CMLS, Cell. Mol. Life Sci. 55 (1999) 735–750
1420-682X/99/050735-16 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

The molecular basis and clinical aspects of Peutz-Jeghers syndrome

A. Hemminki

Department of Medical Genetics, Haartman Institute, P.O. Box 21, FI-00014 University of Helsinki (Finland), Fax + 358 9 1912 6677, e-mail: Akseli.Hemminki@Helsinki.Fi

Received 12 October 1998; received after revision 30 November 1998; accepted 30 November 1998

Abstract. Peutz-Jeghers syndrome (PJS) is a classic, but not widely known hereditary trait [1, 2]. Its clinical hallmarks are intestinal hamartomatous polyposis and melanin pigmentation of the skin and mucous membranes. In addition, PJS predisposes to cancer [3, 4]. The most common malignancies are small intestinal, colorectal, stomach and pancreatic adenocarcinomas. Other cancer types that probably occur in excess in PJS families include breast and uterine cervical cancer, as well as testicular and ovarian sex cord tumors. The relative risk of cancer may be as high as 18 times that of the general population, and the cancer patients' prognosis is reduced. Recently, the predisposing locus was mapped to 19p13.3 using a novel method [5]. Subsequently, the causative gene was shown to be *LKB1* (a.k.a. *STK11*), a serine/threonine kinase of unknown function [6]. Although preliminary reports seem to suggest a minor role for *LKB1* in sporadic tumorigenesis [7–12], further investigations are needed.

Key words. Peutz-Jeghers; hamartoma; polyposis; hereditary cancer; serine/threonine kinase; LKB1; STK11.

The Peutz-Jeghers syndrome

The first report describing Peutz-Jeghers syndrome (PJS) was probably by a London surgeon, Jonathan Hutchinson, in 1896 [13]. He reported identical twins with spots on the lips and buccal mucosa. However, the first investigator to recognize the connection between 'polyps and spots' was Dr. Peutz in 1921 [1]. In 1949 Drs. Jeghers, McKusick and Katz realized the autosomal dominant inheritance and made the syndrome better known to the scientific community [2]. Originally, the increased risk of cancer associated with the syndrome was not recognized, and interest in PJS was mainly due to the morbidity and mortality caused by the intestinal hamartomas and the sometimes conspicuous melanin pigmentation.

PJS and the other intestinal polyposes are inherited in an autosomal dominant manner. The frequency is not known, since its malignant potential has been recognized only recently, and there are no accurate registries that would give a reliable figure. An estimate has placed the frequency between 1:8300 and 1:29,000 births [14], whereas in Finland it has been estimated to be between 1:50,000 and 1:100,000 (H. Järvinen, unpublished). Improved awareness and proper registries may improve the accuracy of these figures.

Although PJS is a classic syndrome, interest in determining the genetic defect has only recently been created, since it has been realized, that the cancerogenic potential of the hereditary mutation is actually quite significant [3, 4, 15]. In 1995, Bali and colleagues reported evidence of linkage of the syndrome to chromosome 1 [16]. However, this preliminary finding could not be confirmed with analysis of additional markers (C. Amos, unpublished). Markie and colleagues described a PJS patient with a pericentric inversion in chromosome

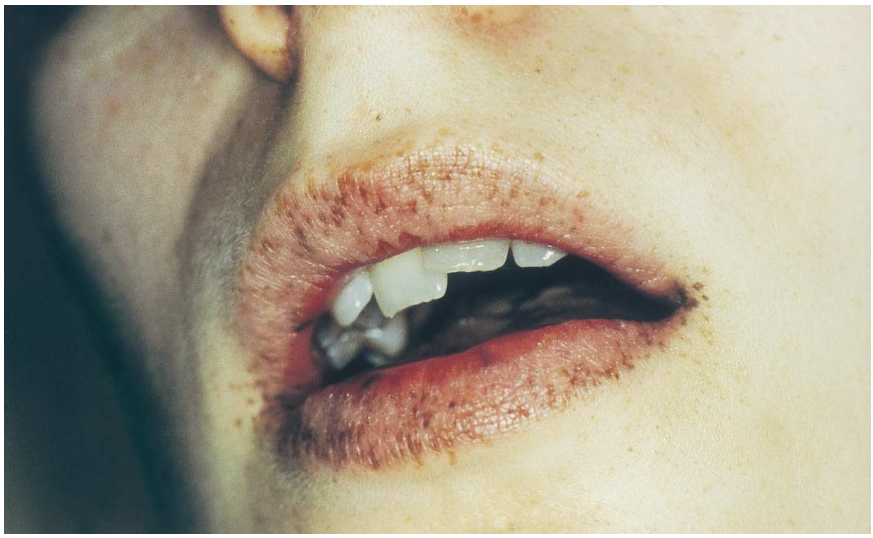

Figure 1. A Peutz-Jeghers patient showing perioral mucocutaneous pigmentation.

6, proposing candidate loci [17], but no linkage was detected in PJS families [18]. In 1997 the predisposing locus was shown to be in 19p13.3 with linkage analysis targeted with comparative genomic hybridization and microsatellite loss of heterozygosity analysis [5]. The next year the causative gene was shown to be *LKB1*, a previously known but unmapped serine/threonine kinase of unknown function [6].

Clinical aspects of PJS

The age of onset of the pigmentation (fig. 1) is usually in the first decade. However, it varies from 15 days [19] to no pigmentation at all. In a large Japanese series, the average age of diagnosis was 23 years in males and 26 in females. The presenting clinical symptoms were obstruction (43%), abdominal pain (23%), rectal bleeding (14%) and extrusion of polyp (7%). Intussusception occured in 47% of patients [20]. The penetrance of the genotype into a phenotype seems to be high. In most studies, no cases of nonpenetrance have been seen [5, 21–23], but one case has been reported [24]. However, as similar pigmentation occurs in up to 15% of the general population [25], the diagnosis should be based on detection of polyps and histological examination to confirm that at least some of the polyps represent typical PJS hamartomas (table 1).

In PJS, polyps can arise anywhere in the gastrointestinal tract, but are most common in the jejunum. In three large series with a total of 404 patients, 78% of patients had polyps in their small intestine, 42% in their colon, 38% in their stomach, and 28% had polyps in their rectum [20, 26, 27]. More rarely, polyps have been reported in the mouth, esophagus, ureter, bladder, renal pelvis, bronchus, nose, maxillary sinuses and breasts [28–33]. Classically, the polyps are hamartomatous in appearance, but the histology varies. In the intestine as well as elsewhere, adenomatous and hyperplastic polyps can be found in addition to hamartomatous polyps, and mixtures of different histologies are not uncommon. The polyps are covered with the epithelium that would usually be present at the respective site. The typical histological picture of a hamartomatous polyp is that of a pedunculated polyp consisting of disorganized glandular tissue resembling adenomatous change, but distinct [34]. There is a connective tissue core infiltrated by smooth muscle. Although it can be demonstrated with immunofluorescent techniques that there are smooth

Table 1. Features suggesting Peutz-Jeghers syndrome.

- The presence of multiple hamartomatous polyps with the characteristic smooth muscle component present
- Characteristic melanin pigmentation found on the buccal mucosa, around the mouth, and anus or palms of hands or feet
- A family history of verified PJS
- Direct detection of a loss-of-function mutation in *LKB1*

If two criteria are fulfilled, the patient is likely to be or become affected with the syndrome, and should be enrolled in a screening protocol. According to current data, direct detection of a loss-of-function mutation in *LKB1* alone makes a patient a likely PJS candidate. Table modified from [90].

muscle fibers present also in juvenile and adenomatous polyps, often the histological picture of a Peutz-Jeghers hamartoma is easy to distinguish because the smooth muscle component is dramatic and can be seen even with a regular hematoxylin-eosin staining [35]. Cysts lined with an increased number of clear, gobletlike cells often form in the glandular parts of the polyps. Sometimes the hamartomas are intramural, and may resemble enteritis cystica profunda [36]. It has been reported that the number of polyps may decrease with advancing age [37].

The expression and penetrance of the mucocutaneus pigmentation varies, and it seems to get less pronounced with advancing age, sometimes even disappearing completely. The lips and oral area are the most common place for the spots (in 94% of patients), and they may be more constant in the buccal mucosa than elsewhere [2]. Also, the buccal mucosal spots can be useful in differential diagnosis between Peutz-Jeghers and ordinary freckles, as the latter are not regularly found on mucosal membranes [38], although another study reports buccal mucosal pigmentation spots in 5.6% of healthy young adults [25]. Other common loci for the spots include the hands (74%), and feet (62%), while in 21% spots also appear elsewhere [20]. It remains to be seen whether the type of mutation contributes to the amount or location of the pigment spots. In conclusion, pigmentation should not form the basis of a PJS diagnosis, as similar pigmentation is not rare in the unaffected population [25].

Psoriasis and PJS have been described in the same patient [39]. In addition to the usual loci, the melanin spots were localized also in psoriatic plaques in sites not usually seen in PJS patients: scalp, elbow, buttocks and legs. The investigators proposed that the formation of melanin spots in PJS patients might be connected to inflammation, as the melanin pigmentation seems to arise in areas of frequent trauma. Inflammation may block transfer of melanin granules from melanocytes to keratinocytes, leading to pigment spots. The spots would then fade when the inflammation would subside. The histological picture of PJS melanin spots is distinctive, with an increased number of melanosomes in melanocytes with long dendrites, and a reduced amount of melanosomes in keratinocytes [40]. This suggests a transport block either at the melanocyte of keratinocyte membrane, but further investigations are needed to clarify the issue. A likely hypothesis is that the gene has something to do with growth control in general [30], which would be in accord with the function of many other tumor suppressors.

A family segregating PJS and polycystic kidney disease has been reported [41]. The cosegregation of the phenotypes was not complete, and the family was relatively small, so it is not clear whether this represents a chance finding or is due to the genes residing close to each other.

Cancer risk in PJS

It was originally proposed that malignant change is very common in PJS polyps, but the matter has been correctly disputed mainly because early reports probably mixed hamartomatous tissue with adenomatous [26, 27, 42–45]. After the possibility of misdiagnosis was recognized and excessive scepticism avoided, true malignant change has been reported in hamartomas. In parallel to the well-documented adenoma-carcinoma sequence in colorectal cancer [46], the reports of adenomatous and carcinomatous change in PJS polyps suggests a hamartoma-adenoma-carcinoma sequence [4, 30, 31, 43, 47–49]. Furthermore, clonal deletions have been detected in PJS hamartomas [5], supporting the view that intestinal hamartomas can be considered preneoplastic lesions—at least in PJS. The rate of malignant change seems much lower than with adenomas.

Despite the dispute regarding hamartomas and malignant change, an increased risk of intestinal cancer was suggested early [20, 49]. The matter was not settled until the report of Giardiello and colleagues, who had followed 31 PJS patients for 12 years and found an 18-fold relative risk of intestinal as well as extraintestinal cancer [3]. The median age of diagnosis for PJS was 17 years, whereas cancer was detected 25 years later on average. Spigelman and colleagues followed a large number of PJS patients and found that the risk of dying from cancer was 48% by the age of 57. According to this report, the survival of PJS cancer patients is strikingly lower than usual. All cancers were lethal at an average age of 39, except one female patient with basal cell carcinoma [4]. The relative risks of dying from cancer were 13 and 9 for gastrointestinal and extraintestinal cancers, respectively.

In a recent study with 34 patients, the overall relative risk for the development of cancer was 9.9 [15]. Interestingly, the risk was 18.5 for women and 6.2 for men. Fifty percent of the women in this study developed gynecologic or breast cancer, and their relative risk for intestinal cancer was as high as 150.9, whereas for both sexes it was 50.5. The mean age of cancer diagnosis was 39.4 years.

The cancer types that convincingly seem to occur more commonly in PJS mutation carriers than in the general population are colon, stomach, small intestinal and pancreatic cancer. Also, breast (often bilateral), ovarian (granulosa or Sertoli cell), testicular (Sertoli cell) and uterine cervical cancers may occur more frequently in PJS than in the average population [3, 4, 15, 30, 50–54]. Adenoma malignum is a peculiar variant of cervical

Table 2. Cancer cases reported in PJS patients.

Gastrointestinal	No.	Average age of diagnosis if available	Extraintestinal	No.	Average age of diagnosis (years)
Duodenum	5	40.2	ovary	8	29
Jejunum	5	35.3	fallopian tube	1	40
Duodenojejunal flexure	2	33	endometrium	2	n.a.
			uterine cervix*	13	37.4
Undefined small intestinal	10	n.a.	breast	22	43.9
(Total small intestinal)	39	34.9	testis	1	7 months
			prostate	1	66
			lung	9	50.8
Esophagus	1	33	gall bladder	1	n.a.
Stomach	17	33.5	biliary tree	1	6
Pancreas	8	52.5	liver	1	n.a.
Colorectal	31	46.6	basal cell	1	39
			thyroid	2	31.5
			osteosarcoma	1	n.a.
			leiomyosarcoma	2	34
			multiple myeloma	2	62.5
			unknown primary	7	50.3

Mean age of cancer diagnosis: 40.5 years.
Total number of cancer cases reported: 181.
Total number of patients in studies (cross-sectional and follow-up studies): 513.
Summary from [3, 4, 15, 20, 31, 36, 49–51, 53, 54, 73, 90, 127, 128]. Please note that some of the cases result from cross-sectional studies, whereas others are from case series or follow-up studies, so the numbers should not be considered epidemiologically applicable. What can be seen is the relative excess of certain cancers such as small intestinal, stomach and pancreatic cancers, compared with common cancer types such as lung cancer. A factor that further disturbs analysis is that some patient groups were only followed up for intestinal cancer, but mostly all cancer cases were reported. Only histologically verified cases are included. The underscored cancer types may occur in excess in PJS (A. Hemminki, unpublished).
*Including 4 cases of adenoma malignum.

adenocarcinoma and relatively often seen in PJS patients, although it is very rare otherwise [51]. There has been one report of breast cancer arising in a fibroadenoma in a PJS patient [31], suggesting a premalignant role for this lesion. Also, melanoma developing in a rectal pigment lesion of a PJS patient has been reported [55]. As this is the only report of melanoma within a PJS pigment spot, it is unlikely that the spots predispose to malignancy.

Sex cord tumors with annular tubules (SCTAT) are very common in PJS patients, and it has been suggested that they can be found in all female PJS patients if enough sampling is done [51]. These peculiar tumors are usually multifocal and bilateral, and can undergo differentiation into granulosa cell or Sertoli cell tumors. Granulosa cell tumors and Sertoli cell tumors are usually benign, but can undergo malignant change. Hormone production causing gynecomastia or endometrial hypertrophy by any of these three tumor types is not uncommon, although SCTATs are usually inactive. Other nonmalignant tumors that have been described in Peutz-Jeghers patients include fibroadenoma of the breast, various cystadenomas of the ovaries and colloid nodules of the thyroid [31, 53]. Also, a PJS patient with multiple bronchial adenomas has been reported [53]. A summary of cancer cases reported in PJS families is presented in table 2.

Other intestinal cancer syndromes

PJS is a member of a group of hereditary diseases called the polyposes (see table 3), characterized by intestinal polyps and often extraintestinal manifestations as well. Most of the syndromes in this group have been the subject of intense interest recently, and the genetic background for most of them has been revealed. Interestingly, the identification of a causative gene and demonstration of mutations has often clarified the clinical picture, as it has become possible to determine which clinical entities are actually variants of the same syndrome.

Familial adenomatous polyposis (FAP) may be the best known and most common polyposis with a frequency of ca. 1:7000 live births [46]. It is characterized by thousands of polyps, mainly in the large intestine, some of which eventually progress to adenoma and carcinoma. Cowden syndrome (CS) is caused by mutations in the *PTEN* gene, a tumor suppressor inactivated in various sporadic tumor types as well [56–62]. CS patients develop hamartomas of various organs, including trich-

Table 3. Hereditary intestinal cancer syndromes.

Syndrome	Gene and locus	Characteristics	Variants
Polyposes			
Familial adenomatous polyposis	*APC* (5q21)	thousands of colonic adenomas, cancer risk 100% if untreated	Turcot's syndrome (brain cancer), Gardner's syndrome (extraintestinal tumors)
Peutz-Jeghers syndrome	*LKB1* (19p13.3)	intestinal hamartomas, melanin spots, elevated cancer risk	
Juvenile polyposis	*SMAD4* (18q21.1)	juvenile polyps (adenomatous and hamartomatous features), elevated cancer risk	some families may have mutations in *PTEN*; hereditary mixed polyposis: putative location on 6q
Cowden syndrome	*PTEN* (18q21.1)	intestinal polyps, hamartomatous and adenomatous, skin tumors	Bannayan-Zonana (malformations)
Neurofibromatosis type 1	*NF1* (17q11.2)	multiple peripheral neurofibromas present also in the submucosa of intestinal walls	
Inflammatory polyposis: ulcerative colitis	unknown, HLA association possible	chronic inflammation causes for-mation of polyps, elevated cancer risk; genetic background unsettled, multifactorial inheritance likely	
Non-polyposes			
Hereditary nonpolyposis colorectal cancer	*MSH2* (2p16), *MLH1* (3p21), *PMS1* (2q32), *PMS2* (7p22), *MSH6* (2p16), *TGFβRII* (3p22)	cancer family syndrome: increased risk of colorectal, endometrial, stomach, biliary tract, ureteral, small intestinal and breast cancers	Turcot's syndrome (colorectal and brain cancer), Muir-Torre syndrome (colorectal and skin cancer)

Table modified from [90].

lemmomas of the skin, benign and malignant tumors of the breast, thyroid, intestine and brain. Up to 50% of women with CS develop breast cancer [56]. The intestinal polyps in CS have varied histologies, but often hamartomas are present. However, the hamartomas lack the smooth muscle infiltration of polyp stroma that it characteristic of PJS.

Solitary juvenile polyps are common in young individuals, but juvenile polyposis (JP) is rare. The genetic background of the syndrome is a bit unsettled, as a few families have been reported to segregate *PTEN* mutations [63], whereas in a large number *PTEN* has been excluded [64]. The main gene for JP seems to be *DPC4*/*SMAD4* [65]. The histology of juvenile polyps resembles the polyps found in CS, but extraintestinal manifestations usually help in differential diagnosis. Also, JP usually presents at an earlier age. Traditionally, no extraintestinal features have been linked to JP, but recently it has been suggested that various developmental defects may commonly occur [66]. Skin and skeletal abnormalities were most common, especially telangiectasies and hypertelorism.

Hereditary nonpolyposis colorectal cancer (HNPCC) is the most common of the intestinal cancer syndromes, with a frequency of at least 1:1000 live births [67]. Thus, HNPCC accounts for a minimum of 2% of all colorectal cancers, one of the most common cancer types in Western societies. As the name implies, polyps do not occur as commonly in HNPCC as in the polyposes. The syndrome is characterized by a high risk of colorectal, endometrial and other cancer types. It is caused by germline defects in mismatch repair genes which can been observed as replication errors (RER, a.k.a. microsatellite instability) in tumor tissue [68].

Localization of the PJS gene

As previous attempts to localize the PJS gene had failed [16–18], a novel approach was developed [5]. It was hypothesized that the hamartomas commonly found in PJS patients would be clonal tissue but premalignant, so that the putative changes would not be too numerous to complicate interpretation of results. This is contrary to earlier reports describing hamartomas as nonclonal tissue with little or no malignant potential [26, 27, 34, 42–45]. In addition, it was thought the gene would be a tumor suppressor, and according to the Knudson model [69, 70], the wild-type allele should then be lost in tumor tissue. Since comparative genomic hybridization (CGH) was to be used for localization of the losses, it was also thought that at least some of the losses would be large enough to be detected (over 10 mb).

CGH is a screening method for detecting genomic gains and losses in tumor tissue [71, 72]. Briefly: equal amounts of fluorescently labeled tumor and normal tissue are hybridized to a normal metaphase spread,

and the respective signal intensities are compared with a fluorescent microscope coupled to a computer-based digital image analysis system. The ratio of tumor to normal tissue is calculated for each chromosomal locus. An advantage of the method is that the whole genome can be analyzed simultaneously, but disadvantages include low sensitivity, especially for deletions. Also, many regions (centromeric and telomeric areas) cannot be reliably analyzed.

Sixteen polyps were obtained from a single PJS patient, and CGH was performed on these. Overall, the karyotypes of the hamartomas looked quite benign, confirming the hypothesis of hamartomas being not very malignant. However, in 6 out of the 16 polyps, it looked like the end of the short arm of chromosome 19 was lost [5], thus suggesting clonality. The changes were detected in the glandular part of the polyp, whereas deletions were not seen in the stromal part with the smooth muscle (A. Hemminki, unpublished). This may suggest that only the glandular part is neoplastic and the muscle reactive, but further investigations are necessary, since muscle tissue has less genetic material (a smaller nuclei to cytosol ratio) than glandular tissue, and thus contaminating cells may disturb the analysis more. Interestingly, there has been a report describing sarcoma within a PJS polyp [73], perhaps suggesting that the stroma may also become malignant.

The hamartoma-adenoma-carcinoma sequence

As most of the cancers arising in PJS patients are adenocarcinomas, the detection of clonal changes in the glandular part of the polyps is not surprising. It is well known that the intestinal epithelium can progress to adenoma, which can then become carcinoma [46]. In contrast, hamartomas have traditionally been considered benign lesions with little or no potential for malignant transformation [26, 27, 34, 42, 45]. Based on observation of adenomatous or carcinomatous foci within PJS hamartomas, the possibility of a hamartoma-adenoma-carcinoma sequence was suggested previously [4, 30, 31, 43, 47–49]. The study by Hemminki and colleagues provided the first evidence of clonal genetic changes detected in hamartomas, which supports the notion of their being premalignant lesions [5]. A similar situation is seen with 'regular' intestinal adenomas, as it has been shown that *APC* mutations are often seen in the smallest adenomas and even in aberrant crypt foci, but not in hyperplastic or inflammatory polyps [74–77].

Currently it is not clear if most cases of cancer in PJS patients develop from premalignant lesions. The number of polyps may decrease with advancing age [37], whereas cancer is most often detected in the third and fourth decades (table 2). This may suggest that hamartomas and the development of cancer are not always connected. However, the more likely but unproven hypothesis is that, as a result of loss of both alleles of *LKB1*, growth control is decreased, and premalignant lesions (hamartomatous or other) can form. Then, in the course of years or decades, additional mutations provide the malignant phenotype.

Microsatellite LOH analysis of PJS polyps

The power of CGH as a screening method was effectively demonstrated when deletions in 19p13.3 were seen as the only recurrent change in PJS polyps [5]. However, microsatellite analysis was necessary, as CGH analysis is often disturbed by artefacts, especially in telomeric and centromeric areas. Also, 19 is not the easiest chromosome to analyze because of its high GC content and the indirect deoxy-uridine-triphosphate (dUTP)-labeling method used.

Three of the six polyps that had shown loss of genetic material in the CGH experiments were chosen for loss of heterozygosity (LOH) analysis. In all cases, the losses could be confirmed, and the most frequently lost area could be determined to be the very end of chromosome 19p. However, the LOH was not convincing until microdissection of the hamartomas was performed with selective ultraviolet radiation fractionation (SURF) [5]. SURF is a quick and easy microdissection technique based on labeling of interesting areas with a micromanipulator under a microscope. Then UV light is applied to destroy the remaining tissue [78]. The need for microdissection to get convincing LOH supports the notion that only the glandular epithelium is clonal.

LOH was detected in the glandular epithelium, particularly in cystic areas, where the amount of clear, gobletlike cells was clearly increased. The degree of LOH seemed to correlate with the amount of gobletlike cells in the specimen extracted (A. Hemminki, unpublished). However, a systematic study is needed to confirm this. In every case the loss affected the wild-type chromosome that the patient had inherited from the unaffected parent. This supports the tumor suppressor gene hypothesis and also increased confidence that the LOH results were nonrandom.

Linkage analysis in PJS families and the question of heterogeneity

To confirm the presence of a PJS locus in 19p13.3, 12 well-characterized PJS families were analyzed with the same markers that had shown LOH. All informative families showed linkage to the most telomeric marker, D19S886, and no cases of nonpenetrance were ob-

served. A neighboring marker on the centromeric side, D19S565, showed recombinations, limiting the candidate area to approximately 6.5 cM. The pairwise logarithm of odds (lod) score was 5.4 for D19S886. Considering that only one locus was analyzed, the finding was unambiguous. Multipoint linkage calculations gave a conclusive lod score of 7.00 at D19S886 [5]. Linkage disequilibrium was not detected in families of Finnish or other origin (A. Hemminki, unpublished).

Later, linkage of PJS to 19p13.3 was confirmed in five studies [21–24, 79], although additional loci have been suggested. Olschwang and colleagues report three families that might be unlinked to 19p13.3 [24], and Mehenni and colleagues report positive lod scores for a locus in 19q in a large Indian family [22]. This family also shows a lod of over 2 for the 19p locus, but no *LKB1* mutation was found [80]. Boardman and colleagues report two families which seem unlinked to *LKB1* [79]. One family shows lod scores to a locus slightly centromeric to *LKB1*, whereas one family seems clearly unlinked. When *LKB1* is analyzed in the possibly unlinked families, the issue of heterogeneity may be clarified.

One possible explanation for the unlinked families is misdiagnosis. Misdiagnosis of PJS may be common, especially in new cases or when the diagnosis is not made in collaboration between an experienced clinician and pathologist. Diagnosis should not be based solely on pigmentation, as this can vary even within the same family, and may be totally absent in affected individuals. Also, pigmentation similar to the PJS type can be seen as a normal variant in up to 15% of healthy young adults [25]. Histological evidence of smooth muscle infiltration of the stromal core of a hamartomatous polyp should form the basis of the diagnosis. As an extrapolation based on Knudson's two-hit hypothesis, solitary Peutz-Jeghers polyps may form because of sporadic inactivation of both alleles of the PJS gene. For the diagnosis of Peutz-Jeghers syndrome to be made, multiple polyps should be seen with or without mucocutaneous melanin pigmentation. See table 1 for features suggesting PJS.

Identification of the PJS gene

The original region candidate region for the PJS gene was limited between the 19p telomere and marker D19S565, a distance of 6.5 cM, which translates into about 3 mb in this region [5]. Using approximately 30 families and 15 novel markers, the critical region could be reduced to 800 kb [6]. 19p13.3 is one of the most gene-rich regions in the human genome, and the 800 kb can be estimated to contain about 40 genes. This is considerably less than the 160 genes that would have been inside the original area. Direct complementary DNA (cDNA) selection was performed to produce sequence from approximately 40 genes mapping inside the final candidate region of 800 kb [6]. Some of these gave direct homology to previously known, but unmapped genes.

Novel genes and genes that had been previously mapped to the candidate area by Lawrence Livermore National Laboratories (LLNL) [81, 82] were sequenced from PJS patients. Expressed sequence tags (ESTs) from the Human Transcript Map [83] were further mapped with the help of a cosmid contig produced by LLNL [81, 82]. The novel sequences and ESTs were further elongated by doing database searches in GenBank and sequence assembly. Figure 2 illustrates the protocol used to create and analyze candidate genes in cloning the PJS gene [6].

After about 600 kb of sequencing of candidate genes, reverse transcriptase polymerase chain reaction (RT-PCR) experiments were being done on the 29th transcript, and a double band was seen in the sample from the original proband whose polyps had led to localization of the gene. The gene was *LKB1*, a serine/threonine protein kinase [6]. It had been identified and submitted to GenBank previously, but had not been localized, nor was anything known about its substrates or regulation [84]. When the coding region of the gene was sequenced from PJS patients, 11 out of 12 families showed mutations, most of which were truncating [6]. Genomic PCR reactions confirmed each mutation and segregation of the mutation with the phenotype in families. None of the presumed mutations could be found in control individuals [6]. Using the linkage information obtained from the PJS consortium [6], another group was almost

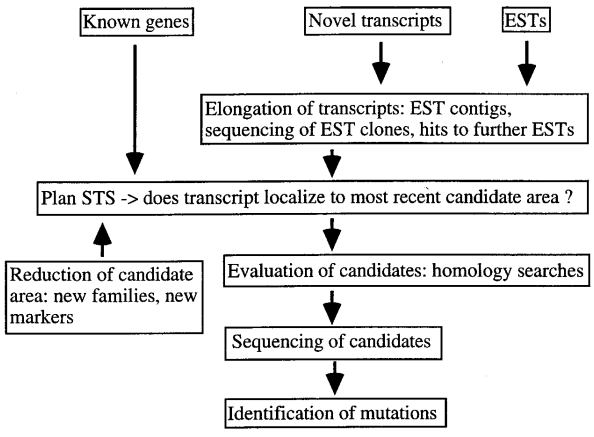

Figure 2. Flow chart of the strategy to identify the PJS gene. Adapted from [90].

simultaneously able to find *LKB1* mutations in PJS patients [85].

LKB1, a serine/threonine kinase, is the Peutz-Jeghers gene

Homology searches to the GenBank nonredundant gene database [86] returned a large number of human and other genes that were homologous to the kinase domain of *LKB1* (codons 50–337), but only one gene had any homology outside it. This was a *Xenopus* (frog) serine/threonine kinase *XEEK1* (for *Xenopus* egg and embryo kinase) [87]. Xeek1 has 83.7% amino acid identity to Lkb1 [88]. In addition, a mouse EST showed high homology to Lkb1 [89]. Xeek1 has been reported to be a 432-amino acid cytosolic protein with narrow substrate specificity and may be phosphorylated by protein kinase A. It is expressed in *Xenopus* oocytes and fertilized eggs but much less in later embryonic stages. However, mature *Xenopus* tissues were not examined [87]. The closest yeast homolog was Snf1/AMPK with 35% identity, but this protein may not be the yeast counterpart of Lkb1, since Lkb1 is not the closest human homolog of yeast Snf1.

The expression of *LKB1* was studied using commercial multiple tissue Northern blot filters I and II (Clontech). Expression was detected in all tissues examined, with the strongest signals seen with testis and skeletal muscle [90]. To confirm the Northern blot results, RT-PCR experiments were done on cDNA from various tissues, and the same ubiquitous expression was seen [6].

LKB1 mutations in Peutz-Jeghers families and sporadic cases

When 12 PJS families linked to 19p13.3 were analyzed for *LKB1* mutations, 11 were found [6] (table 4). There were four single-base substitutions leading to immediate stop codons, three small deletions or insertions causing frameshifts and premature stop codons, two larger deletions of 188 bp and 174 bp, one 9-bp deletion causing alteration of isoleucine-arginine-glutamine-histidine to asparagine and one missense type change (leucine to proline). All of the mutations were found in the conserved kinase domain of the gene, and were not found in controls. The truncating nature of the mutations fits well with the finding of deletions in the hamartomas [5] and seems to indicate that the healthy Lkb1 protein serves a tumor-suppressing function. The 12 families used in the primary panel for mutation detection were known to be linked to 19p13.3. Thus the results do not exclude the possibility of additional PJS genes existing elsewhere in the genome. All of the mutations occurred within the conserved kinase domain, and 7 of the 11 mutations occured within exon 1, suggesting a hotspot area.

Jenne and colleagues reported five *LKB1* mutations in PJS patients [85]. Three of the five patients had frameshift mutations leading to stop codons, one was a nonsense point mutation and one was a four-exon deletion. The authors suggest de novo identification of the PJS gene (renamed *STK11*), although *LKB1* had been submitted to GenBank in 1996 by Nezu and colleagues [84]. Jenne and colleagues considered *LKB1* a likely candidate gene because of strong linkage equilibrium between the PJS locus and D19S886. In contrast, linkage disequilibrium is not seen in Finnish families or in families of other origins (A. Hemminki, unpublished), nor has it been reported in the literature confirming linkage [21–24].

Other investigators have also found *LKB1* mutations in PJS families (see table 4 for mutations reported to date). Nakagawa and colleagues report 10 mutations in 15 families [91]. One mutation was found in two families, but a founder effect is unlikely since one family is caucasian and the other Japanese. Six of the 9 different mutations were frameshift or nonsense causing premature stop codons, whereas two were splice junction changes and one was an inframe deletion of one codon. Three of the five frameshift mutations occured in a mononucleotide repeat (CCCCCC) at codons 279–281, and two additional mutations occurred within exon 6. None of the mutations described by Hemminki and colleagues hit these codons, but it may represent another hotspot.

Ylikorkala, Avizienyte and others analyzed 33 unrelated PJS cases, 20 of which were familial, 8 sporadic (no family history, but polyposis and/or pigmentation) and in 5 cases the family history was unknown [92]. *LKB1* mutations were detected in 12/20 familial cases, 4/8 sporadic and 3/5 in the cases with unknown family history. Most of the mutations described (12/19) cause frameshifts and/or premature stop codons within the kinase domain and thus strongly support a tumor suppressor function for the protein. In addition, two novel missense changes, one small inframe deletion within the kinase domain, three splice site changes and one large genomic rearrangement are reported. The authors also investigated the consequences of some of the less dramatic mutations. Cases SL8, SL25 and SL26 (numbers 1, 5 and 6 in table 4) were all shown to cause loss of autophosphorylation seen with wild-type Lkb1.

Boardman and colleagues report *LKB1* mutations in three out of six PJS families [79]. Two of these are truncating, whereas one is missense. In addition, the authors analyzed nine sporadic PJS cases, three of which showed germline *LKB1* mutations. One of the familial cases and one sporadic case had the same 1-bp insertion near the putative mutation hotspot repeat in

Table 4. Mutations of *LKB1* reported in PJS patients' germlines.

No.	Patient/family	Nucleotide change*	Predicted consequence*,†	Exon	Family history	Ref
1	SL8	loss of exon 8 (307–370)	fs, stop at 404	8	yes	6
2	SL12	G to T at 57	glutamic acid to stop	1	yes	6
3	SL14	29 bp del 66-75	fs, stop at 152	1	yes	6
4	SL20	G to T at 70	glutamic acid to stop	1	yes	6
5	SL25	T to C at 67	leucine to Proline	1	yes	6
6	SL26	9 bp del 303–306	Ile-Arg-Gln-His to Asn	7	yes	6
7	SL27	C to G at 60	tyrosine to stop	1	yes	6
8	SL28	1 bp ins at 55–57	fs, stop at 162	1	yes	6
9	SL29	loss of exons 2–3 (98–155)	truncated protein	2–3	yes	6
10	SL31	2 bp del at 277-278	fs, stop at 283	6	yes	6
11	SL32	A to T at 84	lysine to stop	1	yes	6
12	A	inv/del 156–307	truncated protein	4–7	yes	85
13	D	AG to AA at 5′ splice site	skipping of exon 4?	3-4	yes	85
14	B	4 bp del at 240	fs, stop at 285	5	unknown	85
15	MA	C to A at 252	tyrosine to stop	6	yes	85
16	FA	del G at 280	fs, stop at 286	6	unknown	85
17	N	ins GC at 38	fs, stop at 51	1	yes	91
18	G	AG to AC at 5′ splice site	skipping of exon 2?	1–2	yes	91
19	L	del C at 140	fs, stop at 160	3	yes	91
20	E	del C at 245	fs, stop at 286	5	yes	91
21	J	GTA to GTT at 3′ splice site	aberrant splicing?§	5–6	yes	91
22	M	CA to AG at 246–247	tyrosine to stop	6	yes	91
23	K	3 bp del at 247	del asparagine	6	yes	91
24	B	del C at 281	fs, stop at 286	6	yes	91
25	C	ins C at 281	fs, stop at 284¶	6	yes	91
26	P	ins C at 281	fs, stop at 284¶	6	yes	91
27	P9	2 kb genomic del?	aberrant/absent prot.	?	yes	92
28	P13	del A at 53	fs, stop at 63	1	yes	92
29	P16	T to C at 157	phenylalanine to serine	4	yes	92
30	P20	4 bp del at 264	fs, stop at 285	6	yes	92
31	P21	C to T at 152	glutamine to stop	3	yes	92
32	P22	AG to AC at 5′ splice site	skipping of exon 8?‖	7–8	yes	92
33	P23	8 bp del at 258	fs, stop at 262	6	yes	92
34	P25	A to T at 84	lysine to stop‡	1	yes	92
35	P27	14 bp del at 249	fs, stop at 260	6	yes	92
36	P28	C to T at 86	arginine to stop	1	yes	92
37	P29	del A at 305	fs, stop at 335	7	yes	92
38	P32	9 bp del at 137	Gln-Glu-Met-Leu to Leu	3	yes	92
39	P3	20 bp del at 202	fs, stop at 258	5	no	92
40	P8	del C at 281	fs, stop at 286	6	no	92
41	P14	GTA to GTT at 3′ splice site	aberrant splicing?§	5–6	no	92
42	P15	A to T at 181	asparagine to tyrosine	4	no	92
43	P10	G to A at 308	tryptophan to stop	8	unknown	92
44	P11	AG to AC at 5′ splice site	skipping of exon 8?‖	7–8	unknown	92
45	P12	C to T at 220	Glutamine to stop	5	unknown	92
46	BF1**	5 bp del	fs, stop codon	8	yes	79
47	BF2	1 bp ins at 283	fs, stop codon††	6	yes	79
48	BF3	G to T	arginine to serine		yes	79
49	BS1	1 bp ins at 283	fs, stop codon††	6	no	79
50	BS2		altered splice acceptor	5–6	no	79
51	BS3	C to T	histidine to tyrosine		no	79
52	PJF263	del 4 bp at 262	fs, stop at 286	6	yes	93
53	PJF512	del 6 bp at 175	in-frame del Lys-Asp	4	yes	93
54	PJ1	G to A at 251	glysine to alanine	6	no	93
55	PJG42	C to T at 304	arginine to tryptophan	7	no	93
56	PJS01	del 18 bp, ins 6 bp at 50–53	del Leu-Met-Gly-Asp	1	yes	80
57	PJS02	ins A	fs, stop codon	4	yes	80
58	PJS03	intronic ins A	altered splicing?	5–6	yes	80
59	PJS04	G to T at 308	tryptophan to cysteine	8	yes	80
60	PJS05	G to A at 526	aspartic acid to asparagine	4	yes	80
61	PJS06	del G at 301	fs, stop codon	7	yes	80
62	PJS09	del 52 bp	altered protein	7	yes	80

* The numbers denote codons of *LKB1*. † fs, frameshift. ‡ Families SL32 and P25 have same mutation. § Family J and case P14 have same mutation. ¶ Families C and P have same mutation. ‖ Family P22 and case P11 have same mutation. ** The cases BF1–BF3 and BS1–3 refer to uncoded cases published by Boardman et al. †† Family BF2 and case BS1 have same mutation.

exon 6. Resta and others studied nine PJS families for *LKB1* mutations and found four: one frameshift, a deletion of two amino acids and two missense changes within the kinase domain [93].

Mehenni and colleagues reported seven mutations in their nine PJS families [80]. Two mutations created frameshifts and premature stop codons, two mutations were likely to cause splicing defects, whereas one was a deletion combined with an insertion and two mutations were of the missense type. An autophosphorylation assay similar to the one used by Ylikorkala and colleagues [92] again showed autophosphorylation of the wild-type Lkb1, but not of the three missense mutations studied (D176N, W308C, L67P). In addition, three-dimensional computer analysis was performed on one mutation (D176N), and this mutation was presumed to prevent proper catalysis at the active site.

LKB1 is a serine/threonine protein kinase with unknown functions and substrates

Upon discovery of mutations in *LKB1*, homology searches were done. The kinase domain that makes up the majority of the gene was similar to a large number of various serine/threonine kinases. However, outside the kinase domain, no human homologs were found. A frog kinase, Xeek1, and a mouse EST were found highly homologous to Lkb1 [6]. Various other kinases are involved in hereditary and sporadic carcinogenesis, but *LKB1* is the first serine/threonine kinase whose inactivating germline mutations cause a cancer susceptibility syndrome. The mutations in *LKB1* are inactivating, and the wild-type allele is knocked out during hamartoma formation, so the Knudson 'two-hit' hypothesis is fulfilled, suggesting tumor suppressor function. To study the function and interactions of Lkb1, investigators performed an in vitro kinase assay [92]. Autocatalytic phosphorylation was detected, but common substrates like myelin basic protein, histone H1 or RNA polymerase II were not implicated.

The role of *LKB1* in development is an interesting, but very much unsettled issue. The frog homolog *XEEK1* is expressed in early embryological phases, but the expression seems to decrease during later phases, although mature tissues have not been examined [87]. There have been a few reports discussing the possible connection between developmental abnormalities and PJS. A family with polycystic kidney disease partially cosegregating with PJS [41], a father and son with PJS and polydactyly [94], and single patients with congenital heart defects [19] or cleft lip in combination with PJS [95] have been reported. The kinase function of Lkb1 does not exclude a role for the protein during development, but further evidence must be gathered.

Cowdon syndrome (CS) and Bannayan-Zonana syndrome are similar traits characterized by hamartoma formation as well as extraintestinal features and are caused by mutations in *PTEN*, also known as *MMAC1* [56, 57, 61]. *PTEN* stands for phosphatase and tensin homolog and is known to be regulated by transformin growth factor-β (Tgfβ), suggesting a signal transduction pathway for *PTEN* [96]. Because kinases add phosphate groups to enzymes, and phosphatases remove them, and the phenotypes of PJS and CS have common features, it is tempting to speculate about functional connection. However, currently there is no evidence supporting such a theory.

Other kinases in carcinogenesis

The Tgfβ receptor II (TgfβrII) is a serine/threonine kinase that functions as a transmembrane receptor for Tgfβ together with TgfβrI. Tgfβ is an important inhibitory regulator of growth of various cell types. In HNPCC cancers the gene coding for TgfβrII is often somatically inactivated. This seems to be associated with adenoma to carcinoma progression, providing growth advantage to the tumors [97–99]. Interestingly, there is a report of a germline TGFβRII mutation in an RER($-$) HNPCC family [100]. Further molecules in the Tgfβ signaling pathway include Smad2, Smad3 and especially Smad4, which may be an important tumor suppressor inactivated during the progression of colorectal cancer [101]. It has recently been shown that at least a portion of JP families have germline mutations in *SMAD4* (not a kinase), further underlining the importance of this pathway in colon tumorigenesis [65].

Other kinases that are important in hereditary cancer include *RET*, a tyrosine kinase whose mutations cause multiple endocrine neoplasia (MEN, types 2a and 2b and familial medullar thyroid carcinoma) [102]. Cyclin-dependent kinase 4 (*CDK4*) is inactivated in the germline of a subset of familial melanoma families [103], and activating missense germline mutations in the tyrosine kinase domain of *MET* cause hereditary papillary renal carcinoma [104].

LKB1 mutations in sporadic cancers

The germline mutations that cause PJS result in inactivation of the protein product, thus suggesting a tumor suppressor role for the gene. However, a useful current definition for tumor suppressors is 'genes that sustain loss-of-function mutations in the development of cancer' [105]. This criterion is not fulfilled before it is known whether *LKB1* is inactivated in cancer tissue. Since inactivation of both alleles of the gene in hamar-

toma has been observed [5], it is not unlikely that mutations will be found also in cancers, at least in PJS patients.

Recent evidence does not suggest a very prominent role for *LKB1* in sporadic breast, colorectal or testicular tumorigenesis [7, 8, 11]. The only variant found in these studies was a missense-type change found in a testicular tumor [7]. Interestingly, the tumor was of a mixed histology with features of both seminoma, immature and mature teratoma. Perhaps this is parallel with the mixed histology often seen in PJS intestinal polyps. The finding was strengthened by biochemical kinase assay performed with the mutation [92]. Only minimal activity was detected with the mutant allele.

Contrary to the observations discussed above, frequent somatic mutations have been reported in left-sided Korean colon cancers [9]. In this study, LOH in the *LKB1* region was seen in 52.6% (10/19) of samples, and in 7 cases mutations were detected. No mutations were detected in right-sided colorectal cancer, but in 53.8% of the left-sided cases *LKB1* was mutated. In all but one noninformative case, both alleles seemed to be lost. The gene was found mutated also in 2/7 left-sided adenomas. Eight of the 9 mutations were missense, and one was frameshift, actually the same mutation detected earlier in a PJS family. Eight out of these 9 mutations were located within the conserved kinase domain supporting their relevance. While the findings of these authors are in contrast to the findings of Avizienyte, Wang and colleagues, who between them analyzed 105 colorectal cancer samples [7, 11], in theory it may be possible that certain environmental or genetic factors may predispose a certain population to certain mutations.

In two unpublished series from Finland and Britain, LOH in the PJS region has been observed in 26% and 20% of colorectal cancer samples, respectively [7, 11]. Resta and colleagues also studied LOH at D19S886 and found it in 19.2% [93]. The authors did single strand conformation polymorphism analysis (SSCP) of 72 colorectal cancer samples and found one missense change (proline to histidine at codon 314). This was not in any of the samples showing LOH.

In a recent article, an *LKB1* missense-type mutation was found in a gastric cancer [10]. Although the change is not very dramatic (proline to leucine), it does occur within the kinase domain. In addition to this mutation the authors report non-amino acid-altering changes that were not present in the germline. The significance of these silent changes remains uncertain. Constant deletions at 19p13.3 have been seen in adenoma malignums [106], tumors seen often in PJS, but rare otherwise [51]. However, the most commonly lost regions seem to reside 10 mb proximal to *LKB1*. Interestingly, a PJS family has been reported to show positive lod scores to this region with a likely recombination to the *LKB1* locus [79].

In addition to the cancer types discussed above, Avizienyte and colleagues studied a large number of different tumor types: 6 melanoma and 8 myeloma cell lines, 12 pancreatic, 8 gastric, 12 ovarian granulosa cell, 26 cervical, 18 lung, 24 soft tissue and 19 renal neoplasms were studied [12]. In a cervical adenocarcinoma, a somatic frameshift and a transversion leading to a stop codon were noted at codon 335. In a lung adenocarcinoma, a somatic aspartic acid to valine transversion was seen at codon 194, with LOH of the normal allele. In addition, a silent C125T change was seen in a pancreatic cancer. These findings support previous reports indicating that *LKB1* mutations are not common in sporadic tumors, but that they do sometimes occur. The two cases with mutations of both alleles provide additional evidence that *LKB1* acts as a tumor suppressor and is recessive at the cellular level.

It is important to investigate whether *LKB1* is mutated in sporadic tumors. Rare hereditary syndromes have often led to the discovery of growth regulatory genes whose inactivation is very important in the propagation of various common and sporadic tumor types. Examples include *APC*, which was found as a result of studies on familial adenomatous polyposis, *RB1* (hereditary retinoblastoma), *CDKN2D* (hereditary melanoma), *VHL* and *WT1* (familial kidney cancer) all of which have proved important tumor suppressor genes. However, not all cancer susceptibility genes are mutated in the corresponding sporadic tumors. For instance, few coding region mutations have been reported in the breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* [107–110] or the mismatch repair genes responsible for HNPCC [111–113]. Nevertheless, there is some recent evidence that suggests the involvement of both HNPCC and BRCA genes in their sporadic counterparts, not by somatic coding region mutations, but rather by methylation of the promoter, causing an absence of the transcript [114–118]. Indeed, up to 90% of sporadic colorectal cancer with the RER + phenotype may be caused by promoter methylation of *MLH1*, an HNPCC gene [116]. This may mean that epigenetic mechanisms play an important role in the development of very common types of cancer.

In addition to methylation defects, there are various types of mutations which cannot be seen with the techniques used in the studies discussed above. For example, large genomic deletions and promoter region mutations would go unseen in SSCP or genomic sequencing analysis. Future studies will show if such factors play a role in *LKB1*-related carcinogenesis.

Patient management

Because PJS syndrome is relatively rare, the diagnosis and recommended follow-up are probably often neglected. Few registries have been set up for follow-up of patients, since the syndrome's malignant potential has only recently become evident. Given the dramatic increase in patients' risk of cancer and the diminished survival associated with these cancers, diagnosis combined with regular follow-up of affected family members should become routine clinical practice. In a study by Spigelman and colleagues all patients affected with cancer died from it, with the exception of one patient with facial basalioma [4]. Another well-executed investigation found the PJS patients' cancer risk to be 18 times that of the general population [3]. Additional support for the need for aggressive screening is provided by the morbidity caused by the polyps if left untreated. The cumulative number of polyps that arise in PJS patients can range from a few to hundreds, while the average number of polypectomies may be between 5 and 10, although prospective studies have not been done. Typically, the polyps cause abdominal pain, bleeding and obstruction, which can be life-threatening. The features in table 1 can be used for detecting the patients that probably are or will be affected with PJS.

The most common malignancies seem to be stomach, small intestinal, pancreatic and colorectal adenocarcinomas (table 2). Thus gastroduodeno- and colonoscopies and removal of all polyps should be performed regularly. The lower small intestine remains problematic, as its endoscopic evaluation is difficult with currently used routine noninvasive methods. However, at laparotomy, a combined endoscopic and surgical approach can visualize the whole small intestine [28, 119, 120]. Better equipment and methods for direct jejunoileal endoscopy (push enteroscopy) are under development, and the method has been used successfully for screening of Peutz-Jeghers patients, although the whole small intestine could not be viewed [121, 122]. Traditional barium double-contrast radiology is probably effective with larger polyps, which can then be removed at laparotomy. However, small polyps can easily be missed. These approaches could be combined with abdominal and pelvic ultrasound and meticulous examination of the breasts and testes to detect tumors as early as possible. The interval between examination of PJS patients needs to be further evaluated, but in the meanwhile, biannual gastro-, colono- and enteroscopies combined with annual hemoglobin, abdominal and vaginal ultrasound, gynecological examination with cervical smear, and perhaps also testicular examination seem indicated due to the highly aggressive nature of the cancers [30, 90, 120, 123, 129] (table 5, H. Järvinen, unpublished). There may be little value in mammography for women under 35 because of the density of the normal breast tissue in young women, so it may be best to start these at 35 [124]. Careful palpation and ultrasound are more useful in women under 35.

The youngest affected patient reported has been 15 days [19], but presentation with polyps in the second decade is more common. Often, in previously known PJS families, genetic tests are not necessary for diagnosis of affected or unaffected, since the typical pigmentation is often visible during the first years of life. However, in new cases, or in families with little pigmentation, direct testing of *LKB1* mutations may be useful. Current ethical views do not favor genetic testing of minors, but in PJS there are multiple case reports of fatal cancer arising in very young individuals. For example, there has been stomach cancer reported in patients aged 13, 14 and 17, ovarian cancer at 19, 21 and 22, testicular cancer at 7 months, and intestinal cancer at 26 (twice) and 27 [4, 20, 36, 49, 53]. The situation with PJS is similar to the situation with FAP, where young individuals are at risk of cancer before adulthood. Screening of gene carriers in such a setting is usually not considered unethical, and since only half of family members have the genetic defect, genetic testing may be useful to avoid psychologically exhausting screening of unaffected individuals [125]. Until more thorough studies are done on the subject, a starting age of 14 to 16 might be suggested for testing and screening of the affected individuals. There is no evidence that few symptoms would mean a smaller cancer risk.

Table 5. Summary of screening protocol suggested for patients 14–16 years and older.

- Hemoglobin concentration: annually
- Abdominal ultrasound: annually
- Gynecological and breast examination: annually
- Cervical smears: annually
- Vaginal ultrasound: annually
- Testicular examination, ultrasound if clinical symptoms: annually
- Colonoscopy: biannually
- Gastroduodenoscopy: biannually
- Small intestinal double-contrast radiology or push enteroscopy: biannually
- Mammography: biannually starting from 35, annual after 50

Summary from [30, 90, 120, 123, 129]. In addition, unpublished data by Drs. Heikki Järvinen and Akseli Hemminki are presented.

Conclusions

The identification of *LKB1* as the gene responsible for PJS, a cancer susceptibility syndrome, opens the field for a great deal of protein research. Almost nothing is known about the cellular location, functions, regulation or partners of the Lkb1 protein. It is possible, that Lkb1 is a member of a novel signal transduction pathway in the cell. Based on current results obtained with SSCP and direct sequencing, *LKB1* does not seem to be commonly involved in the carcinogenesis of common sporadic cancers. However, as LOH is seen in 19p13.3, and a few mutations have been found, it may play a role. Also, it is possible that the gene is important in a specific type of cancer. Methylation defects of *LKB1* in tumor tissue have not been studied.

Identification of the gene causative for the syndrome makes direct and specific diagnosis of affected individuals possible. The small size of the gene and the usually truncating nature of the mutations facilitate molecular genetic diagnosis. However, due to the often conspicuous phenotype, genetic testing is not always necessary to recognize the affected individuals. However, in new PJS families and in borderline cases with little pigmentation or few polyps, a genetic test can be useful.

Now that the very malignant nature of the syndrome has been realized, it is important that patients be diagnosed and enrolled in screening programs. National or regional registries would facilitate effective screening, which would also help to assess the true frequency and consequences of the syndrome. In HNPCC it has been shown that regular screening significantly reduces morbidity and mortality of patients [126]. Although such an investigation has not been reported for PJS, it is not unlikely that similar results could be obtained.

Acknowledgments. The author thanks Dr. Heikki Järvinen for the PJS patient photo. The author has received support from the Finnish Cultural Fund (Uudenmaan rahasto), the Päivikki and Sakari Sohlberg Foundation, the Maud Kuistila Foundation, the Emil Aaltonen Foundation and the Ida Montin Foundation.

1 Peutz J. L. (1921) A very remarkable case of familial polyposis of mucous membrane of intestinal tract and accompanied by peculiar pigmentations of skin and mucous membrane [Dutch]. Nederlands Tijdschrift voor Geneeskunde. **10:** 134–146
2 Jeghers H., McKusick V. A. and Katz K. H. Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits. New Engl. J. Med. **241:** 992–1005, 1031–1006
3 Giardiello F. M., Welsh S. B., Hamilton S. R., Offerhaus G. J., Gittelsohn A. M., Booker S. V. et al. (1987) Increased risk of cancer in the Peutz-Jeghers syndrome. New Engl. J. Med. **316:** 1511–1514
4 Spigelman A. D., Murday V. and Phillips R. K. (1989) Cancer and the Peutz-Jeghers syndrome. Gut **30:** 1588–1590
5 Hemminki A., Tomlinson I., Markie D., Jarvinen H., Sistonen P., Bjorkqvist A. M. et al. (1997) Localization of a susceptibility locus for Peutz-Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis. Nature Genet. **15:** 87–90
6 Hemminki A., Markie D., Tomlinson I., Avizienyte E., Roth S., Loukola A. et al. (1998) A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature **391:** 184–187
7 Avizienyte E., Roth S., Loukola A., Hemminki A., Lothe R. A., Stenwig A. E. et al. (1998) Somatic mutations in LKB1 are rare in sporadic colorectal and testicular tumors. Cancer Res. **58:** 2087–2090
8 Bignell G. R., Barfoot R., Seal S., Collins N., Warren W. and Stratton M. R. (1998) Low frequency of somatic mutations in the LKB1/Peutz-Jeghers syndrome gene in sporadic breast cancer. Cancer Res. **58:** 1384–1386
9 Dong S. M., Kim K. M., Kim S. Y., Shin M. S., Na E. Y., Lee S. H. et al. (1998) Frequent somatic mutations in serine/threonine kinase 11/Peutz-Jeghers syndrome gene in left-sided colon cancer. Cancer Res. **58:** 3787–3790
10 Park W. S., Moon Y. W., Yang Y. M., Kim Y. S., Kim Y. D., Fuller B. G. et al. (1998) Mutations of the STK11 gene in sporadic gastric carcinoma. Int. J. Oncol. **13:** 601–604
11 Wang Z.-J., Taylor F., Churchman M., Norbury G. and Tomlinson I. (1998) Genetic pathways of colorectal carcinogenesis rarely involve the PTEN and LKB1 genes outside the inherited hamrtoma syndromes. Am. J. Pathol. **153:** 363–366
12 Avizienyte E., Loukola A., Roth S., Hemminki A., Salovaara R., Arola J. et al. LKB1 somatic mutations in sporadic tumors. Am. J. Pathol., in press
13 Hutchinson J. (1896) Pigmentation of lips and mouth. Arch. Surgery **7:** 290
14 Mallory S. B. and Stough D. B. (1987) Genodermatoses with malignant potential. Dermato. Clin. **5:** 221–230
15 Boardman L. A., Thibodeau S. N., Schaid D. J., Lindor N. M., McDonnell S. K., Burgart L. J. et al. (1998) Increased risk for cancer in patients with the Peutz-Jeghers syndrome. Ann. Intern. Med. **128:** 896–899
16 Bali D., Gourley I. S., McGarrity T. J., Spencer C. A., Howard L., Frazier M. L. et al. (1995) Peutz-Jegher's syndrome maps to chromosome 1p. In: American Society of Human Genetics, abstract 1067
17 Markie D., Huson S., Maher E., Davies A., Tomlinson I. and Bodmer W. F. (1996) A pericentric inversion of chromosome six in a patient with Peutz-Jeghers' syndrome and the use of FISH to localise the breakpoints on a genetic map. Hum. Genet. **98:** 125–128
18 Tomlinson I. P., Olschwang S., Abelovitch D., Nakamura Y., Bodmer W. F., Thomas G. et al. (1996) Testing candidate loci on chromosomes 1 and 6 for genetic linkage to Peutz-Jeghers' disease. Ann. Hum. Genet. **60:** 377–384
19 Fernandez Seara M. J., Martinez Soto M. I., Fernandez Lorenzo J. R., Trabazo S., Gamborino E. and Forteza Vila J. (1995) Peutz-Jeghers syndrome in a neonate. J. Pediatr. **126:** 965–967
20 Utsunomiya J., Gocho H., Miyanaga T., Hamaguchi E. and Kashimure A. (1975) Peutz-Jeghers syndrome: its natural course and management. Johns Hopkins Med. J. **136:** 71–82
21 Amos C. I., Bali D., Thiel T. J., Anderson J. P., Gourley I., Frazier M. L. et al. (1997) Fine mapping of a genetic locus for Peutz-Jeghers syndrome on chromosome 19p. Cancer Res. **57:** 3653–3656
22 Mehenni H., Blouin J.-L., Radhakrishna U., Bhardwaj S. S., Bhardwaj K., Dixit V. B. et al. (1997) Peutz-Jeghers syndrome: confirmation of linkage to chromosome 19p13.3 and identification of a potential second locus, on 19q13.4. Am. J. Hum. Genet. **61:** 1327–1334
23 Nakagawa H., Koyama K., Tanaka T., Miyoshi Y., Ando H., Baba S. et al. (1998) Localization of the gene responsible for Peutz-Jeghers syndrome within a 6-cM region of chromosome 19p13.3. Hum. Genet. **102:** 203–206
24 Olschwang S., Markie D., Seal S., Neale K., Phillips R., Cottrel L. S. et al. (1998) Peutz-Jeghers disease: most families compatible with linkage to 19p13.3, but evidence for a second locus at a different site. J. Med. Genet. **35:** 42–44
25 Westerman A. M., Chong Y. K., Entius M. M., Wilson J. H. P., Velthuysen V., Lindhout D. et al. (1997) In: First joint meeting of ICG-HNPCC and LCPG, Noordwijk, The Netherlands

26 Bartholomew L. G., Dahlin D. C. and Waugh J. M. (1957) Intestinal polyposis associated with mucocutaneus melanin pigmentation (Peutz-Jeghers syndrome). Gastroenterology **32:** 434–451

27 Bartholomew L. G., Moore C. E., Dahlin D. C. and Waugh J. M. (1962) Intestinal polyposis associated with mucocutaneous pigmentation. Surg. Gyn. Obstet. **115:** 1–11

28 De Facq L., De Sutter J., De Man M., Van der Spek P. and Lepoutre L. (1995) A case of Peutz-Jeghers syndrome with nasal polyposis, extreme iron deficiency anemia and hamartoma-adenoma transformation: management by combined surgical and endoscopic approach. Am. J. Gastroenterol. **90:** 1330–1332

29 Dormandy T. L. (1957) Gastrointestinal polyposis with mucocutaneous pigmentation (Peutz-Jeghers syndrome). New Engl. J. Med. **256:** 1093–1103

30 Phillips R. K. S., Spigelman A. D. and Thomson J. P. S. (1994) Familial Adenomatous Polyposis and Other Polyposis Syndromes, Edward Arnold, London

31 Burdick D. and Prior J. T. (1982) Peutz-Jeghers syndrome. A clinicopathologic study of a large family with a 27-year follow-up. Cancer **50:** 2139–2146

32 Jancu J. (1971) Peutz-Jeghers syndrome. Involvement of the gastrointestinal and upper respiratory tracts. Am. J. Gastroenterol. **56:** 545–549

33 Sommerhaug R. G. and Mason T. (1970) Peutz-Jeghers syndrome and ureteral polyposis. J. Am. Med. Assoc. **211:** 120–122

34 Rosai J. (ed.) (1996) Ackerman's Surgical Pathology, 8th ed., Mosby, St. Louis, MO

35 Fulcheri E., Baracchini P., Pagani A., Lapertosa G. and Bussolati G. (1991) Significance of the smooth muscle cell component in Peutz-Jeghers and juvenile polyps. Human Pathol. **22:** 1136–1140

36 Dippolito A. D., Aburano A., Bezouska C. A. and Happ R. A. (1987) Enteritis cystica profunda in Peutz-Jeghers syndrome. Report of a case and review of the literature. Dis. Colon Rectum **30:** 192–198

37 Mathus-Vliegen L. Follow-up of families with Peutz-Jeghers syndrome (1997) In: First joint meeting of ICG-HNPCC and LCPG, Noordwijk, The Netherlands

38 Rustgi A. K. (1994b) Hereditary gastrointestinal polyposis and nonpolyposis syndromes. N. Engl. J. Med. **331:** 1694–1702

39 Banse-Kupin L. A. and Douglass M. C. (1986) Localization of Peutz-Jeghers macules to psoriatic plaques. Arch. Dermatol. **122:** 679–683

40 Yamada K., Matsukawa A., Hori Y. and Kukita A. (1981) Ultrastructural studies on pigmented macules of Peutz-Jeghers syndrome. J. Dermatol. **8:** 367–377

41 Kieselstein M., Herman G., Wahrman J., Voss R., Gitelson S., Feuchtwanger M. et al. (1969) Mucocutaneous pigmentation and intestinal polyposis (Peutz-Jeghers syndrome) in a family of Iraqi jews with polycystic kidney disease. With a chromosome study. Israel J. Med. Sci. **5:** 81–90

42 Linos D. A., Dozois R. R., Dahlin D. C. and Bartholomew L. G. (1981) Does Peutz-Jeghers syndrome predispose to gastrointestinal malignancy? A later look. Arch. Surg. **116:** 1182–1184

43 Perzin K. H. and Bridge M. F. (1982) Adenomatous and carcinomatous changes in hamartomatous polyps of the small intestine (Peutz-Jeghers syndrome): report of a case and review of the literature. Cancer **49:** 971–983

44 Shepherd N. A., Bussey H. J. and Jass J. R. (1987) Epithelial misplacement in Peutz-Jeghers polyps. A diagnostic pitfall. Am. J. Surg. Pathol. **11:** 743–749

45 Dozois R. R., Judd E. S., Dahlin D. C. and Bartholomew L. G. (1969) The Peutz-Jeghers syndrome. Is there a predisposition to the development of intestinal malignancy. Arch. Surg. **98:** 509–517

46 Kinzler K. W. and Vogelstein B. (1996) Lessons from hereditary colorectal cancer. Cell **87:** 159–170

47 Hizawa K., Iida M., Matsumoto T., Kohrogi N., Yao T., Fujishima M. et al. (1993a) Neoplastic transformation arising in Peutz-Jeghers polyposis. Dis. Colon Rectum **36:** 953–957

48 Defago M. R., Higa A. L., Campra J. L., Paradelo M., Uehara A., Torres Mazzucchi M. H. et al. (1996) Carcinoma in situ arising in a gastric hamartomatous polyp in a patient with Peutz-Jeghers syndrome. Endoscopy **28:** 267

49 Foley T. R., McGarrity T. J. and Abt A. B. (1988) Peutz-Jeghers syndrome: a clinicopathologic survey of the 'Harrisburg family' with a 49-year follow-up. Gastroenterology **95:** 1535–1540

50 Riley E. and Swift M. (1980) A family with Peutz-Jeghers syndrome and bilateral breast cancer. Cancer **46:** 815–817

51 Young R. H., Welch W. R., Dickersin G. R. and Scully R. E. (1982) Ovarian sex cord tumor with annular tubules: review of 74 cases including 27 with Peutz-Jeghers syndrome and four with adenoma malignum of the cervix. Cancer **50:** 1384–1402

52 Wilson D. M., Pitts W. C., Hintz R. L. and Rosenfeld R. G. (1986) Testicular tumors with Peutz-Jeghers syndrome. Cancer **57:** 2238–2240

53 Dozois R. R., Kempers R. D., Dahlin D. C. and Bartholomew L. G. (1970) Ovarian tumors associated with the Peutz-Jeghers syndrome. Ann. Surg. **172:** 233–238

54 Tomlinson I. P. M. and Houlston R. S. (1997) Peutz-Jeghers syndrome. J. Med. Genet. **34:** 1007–1011

55 Wong S. S. and Rajakulendran S. (1996) Peutz-Jeghers syndrome associated with primary malignant melanoma of the rectum. Br. J. Dermatol. **135:** 439–442

56 Liaw D., Marsh D. J., Li J., Dahia P. L. M., Wang S. I., Zheng Z. et al. (1997) Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nature Genet. **16:** 64–67

57 Marsh D. J., Dahia P. L. M., Zheng Z., Liaw D., Parsons R., Gorlin R. J. et al. (1997) Germline mutations in PTEN are present in Bannayan-Zonana syndrome. Nature Genet. **16:** 333–334

58 Guldberg P., Straten P. T., Birck A., Ahrenkiel V., Kirkin A. F. and Zeuthen J. (1997) Disruption of the MMAC1/PTEN gene by deletion or mutation is a frequent event in malignant melanoma. Cancer Res. **57:** 3660–3663

59 Kong D., Suzuki A., Zou T.-T., Sakurada A., Kemp L. W., Wakatsuki S. et al. (1997) PTEN1 is frequently mutated in primary endometrial carcinomas. Nature Genet. **17:** 143–144

60 Li J., Yen C., Liaw D., Podsypanina K., Bose S., Wang S. I. et al. (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast and prostate cancer. Science **275:** 1943–1947

61 Steck P. A., Pershouse M. A., Jasser S. A., Alfred Yung W. K., Lin H., Ligon A. H. et al. (1997) Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nature Genet. **15:** 356–362

62 Teng D. H.-F., Hu R., Lin H., Davis T., Iliev D., Frye D. et al. (1997) MMAC1/PTEN mutations in primary tumor specimens and tumor cell lines. Cancer Res. **57:** 5221–5225

63 Olschwang S., Serova-Sinilnikova O. M., Lenoir G. and Thomas G. (1998) PTEN germline mutations in juvenile polyposis coli. Nature Genet. **18:** 12–14

64 Marsh D. J., Roth S., Lunetta K. L., Hemminki A., Dahia P. L. M., Sistonen P. et al. (1997) Exclusion of PTEN and 10q22-24 as the susceptibility locus for juvenile polyposis syndrome. Cancer Res. **57:** 5017–5021

65 Howe J. R., Roth S., Ringold J. C., Summers R. W., Järvinen H. J., Sistonen P. et al. (1998) Mutations in the Smad4/DPC4 gene in juvenile polyposis. Science **280:** 1086–1088

66 Desai D. C., Murday V., Phillips R. K. S., Neale K. F., Milla P., Hodgson S. V. et al. (1998) A survey of phenotypic features in juvenile polyposis. J. Med. Genet. **35:** 476–481

67 Aaltonen L. A., Salovaara R., Kristo P., Canzian F., Hemminki A., Peltomäki P. et al. (1998) Incidence of hereditary

nonpolyposis colorectal cancer, and molecular screening for the disease. New Engl. J. Med. **338:** 1481–1487
68 de la Chapelle A. and Peltomäki P. (1995) Genetics of hereditary colon cancer. Ann. Rev. Genet. **29:** 329–348
69 Knudson A. G. (1993) Antioncogenes and human cancer. Proc. Natl. Acad. Sci. USA **90:** 10914–10921
70 Knudson A. G. (1971) Mutation and cancer: statistical study of retinoblastoma. Proc. Natl. Acad. Sci. USA **68:** 820–823
71 Kallioniemi A., Kallioniemi O.-P., Sudar D., Rutovitz D., Gray J. W., Waldman F. et al. (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science **258:** 818–821
72 Kallioniemi O.-P., Kallioniemi A., Piper J., Isola J., Waldman F. M., Gray J. W. et al. (1994) Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer **10:** 231–243
73 Patterson M. J. and Kernen J. A. (1985) Epithelioid leiomyosarcoma originating in a hamartomatous polyp from a patient with Peutz-Jeghers syndrome. Gastroenterology **88:** 1060–1064
74 Boland C. R., Sato J., Appelman H. D., Bresalier R. S. and Feinberg A. P. (1995) Microallelotyping defines the sequence and tempo of allelic losses at tumour suppressor gene loci during colorectal cancer progression. Nature Med. **1:** 902–909
75 Jen J., Powell S. M., Papadopoulos N., Smith K. J., Hamilton S. R., Vogelstein B. et al. (1994) Molecular determinants of dysplasia in colorectal lesions. Cancer Res. **54:** 5523–5526
76 Powell S. M., Zilz N., Beazer-Barclay Y., Bryan T. M., Hamilton S. R., Thibodeau S. N. et al. (1992) APC mutations occur early during colorectal tumorigenesis. Nature **359:** 235–237
77 Smith A. J., Stern H. S., Penner M., Hay K., Mitri A., Bapat B. V. et al. (1994) Somatic Apc and K-RAS codon 12 mutations in aberrant crypt foci from human colons. Cancer Res. **54:** 5527–5530
78 Shibata D., Hawes D., Li Z.-H., Hernandez A. M., Spruck C. H. and Nichols P. W. (1992) Specific genetic analysis of microscopic tissue after selective ultraviolet radiation fractionation and the polymerase chain reaction. Am. J. Pathology **141:** 539–543
79 Boardman L., Schwartz D., Couch F., Burgart L. and Thibodeau S. (1998). STK11 mutations occur in some but not all cases of Peutz-Jeghers syndrome. American Society of Human Genetics, meeting abstract Denver, CO, USA
80 Mehenni H., Gehring C., Nezu J.-I., Oku A., Shimane M., Rossier C. et al. (1998) Loss of LKB1 kinase activity in Peutz-Jeghers syndrome and evidence for allelic and locus heterogeneity. Am. J. Hum. Genet. **63:** 1641–1650
81 Lawrence Livermore National Laboratories (1997) http://www-bio.llnl.gov/
82 Ashworth L. K. (1995) An integrated metric physical map of human chromosome 19. Nature Genet. **11:** 422–427
83 Schuler G. D., Boguski M. S., Stewart E. A., Stein L. D., Gyapay G., Rice K. et al. (1996) A gene map of the human genome. Science **274:** 540–546
84 Nezu J. (1996) Molecular cloning of a novel serine/threonine protein kinase expressed in human fetal liver (direct submission to GenBank, unpublished), http://www.ncbi.nlm.nih.gov/irx/cgi-bin/birx_doc?genbank + 65606
85 Jenne D. E., Reimann H., Nezu J.-I., Friedel W., Loff S., Jeschke R. et al. (1998) Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nature Genet. **18:** 38–43
86 GenBank, http://www.ncbi.nlm.nih.gov/index.html
87 Su J.-Y., Erikson E. and Maller J. L. (1996) Cloning and characterization of a novel serine/threonine protein kinase expressed in early *Xenopus* embyos. J. Biol. Chem. **271:** 14430–14437
88 NCBI BLAST, http://www.ncbi.nlm.nih.gov/BLAST/
89 Ko M. S. H. Systematic analyses of mouse genes expressed in embryo implantation site (unpublished), http://www.ncbi.nlm.nih.gov/irx/cgi-bin/birx_doc?genbank + 431188
90 Hemminki A. (1998) Inherited Predisposition to Gastrointestinal Cancer: The Molecular Backgrounds of Peutz-Jeghers Syndrome and Hereditary Non-polyposis Colorectal Cancer. Hakapaino, Helsinki, Finland
91 Nakagawa H., Koyama K., Miyoshi Y., Ando H., Baba S., Watatani M. et al. (1998b) Nine novel germline mutations of STK11 in ten families with Peutz-Jeghers syndrome. Hum. Genet. **103:** 168–172
92 Ylikorkala A., Avizienyte E., Tomlinson I. P. M., Tiainen M., Roth S., Loukola A. et al. (1999) Mutations and impaired function of LKB1 in familial and non-familial Peutz-Jeghers syndrome and a sporadic testicular cancer. Hum. Mol. Genet. **8:** 45–51
93 Resta N., Simone C., Mareni C., Montera M., Gentile M., Susca F. et al. (1998) STK11 mutations in Peutz-Jeghers syndrome and sporadic colon cancer. Cancer Res. **58:** 4799–4801
94 Nijhawan M., Nijhawan S., Verma A. and Bhargava M. (1994) Peutz-Jegher's syndrome with polydactyly. Indian J. Gastroenterol. **13:** 72
95 Hagstrom W. J. and Parsons R. W. (1986) Is there an association between Peutz-Jeghers syndrome and cleft lip? Plastic Reconstr. Surg. **78:** 698
96 Li D.-M. and Sun H. (1997b) TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor β. Cancer Res. **57:** 2124–2129
97 Akiyama Y., Iwanaga R., Saitoh K., Shiba K., Ushio K., Ikeda E. et al. (1997a) Transforming growth factor beta type II receptor gene mutations in adenomas from hereditary nonpolyposis colorectal cancer. Gastroenterology **112:** 33–39
98 Markowitz S., Wang J., Myeroff L., Parsons R., Sun L., Lutterbaugh J. et al. (1995) Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science **268:** 1336–1338
99 Parsons R., Myeroff L. L., Liu B., Willson J. K., Markowitz S. D., Kinzler K. W. et al. (1995b) Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. Cancer Res. **55:** 5548–5550
100 Lu S. -L., Kawabata M., Imamura T., Akijama Y., Nomizu T., Miyazona K. et al. (1998) HNPCC associated with germline mutation in the TGF-B type II receptor gene. Nature Genet. **19:** 17–18
101 Takaku K., Oshima M., Miyoshi H., Matsui M., Seldin M. F. and Taketo M. M. (1998) Intestinal tumorigenesis in compound mutant mice of both Dpc4 (Smad4) and Apc genes. Cell **92:** 645–656
102 Eng C. (1996) Seminars in Medicine of the Beth Israel Hospital, Boston: The RET proto-oncogene in multiple endocrine neoplasia type 2 and Hirschsprung's disease. New Engl. J. Med. **335:** 943–951
103 Zuo L., Weger J., Yang Q., Goldstein A. M., Tucker M. A., Walker G. J. et al. (1996) Germline mutations in the p16ink4a binding domain of CDK4 in familial melanoma. Nature Genet. **12:** 97–99
104 Schmidt L., Duh F.-M., Chen F., Kishida T., Glenn G., Choyke P. et al. (1997) Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nature Genet. **16:** 68–73
105 Haber D. and Harlow E. (1997) Tumour-suppressor genes: evolving definitions in the genomic age. Nature Genet. **16:** 320–322
106 Lee J. Y., Dong S. M., Kim H. S., Kim S. Y., Na E. Y., Shin M. S. et al. (1998) A distinct region of chromosome 19p13.3 associated with the sporadic form of adenoma malignum of the uterine cervix. Cancer Res. **58:** 1140–1143

107 Lancaster J. M., Wooster R., Mangion J., Phelan C. M., Cochran C., Gumbs C. et al. (1996) BRCA2 mutations in primary breast and ovarian cancers. Nature Genet. **13:** 238–240
108 Futreal P. A., Liu Q., Shattuck-Eidens D., Cochran C., Harshman K., Tavtigian S. et al. (1994) BRCA 1 mutations in primary breast and ovarian carcinomas. Science **266:** 120–122
109 Teng D. H.-F., Bogden R., Mitchell J., Baumgard M., Bell R., Berry S. et al. (1996) Low incidence of BRCA mutations in breast carcinoma and other cancers. Nature Genet. **13:** 241–244
110 Miki Y., Katagiri T., Kasumi F., Yoshimoto T. and Nakamura Y. (1996) Mutation analysis in the BRCA2 genein primary breast cancers. Nature Genet. **13:** 245–247
111 Wu Y., Nyström-Lahti M., Osinga J., Looman W. G., Peltomäki P., Aaltonen L. A. et al. (1997) MSH2 and MLH1 mutations in sporadic replication error-positive colorectal carcinoma as assessed by two-dimesional DNA electrophoresis. Genes Chromosomes Cancer **18:** 269–278
112 Konishi M., Kikuchi-Yanoshita R., Tanaka K., Muraoka M., Onda A., Okumura Y. et al. (1996) Molecular nature of colon tumors in hereditary nonpolyposis colon cancer, familial polyposis, and sporadic colon cancer. Gastroenterology **111:** 307–317
113 Liu B., Nicolaides N. C., Markowitz S., Willson J. K. V., Parsons R. E., Jen J. et al. (1995) Mismatch repair defects in sporadic colorectal cancers with microsatellite instability. Nature Genet. **9:** 48–55
114 Dobrovic A. and Simpendorfer D. (1997) Methylation of the BRCA1 gene in sporadic breast cancer. Cancer Res. **57:** 3347–3350
115 Kane M. F., Loda M., Gaida G. M., Lipman J., Mishra R., Goldman H. et al. (1997) Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res. **57:** 808–811
116 Thibodeau S. N., French A. J., Cunningham J. M., Tester D., Burgart L. J., Roche P. C. et al. (1998) Microsatellite instability in colorectal cancer-different mutator phenotypes and the principal involvement of hmlh1. Cancer Res. **58:** 1713–1718
117 Thibodeau S. N., French A. J., Roche P. C., Cunningham J. M., Tester D. J., Lindor N. M. et al. (1996) Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. Cancer Res. **56:** 4836–4840
118 Cunningham J. M., Christensen E. R., Tester D. J., Kim C. Y., Roche P. C., Burgart L. J. et al. (1998) Hypermethylation of the hmlh1 promoter in colon cancer with microsatellite instability. Cancer Res. **58:** 3455–3460
119 van Coevorden F., Mathus-Vliegen E. M. and Brummelkamp W. H. (1986) Combined endoscopic and surgical treatment in Peutz-Jeghers syndrome. Surg. Gyn. Obstet. **162:** 426–428
120 Rebsdorf Pedersen I., Hartvigsen A., Fischer Hansen B., Toftgaard C., Konstantin-Hansen K. and Bullow S. (1994) Management of Peutz-Jeghers syndrome. Experience with patients from the Danish Polyposis Register. Int. J. Colorectal Dis. **9:** 177–179
121 Rossini F. P. and Pennazio M. (1996) Enteroscopy and Peutz Jeghers syndrome. Am. J. Gastroenterol. **91:** 2252–2253
122 Rossini F. P., Arrigoni A. and Pennazio M. (1996) Clinical enteroscopy. J. Clin. Gastroenterol. **22**: 231–235; discussion 235–236
123 Spigelman A. D. and Phillips R. K. (1989) Management of the Peutz-Jeghers patient. J. Roy. Soc. Med. **82:** 681
124 Parker M. C. and Michell M. J. (1996) Polyposis: the Peutz-Jeghers syndrome. Br. J. Surg. **83:** 874–875
125 Harper P. S. and Clarke A. J. (1997) Genetics, Society and Clinical Practise, pp. 15–29, BIOS Scientific Publishers, Oxford
126 Järvinen H. J., Mecklin J.-P. and Sistonen P. (1995) Screening reduces colorectal cancer rate in families with hereditary nonpolyposis colorectal cancer. Gastroenterology **108:** 1405–1411
127 Laughlin E. H. (1991) Benign and malignant neoplasms in a family with Peutz-Jeghers syndrome: study of three generations. South. Med. J. **84:** 1205–1209
128 Hizawa K., Iida M., Matsumoto T., Kohrogi N., Kinoshita H., Yao T. et al. (1993) Cancer in Peutz-Jeghers syndrome. Cancer **72:** 2777–2781
129 Spigelman A. D., Arese P. and Phillips R. K. S. (1995) Polyposis: the Peutz-Jeghers syndrome. Br. J. Surg. **82:** 1311–1314