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REVIEW ARTICLE
Protean PTEN: Form and Function

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Germline mutations distributed across the *PTEN* tumor-suppressor gene have been found to result in a wide spectrum of phenotypic features. Originally shown to be a major susceptibility gene for both Cowden syndrome (CS), which is characterized by multiple hamartomas and an increased risk of breast, thyroid, and endometrial cancers, and Bannayan-Riley-Ruvalcaba syndrome, which is characterized by lipomatosis, macrocephaly, and speckled penis, the *PTEN* hamartoma tumor syndrome spectrum has broadened to include Proteus syndrome and Proteus-like syndromes. Exon 5, which encodes the core motif, is a hotspot for mutations likely due to the biology of the protein. *PTEN* is a major lipid 3-phosphatase, which signals down the PI3 kinase/AKT pro-apoptotic pathway. Furthermore, PTEN is a protein phosphatase, with the ability to dephosphorylate both serine and threonine residues. The protein-phosphatase activity has also been shown to regulate various cell-survival pathways, such as the mitogen-activated kinase (MAPK) pathway. Although it is well established that PTEN's lipid-phosphatase activity, via the PI3K/AKT pathway, mediates growth suppression, there is accumulating evidence that the protein-phosphatase/MAPK pathway is equally important in the mediation of growth arrest and other crucial cellular functions.

Introduction

Prior to 1996, when the susceptibility gene for Cowden syndrome (CS [MIM 158350]) was mapped to 10q22-q23 (Nelen et al. 1996), the molecular bases of the inherited hamartoma-tumor syndromes were obscure. CS is an autosomal dominant disorder that is characterized by multiple hamartomas that affect derivatives of all three germ layers and by a risk of breast, thyroid, and endometrial neoplasias (Appendix A) (Eng 2000). Germline mutations in *PTEN/MMAC1/TEP1* (MIM 601728), a tumor-suppressor gene located on 10q23, have since been found in 80% of probands with CS (Liaw et al. 1997; Marsh et al. 1998*b*). *PTEN* encodes a lipid dual-specificity phosphatase and is the major 3-phosphatase in the phosphoinositol-3-kinase (PI3K)/AKT pro-apoptotic pathway (Li and Sun 1997; Li et al. 1997; Steck et al. 1997; Maehama and Dixon 1998; Stambolic et al. 1998). This represents the first phosphatase gene that has been implicated in the etiology of an inherited cancer syndrome. Subsequently, the clinical spectrum of disorders that are associated with germline *PTEN* mutations has expanded to include seemingly disparate syndromes.

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Identification of *PTEN*

PTEN was first identified in 1997 by three independent groups, each of which had slightly different strategies. Two groups used positional-cloning approaches to map this gene to 10q23 (Li et al. 1997; Steck et al. 1997); sequence analysis showed a large region of homology to chicken tensin, bovine auxilin, and a protein tyrosine-phosphatase domain, from which the name "PTEN" was coined (for phosphatase and tensin homolog, deleted on chromosome 10 [ten]). A third group (Li and Sun 1997) identified PTEN by searching for genes with its biochemical properties. Li and Sun searched for novel human protein tyrosine phosphatases by using two different methods (Li and Sun 1997). By searching GenBank for entries that contain phosphatase motifs and using a PCR-based approach to screen a human cDNA library, they identified TEP1 (transforming growth factor [TGF]–regulated and epithelial cell-enriched phosphatase), which was also shown to be identical to PTEN. TEP1 was shown to dephosphorylate an in vitro substrate for tyrosine phosphatases (phosphotyrosyl-RCML). Furthermore, Li and Sun demonstrated that when the

essential cysteine in the tyrosine-phosphatase motif was mutated, a mutation that occurs in CS, the phosphatase activity was abolished (Li and Sun 1997).

CS

CS exhibits variable expressivity, which makes the diagnosis of CS and the accurate measurement of its incidence a challenge. Before the identification of the susceptibility gene, the incidence was thought to be 1/1,000,000 (Nelen et al. 1996). However, after identification of *PTEN* as the gene for CS (Liaw et al. 1997), a molecular-based study revealed the incidence to be ⩾1/200,000 (Nelen et al. 1997, 1999), although this too is likely to be an underestimate. Because CS is underdiagnosed, a true count of the proportion of isolated cases (defined as having no obvious family history) and familial cases (defined as having two or more related affected individuals) cannot be performed. From the literature and the experience of major CS centers in the world, the majority of CS cases appear to be isolated. As a broad estimate, ~10%–50% of CS cases are familial (Marsh et al. 1999).

Because of the lack of uniform diagnostic criteria for CS prior to 1995, the International Cowden Consortium arrived at a set of consensus operational diagnostic criteria culled from published data and expert opinion (Nelen et al. 1996; Eng 1998). This consortium represented a group of centers, mainly in North America and Europe, that were interested in systematically studying this syndrome to localize the susceptibility gene. These diagnostic criteria have been revised recently, in the context of new data, and are reflected in the practice guidelines of the U.S.-based National Comprehensive Cancer Network (NCCN) Genetics/High Risk Panel (Appendix B) (NCCN 1999; Eng 2000).

More than 90% of individuals affected with CS are believed to manifest a phenotype by the age of 20 years (Nelen et al. 1996; Eng 2000). By the end of the 3d decade of life (i.e., at 29 years of age), 99% of affected individuals are believed to have developed at least the mucocutaneous signs of the syndrome, although any of the other clinical features could also be present (Appendixes A and B). The most commonly reported manifestations are mucocutaneous lesions, thyroid abnormalities, fibrocystic disease, and carcinoma of the breast, multiple, early-onset uterine leiomyoma, and macrocephaly (specifically, megalencephaly) (Appendix A) (Starink et al. 1986; Hanssen and Fryns 1995; Mallory 1995; Longy and Lacombe 1996; Eng 2000). Mucocutaneous trichilemmomas and papillomatous papules are considered diagnostic of the syndrome (Appendix B).

The two most well-documented component malignancies of CS are carcinomas of the breast and epithelial thyroid gland (Starink et al. 1986). In women with CS, lifetime risks of breast cancer are estimated to be 25%–50% (Starink et al. 1986; Hanssen and Fryns 1995; Longy and Lacombe 1996; Eng 1997) in contrast to the 11% in the general population. The mean age at diagnosis is likely 10 years earlier than that in the general population, at ~38–48 years of age, with a range of 14–65 years of age (Starink et al. 1986; Longy and Lacombe 1996). Until genotype-phenotype analyses were performed with the discovery of the susceptibility gene, it was thought that male breast cancer was not a component of CS. However, male breast cancer does occur in *PTEN*-mutation–positive CS, but with unknown frequency (Marsh et al. 1998*b;* Fackenthal et al. 2001). The lifetime risk of epithelial thyroid cancer can be as high as 10% in males and females with CS. It is unclear if the age at onset is truly earlier than that of the general population. Histologically, CS-associated thyroid cancer is predominantly follicular carcinoma, although papillary histology has also been rarely observed (Starink et al. 1986; Hanssen and Fryns 1995; Longy and Lacombe 1996; C. Eng, unpublished data). After identification of *PTEN* as the susceptibility gene, preliminary data suggested that endometrial carcinoma is a component cancer of CS (Marsh et al. 1998*a*; De Vivo et al. 2000; Eng 2000). Its frequency in mutation carriers is as yet unknown.

Germline *PTEN* Mutations Cause CS

Germline mutations in *PTEN,* have been found in CS (Liaw et al. 1997; Lynch et al. 1997; Nelen et al. 1997, 1999; Tsou et al. 1997; Marsh et al. 1998*b*). Although the original linkage study mapped CS to 10q22-q23 without genetic heterogeneity (Nelen et al. 1996), one subsequent study suggested that rare locus heterogeneity may exist (Tsou et al. 1997). It is unclear if *BMPR1A,* which is on 10q22 and encodes a bone morphogenic protein receptor belonging to the TGF-β–receptor superfamily, is a susceptibility gene for juvenile polyposis syndrome (Eng 2001; Howe et al. 2001), is also a rare susceptibility gene for CS (Zhou et al. 2001*b*). *MINPP1,* another gene that maps to 10q23 upstream from *PTEN* and that encodes a phosphatase, has been excluded as a locus for CS (Dahia et al. 2000).

When CS is strictly defined by the operational diagnostic criteria of the International Cowden Consortium, 80% have been found to harbor germline *PTEN* mutations (Marsh et al. 1998*b*). Approximately two-thirds of these mutations were found in exons 5, 7, and 8 (fig. 1 and table 1). Approximately 40% of all CS germline mutations are located in exon 5, although exon 5 represents only 20% of the coding sequence. Genotype-phenotype analyses revealed an association between the presence of germline mutations and malignant breast disease (Marsh et al. 1998*b*). In other words, more ma-

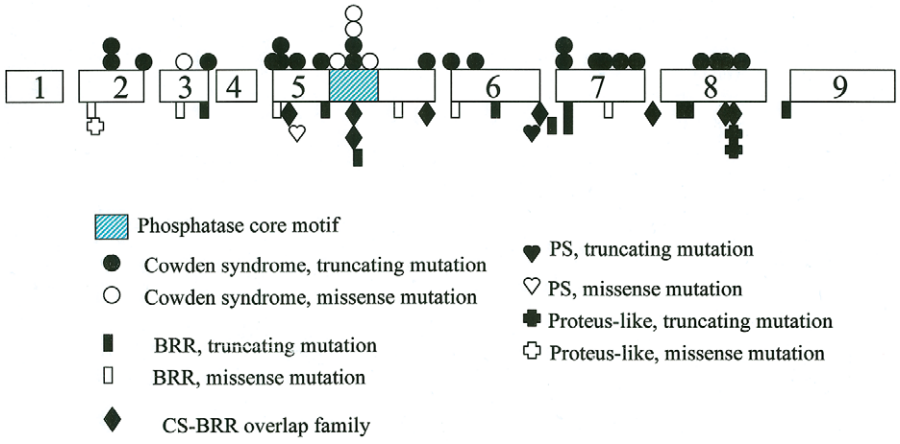

Figure 1 Germline *PTEN* mutations in CS, BRRS, PS, and Proteus-like syndromes

lignant breast disease occurred in the 80% of families with CS diagnosed by the International Cowden Consortium criteria and who were mutation positive, compared to the 20% of families who also met International Cowden Consortium criteria but were mutation negative. In addition, missense mutations and those within and 5′ to the phosphatase core motif appear to be associated with involvement of five or more organs, a surrogate phenotype for severity of disease (Marsh et al. 1998*b*). Another group examined families for germline *PTEN* mutations and found mutations in only 13 (~50%) probands (Nelen et al. 1999). They could not find any clear genotype-phenotype associations, almost certainly due to their small sample size and consequent lack of statistical power. The differences in mutation frequency between the studies by Marsh et al. (1998*b*) and Nelen et al. (1999) include a decreased stringency of ascertainment by the International Cowden Consortium criteria and, perhaps, small sample size in the latter study.

Clinical Spectrum of *PTEN*-Defined Syndromes: The Concept of the *PTEN* Hamartoma-Tumor Syndrome (PHTS)

Bannayan-Riley-Ruvalcaba Syndrome (*BRRS*)

Germline *PTEN* mutations in families with BRRS (MIM 153480), characterized by macrocephaly, lipomatosis, hemangiomatosis and speckled penis (Gorlin et al. 1992), have also been found (fig. 1) (Marsh et al. 1997*a*). Thus, at least a subset of patients with BRRS and CS may be considered allelic. In contrast to patients with CS, 60% of patients with BRRS were found to have germline *PTEN* mutations (Marsh et al. 1999). These mutations included one with a cytogenetically detectable deletion of 10q23, encompassing *PTEN,* and a translocation involving 10q23 (Arch et al. 1997; Ahmed et al. 1999; Marsh et al. 1999). The mutational spectra of BRRS and CS seem to overlap, lending formal proof that CS and BRRS are allelic (Marsh et al. 1999). Thus, it has been suggested that syndromes that are characterized by the presence of germline *PTEN* mutations may be grouped by molecular definition and referred to as the "PHTSs" (Marsh et al. 1999).

Genotype-phenotype association analyses revealed several correlations in BRRS (Marsh et al. 1999). The presence of germline *PTEN* mutations was found to be correlated with the presence of breast tumors, the presence of breast fibroadenomas, and lipomatosis. In other words, there was a higher frequency of breast tumors, fibroadenomas, and lipomas among the group of patients with BRRS, all of whom met published diagnostic criteria (Gorlin et al. 1992) and were found to be mutation positive, compared to the 40% of patients with BRRS, who also met diagnostic criteria but were mutation negative. In this analysis, there were nine families with clinical overlap of CS and BRRS; eight of these nine families were found to harbor germline *PTEN* mutations (Marsh et al. 1999). This high frequency of mutation positivity among families with overlap continues to hold true, since such families continue to be accrued and subjected to mutation analysis (Celebi et al. 1999; Wanner et al. 2001; X. P. Zhou and C. Eng, unpublished data).

One report suggested that germline *PTEN* mutations occur only in familial BRRS but not isolated (i.e., nonfamilial) BRRS (Carethers et al. 1998). However, this finding has not held true in multiple other studies (Arch et al. 1997; Longy et al. 1998; Zori et al. 1998; Marsh et al. 1999). In the largest single series of patients with isolated BRRS, 7 of 16 unrelated probands with isolated BRRS were found to have germline intragenic *PTEN* mutations (Marsh et al. 1999). If we also considered

Table 1

Missense Mutations in *PTEN* Tested for Lipid Phosphatase Activity

Mutation	Domain	Phosphatase Activity[a]
S10N	Phosphatase	Full
Y16C	Phosphatase	Null
G20E	Phosphatase	Partial[b]
Y27S	Phosphatase	Null
L42R	Phosphatase	Full
H61R	Phosphatase	Null
Y68R	Phosphatase	Null
C71Y	Phosphatase	Null
H93Y	Phosphatase/active core	Null
C105F	Phosphatase	Null
D107Y	Phosphatase	Null
L112P	Phosphatase	Null
L112R	Phosphatase	Null
A121	Phosphatase	Null
C124R	Phosphatase/active core	Null
G129R	Phosphatase/active core	Null
G129E	Phosphatase/active core[c]	Null
R130G	Phosphatase/active core	Null
R130L	Phosphatase/active core	Null
R130Q	Phosphatase/active core	Null
V133I	Phosphatase	Null
M134L	Phosphatase	Partial[b]
C136	Phosphatase	Null
Y155	Phosphatase	Null
G165R	Phosphatase/active core	Null
S170N	Phosphatase	Null
S170R	Phosphatase	Null
R173C	Phosphatase	Null
R173H	Phosphatase	Null
R173P	Phosphatase	Null
Y174N	Phosphatase	Null
S227F	C2	Partial[b]
G251C	C2	Null
K289E	C2	Full
D331G	C2	Partial
F341V	C2	Null
K342N	C2	Partial[b]
V343E	C2	Null
L345Q	C2	Null
F347L	C2	Partial
V369	C terminus	Full
T401	C terminus	Full

NOTE.—Adapted from Han et al. (2000).

[a] Lipid-phosphatase activity toward inositol-1,3,4,5-tetraphosphate.

[b] Full-lipid-phosphatase activity to PIP3.

[c] Protein-phosphatase active.

large rearrangements, 9 of 18 patients with isolated BRRS harbored a germline *PTEN* alteration. This frequency of mutation-positive cases is not significantly different from that obtained for familial BRRS.

Currently, it is unclear if the 40% of BRRS without germline *PTEN* mutations have large rearrangements affecting *PTEN* or if genes other than *PTEN* may also be responsible for BRRS. *MINPP1* has also been excluded as a susceptibility gene for *PTEN*-mutation–negative BRRS (Dahia et al. 2000).

Proteus Syndrome (PS) and Proteus-like Syndromes

Although PS (MIM 176920) had always been considered part of the genetic differential diagnosis of CS, it was often discarded as being part of PHTS. The mosaic distribution of affected tissues in PS and its sporadic occurrence strongly suggested either somatic mutation or germline mosaic mutation as its etiology. Interestingly, during the ascertainment of probands with the minimal clinical features of hamartomas, lipomas and overgrowth, an individual with a Proteus-like syndrome was found to carry a germline *PTEN* R335X mutation and a second-hit germline mosaic R130X mutation in his affected tissues (Zhou et al. 2000). This individual was born of normal parents and had marked hemihypertrophy, macrocephaly, epidermoid nevi, lipomas, and progressively worsening arterioventricular malformations, features that are reminiscent of PS but do not meet the full published criteria for the clinical diagnosis of PS (Biesecker et al. 1999). This individual with a Proteus-like syndrome also did not meet the criteria for the diagnosis of either CS or classic BRRS.

Given these observations, a series of nine unrelated patients with isolated PS and five individuals with Proteus-like syndromes were analyzed for the presence of germline *PTEN* mutations (fig. 1) (Zhou et al. 2001*a*). Of the nine patients with classic PS who met the published clinical criteria (Biesecker et al. 1999), two were found to have germline *PTEN* mutations, W211R and C211X, neither of which have previously been found in CS or BRRS (Zhou et al. 2001*a*). Three of the five individuals with a Proteus-like syndrome were also found to have germline *PTEN* mutations. Interestingly, R335X, which has also been found in CS and BRRS, was found in the germline of two of these individuals. The third, M35T, is, thus far, unique. There is at least one other small series of individuals with PS that was examined for the presence of *PTEN* mutations (Barker et al. 2001). Among eight individuals with PS, no mutations were found. This negative result is almost certainly due to small sample size and/or may be secondary to the mutation-scanning method chosen, conformation-specific gel electrophoresis, which has been shown to be relatively insensitive even in the best of hands (Eng et al. 2001). Together, these data suggest that subsets of both PS and Proteus-like syndromes may be considered PHTS. It is also clear that other susceptibility genes will be found for PS and Proteus-like syndromes.

Although *PTEN*-mutation–positive CS, BRRS, PS, and Proteus-like syndromes are considered in the PHTS continuum, the phenotypes associated with the same mutation can vary quite remarkably (Appendix A). This is particularly pronounced for the hotspot mutations on CpG islands, R233X, R235X, and R335X.

Other Clinical Syndromes?

Germline *PTEN* mutations have been uncovered in a single case of isolated hydrocephaly with VATER association (Reardon et al. 2001) and an individual with megalencephaly with autistic features (Dasouki et al. 2001). VATER association comprises vertebral and anal malformations, tracheoesophageal atresia, and radial and renal malformations. VATER associated with hydrocephaly is a distinct entity from VATER association alone, and, unlike VATER, familial cases have been reported (Iafolla et al. 1991; Devriendt et al. 1995; reviewed in Reardon et al. 2001). In the latter case, it is unclear if the autistic features are part of PHTS or if it is only the megalencephaly that is germane. Both of these types of clinical presentations are extremely rare, and further investigation is required to determine if these clinical syndromes are also prominent members of PHTS.

Clinical Syndromes That Are Not PHTS

The molecular classification of PHTS is important in two ways. First, the broadening phenotypic spectrum of PHTS yields clues to fundamental insights into the structure-function relationship of *PTEN*. Second, the PHTS molecular classification of clinical syndromes is important from a clinical management point of view. Genotype-phenotype analyses have suggested that the presence of germline mutations, in CS or BRRS, is associated with cancer, at least breast cancer (Marsh et al. 1998*b,* 1999). Thus, a conservative clinician would recommend cancer surveillance for all individuals with PHTS, as is advocated for classic CS, irrespective of their clinical diagnosis (Eng 2000).

There are several inherited hamartoma-tumor syndromes that do not belong to PHTS. Peutz-Jeghers syndrome (PJS [MIM 175200]) is an autosomal dominant inherited cancer syndrome characterized by gastrointestinal hamartomatous polyposis, peroral pigmentation, and a risk of gastrointestinal and breast cancers. Its susceptibility gene is *LKB1/STK11,* on 19p, encoding a nuclear serine threonine kinase (Hemminki et al. 1997, 1998; Jenne et al. 1998). *PTEN* has been excluded as a locus in PJS (D. J. Marsh and C. Eng, unpublished data).

Juvenile polyposis syndrome (JPS [MIM 174900]) is a clinical diagnosis of exclusion, and there has been some confusion whether JPS is a PHTS (Eng and Ji 1998). JPS is an autosomal dominant disorder characterized by gastrointestinal hamartomatous polyps ("juvenile polyps") and a risk of gastrointestinal cancers. Initial confusion stemmed from a paper that described germline *PTEN* mutations in two individuals who had been reported to have JPS (Olschwang et al. 1998). It became clear, however, that the insufficiently detailed clinical descriptions that were provided for these patients strongly suggested that these individuals had CS or BRRS (Eng and Ji 1998). Similarly, the title of one report referred to germline mutations in individuals with JPS, but it was obvious, from the text, that all of these individuals had CS (Lynch et al. 1997). Even recently, a small study reported that *PTEN* may be a rare *JPS*-susceptibility gene (Huang et al. 2000), but, again, insufficient clinical detail was given in this report to determine if these individuals had features of either CS or BRRS. At least one series systematically examined this issue. The first was a single hospital-based series that looked for germline *PTEN* mutations in diagnoses of JPS (Kurose et al. 1999). In this series, one individual was found to harbor a germline *PTEN* mutation. When that individual was recalled for thorough examination, classic cutaneous features of CS were found (Kurose et al. 1999). *PTEN* was formally excluded as a *JPS*-susceptibility gene (Marsh et al. 1997*b*), and we now know that germline mutations in *MADH4* on 18q and *BMPR1A* on 10q21-q22 account for 40%–60% of JPS (Howe et al. 1998*a,* 1998*b,* 2001; Zhou et al. 2001*b*).

Overall, the data to date suggest that JPS is not a PHTS. However, the discovery of a germline *PTEN* mutation in an individual considered to have JPS should raise the suspicion that the clinical diagnosis is incorrect and that such an individual be medically managed in the same manner as all patients with PHTS.

Murine Models

Animal models of human disease are helpful when they faithfully recapitulate the human disease and/or when they can be utilized for fundamental research that can elucidate the biology of the gene in question. Three different groups have generated *Pten*-knockout mice. All three groups targeted exon 5 in the targeting construct (Di Cristofano et al. 1998; Suzuki et al. 1998; Podsypanina et al. 1999), and two of these groups also deleted extra exons (i.e., exons 3–5 [Suzuki et al. 1998] and exons 4 and 5 [Di Cristofano et al. 1998]). The third group targeted a frameshift mutation within the phosphatase motif (Podsypanina et al. 1999). These groups all reported that homozygous knockout of *Pten* was embryonic lethal within a range of embryonic days 6.5–9.5. Beyond this, however, the groups observed varied phenotypes in the heterozygous mice. Few features of CS or BRRS have been described in these models, although each model contains features that are reminiscent of these disorders. For example, one model is characterized by abnormal development of the three germinal layers, as well as skin hyperkeratosis and papillary-like thyroid carcinoma in heterozygous mice (Di Cristofano et al. 1998). Superficially, one may imagine that these

are features found in human CS; however, they are not (e.g., papillary thyroid carcinoma is rarely observed in *PTEN*-mutation–positive CS or BRRS). In their mouse model, Suzuki et al. (1998) found defects in the generation of mesodermal lineages, as well as hamartomatous colonic polyps, which are not particularly reminiscent of the human counterparts typical in CS or BRRS. They also observed thymic lymphomas, which are not components of either CS or BRRS. Interestingly, with long follow-up, these mice developed breast cancer and endometrial cancers, both of which are components of CS (Stambolic et al. 2000). With follow-up, pheochromocytomas also developed, although, to date, these neuroendocrine tumors have never been observed in CS or BRRS. Finally, Podsypanina et al. (1999) observed follicular and papillary thyroid tumors and atypical hyperplasia of the endometrium, features that, again, are reminiscent of CS/BRRS but are not similar when examined in histologic detail. The type of mutation or differences in the genetic background of the mice may contribute to the differences observed in each model. The lack of significant similarity to CS and BRRS raises questions to the usefulness of these models in terms of representing the clinical conditions. Nonetheless, these models should prove useful, particularly when crossbred with other knockout mice and/or for fundamental biochemical studies, in elucidating information about the pathways with which PTEN interacts in situ.

PTEN Structure: Does It Explain Phenotype?

Clinical and genetic analyses have revealed that *PTEN* mutations result in an ever-widening spectrum of phenotypic features. Does the structure of PTEN yield clues to explain this? Analysis of the crystal structure of PTEN has identified two major domains (fig. 2).

The C-Terminal Domain

The C-terminal domain, in which ⩾43% of *PTEN* mutations occur, is composed of antiparallel β-sheets that are linked together by short α-helices (Lee et al. 1999). This domain contains many important subdomains that are common to other signal-transducing molecules. First, PTEN contains a C2 domain, which is associated with phospholipid-binding regions (Lee et al. 1999). C2 domains have been identified in many proteins involved in signal transduction and membrane localization (Rizo and Sudhof 1998). Indeed, the C2 domain in PTEN has been shown to have affinity for phospholipid membranes in vitro (Rizo and Sufhof 1998). Many germline *PTEN* mutations lie within the C2 domain, suggesting its functional significance (figs. 1 and 2 and table 1). However, germline mutations within the C2 domain have been found for the entire

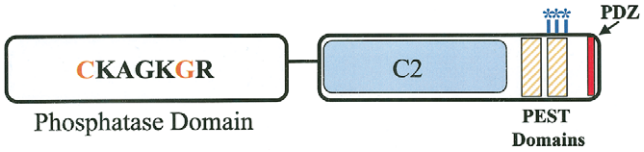

Figure 2 Protein domains of PTEN. The N-terminal phosphatase domain (amino acids 1–185) is shown with the catalytic core. The missense mutations which have been crucial for the elucidation of the cellular role of PTEN are highlighted in orange. Mutations at C124 render a lipid- and protein-phosphatase–inactive protein, whereas mutations at G129 result in a lipid-phosphatase–inactive yet protein-phosphatase–active PTEN. The C-terminal domain (amino acids 186–403) contains the lipid-binding C2 domain (amino acids 186–351); PEST domains (amino acids 350–375 and 379–396), which regulate protein stability; and the PDZ domain, which is important in protein-protein interactions. The CK2 phosphorylation sites (S380, T382, and T383), which are important for stability, are indicated by the blue asterisks (*).

spectrum of PHTS phenotypes (figs. 1 and 2), without an obvious functional genotype-phenotype correlation.

Another feature of the C terminus is a PDZ-binding motif, which interacts strongly with the phosphatase domain (described below; fig. 2) by both hydrogen bonding and hydrophobic interactions (Lee et al. 1999). In addition, PDZ domains are significant regions for protein-protein interactions (Fanning and Anderson 1999; Kay et al. 2000), which play a vital role in cellular signal transduction. Removal of the PDZ domain reduces the ability of PTEN to inhibit one of its substrates, AKT (Wu X. et al. 2000), suggesting that PTEN interactions may be important in regulating PTEN activity. The C-terminal tail also contains PEST sequences, which are critical for PTEN stability (Georgescu et al. 1999). PEST sequences target proteins for short intracellular half-lives and protein degradation. Paradoxically, deletion of these regions leads to decreased protein expression versus the expected increase (Georgescu et al. 1999). Nonetheless, these studies point out that the PEST regions are necessary for PTEN stability.

The C-terminal tail also contains several phosphorylation sites located in the last 50 amino acids. Although this region or "tail" of PTEN is unnecessary for phosphatase activity and cell-growth suppression (Vazquez et al. 2000), it is critical for protein stability. Protein stability is dependent on the phosphorylation of S380, T382, and T383. Mutations of these sites reduced both the protein half-life and the PTEN levels (Vazquez et al. 2000). Torres and Pulido (2001) demonstrated that CK2, the protein kinase, phosphorylates *PTEN* in vivo on serine residues 370, 380, and 385 and on threonine residue 383. Furthermore, phosphorylation-defective mutants have decreased protein stability and provided evidence that dephosphorylated PTEN is degraded by

proteasome-mediated mechanisms (Torres and Pulido 2001). Together, these data suggest that protein phosphorylation plays an important role in the regulation of PTEN by influencing protein levels. PTEN phosphorylation has also been shown to cause a conformational change that masks the PDZ domain, reducing PTEN's ability to bind to PDZ-domain–containing proteins (Vazquez et al. 2000).

It is interesting to note that, to date, no germline or somatic mutations have been detected specifically in the PDZ, PEST, or phosphorylation sites, although there are many mutations, both germline and somatic, that would truncate the protein before these features. There are two obvious explanations for these observations. This may suggest that the presence of dominant negative mutations within each of these domains may be embryonic lethal while haploinsufficiency is viable, although it results in the PHTS phenotypes. It is difficult to imagine that the alternate hypothesis—that is, that they are not germane to carcinogenesis—could be true.

The N-Terminal Domain

The N-terminal domain contains the enzymatic side of PTEN (i.e., its phosphatase domain), which is composed of β-sheets surrounded by α-helices (Lee et al. 1999). The majority of *PTEN* mutations occur within this domain. Analysis of the crystal structure revealed that the PTEN phosphatase domain, although similar to those of other protein phosphatases, has a slightly larger active site. This enlarged active site allows for the accessibility of phospholipid substrates (Lee et al. 1999), thereby making PTEN a unique phosphatase. The active site is also a mutational hotspot, with ~31% of germline and somatic mutations occurring in exon 5, which encodes the catalytic core. That 40% of germline mutations in CS lie in exon 5, which represents 20% of the coding sequence, undoubtedly reflects the biology of the resulting protein.

PTEN, a Dual-Specificity Phosphatase

Since the discovery of PTEN in 1997, abundant data have been presented that show that PTEN is a tumor suppressor in vitro and in vivo. PTEN can be regarded as a dual-specificity phosphatase on several levels. In particular, recombinant PTEN has been shown to dephosphorylate protein substrates in vitro on serine, threonine, and tyrosine residues (Myers et al. 1997). In this manner, PTEN is a dual-specificity *protein* phosphatase. One proposed PTEN substrate is focal adhesion kinase (FAK); PTEN interacts with FAK and decreases its tyrosine phosphorylation levels (Gu et al. 1998; Tamura et al. 1998). Dephosphorylation of FAK inhibits cell spreading, suggesting that the protein-phosphatase activity of PTEN may be important in the regulation of cellular interactions. This, however, may be cell line–specific, since others have been unable to reproduce this work.

In 1998, Maehama and Dixon reported another PTEN substrate. Previous work had shown that the PTEN mutant G129E, a mutation found in at least two families with CS, still had the ability to dephosphorylate peptide substrates in vitro (Maehama and Dixon 1998). This suggested that PTEN may also have a nonproteinaceous target in vivo. Maehama and Dixon observed that the overexpression of PTEN reduced the cellular levels of phosphoinositol 3,4,5-triphosphate (PIP3) in response to insulin. This occurred without a change in the activity of PI3K, the kinase that phosphorylates phosphoinositol-diphosphate. When the G129E mutant was expressed in cell lines, PIP3 levels increased. Together, these data suggested that PTEN was capable of dephosphorylating cellular phospholipids. Meahama and Dixon expanded their observations by showing that PTEN catalyzes, in vivo and in vitro, the removal of phosphate from the D3 position of the inositol ring. Therefore, PTEN is also a dual-specificity phosphatase in the sense that it dephosphorylates *protein* substrates in addition to *lipid* substrates.

PTEN Regulation of the PI3K Pathway

Phosphoinositide second messengers play an important role in signal transduction pathways that regulate cell growth, differentiation, apoptosis, metabolism, actin rearrangements, and membrane trafficking. They do this by directly activating enzymes or by directing proteins to various subcellular locations via lipid binding. Abundant data have shown that PIP3 is required for the activation of AKT/PKB, a serine/threonine protein kinase that plays a role in cell survival (Vazquez et al. 2000). PI3K activation, mainly by growth factors, results in the accumulation of PIP3 in cellular membranes (Kapeller and Cantley 1994) resulting in the translocation of AKT from cytoplasmic stores to cellular membranes. On membrane docking, AKT undergoes a conformational change and is phosphorylated by PDK1, thereby stimulating AKT and subsequent signaling via its downstream effectors (fig. 3). This stimulates pathways required for cell survival and proliferation (Downward 1998).

PTEN antagonizes the PI3K/AKT pathway by dephosphorylating PIP3, resulting in a decreased translocation of AKT to cellular membranes and subsequent down-regulation of AKT activation. Indeed, it has been shown that expression of PTEN in cells leads to decreased levels of phospho-AKT, and, therefore, to increased apoptosis (Davies et al. 1998; Myers et al. 1998). In addition, constitutively active, but not wild-type, AKT, can rescue cells from PTEN-mediated G1

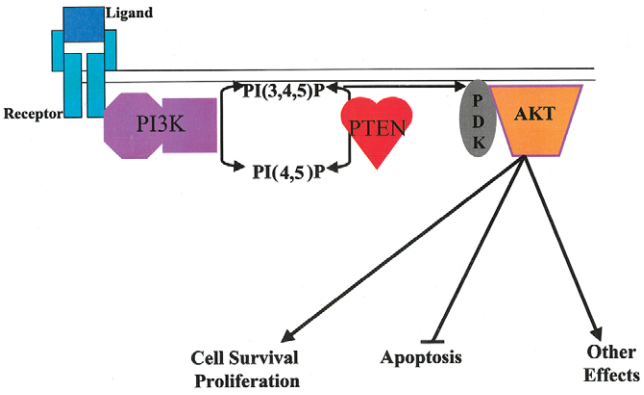

Figure 3 PTEN as a regulator of the PI3K pathway. Ligand binding to membrane receptors results in the activation of PI3K and the subsequent increase in PIP3, which recruits PDK1 to the cellular membrane. PDK1 phosphorylates and activates AKT, which in turn regulates a variety of cellular processes. PTEN dephosphorylates PI3P, lowering its cellular levels and resulting in the down-regulation of AKT.

arrest and apoptosis. Furthermore, PI3K inhibition by LY294002 could mimic the effects of PTEN (Li and Sun 1998). Together, these data demonstrate that PTEN exerts its effect upstream from PI3K.

The effect that PTEN has on the PI3K pathway is phosphatase dependent. C124 is the active-site cysteine of PTEN and has been found to be mutated in at least three probands with CS (Marsh et al. 1998*b*; Bonneau and Longy 2000). The C124S mutation abolishes both the protein and lipid-phosphatase activities of PTEN (Myers et al. 1998). When the PTEN C124S mutant was introduced into cells, phospho-AKT levels remained constant, and cells did not undergo G1 arrest or apoptosis (Myers et al. 1998; Weng et al. 1999, 2001*b*).

Several groups have shown that PTEN coordinates G1 arrest through up-regulation of p27 and concomitant down-regulation of cyclin D1 (Cheney et al. 1999; Bruni et al. 2000; Medema et al. 2000; Persad et al. 2001; Weng et al. 2001*a*). The D cyclins are key regulators of progression through G1 of the cell cycle, whereas p27 is an inhibitor of cyclin-dependent kinases and acts as a negative regulator of the cell cycle. Another factor that influences G1 arrest may be the inhibition of retinoblastoma protein phosphorylation via the effects that PTEN has on the PI3K pathway (Paramio et al. 1999). Use of a G129E mutant has shed light on the mechanisms by which PTEN exerts some of these effects. Similar to C124, G129 is also one of the key residues in the active site of PTEN and has been found to be mutated in at least three probands with CS. Interestingly, this mutation renders the PTEN molecule *lipid*-phosphatase inactive but *protein*-phosphatase active. Weng et al. (2001*a*) used both the G129E and C124S mutants to determine that PTEN down-regulates cyclin D1 expression by its protein-phosphatase activity. In contrast, p27 levels are up-regulated downstream from the lipid-phosphatase activity (Weng et al. 2001*a*). In contrast, p27 levels, as well as p21 and p57 levels, are up-regulated downstream from the lipid-phosphatase activity. Increased transcription of these cyclin-dependent kinase inhibitors occurs in response to PTEN-mediated down-regulation of AKT. This is believed to be mediated by the Forkhead family of transcription factors, and it also contributes to G1 arrest. Together, these data indicate that PTEN's lipid-phosphatase activity is critical to cell function. In light of this, it is interesting to note that 90% of PTEN missense mutations eliminate or reduce lipid-phosphatase activity (table 1), without affecting the ability of the protein to bind to the cellular membrane (Han et al. 2000). Since the PI3K pathway is involved in a variety of pathways, including those for hypoxia-induced protein regulation and obesity (reviewed in Katso et al. 2001; Minet et al. 2001), the phenotypic result of *PTEN* mutation could be compounded by the as-yet-unknown effects of multiple downstream and feedback pathways.

Most naturally occurring mutations are both lipid- and protein-phosphatase inactive (e.g., C124S; see table 1), although a minority are lipid-phosphatase inactive but protein-phosphatase active (e.g., G129E). It is interesting that no naturally occurring mutation that results in lipid-phosphatase–active but protein-phosphatase–inactive PTEN has been identified. This is most likely because a change in the active site that renders PTEN protein-phosphatase inactive would automatically render the protein lipid-phosphatase inactive. However, this does not necessarily mean that PTEN has no other function than to regulate the PI3K pathway, as has been suggested by emerging data (see next section).

Other Roles of PTEN

Although abundant data show that PTEN is a negative regulator of the PI3K/AKT pathway, it is also becoming clear that PTEN may be involved in other cellular functions. For example, FAK has been proposed as a protein substrate of PTEN (Tamura et al. 1998, 1999).

As is the PI3K pathway, the mitogen-activated kinase (MAPK) pathway is another critical pathway for proliferation and differentiation. PTEN also regulates this pathway. PTEN can dephosphorylate Shc, resulting in the inhibition of Grb2 and, ultimately, the down-regulation of MAPK. However, it has also been demonstrated that PTEN can inhibit the MAPK pathway in a Shc-independent manner (fig. 4). First, PTEN may regulate the MAPK pathway by modulating the movement of Gab1 to the plasma membrane. Gab1 contains a pleckstin-homology domain that interacts with PIP3-rich membranes (Ong et al. 2001). By dephosphorylat-

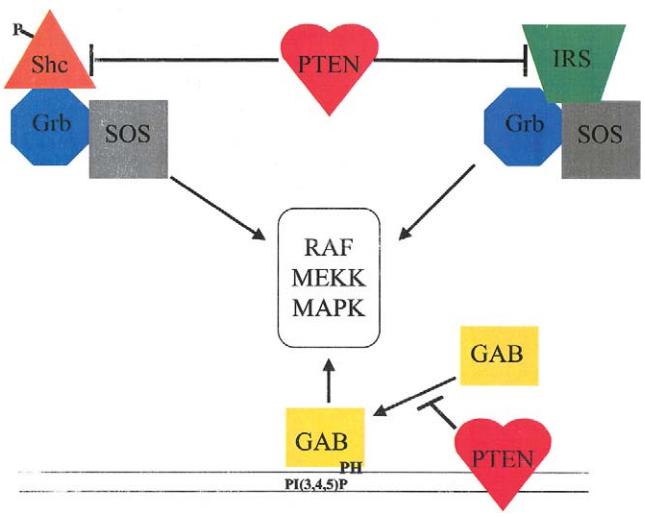

Figure 4 PTEN as a modulator of the MAPK pathway. PTEN can inhibit the activation of MAPK by several mechanisms. By dephosphorylating Shc and/or IRS-1, PTEN prevents the association of these proteins to the Sos:Grb complex, which is required for MAPK activation. Gab interacts with the membrane by binding to PI3P regions via the pleckstrin-homology domain. By decreasing the PI3P levels in the membrane, PTEN inhibits the translocation of Gab to the membrane and its subsequent activation of the MAPK pathways. The entire pathway for MAPK activation and protein-protein interactions has been omitted for clarity.

ing PIP3, PTEN would effectively prevent Gab1 from translocating to the membrane, thus decreasing the activation of MAPK (fig. 4) (Takahashi-Tezuka et al. 1998; Yart et al. 2001). Second, PTEN has been shown to inhibit insulin stimulation of the MAPK pathway. This inhibition results from the dephosphorylation of the insulin-receptor substrate–1 (IRS-1), which inhibits the formation of the IRS-1/Grb2/Sos complex, a complex that is required for MAPK activation. This indicates that PTEN can play a role in insulin signaling (Weng et al. 2001*c*).

That PTEN has a central role in insulin signaling is supported by further evidence: (1) PTEN expression in adipocytes inhibits insulin-stimulated production of 2-deoxyglucose (Nakashima et al. 2000). (2) PTEN expression inhibits Glut4 translocation, which is a key event in insulin signaling (Nakashima et al. 2000); however, this line of evidence may be controversial, since PTEN does not have such an effect in adipose cells (Moser et al. 2001). (3) Insulin-receptor substrate–2 (IRS-2) has recently been shown to increase in the presence of PTEN (Simpson et al., in press). Together, this evidence suggests other roles for PTEN besides the regulation of the PI3K pathway.

An interesting observation—with regard to alternative roles for PTEN besides the regulation of PI3K or MAPK pathway—is that PTEN can be found in the nuclear compartment (Tamura et al. 1998; Gimm et al. 2000*b;* Lachyankar et al. 2000; Perren et al. 2000; Whiteman et al., in press). PTEN lacks a clear nuclear-localization sequence, and the mechanism of its transport into the nuclear compartment is still being elucidated. Immunohistochemistry analysis demonstrated that PTEN expression in the nuclear compartment is higher in normal tissue than in counterpart neoplastic tissue found in the thyroid, endocrine pancreas, and primary cutaneous melanomas (Gimm et al. 2000*b;* Perren et al. 2000; Whiteman et al., in press). The significance of this observation is still under investigation. Because PTEN can regulate transcription via the PI3K pathway, which is found predominantly at the plasma membrane (Datta et al. 1999; Dong et al. 1999), does the localization of PTEN to the nuclear compartment sequester PTEN and reduce PI3K-dependent transcription of pro-proliferative factors? Alternatively, nuclear PTEN could act on lipids in the nuclear membrane. Although the level of phosphoinositol in the nuclear membrane is low, it is present, along with PI3K (D'Santos et al. 1998; Marchisio et al. 1998; Martelli et al. 1999; Metjian et al. 1999). Thus, the trafficking of PTEN to the nuclear compartment may activate transcription of pro-apoptotic pathways, as it does at the plasma membrane. Many transcription factors are regulated by their phosphorylation, and a promising alternative hypothesis is that PTEN may regulate the activity of various transcription factors via direct dephosphorylation in the nucleus. Future investigations into the function of nuclear PTEN should prove to be enlightening.

The Present and the Future

What does the future hold for PTEN research? Undoubtedly, we will learn more about the mechanisms by which PTEN regulates the PI3K, MAPK, and insulin-signaling pathways, but it is naive to think that these are the only pathways that PTEN influences. Enough evidence is currently present to surmise that PTEN may play a role in the regulation of a variety of disease states and metabolic processes. In this era of genomics/proteomics, array analysis will certainly yield more clues to, as well as more puzzles about, the roles that PTEN plays. Indeed, the up-regulation of IRS-2 by PTEN was demonstrated by microarray expression analysis (Simpson et al., in press). Unoki and Nakamura have used global expression analysis to demonstrate that EGR2 and BPOZ are involved in PTEN signaling (Unoki and Nakamura 2001). *EGR2* is part of a multigene family that contains C_2H_2-type zinc-finger proteins (Joseph et al. 1998), and germline mutations in this transcription factor gene have been implicated in Charcot-Marie-Tooth

syndrome (MIM 118200) and congenital hypomyelinating neuropathy (MIM 605253) (Warner et al. 1998). The association between PTEN and EGR2 is interesting, because prominent PTEN expression has been demonstrated in the CNS and neural crest throughout human development (Gimm et al. 2000*a*) and because a subset of patients with CS have signs of neuropathy (C. Eng, unpublished data). Other expression-array studies have implicated PTEN in the regulation of members of the tumor necrosis factor (TNF)–receptor family, TNF-associated genes, and members of both the Mad and Notch signaling families (Hong et al. 2000; Matsushima-Nishiu et al. 2001). Additional array studies will undoubtedly discover more protean pathways, as well as "red herrings," downstream from PTEN.

PTEN appears to be constitutively active. Thus, the regulation of PTEN levels via transcription, translation, and posttranslational means remains to be investigated in depth. We now know that phosphorylation of the C-terminal tail of PTEN by CK2 can negatively regulate the stability of PTEN (discussed in the "C-Terminal Domain" subsection of "PTEN Structure," above). What we do not know is the identity of the phosphatase that dephosphorylates PTEN. Phosphorylation and dephosphorylation are the yin and yang of signal transduction; thus, the phosphatase must be there, yet it remains unidentified. Preliminary data have recently been published on the regulation of PTEN transcription. Activated PPARγ, p53, and EGR1 have been shown to up-regulate PTEN transcription (Patel et al. 2001; Stambolic et al. 2001; Virolle et al. 2001). Factors that may down-regulate transcription have yet to be identified.

Copious amounts of data have demonstrated that PTEN can influence signal transduction via its phosphatase activity. What remains to be determined is if PTEN can influence pathways by other mechanisms. As described above, PTEN contains many signaling domains (e.g., PEST and PDZ). It is interesting to postulate that these domains may also play a role in signal transduction. PTEN has been shown, via yeast two-hybrid studies, to associate with membrane-associated guanylate-kinase inverted (MAGI) proteins, which contain PDZ domains and are localized to tight junctions (Wu X. et al. 2000; Wu Y. et al. 2000). Additional studies suggest that the interaction between PTEN and MAGI may enhance the stability of PTEN (Wu X. et al. 2000). Although this interaction seems to regulate PTEN activity, an appealing proposal is that PTEN may interact with other signaling molecules to modulate specific pathways in a phosphatase-independent manner; however, this needs to be proved.

In this era of genetics, data generated in the laboratory should be translated to the practice of genomic medicine. Already, we are able to use *PTEN*-mutation analysis for predictive testing within *PTEN*-mutation–positive families, and we can use *PTEN* testing as a molecular-diagnostic tool to sort out a group of difficult-to-diagnose inherited hamartoma-tumor syndromes. Classification of these protean clinical syndromes by molecular identity is believed to be useful in the prediction of the development of neoplasias and the recommendation of surveillance. However, the mechanisms by which identical mutations result in phenotypes as diverse as CS, BRRS, and PS is, as yet, unknown. The challenge for the next decade is to determine the gene-gene interactions and gene-environment interactions that arise in different tissue-specific contexts to result in a specific phenotype.

The brave new world would be incomplete without an attempt to utilize molecular-based knowledge to create a novel treatment. Since overwhelming data demonstrate that the PI3K/AKT pathway lies downstream from PTEN and that mTOR (i.e., mammalian target of rapamycin) is downstream from—or, at least, acts in concert with—the PI3K/AKT pathway, rapamycin and its analogs have been hailed as the "magic bullet" that will cure all the ailments of a sick PTEN (Mills et al. 2001; Neshat et al. 2001). However, the PI3K/AKT and mTOR pathways merely lie downstream from PTEN's lipid-phosphatase activity. Virtually all enzymatic activity–abrogating mutations affect the lipid- and protein-phosphatase activity. Thus, it would be predicted that drugs that target only downstream from the lipid-phosphatase activity would not be completely effective and might, in fact, cause harm. Nevertheless, this is an auspicious start. The challenge for the future is to have "designer drugs" that target in a manner that takes into account the genomic, epigenomic, and proteomic milieu. To achieve this, not only should efforts be made in genomic, epigenomic, and proteomic research, which is already happening, but these data must also be linked with comprehensive and meticulous clinical and cellular phenotypic data, which we call "phenomics."

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Appendix A

Common Manifestations of CS

Mucocutaneous lesions (90%–100%):
- Trichilemmomas
- Acral keratoses
- Verucoid or papillomatous papules

Thyroid abnormalities (50%–67%):
- Goiter
- Adenoma
- Cancer (3%–10%)

Breast lesions:
- Fibroadenomas/fibrocystic disease (76% of affected females)
- Adenocarcinoma (25%–50% of affected females)

Gastrointestinal lesions (40%): hamartomatous polyps
Macrocephaly (38%)
Genito-urinary abnormalities (44% of females): uterine leiomyoma (multiple, early onset)

Appendix B

International Cowden Consortium Operational Diagnostic Criteria: 2000 Version

Operational diagnostic criteria are reviewed and revised on a continuous basis, as new clinical and genetic information becomes available. The 1995 and 2000 versions have been accepted by the U.S.-based NCCN High Risk/Genetics Panel.

Pathognomonic criteria (mucocutaneous lesions):
- Facial trichilemmomas
- Acral keratoses
- Papillomatous papules
- Mucosal lesions

Major criteria:
- Breast carcinoma
- Thyroid carcinoma (nonmedullary), especially follicular thyroid carcinoma
- Macrocephaly (megalencephaly; e.g., $\geqslant$97th percentile)
- Lhermitte-Duclos disease (LDD)
- Endometrial carcinoma

Minor criteria:
- Other thyroid lesions (e.g., adenoma or multinodular goiter)
- Mental retardation (e.g., intelligence quotient $\leqslant$75)
- Gastrointestinal hamartomas
- Fibrocystic disease of the breast
- Lipomas
- Fibromas
- Genito-urinary tumors (e.g., renal cell carcinoma or uterine fibroids) or malformation

Operational diagnosis in an individual:
1. Mucocutaneous lesions alone if there are (*a*) six or more facial papules, of which three or more must be trichilemmoma; (*b*) cutaneous facial papules and oral mucosal papillomatosis; (*c*) oral mucosal papillomatosis and acral keratoses; or (*d*) six or more palmar or plantar keratoses
2. Two major criteria, of which one must be macrocephaly or LDD
3. One major and three minor criteria
4. Four minor criteria

Operational diagnosis in a family in which one individual has had a diagnosis of CS:

1. One or more of pathognomonic criteria
2. Any one major criterion with or without minor criteria
3. Two minor criteria

Electronic-Database Information

Accession numbers and the URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for Charcot-Marie-Tooth syndrome [MIM 118200], BRRS [MIM 153480], CS [MIM 158350], JPS [MIM 174900], PJS [MIM 175200], PS [MIM 176920], congenital hypomyelinating neuropathy [MIM 605253], and *PTEN/MMAC1/TEP1* [MIM 601728])

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