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STK11 Domain XI Mutations: Candidate Genetic Drivers Leading to the Development of Dysplastic Polyps in Peutz–Jeghers Syndrome

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ABSTRACT: Peutz–Jeghers syndrome (PJS) is a rare hereditary disorder resulting from mutations in serine/threonine kinase 11 (*STK11*) and characterized by gastrointestinal (GI) hamartomatous polyps, mucocutaneous pigmentation, and an increased risk for specific cancers. Little is known about the genetic implications of specific *STK11* mutations with regard to their role in dysplastic and malignant transformation of GI polyps. Peripheral blood genomic DNA samples from 116 Chinese PJS patients from 52 unrelated families were investigated for *STK11* mutations. Genotype–phenotype correlations were investigated. The mutation detection rate was 67.3% (51.9% point mutations, 15.4% large deletions). Fourteen out of the 25 point mutations identified were novel. Nearly one-third of all mutations, 8/27 (29.6%), were in exon 7, the shortest out of the nine exons. Strikingly, mutations affecting protein kinase domain XI, encoded in part by exon 7, correlated with a 90% (9/10) incidence of GI polyp dysplasia. In contrast, only two out of 17 (11.8%) nondomain XI mutations were linked to polyp dysplasia ($P = 0.0001$). The extent of the association between dysplasia and the development of GI-related cancers is currently unknown but our results highlight a novel *STK11* genotype–phenotype association as the basis for future genetic counseling and basic research studies.
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KEY WORDS: dysplastic polyp; hereditary cancer; malignancy; Peutz–Jeghers syndrome; *STK11*; *LKB1*

Introduction

Peutz–Jeghers syndrome (PJS; MIM #175200) is a rare autosomal dominant disorder characterized by gastrointestinal (GI) hamartomatous polyps, mucocutaneous pigmentation, and an age-dependent increased risk for development of GI and extraintestinal malignancies [Giardiello et al., 2000; Hearle et al., 2006]. The estimated relative cancer risk is 9–18-fold higher than in the general population [Giardiello et al., 1987; Boardman et al., 1998]. The cumulative cancer risk for GI cancers is 20% at the age of 40 years increasing to >70% at the age of 70 years [van Lier et al., 2011]. Mutations of the tumor suppressor gene serine threonine kinase 11/liver kinase B1 (*STK11*; MIM #602216) on chromosome 19p13.3 have been identified as the major cause of PJS [Hemminki et al., 1998; Jenne et al., 1998; Wang et al., 1999].

The attempt to correlate both the type and site of *STK11* genetic mutations with the risk of malignancy has been the focus of many studies and progress in this area would represent an important research and clinical advance. Previous evidence suggested that mutations in exon 3 were associated with a higher cancer risk [Lim et al., 2004] whereas in another study, statistically significant evidence correlated mutations in exon 6 with higher cancer risk [Mehenni et al., 2006]. Schumacher et al. (2005) found that missense mutations in the C terminus and regions VIB-VIII of the protein were more frequently associated with malignancies. On the other hand, in-frame deletions and splice site mutations have been found to be only rarely associated with malignancies, whereas PJS patients with breast cancer were found to predominantly have truncating mutations [Schumacher et al., 2005]. Unfortunately, the analysis of additional sample sets by other groups seeking to validate these findings has not yet provided a clear genotype–phenotype association with malignancy [Hearle et al., 2006].

In general, familial cancer syndromes have provided unique insights into tumor initiation and progression. In direct contrast to the paradigm established by the adenoma–carcinoma sequence of tumor progression in familial adenomatous polyposis, the potential for malignant degeneration of PJS polyps is controversial and evidence of dysplasia occurring in PJS polyps is rare [Jansen et al., 2011]. Shepherd et al. (1987) examined 491 polyps and identified no dysplastic polyps in their cohort of samples. In an independent study by Latchford et al. (2011), in 2,461 polyps, only six were found to contain atypic hyperplasia or dysplasia. Thus, given this very low frequency of dysplasia, the exact role, if any, of the PJS polyp in cancer development is unclear.

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In the present study, we directly interrogated *STK11* gene mutations in a large cohort of 116 Chinese PJS patients representing 52 index cases. Based on the size of this domain, mutations in kinase domain XI of the *STK11* protein were overrepresented in our population when compared with other published studies. Moreover, mutations in this domain correlated with a very high incidence of dysplastic GI hamartomatous polyps. These findings may prove valuable for patient counseling and may provide mechanistic insight into the link between PJS polyp development, dysplasia, and cancer.

Materials and Methods

Patient Recruitment

From 2006 to November 2012, 133 patients from 28 PJS families and 33 sporadic cases were identified from a regional Chinese population for clinical evaluation, performed in Nanfang Hospital. Seventeen patients did not consent to genetic analysis and were therefore excluded from these molecular studies. In total, 116 patients, representing 52 unique PJS index cases, were available for *STK11* sequence analysis. The diagnostic criteria for PJS included the presence of characteristic mucocutaneous pigmentation, the presence of hamartomatous polyps with a core of large tree-like branches of smooth muscle covered with displaced epithelium specific to the involved area of the GI tract, and a family history of PJS. Patients needed to fulfill two of these three criteria for a clinical diagnosis of the disease [Giardiello et al., 1987] and all met criteria recommended by the WHO [Hamilton et al., 2000].

Clinical data were collected retrospectively using a standardized questionnaire and patients' medical records. Endoscopic or surgical polypectomy, including histopathologic examination (reviewed by at least two pathologists), was performed in all patients. In general, patients had endoscopic polypectomy every 1–3 years depending on medical recommendation. The histologic criteria used in this study to define dysplasia in intestinal epithelium on H&E-stained slides were the presence of "enlarged, hyperchromatic nuclei, varying degrees of nuclear stratification and loss of polarity" which is based upon the most recent edition of the WHO [Bosman et al., 2010]. Primary tumors with the diagnoses confirmed by tissue review or pathology reports were eligible for inclusion. All participating PJS patients and their family members gave informed consent for this investigation and the principles outlined in the Declaration of Helsinki were followed. The project was approved for human study by the Medical Ethics Committee, Nanfang Hospital of Southern Medical University.

Detection of Point Mutations

Genomic DNA was extracted from peripheral blood using the QiAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). PCR primers were designed to amplify the *STK11* gene exons and intron–exon boundaries (GenBank NM_000455.4) using the software program Primer 3.0 (http://frodo.wi.mit.edu/primer3/). Sanger sequencing was performed for each index case in the 25 families and 27 sporadic cases. Details of primer sequences and PCR conditions are available upon request. The PCR products were then sequenced in both orientations using an ABI 3730 XL Automated DNA Sequencer (Applied Biosystems, Foster City, CA) with the ABI BigDye Terminator v3.1 cycle sequencing kit. Standard nomenclature (http://www.hgvs.org/mutnomen/) was used to describe sequence variations, with +1 corresponding to the A of the ATG translation initiation codon of GenBank NM_000455.4 for *STK11*. All sequence variants were submitted for deposit in the Leiden Open Variation Database (http://databases.lovd.nl/shared/variants/STK11).

In familial cases, identified mutations were further tested in all available family members to confirm segregation of the mutation with the disease. All variants identified were screened against the dbSNP database to rule out the possibility of these variants representing polymorphisms.

Detection of Large Genomic Deletions by MLPA

A search for large intragenic deletions was performed using the MLPA assay. The MLPA test kit (SALSA P101-B1 *STK11*; MRC-Holland, Amsterdam, The Netherlands) contained 12 paired probes against the *STK11* region (three probes for exon 1, one each for the other eight coding exons, and one probe for the noncoding exon 10 in the 3′ untranslated region of the gene). In addition, it contained four probes localized at 0.6 and 0.9 MB telomeric and one probe at 10 MB centromeric to the *STK11* locus, and another nine probes from other chromosomal regions as controls. Deletion screening was performed according to the manufacturer's protocol. Briefly, 100 ng of genomic DNA in 5 μl TE buffer was heat-denatured and incubated with the probe set for 16 hr at 60°C. The hybridized products were then ligated, amplified by PCR, and separated on an ABI 3130 Genetic Analyzer in conjunction with Genotyper software (version 4.0; Applied Biosystems).

MLPA assay results were analyzed using GeneMarker® HID STR Human Identity Software (Softgenetics LLC, State College, PA). Values of 0.85–1.15 indicate normal results (presence of two copies), and values of 0.35–0.65 or 1.35–1.65 indicate a deletion or duplication, respectively. All identified deletions were confirmed in a second independent reaction and confirmed to segregate within the relevant family members.

Immunohistochemistry

Immunohistochemistry was performed using monoclonal antibodies against Ki-67 (MIB-1; Dako, Glostrup, Denmark; 1:300 dilution), and p53 (DO7; Dako; 1:200 dilution). Sections (4–5 μm) were cut and deparaffinized. Endogenous peroxidase activity was blocked in 0.3% H_2O_2 for 20 min. Slides were submerged in citrate buffer (0.01 M, pH 6.0) and heated in a temperature probe controlled microwave oven for 10 min at 100°C. After cooling for 30 min, 10% normal goat serum in PBS was applied for 20 min. Incubations with primary antibodies were carried out overnight at 4°C and for 1 hr at room temperature. Sections were washed three times with PBS and incubated with HRP-labeled goat anti-mouse IgG (EnVision™+; Dako) for 30 min. Sections were washed four times with PBS. After DAB precipitation, a hematoxylin counterstaining was performed. A known p53-positive CRC was used as a positive control for p53 staining. p53 staining was scored as positive when more than 10% of the cells demonstrated nuclear expression.

Statistical Analysis

Differences between groups were determined using chi-squared (χ^2) test or Fisher's exact test. The statistical analyses were assessed using SPSS 17.0 software (SPSS Inc., Chicago, IL). A value of $P < 0.05$ was considered statistically significant.

Table 1. Baseline Clinical Characteristics of 133 PJS Patients

Characteristic	*N* (Frequency)
Sex	
Male	73 (54.9%)
Female	60 (45.1%)
Mean age of diagnosed	21 years (1–43 years)
Family history of index cases	
Sporadic	33 (54.1%)
Familial	28 (45.9%)
Symptoms	
Abdominal pain	118 (88.7%)
Rectal bleeding	84 (63.2%)
Bowel obstruction	90 (67.7%)
FOB (+)	97 (72.9%)
Anemia	74 (55.6%)
Intussusception	85 (63.9%)
Mean age of the first intussusception	17.29 years (3–68 years)
Cancer history	
Cancer frequency	25 (18.8%, 25/133)
Mean age at cancer onset	37.4 years (n = 27)
Gastrointestinal cancer frequency	18 (66.7%, 18/27)
Breast and gynecological cancer frequency	7 (25.9%, 7/27)
Patients with gastrointestinal dysplastic hamartomas	16 (12%, 16/133)
Mean age at dysplasia diagnosed	30.4 years (16–44 years)

FOB, fecal occult blood.

Results

Clinical Characteristics of Patients with PJS

Our clinical studies focused on 133 Chinese PJS patients from 61 unrelated families (Table 1). Twenty-eight PJS index cases were familial (total 100 familial patients) and 33 were sporadic. The average age of disease diagnosis was 21 years (range, 1–43 years). In all, 85 patients (85/133, 64%) had received at least one laparotomy (range, 1–5) and the average age at first laparotomy was 17.29 years (range, 3–68 years). Polyp-induced complications, primarily intussusception of the small bowel, were the major referring cause for laparotomies.

The overall cancer frequency in our study population was 25/133 (18.8%) and the mean age at cancer onset was 37.4 years ($n = 27$). Of these, 24 cancer patients had a family history of PJS, whereas only one was sporadic. Two patients were diagnosed with two different primary malignancies. From a total of 27 malignancies, GI cancer was noted in 18 cases (18 out of 27, 66.7%). Breast and gynecological cancers were noted in 25.9% (7/27).

Of particular interest, GI dysplastic hamartomas were identified in 16 patients (16 out of 133, 12%) and these were detected at a mean age of 30.4 years. The total number of polyps histologically examined from our 133 patients was 1,427 (Fig. 1). Of these, 17 (1.2%, 17 out of 1,427) were defined as dysplastic. All hamartomas retained the normal topographical expression of Ki67, which was restricted to the crypt base (Fig. 1B). However, the aberrant Ki67 staining was present in hamartomas with dysplastic changes (Fig. 1F and J). Positive p53 staining, suggesting the possible presence of mutant p53 protein, was not observed in hamartomas or low grade dysplasia, but was evident in five high grade dysplasia samples tested (Fig. 1G and K).

STK11 Mutations and Genomic Deletions

We identified germline point mutations in 27 out of 52 (51.9%) of the index cases (Table 2). Twenty-five different point mutations were identified. Of these, 15 were found in familial (15 out of 25, 60%) and 12 in sporadic (12out of 27, 44.4%) cases. We detected eight missense mutations and 17 different truncating mutations. Ten mutations (10 out of 25, 40%) were associated with cancer in the index patient and/or in relatives with PJS. To our knowledge, 14 (14 out of 25, 56%) of these mutations are novel: four deletion, three insertion, two nonsense, two splice site, and three missense mutations. The novel missense mutations were all within the kinase domain and, based on in silico analysis, are all predicted to be pathogenic (PolyPhen2: http://genetics.bwh.harvard.edu/pph2/).

Intriguingly, nearly one-third (eight out of 27, 29.6%) of all point mutations clustered in exon 7. Exon 7 is the shortest of the nine exons encoding just 19 out of the 433 amino acid full-length space protein (Fig. 2). Five out of these eight mutations localize to a highly conserved, putative phosphorylation site (amino acids 297–302, **Arg**-**Phe**-Ser-Ile-Arg-**Gln;** affected residues are highlighted in bold) [Bosman et al., 2010].

We next analyzed for the presence of exonic rearrangements by using MLPA in the 25 PJS probands in whom no mutation was identified by Sanger sequencing. The overall frequency of large deletions was eight out of 52 (15.4%). Three (three out of eight, 37.5%) deletions were associated with cancer in the index patient and/or in relatives, and one was associated with dysplastic changes in a hamartoma (Table 2). The large deletions ranged in size from one to seven exons (Fig. 2). All deletions were predicted to affect the kinase domain of the protein.

Genotype–Phenotype Correlations

By applying both direct sequencing and the MLPA assay, the total mutation detection rate in our population was 67.3% (35 out of 52). A higher frequency of mutations was identified in our patients with a family history of PJS (20 out of 25, 80%) when compared with our sporadic cases (15 out of 27, 55.6%); however, the difference was not statistically significant ($P = 0.06$, $\chi^2 = 3.525$).

Eighteen (18 out of 83, 21.7%) patients with an *STK11* mutation developed a malignancy, which was not statistically different than the cancer frequency in our patients without *STK11* mutations (five out of 33, 15.2%) ($P = 0.426$, $\chi^2 = 0.634$). None of the five splice site mutations or four small in-frame deletions was associated with malignancies in the index patient or their affected relatives. In contrast, all five insertion mutations and three out of four nonsense mutations were associated with malignancies. However, no difference was detected between missense mutations (two out of eight) and large deletions (three out of eight) with regard to cancer risk.

We next sought a possible genotype–phenotype correlation (Table 2). Ten out of the 27 germline mutations (37%) that we identified were present in kinase domain XI (amino acids 277–309). Strikingly, nine out of the ten mutations (90%) were associated with GI tract hamartomatous polyp dysplasia. Conversely, only two out of the remaining 17 (11.8%) mutations, one each in domains I and V, respectively, were associated with dysplastic changes in polyps ($P = 0.0001$). Similarly, the dysplasia risk in those with domain XI mutations (10 out of 133 polyps in total in this group) compared against those without domain XI mutations (five out of 1,112 polyps in total) is statistically significant ($P = 0.0001$). Although there was no difference between these two groups for overall cancer frequencies (50% vs. 35.3%; $P = 0.687$), three out of the five of the domain XI cancers were GI. Two individuals had gastric cancer, and one each had pancreatic, liver, or colorectal cancer.

To assess the possible importance of kinase domain XI mutations in other populations and cancers, we performed a search of two publically available databases. Interrogation of the Human Gene

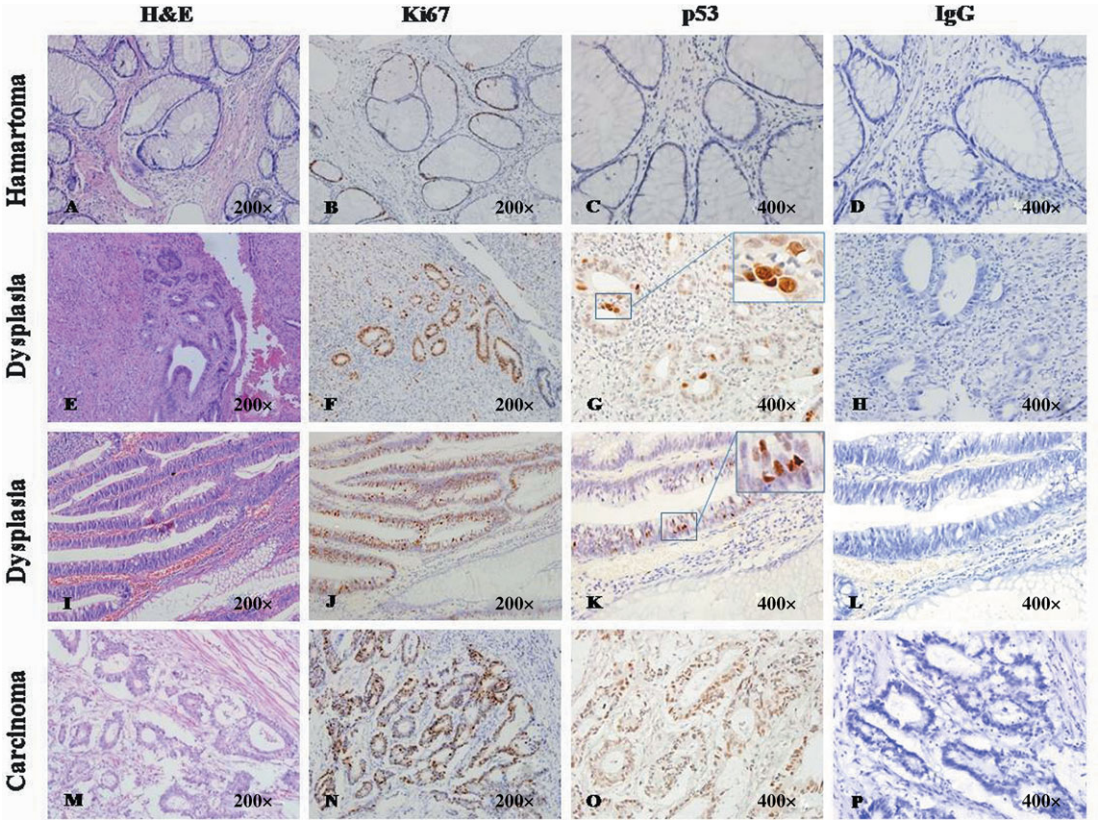

Figure 1. Representative examples of a hamartomatous polyp (**A–D**) and high-grade dysplasia (**E–L**) and carcinoma in hamartomatous polyps (**M–P**), from four individuals. H&E stained micrographs reveal the branching bundles of smooth muscle fibers characteristic of hamartomatous polyps in Peutz–Jeghers syndrome (**A**, **E**, **I**, and **M**). Ki67 staining demonstrates areas of increased proliferation in the dysplastic (**F and J**) and cancerous (**N**) lesions compared with the hamartoma (**B**). Increased p53 staining is evident in a cluster of cells (inset) in high-grade dysplasia (**G and K**) and is present diffusely in carcinoma (**O**).

Mutation Database (HGMD) revealed a total of 22 domain XI mutations from 18 reported studies. Of these, 13 mutations were linked with clinical information and 10 (76.9%) were associated with the development of cancer (Table 3). In addition, mutations in domain XI represent 14.8% (35 out of 237) of all *STK11* mutations in the COSMIC database (including lung, skin, gynecological, and GI cancers) (Table 4).

Discussion

In this study of Chinese PJS patients, we analyzed a total of 133 affected individuals, which included index cases and family members. Direct sequencing of the *STK11* gene combined with the MLPA assay for detection of deletions resulted in a mutation detection rate of 67.3% (35 out of 52). This rate is consistent with the 50%–90% frequency reported by different centers [Jenne et al., 1998; Mehenni et al., 1998; Ylikorkala et al., 1999]. Of the mutations we identified in this present study, 14 were novel and thus extend the spectrum of known disease-causing *STK11* mutations.

While an increased cancer risk is well established for PJS, the molecular stages leading up to cancer development are unclear and the exact role of GI polyps in malignant transformation is debated. In our cohort, 25 patients (18.8%) developed a total of 27 malignancies at an average age of 37.4 years. The age and frequency of malignancies in our patients, while slightly younger and lower, respectively, is consistent with those reported in other studies but difficult to further evaluate given a possible ascertainment bias. For example, van Lier et al. (2010) evaluated 20 cohort studies representing a total of 1,644 patients wherein 349 (21.2%) developed 384 malignancies at an average age of 42 years.

An unexpected finding from our patient cohort was the marked overrepresentation of mutations present within two contiguous exons, exons 6 and 7, which encode *STK11*'s kinase domain XI. Thirty-seven percent (10 out of 27) of the mutations we identified, not including deletions, were present in this domain. In addition, and highly suggestive of a functional link, nearly all of these mutations (nine out of 10) were associated with the presence of areas of dysplasia in GI polyps. As a group, these patients were relatively young (average age of 26.8 years). As follow-up time increases, it will be interesting to note if there is an increase in either the frequency of cancer or dysplasia in this population. This association between kinase domain XI mutations with dysplastic changes was in marked contrast to the low frequency of dysplasia in the other 17 mutation carriers identified in our study. In these, only two had evidence of dysplasia.

In our study, 12% (16 out of 133) of patients have dysplastic polyps. Other groups have recently reported rates of up to 17% (nine out of 52) [Korsse et al., 2013]. The total number of polyps analyzed in our 133 patients was 1,427, of which 17 (1.2%; 17 out of 1427) were diagnosed as dysplastic. This percentage is, in essence, equal to the 1.4% identified by Korsse et al. (2013) in their recent study.

Table 2. *STK11* Gene Mutation Spectrum and Clinical Characteristics of the 35 PJS Families

Family	Exon	Domain	Nucleotide change	Protein change	Dysplastic changes					Detected polyps number		Malignancies		
					Localization	Size (mm)	Age	*M*	*R*	Affected	Member (s)	Member	Type	Age
Point mutations														
1S	1	I	c.151_162del	p.M51_L54del	–	–	–	–	–	11	–	–	–	–
2F*	1	I	c.179dupA	p.Y60*	Colon	30 × 30	25	M1	R1	9	14	Grandfather	Lung	58
3F*	1	I	c.180C > A	p.Y60*	–	–	–	–	–	24	6, 4	Father	Leukemia	36
4S*	Intron 1	III	c.290+1G > A	-	–	–	–	–	–	11	–	–	–	–
5S	Intron 1	III	c.291-1G > A	-	–	–	–	–	–	13	–	–	–	–
6F	2	III	c.308_317del	p.R103Sfs*23	–	–	–	–	–	8	7	–	–	–
7F*	2	IV	c.351dupA	p.Y118Ifs*45	–	–	–	–	–	12	10	Grandfather	Gastric	32
8F	3	V	c.402_403dupTG	p.G135Vfs*27	Small bowel	40 × 35	28	M2	R1	12	Unknown	Mother	Cervix	28
9F	3	VIA	c.455_478dup	p.Q160_Q167dup	–	–	–	–	–	10	12, 9	Father	Colon	42
10S*	4	VIB	c.521A > G	p.H174R	–	–	–	–	–	11	–	–	–	–
11S*	4	VIB	c.526G > A	p.D176N	–	–	–	–	–	15	–	–	–	–
12S*	5	IX	c.725G > A	p.G242E	–	–	–	–	–	13	–	–	–	–
13S*	Intron 5	IX	c.734+1G > A	-	–	–	–	–	–	9	–	–	–	–
14F*	Intron 5	IX	c.734+1G > A	-	–	–	–	–	–	12	6, 14, 10, 4	–	–	–
15S	Intron 5	IX	c.734+2T > C	-	–	–	–	–	–	10	–	–	–	–
16S	6	X	c.783C > G	p.Y261*	–	–	–	–	–	11	–	–	–	–
17F	6	X	c.787_790dup	p.E265Vfs*2	–	–	–	–	–	13	7, 12, 4	Proband	Colon	54
18S	6	XI	c.834_835del	p.C278Wfs*6	Small bowel	15 × 40	16	M1	R2	19	–	–	–	–
19F	6	XI	c.862G > A	p.G288R	Gastric	25 × 35	23	M1	R2	11	7, 8	Father	Gastric	68
20S	7	XI	c.866T > G	p.M289R	Colon	40 × 30	28	M1	R2	14	–	–	–	–
21F*	7	XI	c.890G > A	p.R297K	Small bowel	30 × 30	26	M2	R1	9	Unknown	Mother	Pancreas	52
22S	7	XI	c.891G > C	p.R297S	Small bowel	30 × 40	21	M2	R1	13	–	–	–	–
23F	7	XI	c.892_893insC	p.F298Sfs*20	Small bowel	30 × 20	33	M2	R1	16	3, 4	Father	Gastric	66
24F*	7	XI	c.904C > T	p.Q302*	Colon	≥30	27	M1	R1	12	6	Mother	Liver	45
25F*	7	XI	c.904C > T	p.Q302*	Colon	50 × 45	23	M1	R1	10	12, 9	Father	Colon	61
26F	7	XI	c.898_906del	p.I300_Q302del	Gastric, colon	≥30	44	M1	R2	21	7, 4	Unknown	Unknown	Unknown
27F*	7	XI	c.916C > T	p.H306Y	–	–	–	–	–	8	12	Unknown	Unknown	Unknown
Exonic deletions														
28F	3		c.375-?_464+?del		Colon	25 × 45	24	M1	R1	14	6, 8, 9, 12, 3, 6	–	–	–
29F	4–6		c. 465-?_862+?del		–	–	–	–	–	17	4	Mother	Breast	45
30F	1		c.-1114-?_290+?del		Colon	45 × 50	27	M2	R1	13	12, 8	Mother, Aunt	Breast, Cervix, Colon	42, 27, 45
31F	1		c.-1114-?_290+?del		–	–	–	–	–	12	6, 4	–	–	–
32S	1		c.-1114-?_290+?del		–	–	–	–	–	7	–	–	–	–
33S	1		c.-1114-?_290+?del		–	–	–	–	–	9	–	–	–	–
34F	3–10		c.375-?_1365+? del		–	–	–	–	–	Unknown	9, 5	Proband	Pancreas	39
35S	6		c.735-?_862+?del		–	–	–	M1	–	14	–	–	–	–

Dash "–", no evidence of dysplasia, detected polyps or malignancy. Family history: "Y", at least two affected persons in the family; "S", the index patient is the only known affected individual. If the mutation identified in our families has been previously reported, this is indicated by the asterisk "*": the publication reference of 2F and 7F is Aretz et al. (2005); the reference of 3F is Wang et al. (1999); the reference of 4S, 12S, 13S, and 14F is Olschwang et al. (2001); the reference of 10S is Mehenni et al. (2006); the reference of 11S is Mehenni et al. (1998); the reference of 21F is Westerman et al. (1999); the reference of 24F and 25F is Wang et al. (2011); the reference of 27F is Boardman et al. (2000). "M", methods to detect gastrointestinal dysplastic polyps: "M1", endoscopy; "M2", surgery. "R", reasons underlying timing of investigation: "R1", patient symptoms; "R2", routine surveillance.

To the best of our knowledge, this is the first reported genotype–phenotype correlation between *STK11* mutations and dysplasia. Of possible interest, eight out of these 10 "dysplasia-linked" mutations were novel (Fig. 2) and may explain why this association had not been previously identified in studies of other ethnicities. Further analysis of this cohort, additional studies in independent cohorts of patients including both Chinese and non-Chinese cohorts, and directed molecular studies on these specific kinase domain XI mutations will be required to test this hypothesis. From our current results, it is difficult to distinguish if kinase domain XI mutations contribute directly to the development of dysplasia or contribute through an indirect mechanism related to increased polyp proliferation and growth. Both would be clinically relevant and could have implications on the surveillance and management of PJS patients.

If, as some studies suggest, dysplasia represents a midpoint along a continuum of malignant transformation in PJS [Mehenni et al., 1998; Wang et al., 1999], then it would be predicted that mutations in this domain should also result in a higher association with GI malignant transformation. Although our study was not designed for directly testing this hypothesis, three out of the five cancers associated with these mutations in our population were GI-tract related (Table 2). Future studies will need to be designed to directly test this hypothesis in PJS patients. Intriguingly, Mehenni et al. (2006) reported that mutations in exon 6 of the *STK11* gene were associated with a higher cancer risk. In total, they identified 14 exon 6 mutations associated with the development of cancers and, interestingly, eight mutations were located within kinase domain XI. Cancer development was associated in 77% (10 out of 13) of the *STK11* domain XI mutations present in the HGMD with linked clinical information further suggesting the importance of this domain (Table 3).

We also examined the possibility that domain XI mutations might be enriched in sporadic cancers but could only conclusively determine that these mutations are well represented in cancer sequence databases. Analysis of the COSMIC database did however reveal that of the nine samples representing the large intestine, which possessed an *STK11* mutation, the one sample, which contained a domain XI mutation, was derived from an adenoma with high-grade dysplasia (sample ID COSS1010958; Table 4).

In summary, our results in a large cohort of Chinese PJS patients identified an overrepresentation of mutations within *STK11*'s kinase domain XI and demonstrated that these mutations were strongly

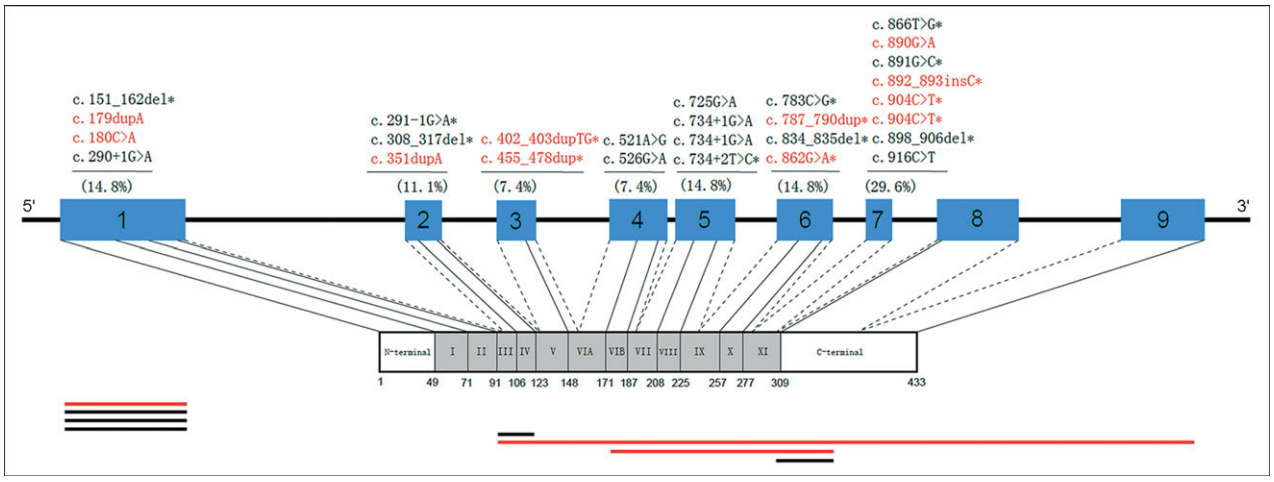

Figure 2. Schematic representation of the *STK11* gene and sites of point mutations and large deletions identified in this study. Coding exons are depicted as blue boxes; exon and intron sizes are drawn to scale. Mutations are shown above the gene, whereas lines drawn on the bottom of the figure represent deletions. Mutations marked with an asterisk (*) are novel including the nonsense mutation, c.904C>T, which we recently reported [Wang et al., 2011]. Mutations associated with cancer in either the index case or family members are highlighted in red.

Table 3. Spectrum of Previously Reported *STK11* Domain XI Mutations and Cancer Associations

Number	Codon	Mutation	Number and type of cancer	Reference
1	279	c.837_842insC	1 AML, 1 duodenum, 1 kidney, 1 pancreas	Schumacher et al. (2005)
2	280	c.842_843insC	1 colon, 1 breast	Amos et al. (2004)
			1 pancreas, 1 kidney, 1 colon	Boardman et al. (2000)
3	281	c.843delC	1 pancreas, 1 kidney	Olschwang et al. (2001)
			1 duodenum	Nakamura et al. (2002)
			1 esophagus, 1 small intestine	Mehenni et al. (2006)
4	282	c.846delC	2 breast	Lim et al. (2003)
5	297	c.891G>T	1 colon	Boardman et al. (2000)
6	297	c.890delG	2 gastric, 2 thyroid, 1 pancreas	Shinmura et al. (2005)
7	304	c.910C>T	2 breast	Lim et al. (2003)
8	304	c.914delA	1 pancreas, 1 small intestine	Ylikorkala et al. (1999)
9	306	c.916C>T	1 thyroid	Boardman et al. (2000)
10	–	c.920+1G>C	1 colon	Ylikorkala et al. (1999)
11	300	c.903delG	0	Mehenni et al. (1998)
12	–	c.920+1delG	0	Scott et al. (2002)
13	–	c.920+978G > C	0	Wei et al. (2003)

Table 4. Somatic Mutations in Domain XI of the *STK11* Gene Associated with Cancer in COSMIC

Number	Codon	Nucleotide change	Protein change	Cancer type (number)
1	279	c.835_836GG > TT	p.G279F	Lung (1)
2	281	c.843C > T	p.P281L	Colon (1), lung (1), ovary (1), stomach (1)
3	290	c.870T > A	p.L290L	Ovary (1)
4	297	c.891G > C	p.R297S	Lung (1)
5	298	c.894C > A	p.F298L	Cervix (1)
6	304	c.910C > T	p.R304W	Cervix (2)
7	304	c.910C>G	p.R304G	Lung (2)
8	308	c.923G > T	p.W308L	Lung (2)

associated with the development of dysplastic GI polyps. Given these findings and the fact that *STK11* mutations in the Chinese PJS population have been primarily limited to case reports, we therefore reexamined this literature [Wang et al., 2000; Zuo et al., 2007; Gao et al., 2010; Liu et al., 2011; Liu et al., 2011; Chen et al., 2012; Liu et al., 2012; Zhao et al., 2012]. Interestingly, seven out of the 23 mutations defined in the eight case reports were in domain XI (30.4%; Table 5). This further supports our hypothesis of a unique role for this kinase domain in this population. Although we did not evaluate *STK11* protein levels to assess the degree of correlation with dysplastic changes, we believe that the *STK11*-XI domain mutations induce the activation of specific signaling pathways rather than affect *STK11* protein levels. This will be explored in future studies. Similarly, for future studies, and based on the hypothesis that specific mTOR inhibitors may provide effective therapy for treatment of both hamartomas and carcinomas in *STK11*-deficient PJS patients [van Veelen et al., 2011], we plan on exploring the possibility that different *STK11* mutations affect mTOR signaling differentially. Taken together, our findings provide a novel testable hypothesis for shedding light on our understanding of the development of cancer in this syndrome. Although the clinical implications of a genotype–phenotype correlation with kinase domain XI mutations and cancer are not entirely clear at this time, if confirmed increased monitoring and surveillance may be recommended for those individuals with these *STK11* mutations. Similarly, these findings may provide insight into the genetic pathways leading from hamartoma development through dysplasia and cancer progression in this syndrome.

Table 5. Spectrum of Previously Reported *STK11* Gene Mutations in the Chinese Population

No.	Domain	Protein change	Reference
1	N-terminal	p.Q16G	Liu et al. (2011)
2	I	p.Y60*	Zuo et al. (2007)
3	I	p.Y60*	Zhao et al. (2012)
4	I	p.Y60*	Zhao et al. (2012)
5	II	p.K84*	Zhao et al. (2012)
6	II	p.C73S	Gao et al. (2010)
7	IV	p.Q123*	Zhao et al. (2012)
8	VIA	p.H154Q	Zhao et al. (2012)
9	VIA	p.Y156Tfs*5	Chen et al. (2012)
10	VIII	p.Q220*	Zhao et al. (2012)
11	IX	p.F234Lfs*3	Liu et al. (2011)
12	IX	p.F234Y	Chen et al. (2012)
13	IX	p.Y246*	Zhao et al. (2012)
14	XI	p.P281Rfs*6	Zhao et al. (2012)
15	XI	p.P281Rfs*6	Wang et al. (2000)
16	XI	p.P281Rfs*6	Wang et al. (2000)
17	XI	p.P281Rfs*6	Chen et al. (2012)
18	XI	p.R297K	Zhao et al. (2012)
19	XI	p.R297S	Zhao et al. (2012)
20	XI	p.W308C	Chen et al. (2012)
21	C-terminal	p.R333Hfs*25	Zuo et al. (2007)
22	C-terminal	p.F354L	Chen et al. (2012)
23	C-terminal	p.T363I	Liu et al. (2012)

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