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ORIGINAL ARTICLE

Molecular and Clinical Characteristics in 46 Families Affected with Peutz–Jeghers Syndrome

Hamid Mehenni · Nicoletta Resta · Ginevra Guanti · Louisa Mota-Vieira · Aaron Lerner · Mohammed Peyman · Kim A. Chong · Larbi Aissa · Ali Ince · Angel Cosme · Michael C. Costanza · Colette Rossier · Uppala Radhakrishna · Randall W. Burt · Didier Picard

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Abstract Germline mutations of the tumor suppressor gene *LKB1/STK11* are responsible for the Peutz–Jeghers syndrome (PJS), an autosomal-dominant disorder characterized by mucocutaneous pigmentation, hamartomatous polyps, and an increased risk of associated malignancies. In this study, we assessed the presence of pathogenic mutations in the *LKB1/STK11* gene in 46 unrelated PJS families, and also carried genotype–phenotype correlation in regard of the development of cancer in 170 PJS patients belonging to these families. All *LKB1/STK11* variants detected with single-strand conformational polymorphism were confirmed by direct sequencing, and those without *LKB1/STK11* mutation were further submitted to Southern blot analysis for detection of deletions/rearrangements. Statistical analysis

H. Mehenni · D. Picard
Unité de Gastroentérologie & Hépatologie, Centre Médico-Chirurgical Rond-Point-Plainpalais et Département de biologie cellulaire, Unité de recherche des maladies prédisposant aux cancers gastro-intestinaux, Université de Genève, Genève, Switzerland

N. Resta · G. Guanti
Sezione di Genetica Medica Dip. di Biomedicina dell'Età Evolutiva, Università di Bari, Italy

L. Mota-Vieira
Unidade de Genética e Patologia Moleculares, Hospital De Ponta Delgada, Ponta Delgada, Açores, Portugal

A. Lerner
Department of Pediatrics, Haifa Hospital, Haifa, Israel

M. Peyman
Shariati Hospital and Islamic Azad University of Najafabad, Iran

K. A. Chong
Instituto da Criança, Hospital das Clinicas da faculdade de medicina da Universidade de Sao Paulo, Brazil

L. Aissa
Service de chirurgie generale, CHU Ibn-Rochd, Casablanca, Morocco

A. Ince
Gastroenterology Clinic, Haydarpasa Numune Education and Training Hospital, Istanbul, Turkey

A. Cosme
Servicio de Digestivo, Hospital Donostia, Edificio Aranzazu, San Sebastian, Spain

M. C. Costanza
Division of Clinical Epidemiology, Geneva University Hospitals, Geneva, Switzerland

C. Rossier
Department of Genetic Medicine and Development, University of Geneva Medical School and University Hospitals, Geneva, Switzerland

U. Radhakrishna
Green Cross Blood Bank & Genetic Research Centre, Paldi, Ahmedabad, India

R. W. Burt
Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah

H. Mehenni (✉)
Centre Medical du Rond-point de Plainpalais, Unité de Gastroentérologie & Hépatologie, 12-14 rue de Carouge, 1205 Genève, Switzerland
e-mail: mehenni@cellbio.unige.ch

for genotype–phenotype correlation was performed. In 59% (27/46) of unrelated PJS cases, pathogenic mutations in the *LKB1*/*STK11* gene, including 9 novel mutations, were identified. The new mutations were 2 splice site deletion–insertions, 2 missenses, 1 nonsense, and 4 abnormal splice sites. Genotype–phenotype analysis did not yield any significant differences between patients carrying mutations in *LKB1*/*STK11* versus those without mutations, even with respect to primary biliary adenocarcinoma. This study presents the molecular characterization and cancer occurrence of a large cohort of PJS patients, increases the mutational spectrum of *LKB1*/*STK11* allelic variants worldwide, and provides a new insight useful for clinical diagnosis and genetic counseling of PJS families.

Keywords Cancer · Genotype–phenotype analysis · Pathogenic *LKB1*/*STK11* gene mutations · Peutz–Jeghers syndrome

Pathologic germline mutations in the *LKB1*/*STK11* gene lead to Peutz–Jeghers syndrome (PJS; MIM 17500), which is a dominantly inherited disorder occurring in 1 of every 120,000 births [1]. PJS is characterized by peroral pigmentation and hamartomatous polyposis and predisposes to different cancers in various organs [2–4].

The first locus responsible for PJS was mapped to chromosome 19p13.3 by the combination of comparative genomic hybridization, loss of heterozygosity, and by targeted linkage analysis [5]. Subsequently, direct cDNA selection, positional cloning experiments, and mutation analysis of candidate genes revealed that the gene responsible for PJS encodes for a serine/threonine kinase termed *LKB1* or *STK11* [6, 7], which has growth-suppressing activity [8]. It has been shown that mutations result in inactivation or loss of function in LKB1/STK11 [9, 10] and that the genetic defect cannot be linked to the 19p13.3 locus in all PJS families, and when linked to this locus, not all the patients have detectable mutations in the *LKB1*/*STK11* gene [9, 11, 12]. There is evidence for a second PJS locus at 19q13.4 [13], which was reinforced by the absence of mutations in the *LKB1*/*STK11* gene in those families that showed linkage to this locus [9]. Recently, additional evidence implicating this locus has been accumulated by the findings of a translocation (11:19) (q13:q13.4) involving this region in a PJS polyp from the small bowel of a newborn girl [14]. Taken together, these data suggest that Peutz-Jeghers syndrome can be caused by mutations in more than one gene, which often complicates genetic counseling [12, 15].

To assess contributions of *LKB1*/*STK11* gene mutations to PJS, we analyzed a cohort of 46 unrelated PJS families. Mutation analysis was performed using single-strand conformational polymorphism (SSCP) and subsequently all variants were subjected to DNA sequencing for confirmation and characterization. All families that did not have any obvious change in the *LKB1*/*STK11* gene were further studied using Southern blot analysis to detect deletions and/or rearrangements. Moreover, genotype–phenotype correlation in regard of the development of cancer were also analyzed in a total of 170 PJS affected individuals belonging to these families.

Patients and methods

Families and patients

A total of 46 unrelated families exhibiting mucocutaneous pigmentation and hamartomatous polyposis were referred by gastroenterologists, gastrointestinal surgeons, and geneticists to the laboratory for genetic analysis. The histologic aspects of PJS polyps were unambiguous in all cases. Clinical data were collected using a questionnaire and through access to patients' medical records. Characteristic features defining the clinical diagnosis were age at first symptoms, initial symptoms, presence of pigmentation, localization of the polyps, histology of the polyp biopsy, presence of cancer, histology type, organ involved, age at cancer diagnosis, and the follow-up period (Table 1). Criteria for exclusion were absence of hamartomatous polyps typical of PJS and presence of only mucocutaneous pigmentation in isolated cases. When $\geq$2 individuals were affected in the same family, they were considered as familial cases. In families with 1 individual with PJS, those who had only gastrointestinal hamartomatous polyposis were also counted as affected members. A PJS patient was defined as a *sporadic case* caused by a de novo mutation only if the parents were clinically, endoscopically, and radiologically free from hamartomatous polyps and mucocutaneous pigmentation and/or if the mutation was known and not present in parents after exclusion of nonpaternity or nonmaternity. The study was performed with ethical committee approval from the relevant authority in each institution. After informed consent, blood samples were obtained from the patients and their relatives. DNA was extracted from 10 mL of peripheral blood using the Gentra kit (Amersham, Buckinghamshire, UK) according to the manufacturer's recommendations.

Single-strand conformational polymorphism analysis, southern blot, and sequencing

A combination of SSCP analysis and direct sequencing was used to screen the 9 *LKB1*/*STK11* exons and flanking intronic sequences in germline genomic DNA extracted from blood leukocytes or tissue samples. Polymerase chain reaction (PCR) and gel conditions have been previously reported [9]. Genomic Southern blots were prepared after endonuclease

digestion with *Eco*RI, *Hin*dIII, and *Bam*HI/*Pst*I, and were probed with a cloned cDNA fragment that comprised the entire coding region of *LKB1*/*STK11* gene.

The100 Caucasians used as controls were all from the same ethnic origin (European) as the missense and splice site mutation carriers. Mutations in the *LKB1*/*STK11* gene identified in the PJS families were coded according to the published sequence of the gene (Genbank accession numbers: exon 1, AF032984; exons 2–8, AF032985; exon 9, AF032986) following the standard nomenclature [16]. STK11 protein sequences of *Homo sapiens* (GenBank accession no. NP 000446), *Mus musculus* (NP 035622), and *Xenopus XEEK1* (Q91604) were obtained from the National Center for Biotechnology Information protein database (available: www.ncbi.nlm.nih.gov/). Alignments were made by using the Clustal W (1.8) multiple sequence alignment program accessed at Baylor College of Medicine Search Launcher (available: www.hgsc.bcm.tmc.edu/SearchLauncher/).

Paternity and maternity testing

Paternity and maternity testing were performed for all families in which *LKB1*/*STK11* mutations were found in sporadic patients and in which parental DNA samples were available. This was done by analysis of ≥ 3 highly informative microsatellite markers (D19S886, D19S878, D19S565), but in the case that these markers were not informative or any suspicion of nonpaternity or nonmaternity is detected, then further markers in other chromosomes were done. We used radioactively labeled primers, and genotypes were determined by gel electrophoresis and autoradiography [13].

Autophosphorylation assays

Autophosphorylation assays were done in the same way as in Mehenni *et al*. [9], except that HA-PCDNA3 (PCDNA3 modified to include a HA-tag), HEK293 cells, monoclonal antibodies to the HA tag (Santa Cruz Biotech), and horseradish peroxidase-conjugated goat anti-mouse kappa light chain antibodies (Bethyl, Montgomery, TX) were used.

DNA isolation from tissue sample

Formalin-fixed and paraffin-embedded tissues were available for study from the biliary adenocarcinoma. The samples were cut into 5-μm sections, mounted on glass slides, and stained with hematoxylin and eosin. Cancer tissue was carefully microdissected using a laser capture microscope. The microdissected tissue was collected into microcentrifuge tube containing 50–200 μL DNA isolation buffer (50 mmol Tris-HCl, pH 8.0, 0.2% Tween-20, and 100 mg/mL proteinase K), and incubated overnight at 56°C. The sample was heated to 96°C for 10 minutes to inactivate the proteinase K. The lymphocyte DNA was used as normal control.

Loss of heterozygosity analysis

Three polymorphic markers spanning a physical distance of ~4 cM flanking *LKB1*/*STK11* gene locus were analyzed for loss of heterozygosity (LOH) analysis. The markers are ordered from telomeric to centromeric (available: www.-genome.wi.mit.edu): D19S886-D19S878-D19S565. PCR conditions for the microsatellite markers have been previously described [13]. PCR products were resolved by electrophoresis and visualized by autoradiography.

Statistical and phenotypic analysis

Statistical comparisons between the various subgroups were made with Fisher's exact test and Student's *t*-test. $P < .05$ was considered statistically significant.

Results

Clinical characteristics of Peutz–Jeghers syndrome patients

To study the prevalence and the nature of germline mutations in 46 unrelated PJS families were analyzed for *LKB1*/*STK11* mutations. These families represent a total of 170 affected individuals; 97 are females and 73 males. The median age of onset of the disease in the probands was 24 years (range, 4–44 years). The clinical details and cancer cases among PJS families are summarized in Table 1. Of the cohort (168 of 170), 99% reported mucocutaneous pigmentation. Moreover, we identified 27 cases of cancer based on histology in 25 affected individuals. Gastrointestinal cancer was noted in 17 cases, including stomach ($n = 3$), small intestine ($n = 5$), colorectal cancer ($n = 8$), and primary biliary cancer ($n = 1$). Extra-intestinal cancer occurred in 10 different patients. Two patients were diagnosed with malignancy at 2 sites. The cancer prevalence per person among affected individuals was 15% (25 of 170). The mean patient age at cancer onset was 36 years (median, 31.5 years; range, 5–58). The median follow-up interval from the diagnosis of PJS to the last routine follow-up examination or death was 20.5 years (range, 7–34 years) for the whole cohort and the compliance rate was 96% (163 of 170).

LKB1/*STK11* germline mutations

Sequencing of the 9 exons and the exon–intron junctions led to the identification of 27 unique pathologic germline mutations including 9 novel mutations (see Table 1). The new

Table 1 Clinical features and mutation spectrum in *LKB1/STK11* gene found in the present PJS cohort

Families	Sex/Age at first symptom of the proband	Familial or sporadic (*n*)	Localization of the polyps	Pigmentation (yes or no)	Tumor (histology, organ)	Age at diagnosis of tumor	Mutation (exon/intron, DNA change)	Mutation type	Protein modification predicted
GE01	F/14	F (2)	ST, SI, nose	Yes	Ovary cystic adenoma mucinous; breast fibrous adenoma	29, 35	Intron 2; IVS2 + 1A>G	Abnormal splicing	Frameshift
GE02[b]	M/38	F (2)	SI	Yes	Basal cell epithelioma	43	Exon4; C.523_528del AAGGAC	Deletion	K175_D176del
GE03[a]	M/31	F (3)	SI, C	Yes	Stomach cancer	58	Intron 5; IVS5 + 5G>A	Abnormal splicing	Frameshift
GE04	M	F (2)		Yes			Exon 2; 334C>T	Nonsense	Q112X
GE05	F/35	F (3)	ST, SI, CR	Yes	Stomach cancer	56	IVS7 + 1 delG	Abnormal splicing	Frameshift
GE06[a]	F/4	F (11)	ST, SI	Yes			Exon 1; 151–168del18, 150–151ins6	Insertion–deletion	50–53 LMGDdel
GE07	M/18	F (5)	SI, CR	Yes	Colon carcinoma	37	Intron 5; IVS5 + 5G>A	Abnormal splicing	Abnormal splicing; truncated protein
GE08[aa]	F/18	F (4)	SI	Yes	SCAT		Exon 8; 924G>T	Missense	W308C
GE09[a]	M/15	F (10)	SI, CR	Yes	Colon and ovarian carcinoma	30, 49	Exon 4; 526G>A	Missense	D176N
GE10[a]	F/19	F (9)	ST, SI, CR	Yes	Colon cancer	42	Exon 7; 903delG	Deletion	FS R301 + 33 new aa
GE11[a]	M/17	F (6)	SI, CR	Yes	Colon carcinoma (2), ovarian cancer	38, 45, 49	Intron 6 & Exon7; IVS6&Ex7; del52 nts	Abnormal splicing	Abnormal splicing; truncated protein
GE12	F/19	F (4)	SI, CR	Yes	Primitive biliary adenocarcinoma; right kidney carcinoma; SCAT of ovary; rectal carcinoma	32, 68,44	Exon 3; 155insG	Insertion	FS: G155 + 6 new aa
GE13	F/19	F (3)	ST, SI, CR	No	Duodenum carcinoma	35	Exon 5; 650insC	Insertion	FS P217 + 45 new aa
GE14	M/30	F (10)	CR	Yes	Colon carcinoma		Intron 3& Exon 4: 2407_2413delCG CAGGinsTGCAC	Abnormal splicing	Truncated protein? Exon skipping?
GE15	F/12	F (9)	SI, CR	Yes	Metastatic gastric cancer	28	Exon 3; 418delC	Deletion	FS M139 + 20 new aa
GE16	F/22	F (7)	SI, C	Yes	Ovarian carcinoma	21	Intron 3& Exon 4; 2412_2413delGG insC	Abnormal splicing	Truncated protein? Exon skipping?
GE17	F/18	F (7)	SI, CR	Yes	Cervix carcinoma; metastatic adrenal cancer	27	No mutation		

Table 1 Continued

GE18	M/14	F (6)	SI, CR	Yes	No		No mutation		
GE19	F/12	F (4)	SI, CR	Yes	No		No mutation		
GE20	M/7	F (5)	ST, SI, C	Yes	No		No mutation		
GE21	F/4	F (2)	ST, SI, CR	Yes	Sertoli cell; ovarian tumor	5	No mutation		
GE22	M/10	F (4)	CR	Yes	No		No mutation		
GE23	F/7	F (6)	ST, SI, C	Yes	No		No mutation		
GE24	M/25	F (2)	ST, SI	Yes	Metastatic duodenum carcinoma	25	No mutation		
GE25	M/42	F (4)	ST, SI, C	Yes	Small intestine adenocarcinoma	42	No mutation		
GE26	F/44	F (3)	SI	Yes	Duodenum adenocarcinoma		No mutation		
GE27	M/23	F (10)	SI, CR	Yes	No		No mutation		
GE28	M/35	F (4)	SI, CR	Yes	No		No mutation		
GE29	F/38	F (6)	ST, SI, C	Yes	Breast (2) and jejunum adenocarcinomas	57, 42,46	No mutation		
GE30	M/15	S	SI	Yes			Exon 6; c.787_790del TTGT	Deletion	Frameshift
GE31[b]	M/39	S	SI	No			Exon7 ; c.910C>T	Missense	R304W
GE32	F/12	S	ST, SI, C	Yes	—	—	Exon 6; c.842_844delCGC insTC	Indels	Frameshift
GE33[b]	F/18	S	ST, SI, C	Yes	—	—	Exon 4 ; c.530T>A	Missense	I177N
GE34[b]	M/10	S	SI, C	Yes			Exon 7 ; 910C>T	Missense	R304W
GE35[b]	M/32	S	ST, SI, C	Yes			r.291_464del	Abnormal splicing	E98_G155 del
GE36	M/10	S	ST, SI, C, nose, sinuses, paranasal	Yes	Colon carcinoma	35	Exon 4; 521A>G	Missense	H174R
GE37	F/5	S	CR	Yes	—	—	Exon 1; c.197–225del of 29nts	Deletion	Frameshift, V65 + 86 new aa
GE38[a]	F/12	S	SI, CR	Yes			Exon 4; 574–575insA	Insertion	FS K191 + 73 new aa
GE39	F/18	S	SI	Yes			Exon 3 ; 449T> G	Missense	V150L
GE40	F/10	S	SI, CR	Yes			Exon 1; 250T>A	Nonsense	K84X

Table 1 Continued

Families	Sex/Age at first symptom of the proband	Familial or sporadic (*n*)	Localization of the polyps	Pigmentation (yes or no)	Tumor (histology, organ)	Age at diagnosis of tumor	Mutation (exon/intron, DNA change)	Mutation type	Protein modification predicted
GE41	M/25	S	SI	Yes			No mutation		
GE42	F/6	S	ST, SI	Yes			No mutation		
GE43	F/18	S	ST, SI, CR	Yes			No mutation		
GE44	M/26	S	SI, CR	Yes			No mutation		
GE45	F/21	S	ST, SI, CR	Yes			No mutation		
GE46	M/9	S	SI, CR	Yes			No mutation		

Abbreviations: ST, stomach; SI, small intestine; C, colon; R, rectum; CR, colorectum; SCAT, sex cord annular tumor; *n* is the number of affected members in the same family (including proband). Cancers reported are those of the proband and/or affected members of the same family. In the column of tumor, the number between brackets indicates the number of the same event observed in different members of the same family.

[a] Family previously reported by Mehenni *et al.* [9].

[b] Family reported by Resta *et al.* [17].

mutations were 2 splice site deletion–insertions, 2 missenses (H174R and V150L), 1 nonsense (Q112X), and 4 abnormal splicings. In addition to the new mutations, 18 mutations were found, consisting of 12 mutations predicted to result in a truncated protein (1 nonsense mutation, 7 frame shift mutations, and 4 mutations in consensus splicing sites), 5 missense mutations, and 1 in-frame deletion of 6 nucleotides. One missense mutation (R304W) was found in 2 independent sporadic cases. There is no evidence that these 2 mutations have a common ancestry [17]. Similar situations have been reported by others [10, 18–20]. In 11 of 17 apparently sporadic cases, the DNA from both parents of the affected individuals did not have these mutations and the possibility of nonpaternity or nonmaternity was excluded using microsatellite markers. These mutations can therefore be considered de novo mutations. In addition, none of the siblings (the mean siblings number in these sporadic PJS cases is 5; range, 1–8) carried the mutation, which makes it unlikely that there was parental mosaicism for these molecular defects.

The identified mutations in the consensus splice sites are not common polymorphisms, because they were not detected in the DNA of the 100 normal Caucasians controls from the same ethnic origin. The unavailability of RNA samples in the carriers of splice site mutations rendered the assessment of the consequences on mRNA level not possible. Missense mutations have occurred within the kinase domain in highly conserved residues of diverse species (human, mouse, xenopus). When assayed by autophosphorylation, the protein kinase activities of the normal and mutant LKB1/STK11s show a complete absence of autophosphorylation, leading to inactivation of the protein kinase of the mutants but not the LKB1/STK11 wild type [9, 21] (Fig. 1).

These pathologic mutations were found in 16 familial and 11 sporadic cases. In the 16 families, the mutations cosegregated well with the disease. No *LKB1/STK11* mutation carriers without phenotypic expression were observed. Moreover, the substitution mutations were not detected in 100 normal Caucasians controls. There was a higher prevalence of *LKB1/STK11* mutations in sporadic PJS patients (64.7%; 11 of 17) compared with familial cases (5.2%; 16 of 29), but the difference was not statistically significant ($P = .55$).

Southern blot analysis in *LKB1/STK11* mutation-negative Peutz–Jeghers syndrome patients

A rare anecdotal case of a PJS patient with a large heterozygous germline deletion of 250 kb including *LKB1/STK11* was reported by Le Meur *et al.* [22]. Furthermore, several gross deletions that escaped the sequencing were also described [7, 10]. Thus, all *LKB1/STK11* mutation-negative probands in the present study ($n = 19$) were analyzed by Southern blot for the possibility of such deletions that

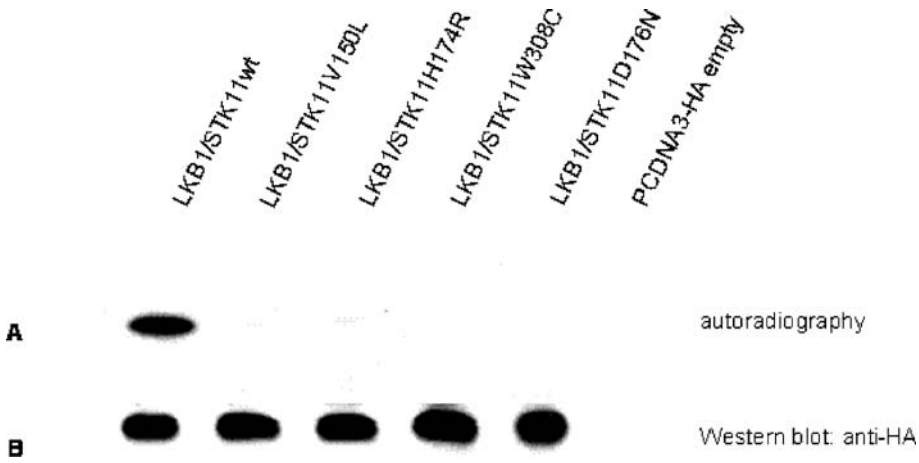

Fig. 1 Autophosphorylation assays of the wild-type and mutant LKB1/STK11 recombinant proteins, as described in patients and Methods. Autophosphorylation assays. **A:** Autoradiography showing that only the *LKB1*/*STK11* wild type is autophosphorylated, whereas all mutants were not autophosphorylated. The PCDNA3-HA vector was used as negative control. **B:** Western blot using anti-HA tag antibodies displaying that equal amounts of the wild-type and different *LKB1*/*STK11* mutant's proteins were used

would not be detected by the combination of SSCP and a sequencing. They comprised 13 of the 29 familial and 6 of the 17 sporadic probands. To assess for gross deletions, the genomic DNA of the 19 *LKB1*/*STK11* mutation negative was digested with EcoRI, HindIII, and BamHI/PstI and subsequently probed with a cloned cDNA fragment that comprised the entire coding region of *LKB1*/*STK11*. Hence, using a Southern blot, no whole-gene or whole-region deletions and small deletions were detected.

Loss of heterozygosity at the *STK11*/*LKB1* locus in the biliary adenocarcinoma

A DNA sample from the biliary carcinoma was evaluated for LOH at the *STK11*/*LKB1* locus using 3 polymorphic microsatellite markers. These markers span approximately 4.0 Mb within chromosome 19p13.3 and D19S886 is localized closest to the *STK11*/*LKB1* (approximately 190 kb telomeric). The results of the LOH analysis are shown in Fig. 2. Allelic loss of 19p13.3 markers was observed in the DNA extracted from biliary adenocarcinoma of the patient with PJS (GE12). This sample (GE12) showed LOH with only 1 marker but not with 2 other markers.

LKB1/*STK11* germline mutation does not correlate with cancer

To assess potential genotype–phenotype correlation, the distributions of cancer development in affected PJS families and sporadic cases with or without *LKB1*/*STK11* mutations were compared. Of 107 clinically affected individuals with *LKB1*/*STK11* mutations, 19 (18%) were diagnosed with cancers. Of 63 clinically affected individuals with symptomatic hamartomatous polyps and mucocutaneous pigmentation but without mutations, 12 (19%) developed cancer. There was no difference in cancer-associated risk between the 2 groups ($P = .84$). Moreover, among the 107 subjects with an *LKB1*/*STK11* mutation, there was no correlation between the type or location of the *LKB1*/*STK11* mutation and the frequency or type of cancer. In fact, there was considerable variability in cancer phenotype among subjects with *LKB1*/*STK11* mutations of all types and locations, even among affected parents and siblings with identical mutations.

Discussion

Of the 46 PJS families in this study, an *LKB1*/*STK11* mutation was identified in 59%, which is similar to the 50–90% frequency reported in other studies of *LKB1*/*STK11* mutations in individuals with PJS [12, 15, 20, 23]. The identification of *LKB1*/*STK11* mutations in only 50–90% of probands (except for the study by Boardman *et al*. [15]) with PJS is attributable to technical limitations of the testing methods presently used and also to genetic heterogeneity [9, 10, 15, 23]. The evidence for the heterogeneity is provided by the linkage studies that excluded the 19p13.3 locus and confirmed the absence of *LKB1*/*STK11* mutations in same families [9, 11, 13, 15]. However, some PJS families are still linked to the 19p13.3 locus despite the absence of mutations in the *LKB1*/*STK11* coding region [12]. Conceivable alternative mechanisms leading to inactivation of the *LKB1*/*STK11* gene include mutations in the regulatory unit or promoter, mutations in a deep intron that was not surveyed in the mutational analysis, and methylation of the promoter region. The latter has yet to be described as a germline mechanism of gene regulation in syndromes where imprinting is not involved. Although mutations in the promoter region in *LKB1*/*STK11* gene seems

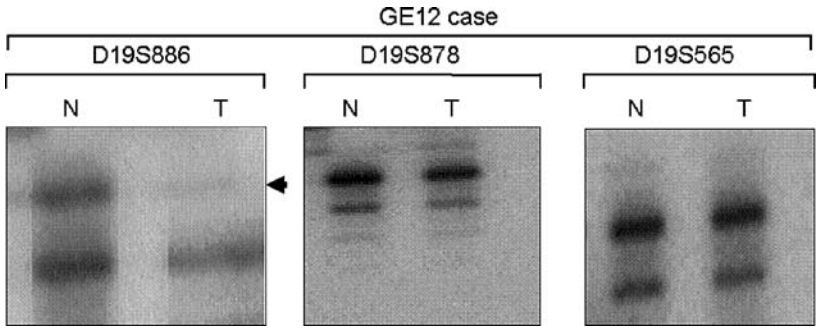

Fig. 2 LOH analysis in the biliary carcinoma. A PJS-associated biliary carcinoma (GE12) exhibits LOH at D19S886 (*arrow*), but not at D19S565 and D19S878

unlikely to contribute to PJS [14, 24], we did not look for mutations in the promoter region of *LKB1*/*STK11* gene in our collection of noncarrier cases; therefore, we cannot exclude such a possibility. Another explanation is the existence of a second gene close to *LKB1*/*STK11* gene that remain to be identified in the PJS families linked to 19p13.3 locus. Among the genes that could be a good candidate for PJS *LKB1*-negative mutation carriers in this latter locus are *BRAF35* and *GIPC3*. *BRAF35* has structural domains similar to those of BAF75, a subunit of the *SWI*/*SNF* complex that was shown to be involved in *LKB1–BRG1* interaction [25]. *GIPC3* gene was shown to modulate WNT signaling [26] where *LKB1* is connected [27, 28]. However, mutation search will definitely clarify whether or not these genes are responsible for PJS *LKB1*-negative carriers. Recently, many LKB1-interacting proteins have been reported [25, 29–32]. One of these proteins could be a good candidate; some have already been excluded [14, 33–35]. In addition, we previously reported that 1 PJS family was linked to 19q.13.4 locus [13] and some of the genes located in this area were excluded [14, 33], but the candidate gene approach is still in progress. Further strategy is to screen the genes that are found upregulated or downregulated in the microarray studies done either on hamartomatous polyps from the knockout *lkb1* mouse [36] or cancer cell lines from human lung cancer with *STK11*/*LKB1* mutations [37]. However, the list of these candidate genes is exhaustive so that 1 gene could emerge as the best candidate.

Among the *LKB1*/*STK11* mutation carriers, there was no correlation between the type or location of the *LKB1*/*STK11* mutation and the presence or type of cancer. This finding is not surprising, given that cancer status can vary markedly between family members sharing the same mutation [9, 10, 23].

A wide range of cancers associated with PJS have been reported in PJS families [38]. Most of these cancers can arise in gastrointestinal organs such as colon, stomach, small intestine, and pancreas or in extra-intestinal organs such as breast, ovaries, testis, and kidney. Interestingly, cancers of the biliary tract were only recently reported in PJS families and to date, germline mutations in the *LKB1*/*STK11* gene associated with primary biliary adenocarcinoma have not been found in PJS families [20], although somatic mutations in the *LKB1*/*STK11* gene in a subset of primary biliary adenocarcinoma (also termed *cholangiocarcinoma*) have been characterized [39, 40]. In one PJS family with an *LKB1*/*STK11* gene insertion mutation of one nucleotide (GE12 family in Table 1) included in the present study, 2 members developed cancer (1 right kidney carcinoma and 1 rectal carcinoma) and third female member developed primary cholangiocarcinoma. Such tumors are rare. Intrahepatic cholangiocarcinoma prevalence ranges from 0.01–0.50% in autopsy series [41] and has a frequency of approximately 10% among primary liver tumors [42]. There were no predisposing factors that could contribute to the development of cholangiocarcinoma such as anatomic anomalies, chronic inflammatory conditions, parasites, hepatolithiasis, autoimmune disease (primary sclerosing cholangitis), nonbiliary cirrhosis, or carcinogens (thorium dioxide that was used before 1955) [41, 42]. We cannot exclude with certainty the possible involvement of other environmental factors in this PJS case.

According to Knudson's model, 2 hits are required for the development of tumors [43]. Consistent with this hypothesis, these somatic mutations would be expected to involve the allele that does not already harbor the germline mutation. Two mechanisms were suggested to explain the inactivation of the second hit; the first is the loss or inactivation of the second copy of the gene through mutations. The second mechanism is the inactivation through the hypermethylation of the CpG island in the promoter region of the gene. This second possibility is less frequent with *STK11*/*LKB1* [44]. Interestingly, LOH at *STK11*/*LKB1* locus has been reported in sporadic cancers originating from the breast, colon, ovary, and pancreas in PJS patients [39, 45–48]. In our biliary adenocarcinoma, we found 1 marker that showed a clear LOH at *LKB1* locus (see Fig. 2), indicating that the PJS gene *STK11*/*LKB1* is a tumor suppressor gene involved in the development of biliary adenocarcinoma. This latter finding is in line with the hypothesis that the PJS gene *STK11*/*LKB1* is a tumor suppressor gene involved in the development of primary biliary adenocarcinoma [39, 40] but contrasting with the recent

study that did not find germline mutations in the DNA of PJS patients with primary biliary adenocarcinoma [20]. In addition, it would be interesting to see if LOH at 19p13.3 locus was present on the DNA of these cancers. Recently, several studies have addressed the question whether LOH near *STK11*/*LKB1* occurs in PJS-associated gastrointestinal cancer [45, 49]. Among these studies, 1 study group investigated 5 PJS-associated gastrointestinal adenocarcinomas (from stomach, small bowel, colon, colon, and pancreas) and 39 gastrointestinal hamartomatous polyps. LOH near *STK11*/*LKB1* was identified in all cancer found in PJS patients [45]. To our best knowledge, this is the first reported case of a PJS patient with an *LKB1*/*STK11* germline mutation who developed primary biliary adenocarcinoma. Therefore, it is likely that PJS families without any *LKB1*/*STK11* germline mutations are not more prone to developing epithelial cancer than families with such mutations, as suggested elsewhere [20].

The present findings should assist the gastroenterologist, gastrointestinal surgeon, and geneticist in counseling families and providing appropriate evaluation and follow-up for patients with PJS. Finally, detailed examination of the clinical and endoscopic phenotype in this cohort should facilitate future studies correlating clinical outcome with molecular pathogenesis.

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