

REVIEW

The lentiginoses: cutaneous markers of systemic disease and a window to new aspects of tumourigenesis

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Familial lentiginosis syndromes cover a wide phenotypic spectrum ranging from a benign inherited predisposition to develop cutaneous lentigines unassociated with systemic disease, to associations with several syndromes carrying increased risk of formation of hamartomas, hyperplasias, and other neoplasms. The molecular pathways involved in the aetiology of these syndromes have recently been more clearly defined and several major cellular signalling pathways are probably involved: the protein kinase A (PKA) pathway in Carney complex (CNC), the Ras/Erk MAP kinase pathway in LEOPARD/Noonan syndromes, and the mammalian target of rapamycin pathway (mTOR) in Peutz-Jeghers syndrome and the diseases caused by *PTEN* mutations. Here we discuss the clinical presentation of these disorders and discuss the molecular mechanisms involved. The presence of lentigines in these diseases caused by diverse molecular defects is probably more than an associated clinical feature and likely reflects cross talk and convergence of signalling pathways of central importance to embryogenesis, neural crest differentiation, and end-organ growth and function of a broad range of tissues including those of the endocrine, reproductive, gastrointestinal, cardiac, and integument systems.

group of disorders that are now slowly but surely being molecularly elucidated, the familial lentiginoses (table 1).

The lentiginoses share multiple lentigines as one of their most prominent clinical features, the lentigen being a hamartomatous melanocytic lesion of the skin clinically almost identical to a freckle but histologically quite distinct.² Peutz-Jeghers syndrome (PJS) is the prototype of these diseases which are almost all inherited in an autosomal dominant manner, have a relatively high rate of de novo cases, and predispose to a variety of neoplasms.² Laugier-Hunziker syndrome (LHS), arterial dissections with lentiginosis (ADL), centropartial and partial unilateral lentiginoses, and LEOPARD and Noonan syndrome with lentigines (NSL) are other lentiginoses.² A number of related disorders may be associated with lentigines: Ruvalcaba-Myhre-Smith or Bannayan-Zonnana syndrome (RMS/BZS), a condition allelic to Cowden disease (CD),³ Schimke immunoosseous dysplasia,⁴ Mulvihill-Smith syndrome (MSS),⁵ Watson syndrome,⁶ McCune Albright syndrome (MAS),⁷ the two types of neurofibromatosis and other phacomatoses,⁸ multiple endocrine neoplasia 2B (MEN 2B), and nevus phacomatosus pigmentovascularis (NPP).⁹ Xeroderma pigmentosum may also be associated with solar lentigines;¹⁰ a number of chromosomal conditions and syndromes predisposing to premature aging or immunodeficiency or associated with DNA and/or chromosomal repair defects may also present with lentigines. However, in most of these conditions, lentigines are either secondary or peripheral to the primary lesions and do not represent hamartomatous proliferation of the melanocytes as is the case in the familial lentiginoses. In this review, we focus on the latter, and among them, the syndromes that have been molecularly elucidated over the last decade: CNC, PJS/LHS, LEOPARD, and NSL and the conditions caused by *PTEN* mutations (RMS/BZS and CD). The argument is made that the affected signalling pathways, protein kinase A (PKA), Ras/Erk MAP kinase, and the mammalian target of rapamycin (mTOR) converge to a complex system of cellular checks and balances that oversee growth, proliferation, and differentiation of many cell types; their perturbation causes a wide array of manifestations, including neoplasms that range from the simple lentigen to aggressive malignancies.

The medical examiner in rural Pennsylvania concluded “this combination of lesions is best explained by the concept of neurocristopathies” when finishing his report on the autopsy of a 19 year old heavily freckled man who died in 1981 due to malignant, metastatic (to his brain) pigmented melanotic schwannoma. The young man had been in and out of the National Institutes of Health (NIH) Clinical Center for a variety of ailments; he had first been diagnosed with a growth hormone producing tumour but his investigation and treatment was complicated by the baffling concurrent diagnosis of testicular tumours and hypercortisolaemia due to adrenal tumours.¹ It was clear that he was affected simultaneously by two rare endocrine conditions, acromegaly and Cushing syndrome, and several physicians had noted his many “freckles” and other pigmented skin lesions, but his disease was not actually diagnosed until years later. In 1995, upon reviewing records of NIH patients, investigators came across his clinical description and it became obvious that he had Carney complex (CNC). CNC belongs to a

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LENTIGO

Lentigines are often divided into two broad categories, simple lentigo and solar lentigo.

Table 1 The main lentiginoses: clinical manifestations and genetics

Disease	MIM	Clinical manifestations	Inheritance	Locus	Gene
Carney complex	160980	Lentiginos, PPNAD cardiac and skin myxoma schwannomas, acromegaly, breast and testicular tumours	AD	17q22–24 2p16	<i>PRKARIA</i> (CNC2) (CNC1) (?)
Peutz-Jeghers	175200	Lentiginos, GI polyps, neoplasia (GI tract, pancreas breast, ovary, uterus)	AD	19p13.3 19q	<i>LKB1/STK11</i> (?)
LEOPARD	151100	Lentiginos, cardiac conduction abnormalities, aneurysms, pulmonic stenosis, cephalo-facial dysmorphism, short stature, sensorineural deafness, mental retardation, skeletal abnormalities	AD	12q22-qter	<i>PTPN11</i>
BRRS/CD	153480	Macrocephaly, lipomatosis, pigmentation of the glans penis, mental retardation, vascular malformations	AD	10q23	<i>PTEN</i>
Lentiginosis	151001	Lentiginos (centrofacial, palmoplantar, trunk)	AD	Unknown	Unknown
	151000	As above in addition to mental retardation, skeletal dysgraphia	AD/sporadic	Unknown	Unknown

BRRS/CD, Bannayan-Riley-Ruvalcaba/Cowden disease; PPNAD, primary pigmented nodular adrenocortical disease.

Macules of café au lait colour that develop with older age are also called lentigo but they are clinically and genetically different lesions. In general, lentiginos associated with the genetic diseases that are being discussed in this review develop at a young age, often increase in number during adolescence, and are not restricted to sun exposed areas, whereas solar lesions often develop after the third decade of life, increase with advancing age, and as the name implies, are found almost exclusively on sun exposed areas.^{11 12} While the descriptive identification appears fairly straightforward, the clinical distinction of simple lentigo is at times more difficult, with lesions often confused with ephelides (freckles). However, several clinical features and histological differences do exist and should aid the clinician in separating these lesions. Although both types are of similar size and appearance and are often described as multiple 4–10 mm, variegated, brown to black macules, lentiginos typically do not darken with sun exposure (as compared to ephelides) and may be distributed on distinct anatomic locations such as the face (around the eyes and on the eyelids, the saddle of the nose, and the perioral areas of the upper and lower lips, crossing the vermilion border in some diseases), palmoplantar regions of the hands and feet, breast nipples, buttocks and genital region (labial, vaginal mucosa, prepuce, and penile mucosa and skin), and less commonly other inner mucosal surfaces (buccal and anal mucosa, and/or the conjunctivae, especially the inner and outer canthal areas).^{2 3} Histologically, while there is some overlap with freckles, lentiginos show prominent rete ridges and melanocytic hyperplasia. In the case of ephelides and solar lentiginos, increased pigmentation results from donation of pigment from otherwise normal (albeit stimulated) melanocytes to adjacent keratinocytes.^{2 11 12} Clinically, lesions on mucosal surfaces, in particular the lacrimal caruncle of the eye, and lesions that cross the vermilion border of the lips are of particular importance as pigmented lesions found in these locations are often heralding features of the familial lentiginos syndromes that are associated with systemic disease.

BENIGN LENTIGINOSES

Centrofacial neurodysraphic (Mendelian Inheritance in Man (MIM) 151000)¹³ and patterned (MIM 151001) lentiginos describe two inheritable conditions that, in keeping with all familial lentiginos syndromes, follow autosomal dominant (AD) inheritance, but in contrast to the other members of this group are not associated with systemic disease.^{14 15} Touraine *et al* described the first syndrome, centrofacial neurodysraphic lentiginos, reporting on a group of 32 patients from 17 families with early onset of facial lentiginos, occasionally found on the upper lip, that faded over time, which was frequently associated with mental retardation.¹⁴ In 1989, O'Neil and James described inherited patterned lentiginos: what distinguished this group of patients was the increased incidence in the African American population

and the description of lentiginos not only limited to the facial region but involving the hands, elbows, and buttocks as well.¹⁶ Interestingly, neither of these syndromes are associated with lesions of the oral mucosa.^{16 17} Several other reports of multiple lentiginos syndromes have since been published, but the significance of these syndromes, and potential overlap with other previously described syndromes, is as yet unknown.^{17 18}

CARNEY COMPLEX

The association of myxomas, spotty skin pigmentation (lentiginos), and endocrine overactivity was first reported by Dr J Aidan Carney in 1985 and subsequently designated as CNC by Bain in 1986¹⁹ and Carney syndrome by MIM¹³ in 1994. With the report of this new syndrome it was realised that the majority of patients previously characterised under the separate diagnoses of LAMB (lentiginos, atrial myxoma, mucocutaneous myxoma, blue nevi) and NAME (nevi, atrial myxoma, myxoid neurofibroma, ephelides) would now be more appropriately classified under CNC. The diagnosis of CNC is made if two of the main manifestations of the syndrome are present²⁰; these need to be confirmed by histology, biochemical testing, or imaging. Alternatively, the diagnosis is made when one of the criteria is present and the patient is a carrier of a known inactivating mutation of the *PRKARIA* gene (see "Molecular mechanism" section below for discussion).^{20–22}

The most common features of CNC include spotty skin pigmentation (fig 1) (lentiginos, freckling, café au lait spots, and blue nevi), myxomas of the heart, skin, and breast, and primary pigmented nodular adrenal cortical disease (PPNAD) associated with an atypical form of Cushing syndrome (CS).^{23 25} The breadth of involved organs in CNC is quite unique; CNC is both a multiple endocrine neoplasia (MEN) (along with MEN-1 and -2) and a cardiocutaneous syndrome (along with LEOPARD and similar conditions). Of the non-cutaneous lesions found in CNC, cardiac myxomas are the most common.^{22 24} These tumours tend to be of a more aggressive nature when compared to sporadic, non-CNC-associated myxomas: unlike the latter, the former may be in any cardiac chamber and may present multiple times, starting at a very young age (even in infancy) and without any predilection for gender (sporadic myxomas are more common in older women and almost always occur in the left atrium as single one-time tumours).²² Historically, cardiac myxomas have been reported to be responsible for more than 50% of the disease specific mortality among CNC patients.²⁴

Endocrine gland involvement can result in growth hormone (GH) secreting pituitary adenomas, thyroid gland disease, corticotropin (ACTH) independent CS secondary to PPNAD, and testicular tumours, in particular, large cell calcifying Sertoli cell tumours (LCCSCT).^{20–24} Overall, PPNAD is the most common endocrine lesion and causes the greatest degree of endocrine associated morbidity (discussed in more

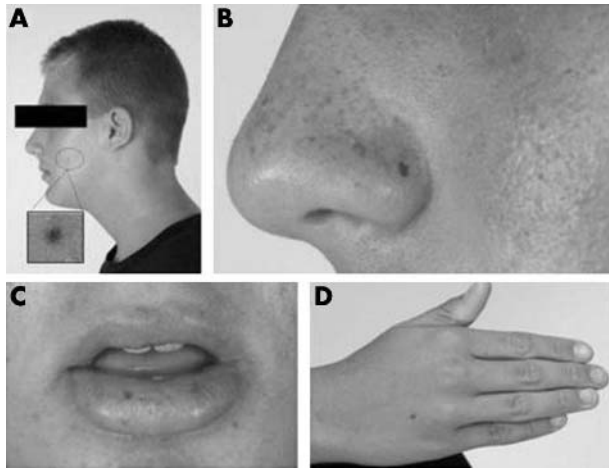


Figure 1 Pigmentation in Carney complex. (A) A blue nevus on the cheek of a patient with the complex. (B) Lentiginos on the sides of the nose. (C) Lentiginos on the vermilion border of the lips and the mucosa. (D) Blue nevus on the dorsal surface of the hand. Unusual pigmented lesions in patients with Carney complex may occur everywhere and are not unusual even in newborns with the disease. (These photographs are reproduced with consent.)

detail below).²⁴ In male patients, however, the occurrence of LCCSCT may supersede PPNAD in number, but not in morbidity, as it is typically a benign lesion most often diagnosed during routine testicular ultrasound when microcalcifications are found.²⁴ Leydig cell tumours and adrenal rests have also been reported.²⁴ Ovarian cysts are often found by sonographic examination as multiple hypoechoic lesions and although usually clinically insignificant, they may occasionally progress to ovarian carcinoma.^{20 26 27}

Thyroid gland disease spans the spectrum from nodular disease to carcinoma, but in contrast to pituitary and adrenal pathology, there does not appear to be an increased risk of hyper- or hypothyroidism. By sonographic examination, more than 60% of children and adults with CNC will be found to have cystic or multinodular disease.²⁸ On biopsy, follicular adenoma is the most common finding, whereas thyroid cancer, follicular or papillary, may develop in up to 10% of CNC patients with preexisting thyroid pathology.²⁸ Of note, recent examination for loss of heterozygosity (LOH) at the CNC locus on chromosome 17 (17q22–24) in sporadic thyroid cancer has found increased loss of this region, supporting the hypothesis that thyroid tissue is susceptible to tumourigenesis induced by *PRKARIA* loss of function.²⁹

Molecular mechanism

Genetic linkage analysis has revealed two distinct loci for CNC, one on chromosome 2p16 (*CNC2*) and the other on chromosome 17q22–24 (*CNC1*).^{30 31} Inactivating mutations of the gene encoding the protein kinase A type I- α regulatory (R1 α) subunit (*PRKARIA*) were identified in patients mapping to chromosome 17 and analysis of 53 of the 70 kindreds registered in the National Institutes of Health-Mayo Clinic collection has revealed that 28 out of the 53 (52.8%) have mutations at the *CNC1* locus.³² The gene responsible for CNC at the chromosome 2p16 locus is unknown and there is at least one large kindred with CNC that does not map to either the *CNC1* or *CNC2* locus.³² At this point, there are no clear phenotypic differences between families mapping to one or the other locus.

The role of R1 α in human tumourigenesis has been explored in several different cancer tissues and cell lines. Enhanced expression of R1 α has been shown to play a role in colorectal, renal, breast, and ovarian cancer, and malignant

osteoblasts, and may be associated with more advanced disease.^{32–39} The notion of reduced R1 α activity had not been investigated prior to the discovery of it being the protein that was defective in CNC; CNC represents the first identified human disease associated with a mutation of the PKA heterotetramer. The majority of mutations in the *PRKARIA* gene result in premature stop codons, with the most frequent mutations found in exons 2, 4, and 6.⁴⁰ Predicted mutant protein products are not found in affected cells secondary to nonsense mRNA mediated decay (NMD) of the mutant sequence.³¹ Biochemically, loss of R1 α leads to increased cAMP stimulated total kinase activity,³¹ thought to be secondary to up regulation of other components of the PKA tetramer,⁴¹ including both type I (*PRKAR1B*) and type II (*PRKAR2A* or *PRKAR2B*) subunits, in a tissue dependent manner,²⁰ but how this leads to increased tumourigenesis is currently unknown.

Initial data supported the role of *PRKARIA* as a “classic” tumour suppressor gene with tumours from CNC patients exhibiting germline mutations and subsequent LOH at the *PRKARIA* locus; however, it now appears that haploinsufficiency of *PRKARIA* may be sufficient for phenotypic expression of increased PKA activity⁴² and the development of certain tumours, such as eyelid myxomas.⁴³ This concept is exemplified in animal models of CNC: whereas mice homozygous for R1 α deletions die early in utero,⁴⁴ transgenic mice with heterogeneous expression of an antisense transgene for exon 2 of *PRKARIA* exhibit many of the phenotypic characteristics of CNC patients, including thyroid follicular hyperplasia and non-dexamethasone suppressible hypercortisolism.^{45 46} Not all of these lesions exhibited consistent losses of the normal R1 α allele.^{46 49}

Examination of the mechanisms associated with loss of R1 α , increased PKA activity, and tumourigenesis are currently underway. PKA is a ubiquitous serine-threonine kinase intimately involved in the regulation of cell growth, including a potential role in chromosome stability.²¹ The cross talk between signal transduction pathways and the tissue specific effects of altered PKA function are inherently quite complex, reflected by at times conflicting data. For example, alterations of 17q and/or the *PRKARIA* locus have been found in both sporadic adrenal and thyroid cancers, yet allelic loss of 17q in cardiac and skin myxomas from CNC patients, with known germline *PRKARIA* mutations, have not been found.^{21 43} Interestingly, CNC myxomas appear to have a more aggressive nature when compared to sporadic, non-CNC-associated myxomas, as discussed previously.

The physiological impact of *PRKARIA* inactivating mutations has been most thoroughly studied in PPNAD, a rare form of ACTH independent CS, which is present in approximately one third of CNC patients. PPNAD often presents in an indolent fashion and may be difficult to diagnose due to an intermittent or cyclical nature of the associated hypercortisolism.²² Diagnosis is established using the 6 day Liddle test as patients with PPNAD show a paradoxical rise in the 24 h urinary free cortisol and/or 17-hydroxysteroids of more than 50% on the second day of high dose dexamethasone administration.²⁴ While this response appears to be pathognomonic for PPNAD, it does not appear to be dependent on the presence of *PRKARIA* mutations as comparative in vitro studies between PPNAD cell lines with and without R1 α deficiency showed increased cortisol secretion in response to dexamethasone associated with increased expression of the glucocorticoid receptor.⁴⁷ The underlying mechanism for this response is not known.

Additional studies aimed at elucidating the inter-relationship between *PRKARIA* status, altered PKA activity, and cellular metabolism are being aggressively pursued. Microarray analysis of R1 α antisense targeted tumour cells



Figure 2 Features of Noonan/LEOPARD syndromes. (A) Pigmented lesions on the face can mimic those of patients with Carney complex, but they tend not to be present on the lips or periorally; they occur mostly as single darkly pigmented spots and look less like freckles. (B) Lentiginos on the shoulders and the back can be extensive; winged scapulae is also an evident deformity in this patient. (C) Skeletal deformities, scoliosis and hyperextensibility, like that shown in this image, are not unusual in patients with this condition. (These photographs are reproduced with consent.)

has recently been shown to change expression of more than 240 genes suggesting that altered regulation of a significant number of downstream targets is likely to contribute to the CNC phenotype.⁴⁸ Investigation of one of the signalling pathways, the mitogen activated protein kinase (MAPK) ERK 1/2 pathway, typically inhibited by PKA in many cells, has recently been reported. In this report, the lymphocytes from CNC patients with known *PRKARIA* mutations showed altered PKA activity and increased ERK 1/2 phosphorylation.⁴² Cell metabolism and cell proliferation studies suggested that altered PKA activity is associated with reversal of PKA mediated inhibition of the MAPK pathway resulting in increased cell proliferation.⁴²

LEOPARD SYNDROME

LEOPARD is also often referred to as multiple lentiginos syndrome (MLS). The acronym, which also describes the pattern of pigmentation (fig 2), was suggested first by Gorlin *et al* in 1969⁵⁰ and reflects the components of this cardiocutaneous disorder: *l*entiginos, *e*lectrocardiographic conduction defects, *o*cular hypertelorism, *p*ulmonary stenosis, *a*bnormal genitalia, *r*etardation of growth, and *s*ensorineural deafness.^{51–52} The diagnosis is established if multiple lentiginos are present in association with at least two other features; if lentiginos are absent, a first degree relative affected with LEOPARD syndrome and three of the other six features are needed for diagnosis.⁵²

As in CNC patients, the lentiginos usually develop in childhood and are often the first clinical manifestation to appear⁵²; they are located primarily on the face and upper trunk, less commonly involving the oral mucosa, extremities, genitalia, or conjunctiva of the eye.⁵³ The appearance and distribution of the lentiginos are very similar to CNC, as is the histological appearance, including melanocytic hyperplasia and elongation of the rete ridges. One important difference is the absence of pigmented spots along the vermilion border of the lips, a finding that is characteristic in CNC and PJS patients. Also, skeletal abnormalities, onychodystrophy, and hyperelastic skin, which are often found in LEOPARD

syndrome patients, are not common in other lentiginos.^{52–53} The craniofacial features of LEOPARD syndrome are generally coarse and include low set and posteriorly rotated ears, hypertelorism with or without ptosis, webbed neck, and mandibular prognathism.⁵² These features, combined with an increased incidence of pulmonic stenosis, show significant phenotypic overlap with Noonan syndrome (MIM 163950); furthermore, hyperelasticity and other features partially overlap with Ehlers-Danlos syndrome subtypes and the arterial dissections with lentiginos (ADL) syndrome (see below).⁵²

Morbidity and mortality associated with LEOPARD syndrome are dependent on the extent of cardiac disease. Multiple congenital heart defects have been reported to include not only pulmonic stenosis (present in 40% of patients) but also subaortic and subpulmonic stenosis, and hypertrophic obstructive cardiomyopathy.⁵³ In addition, conduction abnormalities are common and whether they are a primary defect, or secondary to structural abnormalities, may result in sudden cardiac death.⁵⁴ More recently, a predisposition to widespread, recurrent polyaneurysms has been reported in a patient with genetically confirmed LEOPARD syndrome.⁵⁵ Although unproven, it is interesting to speculate whether patients previously diagnosed with the ADL disorder (MIM 600459) may be more appropriately reclassified as having a variant of LEOPARD syndrome; to our knowledge, these patients have not had genetic testing.⁵⁶

Molecular mechanism

The clinical similarities between LEOPARD and Noonan syndrome (NS), and the series of patients with both lentiginos and NS-like features (NSL), raised the question whether these syndromes could be allelic disorders with varied clinical expression. Some of the patients with NSL had in fact Watson syndrome, a condition that is allelic to neurofibromatosis type I. Still, LEOPARD, NS, and NSL share similar craniofacial features, an increased incidence of sensorineural deafness and cryptorchidism, and associated pulmonic stenosis. In total, up to 10% of NS patients have lentiginos.⁵⁷ In 1996, mutations in the *PTPN11* gene (12q24.1), encoding the non-receptor tyrosine protein tyrosine phosphatase Shp-2 (Src homology 2 domain containing protein tyrosine phosphatase-2), were found to be the cause of NS in 50% of patients.⁵⁸ In 2002, independent research groups published reports linking *PTPN11* mutations to LEOPARD syndrome.^{59–60}

Shp-2 is an important intermediate in several signalling pathways involved in modulating cellular proliferation, differentiation, and migration. Vertebrates have two Shp proteins, Shp-1 and Shp-2, both having two N-terminal domains, N-SH2 and C-SH2, a catalytic protein-tyrosine phosphatase (PTP) domain, and a C-terminal tail.⁶¹ Shp2 is a key regulatory protein in the receptor tyrosine kinase (RTK) signalling pathways whose primary role is to down regulate GTPase activating proteins (GAP) resulting in activation of Ras.⁶¹ In the basal state, the N-SH2 domain of Shp-2 exhibits an inhibitory role over the phosphatase activity by allosterically blocking the binding domain of PTP.⁶¹ To date, the *PTPN11* mutations that have been described in both NS and LEOPARD are believed to be gain of function mutations leading to dysregulated phosphatase activity with subsequent increased inhibition of GTPase which in turn leads to increased Ras activity.^{61–62} In keeping with this, *in vitro* studies of haematopoietic cell lines harbouring *PTPN11* mutations show increased proliferation rates and reduced growth factor requirements.⁶²

How these abnormalities result in human disease and what intermediary molecules and additional downstream targets of Shps are affected by mutations of the *PTPN11* gene are as

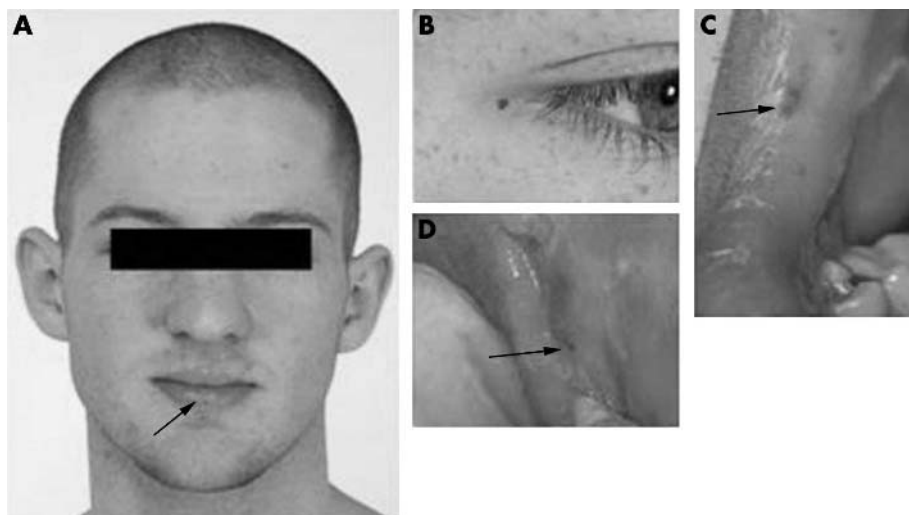


Figure 3 Pigmentation in Peutz-Jeghers syndrome looks similar to that in Carney complex except that single lesions tend to be larger and more pigmented. (A) Lentiginos on the vermillion border of the lips and a darkly pigmented lesion on the mucosa (arrow). (B) Freckling around the eyes with multiple lentiginos. (C, D). Pigmented lesions in the oral mucosa are frequent in patients with this syndrome, perhaps more frequent than in any other lentiginosis. (These photographs are reproduced with consent.)

yet poorly understood. It is of interest to note, however, that SHP-2 has mitogenic effects on vascular smooth muscle and interacts with several key elements of angiogenesis, including the angiopoietin-1 receptor and the signalling cascade of vascular endothelial growth factor (VEGF).⁵⁴

Accumulating evidence suggests that certain germline *PTPN11* mutations play a key role in certain manifestations of LEOPARD, NS, and/or NSL, such as the malformations of the cardiovascular system and predisposition to certain malignancies.⁵⁴ For example, exon 8 mutations were more commonly associated with pulmonary valve stenosis, and exon 7 and 12 mutations were more frequently associated with hypertrophic cardiomyopathy, whereas those of exon 3 were associated with atrial septal defects.⁶³ Certain mutations are also associated with malignancies in both the germline and somatic state: although neoplasms are uncommon in LEOPARD patients, children with NS have an increased incidence of haematological disorders, including juvenile myelomonocytic leukaemia (JMML).⁶² Recent studies indicated that in addition to mutations in the *Ras* oncogene and inactivating mutations of *NF1*, in a mutually exclusive manner, 25–30% of JMML cases harboured somatic *PTPN11* mutations.^{62–64} These mutations are found in similar locations as those found in LEOPARD and NS, but resulted in different amino acid substitutions.⁶¹ This apparent genotype-phenotype correlation was further supported by the observations made in a transgenic mouse model which expressed a heterozygous NS associated *PTPN11* mutation (D61G) developing short stature, craniofacial abnormalities, myeloproliferative disease, and multiple cardiac defects.⁶⁵

PEUTZ-JEGHERS SYNDROME

PJS is a disorder characterised by mucocutaneous lentiginos (lips, buccal mucosa), gastrointestinal (GI) hamartomatous polyps (affecting the small bowel, stomach, and large colon), and an increased risk of developing early onset adenocarcinoma of the GI tract, as well as tumours of the pancreas, breast, thyroid, and reproductive organs.^{66–67} There is significant clinical overlap between PJS and CNC to the point that some patients with CNC in the NIH series had been diagnosed with PJS⁶⁸ (the opposite is less frequent since PJS is a widely known condition, whereas CNC was only recently

described). As in CNC, patients with PJS may have lentiginos of the lips, buccal mucosa, genitals, or the hands and feet (fig 3), which tend to fade in older age² (an important consideration in the evaluation of the older patient with multiple hamartomatous GI polyps) and a number of other skin lesions (mostly compound but also blue and Spitz nevi), thyroid tumours, and an increased incidence of gonadal tumours. Most males with PJS, as patients with CNC, have upon sonographic examination testicular micro-calcifications which reflect the presence of multiple foci of large cell calcifying Sertoli cell tumours (LCCSCTs). Leydig cell tumours are less frequent, as in CNC. LCCSCTs may express aromatase and lead to precocious puberty and gynaecomastia,⁶⁸ clinical findings that are more common in boys with PJS than in those with CNC. Females with PJS develop a variety of gynaecological neoplasms; their high prevalence and histological subtypes are at variance with those of women with CNC.²⁷

It was recently reported that the cumulative life long risk for developing cancer in a patient with PJS exceeds 90%.⁶⁶ There does not appear to be a risk difference between genders, with the exception of gonadal malignancies and breast cancer, which are far more common in females.⁶⁹ The mean age of diagnosis of a first cancer was 42.9 ± 10.2 years in one study.⁶³ The most recent study of 240 patients with PJS (188 familial and 52 sporadic cases were included), all with confirmed genetic defects, showed an age dependent risk of developing cancer as follows: 1% at age 20, 3% at 30, 19% at 40, 32% at 50, 63% at 60, and 81% at 70 years.⁶⁹ Overall, 54 malignancies were diagnosed in 47 carriers; GI (oesophageal, stomach, small bowel, colon, rectum, and pancreas) and breast cancers were the most common. Compared to the general population, the risk of developing colorectal and breast cancer in PJS by age 60 was 42% and 32% versus 1% and 5%, respectively.⁶⁹ Thus, breast cancer risks in women with PJS are comparable to those of women with either *BRCA1* or *BRCA2* mutations. The optimal time to initiate GI and breast cancer surveillance is still somewhat controversial, although most agree that GI screening (by endoscope, colonoscopy, and abdominal CT) and breast examinations with reproductive tract screening (by pelvic ultrasound, cervical cytology, and serum CA125 levels) should start after age 20 and 25, respectively.⁶⁹

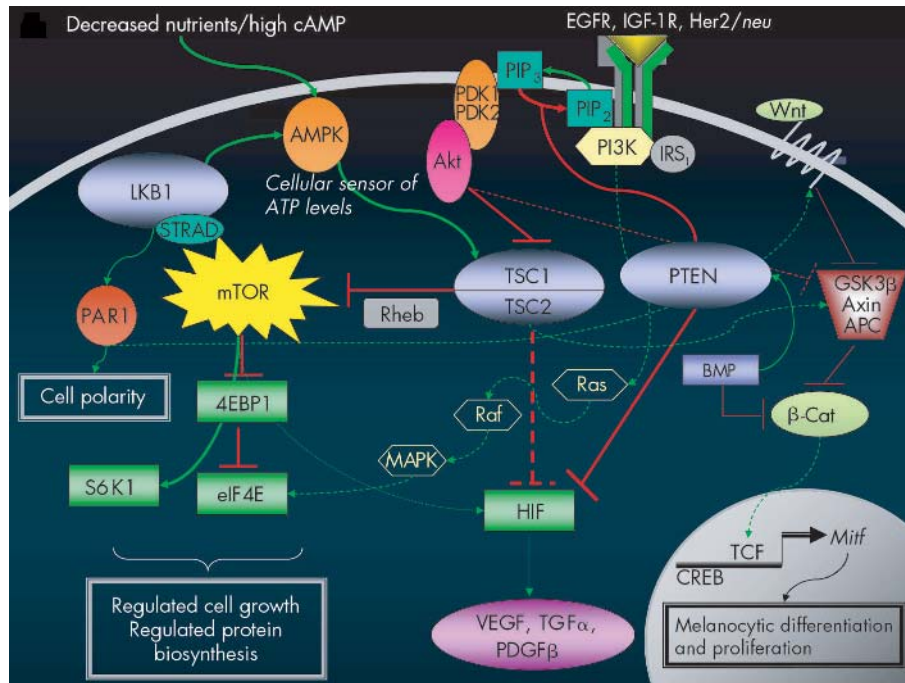


Figure 4 Two independent pathways appear to be critically important in regulating cell growth in response to nutrient supply and mitogenic stimulation: (i) the PKA/PRKAR1A-LKB1 tumour suppressor protein pathway, acting via AMPK, and (ii) the PI3K/AKT pathway. Recent evidence suggests that the tumour suppressor gene complex, TSC1/TSC2, orchestrates the signal from both pathways to the downstream target, mTOR, which in turn regulates the ribosomal protein S6 and 4EBP-1, a repressor of the translational initiation factor eIF4E. In this model, at times of nutrient stress LKB1/AMPK activation of the TSC1/TSC2 complex results in inhibition of mTOR and a decrease in protein synthesis. Under stimulation of mitogenic pathways, PI3K phosphorylates PIP2 to PIP3 resulting in recruitment of AKT to the membrane where it is activated by PDK1. Activated AKT inhibits the TSC1/TSC2 tumour suppressor complex leading to increased mTOR activity. In the later pathway, PTEN antagonises PIP3 action through dephosphorylation, and thus provides an “off” switch for regulating mitogenic pathway induced cellular growth and proliferation. Cross talk of several other pathways appears to play important regulatory roles in the lentiginoses syndromes to include the Ras/MAPK pathway in the regulation of translation, the LKB1 pathway in cellular polarity, the AKT pathway (as well as the TSC1/TSC2 complex) in the regulation of the Wnt/GSK3 β / β -Cat pathway, and the BMP pathway in the regulation of PTEN (see text for further discussion). Lastly, both PTEN and mTOR appear to have negative regulatory effects on VEGF through loss of stabilisation of the hypoxia inducible transcription factor 1 (HIF1).

Molecular mechanism

In 1998, two independent reports identified germline mutations in the gene *LKB1/STK11* on 19p13.3, coding for a serine/threonine kinase, as the cause of PJS in most (but not all) patients.^{70–71} To date mutations in *LKB1* can be found in only 30–80% of patients; linkage to other loci, including 19q13.4, has also been reported but the causative gene(s) have not been identified.^{72–73} *LKB1* appears to function as a classic tumour suppressor following Knudson’s two hit hypothesis, at least as far as the development of GI neoplasms is concerned. Interestingly, *LKB1* also appears to play a role in two additional key regulatory pathways, involved in controlling the polarity of epithelial cells and as the master regulator of AMP dependent kinase, the central sensor of cellular ATP levels and key regulator of cellular energy consumption^{74–77}; *LKB1* may also be interacting with PRKARIA, and is phosphorylated by PKA (fig 4).

The lentiginoses and the mTOR pathway

It has recently been suggested that *LKB1* defects lead to dysregulation of the downstream target mTOR, the mammalian target of rapamycin, a key regulator of protein translation.^{78–79} mTOR is a highly conserved serine/threonine kinase that mediates cellular growth by sensing information on the cellular energy status and mitogenic signals and then coordinating the activity of the translational machinery of the cell through regulation of the ribosomal protein S6 kinases (S6Ks) and the eukaryotic translational initiation factor 4E (eIF4E) binding proteins (4E-BPs).⁸⁰ Dysregulated activation of mTOR is believed to allow growth of cells to

occur at times of reduced nutrient or energy supply and independently of mitogenic stimuli with the “uncoupling” hypothesised to be the underlying mechanism responsible for the formation of hamartomas and neoplasia in PJS and other conditions (fig 5). *LKB1* plays a fundamental role in regulating cellular energy metabolism by down regulating mTOR dependent protein synthesis during times of nutrient stress.⁸¹ This regulatory control is mediated through the direct activation of AMP activated protein kinase (AMPK), a primary sensor of cellular response to reduced ATP levels. AMPK is activated by a variety of stimuli, including oxidative and osmotic damage, hypoxia, and hypoglycaemia.⁸¹ Once activated, AMPK phosphorylates and activates tuberlin (encoded by the tumour suppressor *TSC2* and mutated in tuberous sclerosis type II), resulting in inhibition of mTOR signalling.⁸² At the basal state, *LKB1* protects cells from apoptosis by reducing protein synthesis at times of stress. Loss of *LKB1* function results in dysregulated mTOR mediated protein synthesis^{74–79}; in addition, aberrant *TSC1/TSC2* and/or mTOR signalling in these cells results in increased angiogenesis through activation of hypoxia inducible factor 1 (HIF) and VEGF.⁸³ Consistent with the above, *Lkb1* deficient mice develop intestinal hamartomatous polyps^{84–85} and hepato-cellular carcinoma.⁸⁶ Interestingly, *Lkb1* deficient cells are resistant to *Ras* induced transformation,⁸⁴ as would have been expected from a disruptor of mTOR signalling. While these alterations shed light on aberrant cellular metabolism and may explain why cells without normal *LKB1* function show immortalised growth and decreased apoptosis, they do not fully explain the

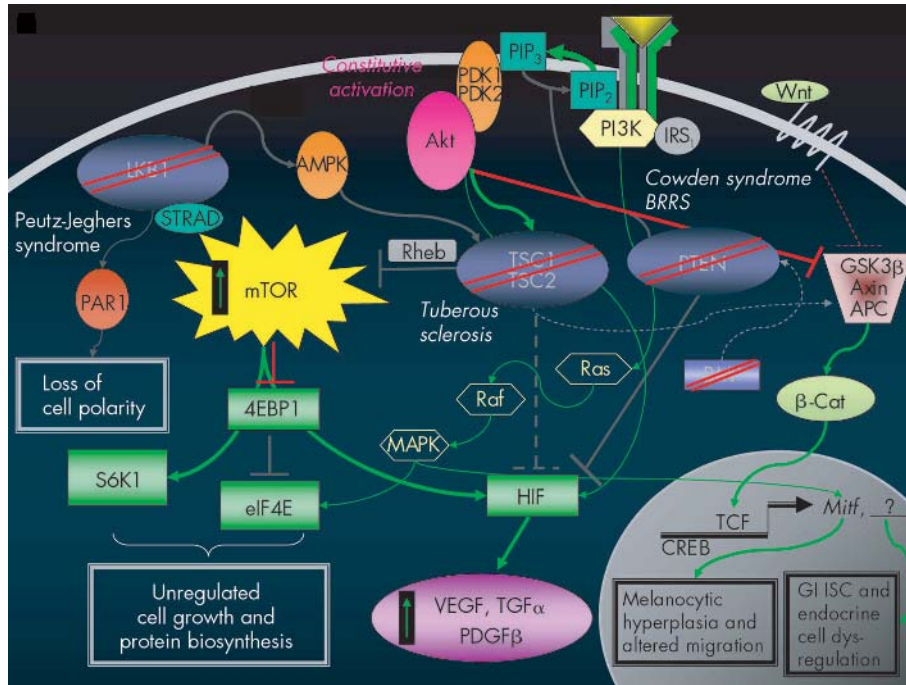


Figure 5 Loss of function of the tumour suppressor proteins LKB1 (or its regulator PKA/PRKAR1A) in Peutz-Jeghers syndrome, and PTEN and BMP in Cowden syndrome/Banayan-Riley-Ruvalcaba (or TSC1/TSC2 in tuberous sclerosis) leads to a proliferative advantage for the affected cells by switching on the protein translational machinery of the cells which advances cellular growth. In addition, LKB1 is required to maintain an organised, polarised epithelium in a cell to cell interaction independent manner; loss of LKB1 leads to loss of cellular polarity and loss of PTEN function to increased levels of PIP3, increased AKT, mTOR, β-catenin, and HIF activity. Accumulating evidence suggests that dysregulated activity of mTOR and the downstream effectors S6K1, 4EBP1, and HIF, as well as down regulation of the GSK3b complex with increased β-Cat activity may be the triggering mechanism for hamartoma formation in the aforementioned syndromes.

apparent tumourigenic affect of *LKB1* mutations. Further study of the *LKB1* pathway and its interaction with other signalling molecules, including the *LKB1* specific adaptor protein STRAD, and other intermediaries (PAR1, PKA, or any of the 13 or more additional kinases of the AMPK subfamily) will need to be completed to more fully understand how *LKB1* choreographs cellular organisation and growth.^{74 76 87}

Laugier-Hunziker syndrome

Laugier-Hunziker syndrome (LHS) is a rare, sporadic disorder, originally described in 1970, that is often confused with PJS due to similar appearance and distribution of hyperpigmented cutaneous and mucocutaneous lesions.^{88 89} Family history and screening for mutations may not aid in distinguishing this disorder from PJS as up to 25% of PJS cases are sporadic, and as previously reviewed, *LKB1* mutations may be found in only 30–80% of cases.⁷⁵ Accurate clinical diagnosis is essential as patients with LHS are not at an increased risk of developing GI tumours and they do not need any invasive GI tract surveillance.⁸⁹

Ruvalcaba-Myhre-Smith, Bannayan-Zonnana syndrome, and Cowden disease (CD)

RMS/BZS and CD along with PJS and juvenile polyposis are a group of inherited disorders that have been previously grouped under the general classification of the familial hamartoma syndromes. With the discovery of mutations in the tumour suppressor gene *PTEN* (10q22–q23) in up to 80% of CD patients and up to 60% of RMS/BZS patients, it has been suggested that these conditions should be all listed under the heading “*PTEN* hamartoma tumour syndromes” (PHTS).^{90–100} Patients with *PTEN* mutations have an increased risk of developing multiple hamartomas in various organ systems such as the breast, thyroid, skin, central nervous system, and GI tract.⁹⁸ Some distinguishing features of RMS/

BZS include delayed motor development, and most germane to our discussion, the presence of lentiginos, especially on the glans penis, known as the “speckled penis”.⁹⁸ The association of macrocephaly, lipomatosis, and speckled penis is also known as the Bannayan-Riley-Ruvalcaba triad.⁹⁶ Hyperpigmentation of the glans penis typically develops during childhood; in 90% of CD patients, mucocutaneous signs develop by 20 years of age.^{98–100} Diagnostic criteria for PHTS, and a thorough review of this topic, were recently published.¹⁰¹ It should be noted that *PTEN* mutations have also been found in several other conditions, including Proteus syndrome (PS; MIM 176920) and Proteus-like disorders,^{102–104} Lhermitte-Duclos disease (LDD), and other rare syndromes.^{105 106}

Molecular mechanism

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a dual specific phosphatase that plays a key role in cell growth, differentiation, apoptosis, membrane trafficking, cellular interactions, and cellular motility.^{99 107 108} The mechanics of how *PTEN* regulates such diverse and key regulatory pathways has been the focus of intense research as early on it was recognised that inactivation of *PTEN/MMAC* (mutated in multiple advanced cancers) effected a large number of cancers and appeared to correlate with advanced disease in CNS glial tumours (glioblastoma multiforme and anaplastic astrocytoma), advanced prostate cancer, and metastatic breast cancer.^{107 108} The early reports of *PTEN*’s inhibitory regulation of the phosphoprotein focal adhesion kinase (FAK) and its effects on decreasing cell spreading and motility have since given way to the ever increasing body of research on the regulator role of *PTEN* on the phospholipids, and of particular interest, the ability of *PTEN* to regulate the plasma membrane inositol phospholipids functioning as the “off” switch for the phosphoinositide 3-kinase (PI3K)

signalling pathway.^{107–109} It has now been suggested that loss of PTEN activity leads to constitutive activation of the cytosolic signalling protein AKT.¹⁰⁹ One of the key downstream targets of AKT is the tuberin-hamartin complex (*TSC1/TSC2*), mutations of which are associated with the hamartomatous syndrome tuberous sclerosis.¹¹⁰ Early evidence suggests that the tuberin-hamartin complex, through inhibition of mTOR signalling, inhibits the 70 kDa ribosomal S6 kinase (S6K1) and eukaryotic initiation factor 4E binding protein (4E-BP1), key mediators of the protein translational machinery.¹¹⁰ In this model, loss of PTEN function results in the constitutive activation of AKT, down regulation of tuberin/*TSC2* and mTOR, and subsequent promotion of cell cycle progression and suppression of apoptosis. Elucidation of this pathway, mediated through mTOR, now provides a link for the previous observation linking the role of mitogenic stimuli in breast, colon, and prostate cancer, as well as in the hamartomatous tumour syndromes PJS, tuberous sclerosis, and CD.¹⁰⁷ A more thorough review of the role of mTOR and translation in cancer pathogenesis has recently been published.^{111–114} The role of the bone morphogenetic proteins (BMP) in regulating PTEN levels was also recently revealed.^{115–117} *Bmpr1a* mutant mice develop intestinal polyps, an effect that is largely due to loss of BMP inhibition of PTEN function and to some extent Wnt signalling cross talk.^{117 118}

SUMMARY: LENTIGINES, A CLINICAL SIGN OF MOLECULAR CONVERGENCE?

Although much remains to be learned, there is compelling evidence to suggest that the apparently different pathways that result in the main lentiginoses are also involved in melanocytic differentiation and migration. Under the direction of several key regulatory signals, including those from the Wnt family of proteins, fibroblast growth factors, and BMPs, neural crest cells differentiate along the dorso-lateral pathway that gives rise to the melanocytes.^{119–121} Interactions with factors such as the microphthalmia associated transcription factor (*Mitf*), mTOR, and possibly *BRAF* and the dickkopf proteins (DKK) may provide a molecular basis as to why the melanocyte is affected in such a way in these disparate disorders.¹²² Figures 4 and 5 provide a summary of what we have learnt in the last 10 years about the lentiginoses, but we are still a long way away from knowing what we should know to treat our patients!

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