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Germline PTEN Mutation Cowden Syndrome: An Under-Appreciated Form of Hereditary Kidney Cancer

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Abstract

Introduction—Cowden syndrome (CS) is a hereditary cancer syndrome associated with a germline mutation in *PTEN*. Patients are predisposed to multiple malignancies including renal cell carcinoma (RCC).

Methods—Patients with CS were evaluated as part of a clinical protocol. Those with a history of RCC underwent review of clinical features, tumor characteristics, and family history. Renal tumors were evaluated for loss of heterozygosity (LOH).

Results—Among 24 CS patients, 4 were identified with RCC (16.7%). Three patients had solitary tumors, two with papillary type I histology and one with clear cell histology. The fourth patient had bilateral, synchronous chromophobe tumors. No patients had a prior family history of RCC. All RCC patients had dermatologic manifestations of CS and had macrocephaly. LOH at the *PTEN* mutation was identified in 4 tumors (80%). No genotype-phenotype association was found, as the same mutation was identified in different RCC histologies.

Conclusion—RCC is an underappreciated feature of CS. As most patients lack a prior family history or a distinctive RCC histology, recognition of the associated non-renal features should target referral for genetic counseling. *PTEN* LOH is common in CS renal tumors. Because loss of PTEN can activate mTOR and mTOR inhibitors are FDA-approved to treat RCC, these agents have clinical potential in RCC associated with CS.

Keywords

PTEN; RCC; Cowden syndrome; hereditary; mTOR

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Introduction

The genetic basis of renal cell carcinoma (RCC) continues to be elucidated, with over a dozen genes implicated in the development of renal tumors.[1,2] Approximately 4-6% of RCC is considered to have a hereditary component, which can often be attributed to a single germline alteration. Several of these conditions, including Von-Hippel Lindau (VHL), Hereditary Papillary RCC (HPRC), Birt-Hogg-Dube (BHD), and Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC), have improved our understanding of sporadic kidney tumors. The genetic basis of many patients with familial renal cancer (FRC), bilateral, multifocal or early-onset RCC has yet to be determined. With increasing availability of next generation sequencing technologies (whole genome/exome sequencing) our understanding of the genetic basis of RCC is expanding, including a class of kidney cancers associated with the *succinate dehydrogenase* (SDH)[3,4] and the MITF transcription factor.[5]

One rare hereditary cancer syndrome, Cowden syndrome (CS), or PTEN hamartoma tumor syndrome, has been recognized for the past half century.[6] This syndrome is inherited in an autosomal dominant pattern with an estimated incidence of 1 in 200,000 individuals.[7] Over a decade ago patients with this syndrome were found to have germline alterations in the *PTEN* gene.[8] Over 70% of CS patients have germline mutations in PTEN. Patients affected with CS develop multiple hamartomas and are at increased risk for breast, endometrial, and thyroid cancers. (Table 1) Dermatologic manifestations such as acral keratosis and facial trichilemmomas are common in CS patients. A clinical diagnosis is made based on a combination of pathognomonic, major, and minor criteria (Table 1).[9] Genitourinary tumors such as kidney and prostate cancer are believed associated with CS, with kidney cancer considered one of the diagnostic criteria. Mester and colleagues reported the first cohort of patients with CS associated RCC (CS-RCC) and estimated these patients had a >30 fold increased risk of developing kidney cancer.[10] However, the overall incidence of RCC in CS was low (4.1%), and no patients had a prior history of RCC. Also unlike many of the other hereditary cancer syndromes such as VHL and HPRC, CS-RCC tumors in this series had variable histology, specifically both papillary and chromophobe types.

To improve the understanding of this hereditary kidney cancer syndrome; we review the clinical features, family history, tumor characteristics, and mechanism of tumorigenesis for CS-RCC.

Methods

Patients affected with CS were evaluated at the National Cancer Institute's (NCI) Center for Clinical Research (CCR) as part of enrollment in a therapeutic trial in the NCI Medical Oncology Branch (*NCT00971789*). On evaluation, patients' medical and family medical history, clinical manifestations, imaging and germline mutation testing were evaluated. All CS patients met clinical diagnostic criteria and had *PTEN* germline mutations confirmed using a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory.[9]

Patients with a history of kidney cancer were further investigated to characterize the features of CS associated RCC. Family pedigrees were created to ascertain the pattern of inheritance and penetrance of kidney cancer. Renal tumor histologic slides were reviewed by a single genitourinary pathologist (MJM). To evaluate for *PTEN* loss of heterozygosity (LOH), microdissection was performed from unstained formalin fixed, paraffin embedded (FFPE) slides of the primary tumor. DNA was extracted from the FFPE tissue with the assistance of an Applied Biosciences RecoverAll kit (Austin, TX). For normal control, peripheral blood samples or normal kidney parenchyma were evaluated when available. For germline mutation analysis, peripheral blood DNA extraction was performed using a Promega/ Maxwell 16 purification kit (Madison, WI). Quantification of extracted DNA was performed using a ThermoScientific Nanodrop 1000 (Wilmington, DE). A Qiagen PCR Kit (Germantown, MD) was used to amplify the sequence at site of the *PTEN* germline mutation using standard primers, and purified DNA products were sequenced bidirectionally using the Big Dye Terminator v.1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and run on an ABI 3130xl Genetic Analyzer. For clear cell RCC cases, *VHL* mutation status was analyzed by sequencing all three exons (primer pairs for *PTEN* and *VHL* available on request). Amplified forward and reverse sequences were evaluated using Sequencher 4.10.01 (Ann Arbor, MI). LOH of *PTEN* at the mutation site was determined by comparing the tumor and normal control to the reference germline sequence.

Data from this cohort was combined with a recently published cohort to gain insight on distribution of histologic subtypes and genotype-phenotype correlations.[10] For each patient, the *PTEN* mutation type (missense, nonsense, deletion, or insertion), exon location, and proximity to known regulatory site were reviewed in relationship to histology.

Results

During the period from 2008 to 2011, a total of 24 patients with CS were evaluated. Four patients (16.7%), 2 men and 2 women, were found to have a history of RCC. Of these four patients, no other family members had a history of RCC, despite other relatives having other types of cancer as well as features suggestive of CS (Figure 1). Patient 4 had previously presented with bilateral renal tumors, as described by Mester, et al.[10], while the remaining three had solitary renal lesions (Figure 2). All patients had renal tumors treated surgically.

All patients had renal tumors indistinguishable from established histologic subtypes.[11] In our series, patients 1 and 2 had papillary type 1 RCC, patient 3 had clear cell RCC and patient 4 had bilateral chromophobe RCC (Figure 3a-d). Combining this series with the recent series reported by Mester, the first 11 CS associated RCC had papillary type I in 6 (54.5%), papillary type II in 2 (18.2%), chromophobe in 2 (18.2%), and clear cell in 1 (9.1%).[10]

Each patient had evidence of macrocephaly, occipito-frontal head circumference measurement >97% for their sex and height (Figure 4A). Pathognomonic dermatologic manifestations were identified in each patient (Figure 4B,C). All patients had a confirmed germline alteration in *PTEN* and met clinical criteria for CS (Table 1). Pathologic staging

demonstrated lesions were T1 or T2, N0M0 (Table 2). Currently no patient has evidence of recurrence (follow-up 1-8 years).

Review of germline *PTEN* mutations in demonstrated that missense, nonsense, and splice site mutations were associated with RCC development (Figure 5A). Several exons were involved, including both functional domains (phosphatase tensin-type and the C2 tensin-type). Specific germline mutations could not be associated with distinct histologic types. Notably, one nonsense mutation in exon 5 (p.R130X) was associated with three tumors in three different individuals that presented with three different histologies (one chromophobe, one papillary type I, and one clear cell) (Figure 5A).

LOH of *PTEN* by PCR was found in 4 of the 5 tumors (80%), including one example from each RCC histology (Figure 5B). The wild-type allele was retained in the remaining tumor (patient 2, papillary type I). However, other mechanisms that could account for loss of wild-type *PTEN* function in this sample such as epigenetic silencing or an additional mutation were not evaluated. Analysis of *VHL* status in the clear cell tumor demonstrated a somatic mutation (c.404delT in exon 2, causing a p.Leu135fs) in a single allele, but no evidence of LOH.

Discussion

Clinicians managing individuals with kidney cancer should be familiar with the hereditary RCC syndromes, as the role of inherited predisposition to kidney cancer is most likely underestimated.[12] The diagnosis of an inherited form of RCC is important in the management of not only the patient, but also for members of the family who may be disease gene carriers. In our hereditary kidney cancer program, affected individuals as well as at-risk family members are routinely screened for the detection of altered germline kidney cancer susceptibility genes, as potentially aggressive renal tumors cancers can be detected at a treatable stage. In addition to RCC screening, other syndromic manifestations can be evaluated and managed. Quality of life may be improved by early detection of disease manifestations, best exemplified by early detection of VHL-associated retinal and endolymphatic sac tumors, respectively.

CS may be a challenging diagnosis for urologic oncologists due to the complexity of a clinical diagnosis that requires a combination of pathognomonic, major, and minor criteria (Table 1).[9] Family history of RCC may not provide an indication of a hereditary component for CS, as patients in the present series, as well as those reported by Mester et al. had no additional family members with RCC.[10] While a family history may allow recognition of a hereditary syndrome, at times it may not. This scenario may occur more frequently in CS than with other hereditary cancer syndromes as the rate of *de novo* germline *PTEN* is estimated to be between 10.7-47.6%.[13] Therefore, clinicians should be aware that family history of RCC or other cancers does not exclude individuals from CS. Those individuals with a personal history or clinical manifestations should be considered for genetic counseling. To aid clinicians and genetic counselors with the identification of patients with CS, a useful nomogram has been developed to predict the presence of a *PTEN* germline alteration (http://www.lerner.ccf.org/gmi/ccscore/).[14]

In our series we observed a higher CS-RCC penetrance (16.7%) than that reported by Mester and colleagues (4.1%).[10] This higher incidence is possibly due to our referral patterns selecting for individuals with more prominent manifestations who may be candidates for a therapeutic trial. CS-RCC penetrance is significantly lower than the kidney cancer penetrance in other hereditary RCC syndromes such as HPRC, where nearly 100% of patients are predicted to develop RCC by their ninth decade.[15] Previous reports of CS-RCC demonstrated an association with chromophobe and papillary RCC. In the current study we determined that numerous histologic subtypes, including clear cell can occur in CS-RCC, in contrast with a number of other hereditary RCC syndromes that have histology-specific phenotypes (e.g., VHL, HPRC).

The genes associated with RCC appear to be linked to common metabolic pathways with PTEN positioned at a central regulatory site (Figure 6). Similar to other hereditary cancer syndromes associated with tumor suppressor genes (*TSC1*, *TSC2*, *FLCN*), dysregulation at upstream regulatory points may be associated with a more variable histology[16,17] Those syndromes associated with tumor suppressor genes responsible for regulatory proteins at distal positions in the RCC metabolic pathway (*VHL*, *FH*, *SDHB*, *SDHC*) may have less variable histology.[18-20]

With some kidney cancer hereditary syndromes, a genotype-phenotype correlation may depend on mutation type and location, best exemplified by the *VHL* mutations; where genotype-phenotype association in VHL patients correlates with propensity to develop pheochromocytomas in patients with missense mutations (Type II VHL). Our preliminary observations do not suggest a correlation of germline mutation with specific histology in CS-RCC. For example, the same *PTEN* mutation (p.R130X) was associated with chromophobe, papillary, and clear RCC (Figure 5B). Similarly, two other germline *PTEN* mutations were associated with both type I and type II papillary RCC (p.D24H and p.I50MfsX4). While other genetic modifiers may contribute to an individual's development of a particular histology, in syndromes such as BHD, several histologic types can be observed with a single genotype, even within the same kidney.[21] Additional genetic events of key regulatory genes may push a tumor towards a particular phenotype. An event such as the loss of one copy of VHL could account for a clear cell phenotype, as observed with BHD cell line UOK257, derived from a clear cell BHD renal tumor where chromosome 3p is deleted.[22] While complete loss of functional VHL is present in the majority of sporadic and hereditary clear cell RCC, this may not be necessary in other hereditary syndromes. Similar to the UOK 257 model, our clear cell CS-RCC did not have detectable LOH despite a *VHL* mutation. While this finding is not consistent with the traditional Knudson two-hit model for *VHL*, it raises the question that perhaps cumulative alterations in an overlapping or different metabolic pathway can cooperate to bring about dysregulation of RCC gene pathways, resulting in RCC tumorigenesis. Several preclinical models support cooperative dysregulation between *PTEN* and *VHL*, with liver hemangioblastomas and renal cysts resulting from mutation of both genes, but not either alone.[23,24] Despite the two-hit model generally considered to have both genetic events happening in consecutive order, recent evidence suggests that haploinsufficiency in tumor suppressor genes can facilitate other genetic events prior to the development of a second hit.[25] Along these lines, in CS-RCC,

PTEN haploinsufficiency could coordinate with secondary events to promote transformation down separate histologic pathways. This requirement for additional genetic alterations for tumorigenesis could also explain the low penetrance of CS-RCC.

Examining the mechanism associated with the development of CS-RCC as an example of hereditary RCC could improve our understanding of sporadic RCC. Although PTEN expression is commonly decreased in RCC, complete loss occurs in less than 10% of sporadic RCC.[26] Mester and colleagues found loss of PTEN by immunohistochemistry in all patients evaluated with CS-RCC.[10] The precise mechanism causing the loss of protein expression was not elucidated, but several mechanisms could underlie the second hit such as LOH, somatic mutation or promoter hypermethylation of the wild-type allele. We demonstrate that LOH, present in four of five of our tumor samples, may be a common mechanism associated with RCC tumorigenesis in with this disease.[10] The remaining tumor may have a secondary event in the *PTEN* wild-type allele, however we did not perform full exon sequencing or epigenetic analysis.

Despite a low incidence of *PTEN* mutations, sporadic clear and non-clear renal tumors frequently have activation of the PI3K/AKT pathway, the downstream targets of PTEN. Targeting this pathway with mTOR agents has shown clinical benefit and 2 agents (everolimus and temsirolimus) are approved for clinical use in RCC. For clear cell RCC, the efficacy of these agents is believed related both to inhibition of the PI3K/AKT pathway as well as its known inhibitory effect on HIF1 translation. In patients with advanced sporadic RCC treated with temsirolimus, pS6 and pAKT are potential biomarkers for response.[27] As both pS6 and pAKT are dysregulated by the loss of PTEN, those with CS-RCC who develop advanced disease may also benefit from mTOR inhibition. Even for patients without metastatic disease, targeting the mTOR pathway in this population may serve as cancer chemoprevention, demonstrated in preclinical animal models.[28]

Detailed characterization on the renal cancer manifestations of a rare disease must be cautioned. The low incidence (1 in 200,000) of CS compounded with the low RCC penetrance limits definitive conclusions on disease biology. Genotype-phenotype relationships could greatly influence the aggressiveness and age of onset. Despite these recognized limitations, our series may aid recognition of clinical features associated with this hereditary cancer syndrome allowing genetic counseling referral and identification of non-urologic disease manifestations in the individuals and affected family members.

Conclusion

CS is a hereditary cancer syndrome associated with dermatologic manifestations and multiple types of cancers including breast, uterine, thyroid, and RCC. Patients often do not have a family history of RCC due to low disease penetrance and a high rate of *de novo* mutation. Recognition of associated cancers, dermatologic features, as well as macrocephaly should lead to consideration for genetic counseling. Classic histologic types including clear cell, chromophobe, and papillary RCC can be seen in CS-RCC. *PTEN* LOH is common in these tumors and may lead to activation of the mTOR pathway. Targeting this pathway may

have an important therapeutic role for those with advanced CS-RCC and/or *PTEN*-deficient sporadic RCC.

Acknowledgments

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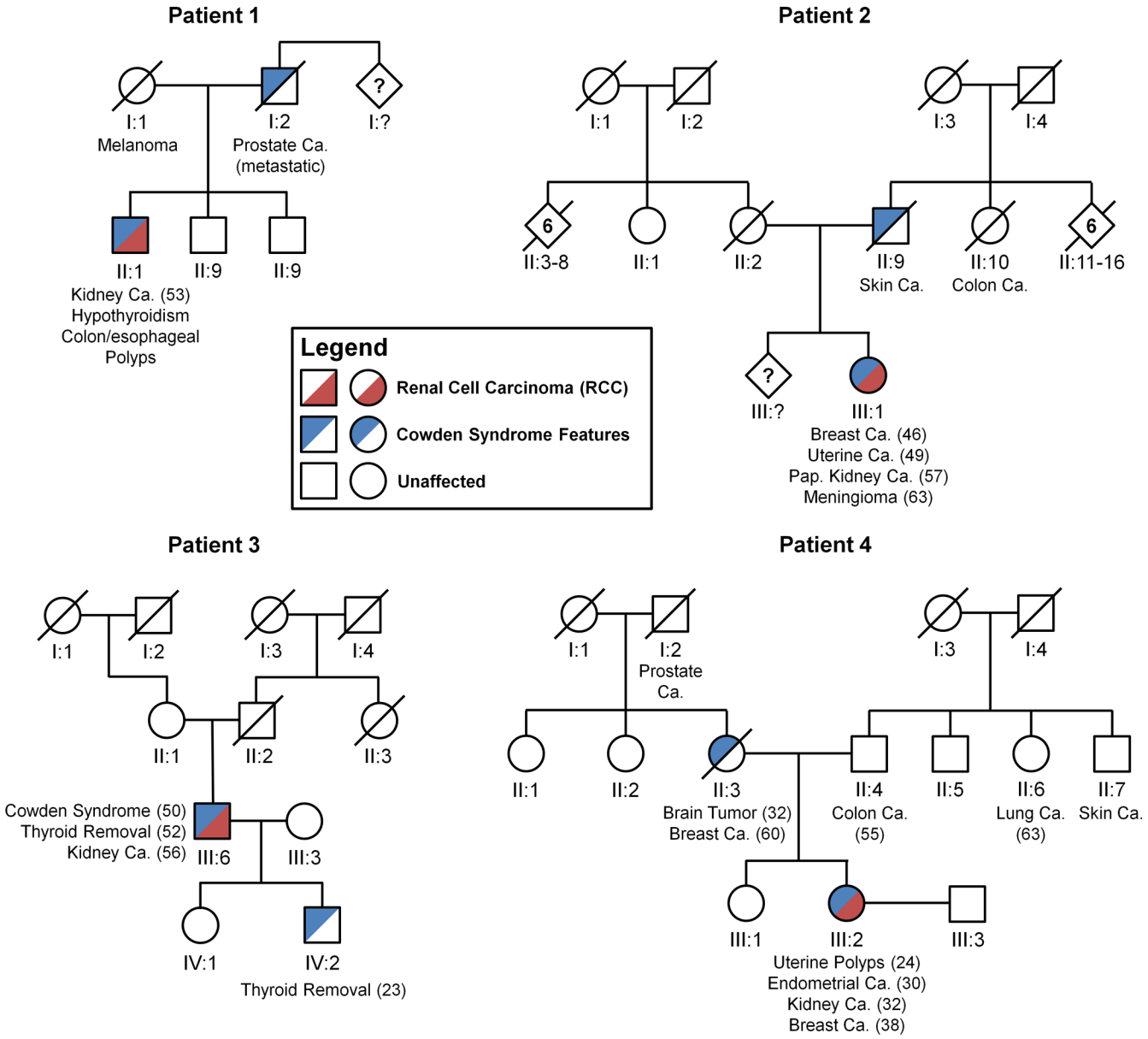

Figure 1. Cowden/*PTEN* Syndrome Family Pedigrees

Cowden/*PTEN* Syndrome families (patients 1-4) are shown with blue segments representing the occurrence of any Cowden Syndrome diagnostic features (described with age of diagnosis) and red segments highlighting the occurrence of Renal Cell Carcinoma (RCC). Ca. – cancer; Pap. – papillary.

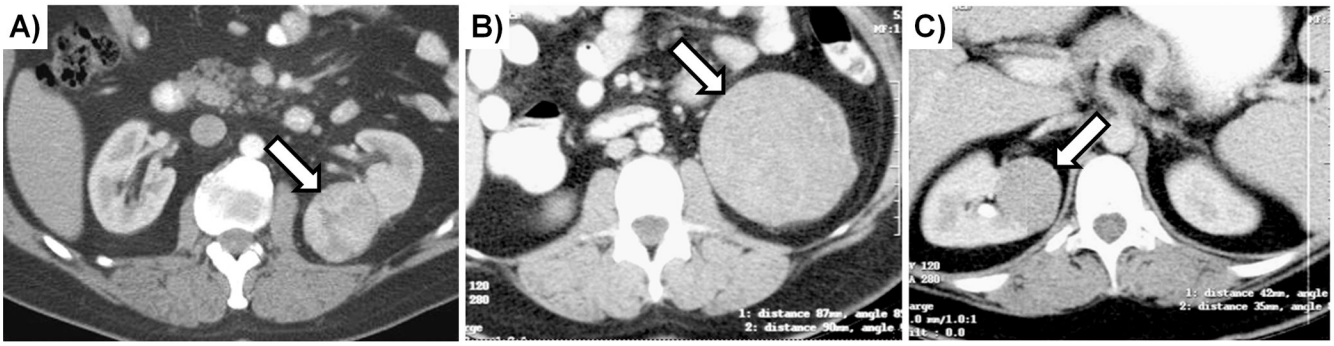

Figure 2. CT Imaging of Cowden/*PTEN* Syndrome Associated RCC (CS-RCC)

A) A contrast-enhanced CT scan of Patient 3 demonstrating a left-sided T1a clear cell RCC. B) & C) Contrast-enhanced CT scans of Patient 4 demonstrated bilateral, multifocal chromophobe RCC, a left-sided T2 and a right-sided T1b respectively.

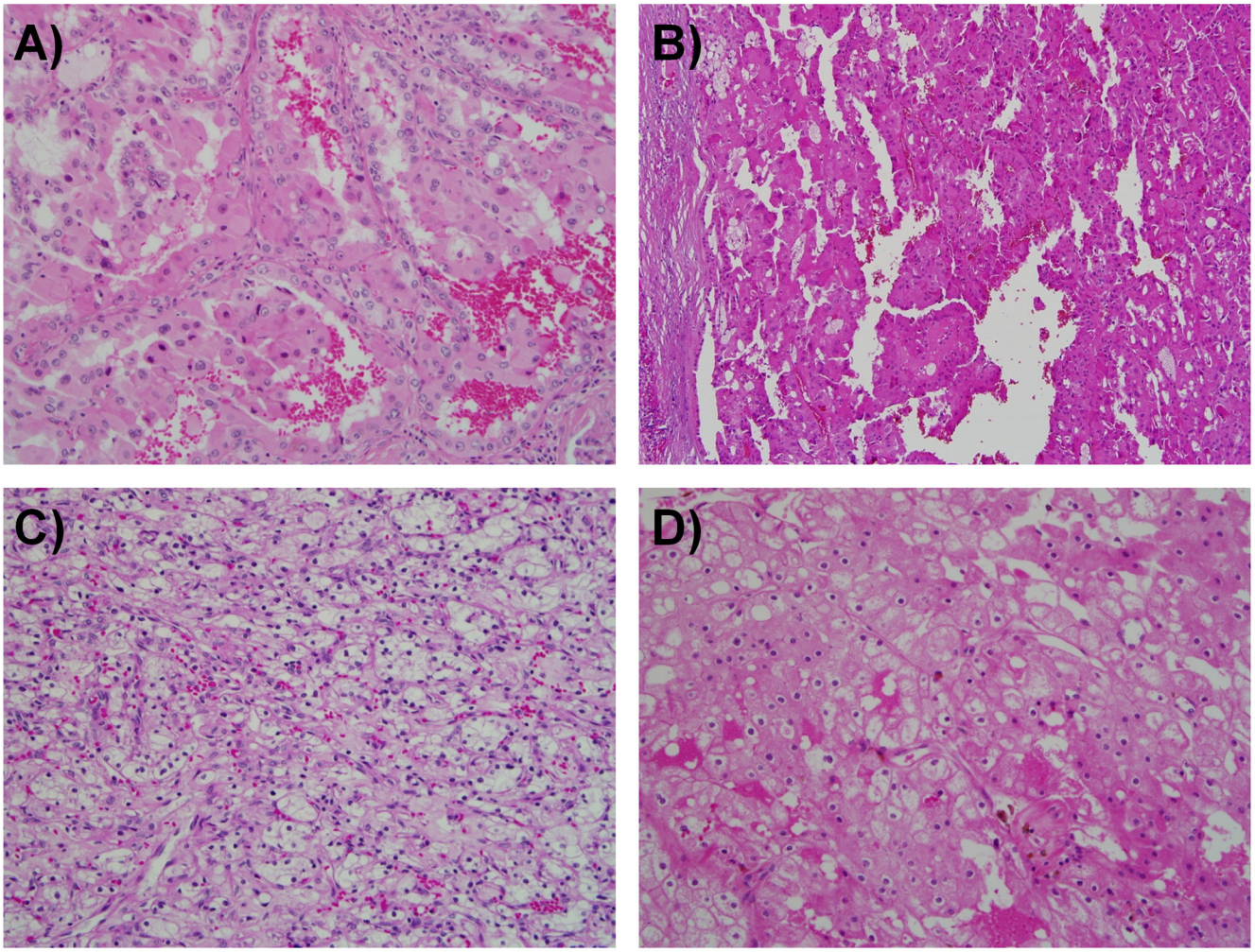

Figure 3. Histology of Cowden/*PTEN* Syndrome Associated RCC (CS-RCC)

Patients 1 (A) and 2 (B) had papillary type 1 RCC, Patient 3 had clear cell RCC (C) and patient 4 had chromophobe RCC (D).

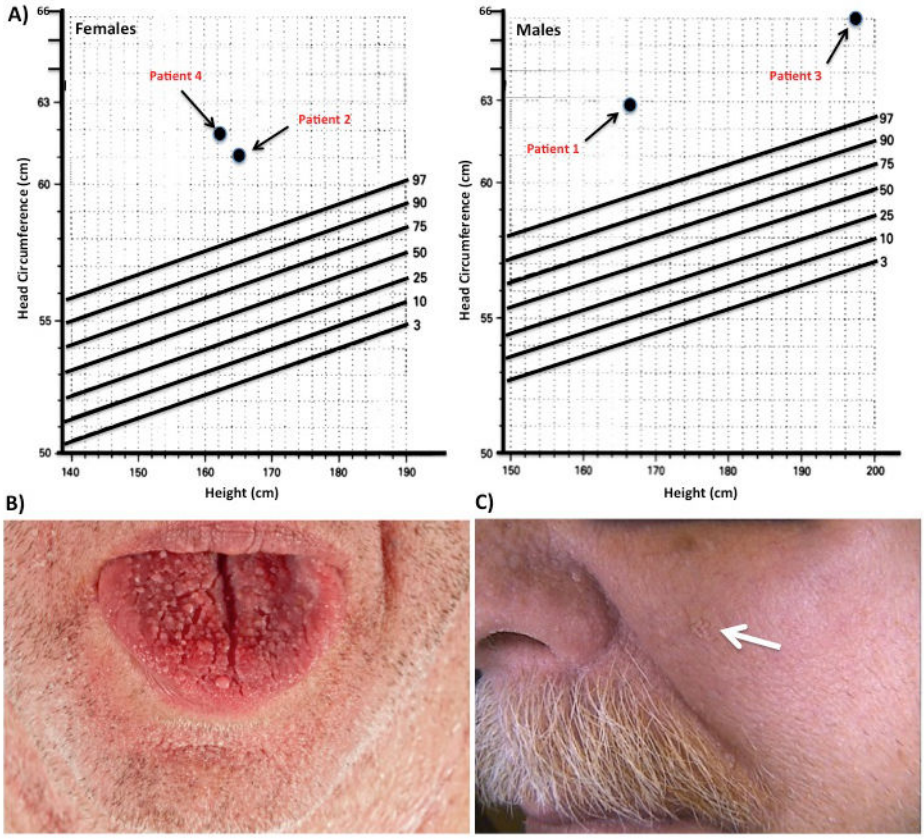

Figure 4. Clinical Manifestations (Head Circumference and Dermatologic Lesions) that may be seen on Physical Exam of Cowden/PTEN Syndrome Patients

A) Measurement of occipito-frontal head circumferences demonstrated that all four patients had macrocephaly (>97% by height). Macrocephaly is considered major criteria for the diagnosis of Cowden Syndrome. (Adapted from Bushby, KM, *et al.*, Arch. Dis. Child. 1992, 67:1286-1287). A variety of dermatologic manifestations are pathopneumonic for Cowden Syndrome present in Patient #1 **B) Oromucosal papillomatous papules creating a cobblestone appearance on the dorsal aspect of the patients tongue C) Cutaneous verroucous papule (white arrow) located at its characteristic distribution, over the central portion of the face.**

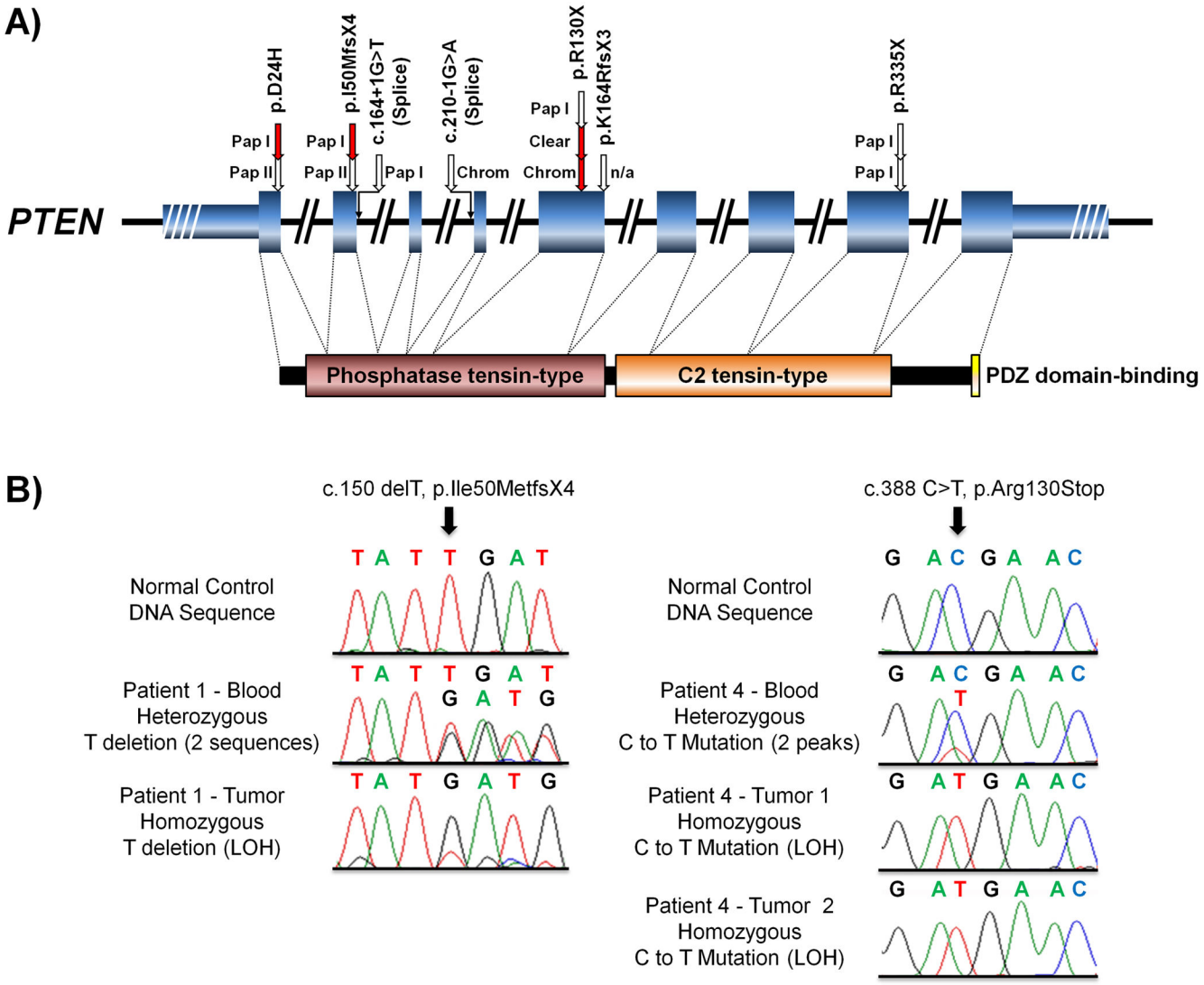

Figure 5. *PTEN* mutation map for CS-RCC and Evidence of *PTEN* loss of heterozygosity (LOH)

A) A schematic of the *PTEN* demonstrates the 9 coding exons (fat blue boxes) and the 5′ and 3′ UTRs (thin blue boxes) with the known functions domains shown below linked to their relevant coding exons (P60484 – http://www.uniprot.org/). The mutations from this report (red arrows) were combined with the recent series reported by Mester and colleagues[10] (white arrows) and mapped to the *PTEN* gene with annotation of the reviewed pathology. Pap I – Papillary Type 1, Pap II – Papillary Type 2, Chrom – Chromophobe, Clear – Clear Cell.

B) The loss of heterozygosity (LOH) was assessed in all tumors and two examples are shown here. The left panel demonstrates normal control sequence followed by heterozygous deletion of a T in the blood DNA of Patient 1, creating a frameshift, and LOH of the frameshift in the tumor DNA. The right panel demonstrates normal control sequence followed by heterozygous substitution of a C for a T in the blood DNA of Patient 4 and LOH of this mutation in the DNA of both the assessed tumors.

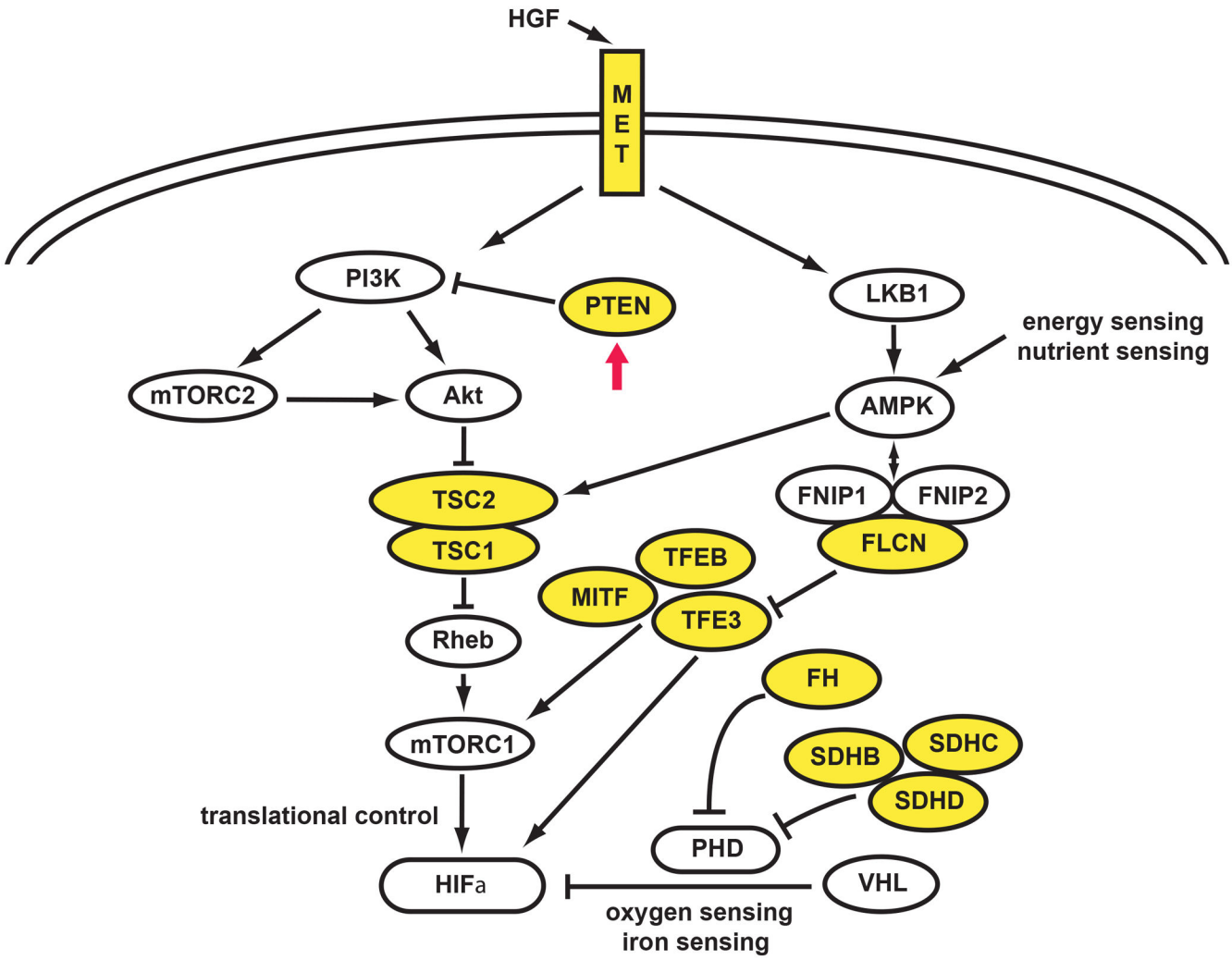

Figure 6. The Kidney Cancer Gene Pathways

Kidney cancer is not a single disease; it is made up of a number of different types of cancer with different histologies, having different clinical courses, responding differently to therapy and caused by different genes (e.g., *VHL*, *MET*, *FLCN*, *FH*, *SDHB/C/D*, *TFE3*, *TFEB*, *MITF*, *TSC1*, *TSC2* and *PTEN*).[2,29] The *PTEN* gene, which is frequently found to be mutated in the germline of patients affected with Cowden Syndrome, encodes for a plasma membrane lipid phosphatase that inhibits the PI3K signaling pathway. Loss of *PTEN* leads to accumulation of phosphatidlyinositol 3,4,5 triphosphate (PIP_3), which activates AKT.[30] Activation of AKT affects the TSC1/2 pathway, resulting in activation of the mTOR pathway. (Adapted from Linehan.[2])

Table 1

Pathognomonic Characteristics	Major Criteria	Minor Criteria
Mucocutaneous Lesions	Breast Cancer	Benign Thyroid Conditions
Mucosal Lesions	Thyroid Cancer	Mental Retardation
Acral Keratoses	Endometrial Cancer	GI Harmatomas
Facial Trichilemmomas	Macrocephaly	Lipomas
Papillomatous Papules	Lhermitte-Dulcos Disease (LDD)	Fibromas
		Renal Cell Carcinoma (RCC)
		Breast Fibrocystic Disease

Diagnostic Criteria	**1**	2 Major Criteria Including LDD
	2	1 Major and 3 Minor Criteria
	3	4 Minor Criteria
	4	Pathognomonic Criteria (Specific combination of lesions)

	Pathognomonic Characteristics	Major Criteria	Minor Criteria
Patient 1	Mucosal Lesions, Papillomatous Papules, Acral Keratoses	Macrocephaly	GI Harmatomas, RCC, Breast Fibrocystic Disease, Benign Thyroid
Patient 2	Trichilemmomas, Mucosal Lesions, Papillomatous Papules, Acral Keratoses	Macrocephaly, Breast Cancer, Uterine Cancer	GI Harmatomas, RCC, Lipomas, Benign Thyroid
Patient 3	Mucosal Lesions, Papillomatous Papules, Acral Keratoses	Macrocephaly, Lhermitte-Duclos Disease, Breast Cancer, Uterine Cancer	GI Harmatomas, RCC, Benign Thyroid
Patient 4	Mucosal Lesions, Papillomatous Papules, Acral Keratoses	Macrocephaly, Lhermitte-Duclos Disease	GI Harmatomas, RCC, Benign Thyroid

Table 2

The diagnosis, genetics and management of the Cowden/PTEN Syndrome Associated RCC (CS-RCC) in our patient cohort.

Patient	Sex	Age	Diagnosis	Size	Mutation	TNM Stage	Histology	Nephrectomy	Follow-Up
1	M	53	Incidental	1.0cm	c.150delT, p.I50MfsX4	T1aN0M0	Papillary Type 1	Radical	NED 5 yrs
2	F	57	Incidental	1.3cm	c.70G>C, p.D24H	T1aN0M0	Papillary Type 1	Partial	NED 8 yrs
3	M	56	Incidental	3.0cm	c.388C>T, p.R130X	T1aN0M0	Clear Cell	Partial	NED 1 yrs
4	F	32	Symptomatic	9.0cm 4.5cm	c.388C>T, p.R130X c.388C>T, p.R130X	T2N0M0 T1bN0M0	Chromophobe Chromophobe	Radical Partial	NED 7 yrs

NED – No Evidence of Disease

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