from small families we did not adjust for familial correlation in the proportional-hazards model. The time intervals in these regression models were the time between birth and first cancer diagnosis for those with cancer and the time between birth and last contact or death for those without cancer. The association between categoric variables was made by using either Fisher exact test or the χ^2 test. A P value of less than 0.05 was considered statistically significant. All statistical analyses were performed by using the statistical software program STATA, Version 7 (Stata Corporation, College Station, TX; http://www.stata.com).

Comparisons of the risk for cancer associated with PJS with that in the general population were based on rates abstracted from the Office of National Statistics database[30] for the year 1999 using the formula (1 − exponential$^{[-\text{cumulative incidence}]}$).[31]

Results

Data on 240 PJS patients (109 male and 131 female cases) in whom a *STK11* mutation had been identified were available for analysis. A total of 188 of the patients were derived from 49 families (mean family size including proband = 4; range = 2–11) and 52 were apparently sporadic cases.

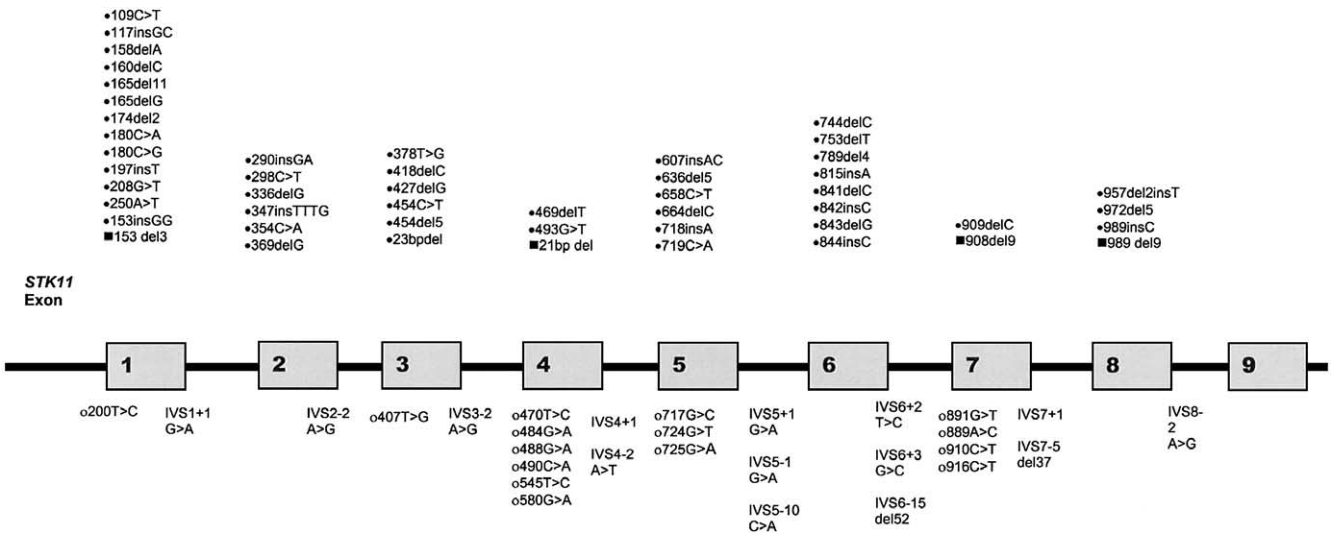

Figure 1. Distribution and type of *STK11* mutations detected in patients with PJS. Exons 1–8 encode the kinase core of *STK11*. Key to mutations: ●, truncating; ■, deletion; ○, missense. Mutations not shown are 1 large deletion and 1 chromosome 11,19 translocation.

STK11 Mutation Spectrum

Seventy-eight of the mutations identified in *STK11* were unique. Figure 1 shows the distribution and type of mutation identified. Forty-five mutations were truncating, leading to the creation of premature stop codons, 14 were within splice sites, 4 were deletions possibly leading to disruption of the kinase activity, and 15 were missense mutations. All of the missense changes led to nonconservative amino acid changes that were highly conserved among human, mouse, and *Xenopus* homologues of *STK11* and resided within the expressed protein kinase core encoded by exons 1–8.[32] In addition to the 78 unique mutations, other mutations included one chromosome 11,19 translocation and one large genomic deletion. Seven families had uncharacterized *STK11* mutations. Mutations were scattered throughout the gene but no mutation in exon 9 was identified in any of the patients. The distribution of mutations within exons 1–8 was not random ($P < 0.001$); truncating mutations were overrepresented in exons 1 and 6 and missense mutations were overrepresented in exons 4 and 7.

Cumulative Cancer Risk

A total of 54 malignant tumors were confirmed in 47 carriers. Seven patients were diagnosed with malignancy at 2 sites. Only one of the malignancies, a cancer of the sigmoid colon, had been diagnosed within the context of a specific cancer surveillance program for PJS patients. Figure 2 details the frequency and spectrum of tumors. Tumors of the gastrointestinal tract and the breast were the most commonly reported sites of malignancies. The carcinomas were subdivided by primary site and the cumulative cancer risks by site were calculated as shown in Table 1.

Among carriers, the risk for developing a first cancer at ages 20, 30, 40, 50, 60, and 70 years was 1%, 3%, 19%, 32%, 63%, and 81%, respectively (Figure 3). The general population risk for cancer by age 60 years is ~8%, hence the risk for cancer in PJS patients is increased by ~8-fold (Table 1). There was no difference in the cumulative cancer risk for all types of cancer between men and women (log-rank test of difference $\chi^2 = 1.31$, with 1 df; $P = 0.25$) or whether cases were familial or nonfamilial (log-rank $\chi^2 = 1.16$, with 1 df; $P = 0.28$).

Figure 4 shows the risk for gastrointestinal (gastroesophageal, small bowel, pancreatic, and colorectal) can-

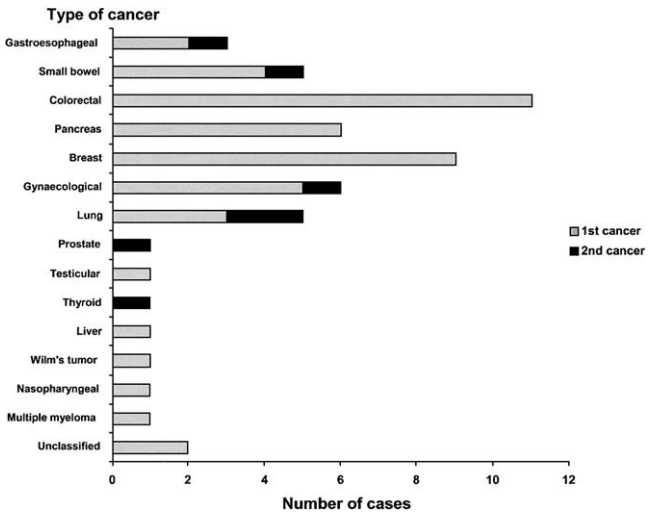

Figure 2. Frequency and spectrum of cancers in *STK11* mutation carriers. □, First cancer; ■, second cancer.

Table 1. Cumulative Cancer Risk By Site and Age in *STK11* Mutation Carriers

Type of cancer	Cancer risk in *STK11* mutation carriers by age (95% confidence limits)						Risk in population at age 60 yr
	20 yr	30 yr	40 yr	50 yr	60 yr	70 yr	
All cancer (ICD C00-C97)	1% (0.3% to 4%)	3% (1% to 7%)	19% (12% to 28%)	32% (23% to 44%)	63% (49% to 78%)	81% (61% to 95%)	8.5%
Gastrointestinal (ICD C15-20,C25)	—	1% (0.1% to 5%)	10% (5% to 18%)	18% (11% to 29%)	42% (26% to 61%)	66% (38% to 92%)	1.3%
Breast (ICD C50)	—	—	8% (3% to 23%)	11% (4% to 27%)	32% (15% to 59%)	—	4.8%
Gynecologic (ICD C53-56)	—	3% (0.7% to 11%)	6% (2% to 18%)	13% (5% to 31%)	13% (5% to 31%)	—	1.6%
Lung (ICD C34)	—	—	1% (0.1% to 6%)	2% (0.6% to 9%)	7% (2% to 25%)	7% (2% to 25%)	0.8%

ICD, International classification of disease.

cer by age. Risks were 1%, 10%, 18%, and 42% at ages 30, 40, 50, and 60 years, respectively. Corresponding general population risks at ages 40 and 60 years in the general population are 0.1% and 1%, respectively (Table 1). Risks were similar in men and women (log-rank test of difference $\chi^2 = 0.26$, with 1 df; $P = 0.61$). Within the gastrointestinal tract, the colorectum was the most commonly affected site of malignancy. The risk for colorectal cancer increased from 1% at age 30 years to 5% at age 40 years and 30% at age 60 years (Table 1). The risk for colorectal cancer was higher in men than women (13 men and 4 women), although the difference did not attain statistical significance (log-rank test of difference $\chi^2 = 1.75$, with 1 df $P = 0.19$). Six patients (3 men and 3 women) were diagnosed with pancreatic cancers. All pancreatic carcinomas were diagnosed between ages 34 and 49 years. The risk for developing pancreatic cancer was 5% at age 40 years, increasing to 8% at age 60 years.

In this series, 9 of the women developed breast cancer. The age at diagnosis ranged from 38 to 62 years. The risk for developing breast cancer was 8% by age 40 years and increased to 32% at age 60 years (Figure 5). The risk for breast cancer in the general population by age 60 is ~5%. Hence, the risk in carriers represents a 7-fold increase in risk.

Five gynecologic cancers (1 uterine, 2 ovarian, and 2 cervical cancers) were diagnosed in patients. Two of the cervical cancers were confirmed histologically as adenocarcinomas. The risk for these cancers was 3% at age 30 years, increasing to 13% by age 60 years. Three cases of lung cancer were reported, which equated to a risk of 7% by age 60 years. Given that the corresponding general population risk at age 60 years is ~1%, this represents a 7-fold increase in risk.

Relationship Between Genotype and Phenotype

We analyzed for risk differences between missense and truncating mutation carriers. Thirty-four carriers with malignancy had truncating mutations and 8 were

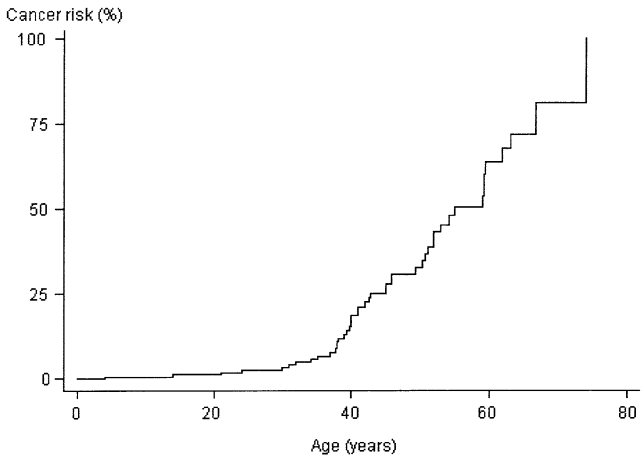

Figure 3. Risk for all cancer in carriers of germline *STK11* mutations. Ages shown are the time between birth and first-cancer diagnosis for those with cancer and the time between birth and either last contact or death for those without cancer.

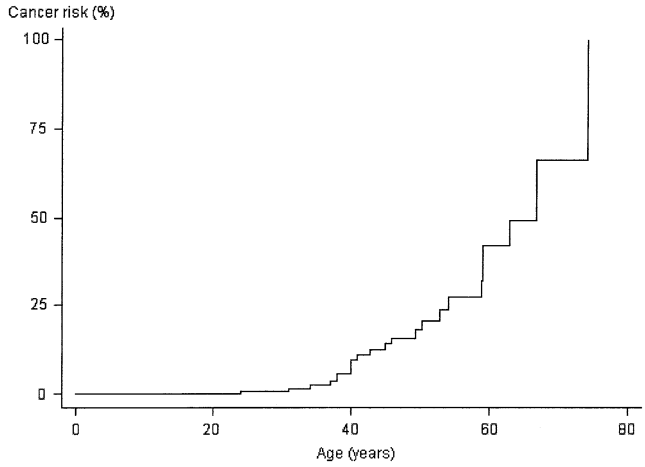

Figure 4. Risk for gastrointestinal cancer (gastroesophageal, small bowel, colorectal, and pancreatic cancers) in carriers of germline *STK11* mutations. Ages shown are the time between birth and first-cancer diagnosis for those with cancer and the time between birth and either last contact or death for those without cancer.

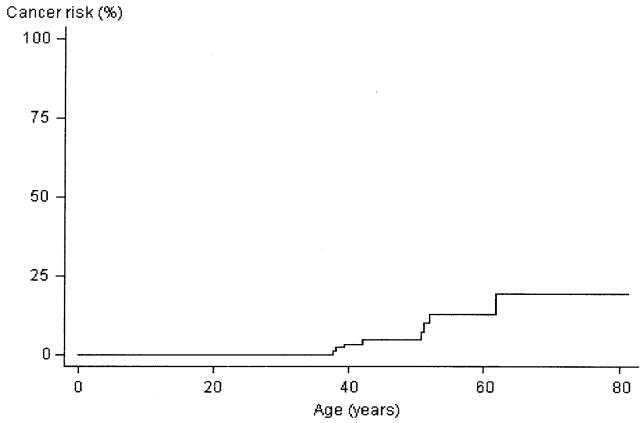

Figure 5. Risk for breast cancer in carriers of germline *STK11* mutations. Ages shown are the time between birth and first-cancer diagnosis for those with cancer and the time between birth and either last contact or death for those without cancer.

associated with missense mutations. Five of the cancers occurred in patients who had uncharacterized *STK11* mutations. There was no difference in the cumulative cancer risk between the mutation types for gastrointestinal (log-rank test of difference $\chi^2 = 1.97$, with 1 df, $P = 0.16$), breast (log-rank $\chi^2 = 0.08$, with 1 df, $P = 0.77$), gynecologic (log-rank test of difference $\chi^2 = 0.2$, with 1 df, $P = 0.66$), and all types of cancers (log-rank test of difference $\chi^2 = 2.48$, with 1 df, $P = 0.12$).

There was some evidence of a relationship between mutation site and risk for all cancers, although this did not attain statistical significance (log-rank test of difference $\chi^2 = 13.41$, with 7 df, $P = 0.06$). Mutations involving exon 3 appeared to be associated with the greatest risk. This was largely attributable to an increased risk for gastrointestinal cancer associated with mutations in this exon (log-rank test of difference $\chi^2 = 18.12$, with 7 df, $P = 0.01$). There was no evidence that these risks were significantly modified by sex or a family history of PJS.

Discussion

In our study of 240 PJS patients with germline *STK11* mutations, the highest absolute cancer risk observed was for carcinomas of the gastrointestinal tract—esophagus, stomach, small bowel, colon, rectum, and pancreas. These cancer types are the principal malignancies identified as standard components of PJS. The major risk for extraintestinal cancer was breast cancer, with the upper confidence limit of the estimated risk being comparable with those associated with mutations in either *BRCA1* or *BRCA2*.[33]

Ascertainment bias is a concern in estimating cancer risks for any rare disorder such as PJS, potentially leading to an overinflated estimate of risk. Although we cannot entirely exclude this factor, the PJS patients on whom we estimated cancer risks were unselected for a diagnosis of cancer both in terms of morphology or primary site, thereby limiting bias. Furthermore, the cancers observed in the patients were not confined to a small number of families.

One of the aims of the present study was to survey for minor cancer types associated with germline *STK11* mutations. In contrast to other inherited cancer syndromes, the cancer risks associated with germline *STK11* mutations are not site specific. Many of the cancers reported in association with PJS develop from hamartomas in which *STK11* functions as a tumor suppressor. STK11, however, also plays a role in a number of pathways involved in controlling cell growth and apoptosis. Therefore, it is likely that the tumorigenic potential of mutations is mediated through alternative mechanisms in some tissues, especially those in which hamartoma development is not a feature. Somatic mutations in *STK11* are common in lung cancer[33] and there is evidence from our analysis, albeit based on small numbers, that germline mutations confer an increased risk for cancer at this site. One caveat to this is the fact that we had no information on smoking histories of the patients to address the confounding factor from this strong covariate.

Single cases of testis, nasopharyngeal, prostate, myeloma, and Wilm's tumor also were observed in the patients with PJS in our study. We cannot exclude the possibility that these observations are simply the result of chance because these cancers are rare in the general population (e.g., Wilm's tumor has an incidence of only ~10 per 100,000 live births).[34] Hence, single cases among 240 PJS patients may be significant, raising the possibility that carrier status may be associated with an increased risk for cancer at these sites.

Around 20% of the germline *STK11* mutations identified in this series of PJS patients were missense changes. This is a higher frequency than in other familial cancer syndromes that predispose to gastrointestinal tract malignancies (i.e., hereditary nonpolyposis colorectal cancer or familial adenomatous polyposis) in which the majority of germline mutations lead to a truncated protein product.[27,35] For some inherited cancer syndromes, a higher cancer risk and earlier age at cancer diagnosis have been associated with missense mutations at critical binding domains rather than with protein-inactivating mutations.[36] In our study, the risk for all cancers was not significantly different between the 2 types of mutation carriers. There was, however, some evidence for a relationship between genotype and phenotype, with muta-

tions in the vicinity of exon 3 being associated with a higher cancer risk.

The substantial cancer risk associated with PJS supports surveillance in patients for the early detection of tumors. Most proposed guidelines advocate upper and lower endoscopy, breast examination, and some form of surveillance for pancreatic (transabdominal and endoscopic ultrasound, abdominal computerized tomography, CA19-9) and gynecologic malignancies (ultrasound, cervical cytology, and CA125). Guidelines, however, differ considerably over when to initiate screening, intervals between screening, and which techniques to use.[38–42] The optimal surveillance strategy is unclear and to date the efficacy of intensive surveillance for cancer associated with PJS has not been established.

To date, all guidelines for cancer surveillance in PJS have been based on risk estimates from retrospective cohort studies without reference to the mutation status of patients.[3–5,43,44] Studies that have estimated cancer risks in PJS generally have not incorporated information on *STK11* mutation status. Those incorporating such information have only analyzed small numbers of patients.[6,24] In our study we have sought to address this issue through analysis of 240 extensively documented PJS patients in whom a germline *STK11* mutation has been identified. The data generated from our study should provide guidance in formulating management policies for patients harboring germline *STK11* mutations. Accepting the caveat that the estimates of cancer risks we have derived may be marginally inflated because of ascertainment, our study showed that the risks for cancer, in particular gastrointestinal and breast cancer, associated with germline *STK11* mutations are high and supports implementation of screening for malignancies at these sites. The risk analyses, however, suggest that cancer surveillance is probably not justified before age 20 years for gastrointestinal cancer and before age 25 years for breast cancer. Although the relative risks for some other cancers may be increased, the absolute risks for these tumors are small. Subjecting patients to regular surveillance for these malignancies is probably not justified on the basis of existing data.

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