

Hereditary and Sporadic Papillary Renal Carcinomas with *c-met* Mutations Share a Distinct Morphological Phenotype

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Germline mutations of *c-met* oncogene at 7q31 have been detected in patients with hereditary papillary renal cell carcinoma. In addition, *c-met* mutations were shown to play a role in 13% of patients with papillary renal cell carcinoma and no family history of renal tumors. The histopathology of papillary renal cell carcinoma with *c-met* mutations has not been previously described. We analyzed the histopathology of 103 bilateral archival papillary renal cell carcinomas and 4 metastases in 29 patients from 6 hereditary papillary renal cell carcinoma families with germline *c-met* mutations and 6 papillary renal cell carcinomas with *c-met* mutations from 5 patients with no family history of renal tumors. Twenty-five sporadic renal tumors with prominent papillary architecture and without somatic *c-met* mutations were evaluated for comparison. All papillary renal cell carcinomas with *c-met* mutations were 75 to 100% papillary/tubulopapillary in architecture and showed chromophil basophilic, papillary renal cell carcinoma type 1 histology. Fuhrman nuclear grade 1–2 was seen in tumors from 23 patients, and nuclear grade 3 was observed focally in 8 patients. Seventeen patients had multiple papillary adenomas and microscopic papillary lesions in the surrounding renal parenchyma. Clear cells with intracytoplasmic lipid and glycogen were focally present in tumors of 94% papillary renal cell carcinoma patients. Clear cells of papillary renal

cell carcinoma had small basophilic nuclei, and clear cell areas lacked a fine vascular network characteristic of conventional (clear) cell renal cell carcinoma. We conclude that papillary renal cell carcinoma patients with *c-met* mutations develop multiple, bilateral, papillary macroscopic and microscopic renal lesions. Renal tumors with *c-met* genotype show a distinctive papillary renal cell carcinoma type 1 phenotype and are genetically and histologically different from renal tumors seen in other hereditary renal syndromes and most sporadic renal tumors with papillary architecture. Although all hereditary and sporadic papillary renal cell carcinomas with *c-met* mutations share papillary renal cell carcinoma type 1 histology, not all type 1 sporadic papillary renal cell carcinomas harbor *c-met* mutations. (Am J Pathol 1999, 155:517–526)

Papillary renal carcinomas (PRC) occur in sporadic and hereditary forms. Sporadic PRC is the second most common carcinoma of the kidney, comprising 10–15% of renal neoplasms^{1–3} and is a predominant tumor type in acquired renal cystic disease.⁴ Sporadic PRC are usually solitary tumors but multiple papillary adenomas were described in the surrounding renal parenchyma in some cases.⁵ Kovacs et al⁶ established criteria for papillary renal neoplasm stipulating that the tumor should be 75% papillary and/or tubulopapillary in architecture to be classified as a PRC. In 1986 Thoenes et al⁷ included PRC in a morphological classification of kidney tumors as a chromophil carcinoma with basophilic, eosinophilic and mixed (basophilic/eosinophilic) histological subtypes. In the two current classifications of renal tumors PRC is included as a separate entity.^{1, 8} Delahunt and Eble⁹ recently suggested subdividing all PRC into type 1 and type 2 tumors which basically correspond to the chromophil basophilic and chromophil eosinophilic carcinoma subtypes, respectively, in Thoenes' classification. PRC

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Table 1. Genetic and Tumor Characteristics of PRC Patients with *c-met* Mutations

Family no. or patient/DNA no.	Family history PRC (yes/no)	<i>c-met</i> mutation	No. patients examined	No. PRC examined	Tumor size range (cm)	Histology
152	Yes	M1149T germline	7	10	0.6–1.5	PRC Type 1
162	Yes	M1149T germline	3	3	0.6–2.0	PRC Type 1
158	Yes	V1206L germline	1	5	1.0–5.0	PRC Type 1
150	Yes	H1112R germline	5	49	0.6–6.0	PRC Type 1
160	Yes	H1112R germline	11	22	1.0–11.0	PRC Type 1
5946	Yes	V1110I germline	2	14	0.6–9.0	PRC Type 1
6088	No	V1110I germline	1	1	2.0	PRC Type 1
6059	No	Y1248D germline	1	2	0.6–0.7	PRC Type 1
6082	No	M1268T germline	1	1	2.2	PRC Type 1
6052	No	H1124D somatic	1	1	2.5	PRC Type 1
6046	No	Y1248C somatic	1	1	2.0	PRC Type 1

PRC, papillary renal carcinoma.

lack chromosome 3p genetic alterations characteristic of clear cell carcinomas in von Hippel-Lindau disease and sporadic conventional (clear) renal carcinoma.¹⁰ Jiang et al¹¹ recently suggested the presence of cytogenetic differences between PRC type 1 and type 2.

Hereditary PRC is a recently described form of familial kidney cancer characterized by a predisposition to develop multiple, bilateral papillary renal tumors^{12, 13} with trisomy of chromosomes 7 and 17.¹⁴ Hereditary PRC is characterized by autosomal dominant transmission with reduced penetrance.^{12, 13, 15} Recently the hereditary PRC gene was localized to chromosome 7q31.1-34 by genetic linkage analysis.¹⁵ Germline missense mutations in the tyrosine kinase domain of the *c-met* proto-oncogene were detected in affected members of 6 of 7 hereditary PRC families.^{15, 16} In addition, *c-met* mutations have been identified in tumors of 13% of PRC patients with no family history of renal tumors.¹⁷ To date 15 different missense mutations have been identified in hereditary and sporadic PRC, located in the tyrosine kinase domain of *c-met*. These mutations have been shown to be transforming when transfected into NIH3T3 cells and cause ligand-independent constitutive phosphorylation of the *c-met* protein.^{16–18} Trisomy 7 harboring non-random duplication of the mutant *c-met* proto-oncogene is thought to play a causative role in the development of multiple renal tumors in hereditary PRC patients.^{19, 20}

The *c-met* proto-oncogene, located at chromosome 7q31, is a member of the receptor tyrosine kinase family of proteins.^{21, 22} Binding of its ligand, hepatocyte growth factor (HGF), to the extracellular portion of *c-met*, triggers autophosphorylation of critical tyrosines in the intracellular tyrosine kinase domain of *c-met*, activating a downstream signaling cascade via an SH2-domain docking site in the COOH-terminus of the protein.²³ Activation of the *c-met*/HGF signaling pathways has been shown to be involved in a number of biological activities including cell motility,²⁴ morphogenic differentiation,²⁵ cell proliferation,²⁶ and invasion.²⁷ In addition, *c-met* has been shown to be overexpressed in a number of human cancers.^{28–30}

The histopathology of PRC harboring *c-met* mutations has not been previously described. We examined the histopathology of multiple, bilateral archival renal tumors and adjacent renal parenchyma in patients from 6 hered-

itary PRC families with germline *c-met* mutations and in patients with PRC with *c-met* mutations and no family history of renal tumors. Sporadic renal tumors with prominent papillary/tubulopapillary architecture and without somatic *c-met* mutations were evaluated for comparison.

Materials and Methods

Patient Characteristics

Twenty-nine patients from 6 hereditary PRC families with germline *c-met* mutations and 5 patients with PRC who had no family history of renal tumors and had a documented mutation in the *c-met* oncogene were included in the study (Table 1). All patients gave informed consent in a protocol approved by the NCI Institutional Review Board or Institutional Review Boards in their country. Hereditary PRC families were from Sweden (family 152), the Netherlands (family 162), Canada (family 160), and the United States (families 158, 150 and 5946).^{13, 15, 16} Hereditary PRC patients included 18 women and 11 men with a mean age 48 years (age range 29–72 years) at the time of diagnosis (Table 2). Most patients with hereditary PRC were asymptomatic at presentation and their tumors were discovered only during screening by computed tomography or magnetic resonance (CT/MRI) imaging. Radiologically, all tumors were relatively hypovascular and enhanced on contrast CT/MRI scans³¹; additional smaller tumors were seen on intraoperative ultrasound or upon sectioning of renal specimens. Five patients underwent partial nephrectomy or tumor enucleation, 8 patients underwent unilateral nephrectomy, 6 patients were treated by bilateral nephrectomy, and kidneys in 2 patients were examined at autopsy. No information about procedure type was available in 6 of 7 patients from family 152. In 2 patients (3740 and 5153) primary kidney tumors were not available for evaluation and metastases obtained at autopsy were examined (Table 2). No information about patients' age, sex, or type of surgery was available on 5 PRC patients with *c-met* mutations who had no family history of renal tumors.

Table 2. Histopathology of PRC with *c-met* Mutations in 34 Patients

Family no. or DNA no.	Patient no.	Patient Age/Sex	No. PRC examined	Architecture*	Histology*	Fuhrman nuclear grade* (1-4)	Clear cells* (%)	Foamy macrophage/psammoma bodies*	Hemorrh/necrosis*	No. PA; no. microscopic lesions	Metastases	
152	T41-76 [†]	42F	2	pap/tubpap/solid	bas/eos [‡]	2-3	10	yes/no	yes/yes	NA	Skeletal muscle	
	N1124-76	51F	1	pap/tubpap/solid	bas	2-3	10	yes/yes	yes/yes	NA		
	4371	53F	1	tubpap/pap	bas	2	40	yes/yes	no/no	NA		
	4374	34F	1	pap	bas	2-3	50	yes/no	yes/yes	NA		
	0260-69	37F	2	pap/tubpap	bas	1-2	30	yes/yes	yes/no	1; 5		
	0690-68 [†]	43M	2	pap/tubpap	bas	1-2	30	no/no	no/no	1; 4		Lung
	4375	29F	1	pap	bas/eos	2	20	yes/no	yes/no	NA		
162	4813	42F	1	pap/solid	bas/eos	2-3	0	yes/no	no/no	NA		
	5227	64F	1	pap	bas	2	70	yes/no	yes/no	NA		
	5228	54F	1	pap	bas	2	50	yes/yes	yes/no	NA		
158	5935	60F	5	pap/tubpap	bas	1-2	30	yes/yes	yes/yes	5; 3	Lymph nodes, lung	
150	4599	45M	40	pap/tubpap/solid/MA-like	bas/eos	1-2	70	yes/yes	no/no	13; 36		
	3740 [†]	72M	2 mets	Kidney NA for evaluation								
	5226	55M	2	pap/tubpap	bas/eos	2-3	50	yes/no	yes/yes	0; 3		
	6092-92	72M	6	pap	bas	2	40	yes/yes	yes/no	none		
160 [§]	3766	40M	1	pap	bas	2	40	yes/no	yes/no	0; 2		
	4967	21M										
	5161	50F	2	tubpap	bas/eos	1-2	70	yes/yes	yes/no	mult; mult		
	5100	42M	3	pap/tubpap	bas	2	20	yes/yes	no/no	mult; mult		
	5126	48M	3	pap/tubpap	bas	1-2	20	yes/yes	yes/yes	mult; mult		
	5153 [†]	57M	2 mets	Kidney NA for evaluation							Lung, skeletal muscle, lymph node	
160	5011	47F	4	pap/tubpap/solid/MA-like	bas	1-2	20	yes/no	yes/yes	0; 1		
	5154	56F	1	pap/tubpap	bas	1-2	10	yes/yes	yes/yes	0; 6		
	5052	36F	1	pap/tubpap/solid	bas	1-2	50	no/no	no/no	0; 2		
	5151	33F	2	pap/tubpap/MA-like	bas/eos	2-3	1	yes/no	no/no	15; 21		
	5020	45M	4	pap/tubpap	bas/eos	2-3	10	yes/yes	yes/yes	3; 9		
	5150	69F	2	pap/tubpap	bas	2	20	yes/no	yes/no	mult; mult		
5946	5946	41F	13	pap/tubpap	bas/eos	2-3	30	yes/yes	yes/yes	15; 29		
2400-74	45F	1	pap/tubpap	bas	1-2	1	yes/no	yes/yes	NA			
6088	6088	NA	1	pap/tubpap	bas	1-2	10	yes/no	yes/no	none		
6059	6059	NA	2	pap/tubpap	bas	1-2	1	yes/no	yes/no	2; 0		
6082	6082	NA	1	pap/tubpap	bas	1-2	1	yes/no	yes/no	none		
6052	6052	NA	1	tubpap	bas	1-2	15	yes/no	no/no	none		
6046	6046	NA	1	tubpap/solid	bas	2	0	yes/yes	yes/no	none		

Age, age at the time of diagnosis; PRC, papillary renal carcinoma; PA, papillary adenoma; hemorrh, hemorrhage; pap, papillary; tubpap, tubulopapillary; MA-like, metanephric adenoma-like; bas, chromophil basophilic (7); NA, not available; mult, multiple.

*Combined histopathological characteristics present in tumors from each patient.

[†]Patients with metastases.

[‡]Eos-eosinophilic cytoplasm when seen was present focally only and all tumors met the criteria of PRC, type 1 (9).

[§]No tumors present in bilateral kidneys on autopsy at age 21.

Morphological Examination of Tumors

One hundred nine PRC from 34 patients with *c-met* mutations were included in the study. Twenty-five renal tumors with prominent papillary/tubulopapillary architecture from 25 sporadic patients with no *c-met* mutations were evaluated for comparison. Formalin-fixed, paraffin-embedded tumors and gross reports were obtained from the files of the Laboratory of Pathology at the National Cancer Institute, NIH, and Laboratory of Immunobiology, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD. In 4 hereditary PRC patients metastases were available for review (Table 2). Tumors were evaluated on hematoxylin and eosin (H&E) stain.

Papillary renal tumors with *c-met* mutations were classified as papillary adenoma (≤ 0.5 cm) or papillary carcinoma based on established histopathological criteria.^{1, 8} Microscopic renal lesions in surrounding renal parenchyma were also evaluated. Tumor histology was evaluated and recorded according to Delahunt and Eble criteria for papillary renal carcinoma as PRC type 1 or PRC type 2⁹ and according to Thoenes criteria for chromophil carcinoma as basophilic, eosinophilic, or basophilic/eosinophilic (mixed)⁷ (Tables 1 and 2).

Mucicarmine stain and immunohistochemistry stains for vimentin (1:40; DAKO, Carpinteria, CA) and *Ulex europaeus* lectin (1:50; Vector, Burlingame, CA) were performed in eight representative PRC. The stains were per-

formed for differential diagnosis between PRC type 1 and other renal tumors with prominent papillary architecture. Mucicarmine stain and *U. europaeus* lectin are thought to be specific for collecting duct carcinoma and are negative in PRC. PRC type 1 have been shown to have significantly greater vimentin staining than PRC type 2.⁹ Three tumors with prominent clear cell component were examined by electron microscopy. For electron microscopic examination, 2.5% glutaraldehyde-fixed, osmium-post-fixed tumor tissue was embedded in Maraglas 655 (Ladd Research Industries, Burlington, VT). Thin sections were stained with uranyl acetate and lead citrate and were reviewed in a Philips CM10 transmission electron microscope.

c-met Germline and Somatic Mutations and Trisomy 7

Mutation analysis of patient tumor and germline DNAs was performed by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP) as described previously³² followed by direct sequencing of PCR products in which aberrant SSCP bands were observed (Figure 1D). Primers and PCR protocols were reported previously.³³ Trisomy 7 in multiple tumors from hereditary PRC patients 4599 and 5161 was documented by FISH (Figure 1C). The trisomy 7 FISH data, trisomy 7 harboring non-random duplication of the mutant *MET* allele and X-chromosome inactivation analysis of multiple renal tumors from these two patients was published in detail previously.¹⁹

Results

Genetic and tumor characteristics of patients from 6 hereditary PRC families with germline *c-met* mutations and of 5 patients with PRC and *c-met* mutations but no family history of renal tumors are shown in Table 1. Family 152 from Sweden and family 162 from the Netherlands had an identical *c-met* mutation, M1149T. Family 150 from the United States (Figure 1A) and family 160 from Canada shared the same mutation, H1112R, with a documented Founder effect.¹⁶ Family 5946 and a PRC patient 6088 with no family history of renal tumors had the same germline mutation, V1110I (Figure 1D).

The number of gross renal tumors examined varied from 1 to 40 per patient (Table 2), and all hereditary PRC patients had multiple bilateral renal lesions clinically and on gross examination. The size of gross tumors ranged from 0.6 to 11 cm (Table 1). Fibrous pseudocapsule was a common feature of PRC more than 0.7 cm in size (see Figure 3A) and was usually absent in smaller renal lesions. The pseudocapsule appeared to be overgrown by the tumor papillae rather than invaded by them, and no desmoplastic reaction was noted in the pseudocapsule around the tumor. A retraction artifact between the pseudocapsule and tumor was common in larger tumors

and created the impression of a "cyst wall" lined by a layer of tumor cells (Figure 3A).

All tumors with *c-met* mutations demonstrated a predominance of PRC type 1 histology by Delahunt and Eble criteria and chromophil basophilic histological subtype by Thoenes criteria (Table 2, Figure 2). All tumors showed papillary/tubulopapillary architecture with slender, short papillae and delicate fibrovascular cores lined by neoplastic cells (Table 2, Figure 2, A and B). Six patients had areas of solid architecture present in their tumors. Areas of "metanephric adenoma-like" architecture with complex papillae resembling glomeruloid bodies (Figure 3B) were present in one member of family 150 (patient 4599) and two members of family 160 (patients 5011 and 5151); both families shared the H1112R mutation. No sarcomatous differentiation was seen in any tumor.

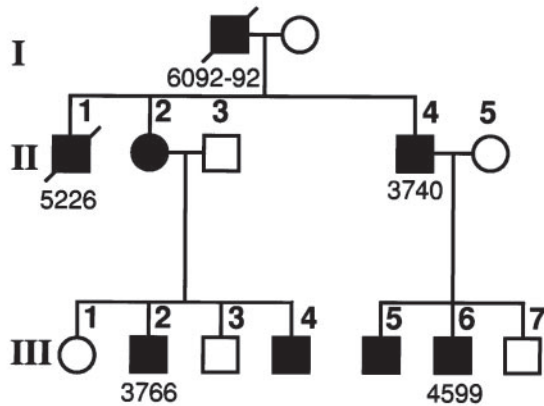
The papillae were lined by a single layer of small cells with small basophilic nuclei and inconspicuous amphophilic cytoplasm (Figures 2 and 3A); however, focal areas of cells with eosinophilic cytoplasm were present in tumors from 9 hereditary PRC patients (Figure 3, B and C). Fuhrman nuclear grade 1-2 predominated in all PRC with *c-met* mutations but focal areas of Fuhrman nuclear grade 3 were seen in tumors from 9 HPRC patients (Table 2, Figure 3C). Nuclear crowding and "grooves" were prominent in some PRC (family 152). Tumors from all but two patients (0690-68 and 5052) demonstrated foamy macrophages in fibrovascular cores, and psammoma bodies were present in tumors from 14 patients (Table 2, Figure 2C). Areas of hemorrhage and necrosis were seen in tumors of 23 and 11 PRC patients, respectively.

Areas of clear cells were common in PRC with *c-met* mutations regardless of size and were seen in renal tumors from 29 patients. The number of clear cells ranged from 1 to 70% (Table 2, Figure 3C). Like clear cells of the conventional (clear) cell RCC, clear cells of PRC contained intracytoplasmic lipid and glycogen demonstrated by electron microscopy (Figure 3D). However, unlike in conventional (clear) cell carcinoma, clear cells of PRC had small basophilic nuclei and the tumor areas composed of clear cells lacked a fine vascular network. Delicate papillae cut in cross-section were seen in clear cell areas of PRC (Figure 3C).

In 16 of 21 PRC patients in whom normal renal parenchyma was available for histological evaluation, papillary renal adenomas (<0.5 cm in size) and/or multiple microscopic papillary renal lesions (<0.5 mm in size) were detected (Table 2, Figure 4, A-C). The number of papillary adenomas ranged from one to 15 on slides available for evaluation, and four patients (5161, 5100, 5126, and 5150) had multiple adenomas recorded at the time of gross examination of kidney specimens. Microscopic renal lesions were common in kidneys of hereditary PRC patients, and patients 4599 and 5946 had 36 and 29 bilateral microscopic lesions, respectively. The microscopic lesions showed the architecture and histology identical to those of papillary adenomas and PRC type 1 (Figure 4B, C). A detailed pathological examination of both kidneys of a 21-year-old man (patient 4967, family 160) who was a H1112R mutation carrier and died of a gunshot wound revealed no renal lesions. Four hereditary

A

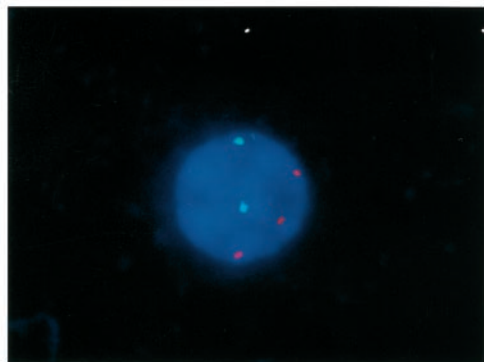
Family 150



B



C



D

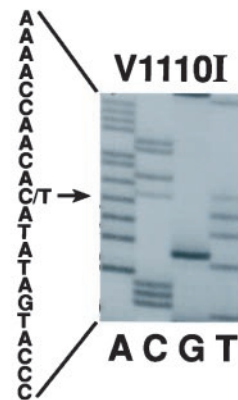


Figure 1. A: Pedigree of hereditary PRC family 150. Solid symbols indicate individuals with renal tumors. Diagonal slash indicates patient is deceased. Patient numbers correspond to individuals listed in Table 2. B: Gross photograph of multiple renal tumors in a nephrectomy specimen from hereditary PRC patient 4599 (family 150) with H1112R mutation (from Zbar et al¹²). The tumors display prominent yellow color. C: Interphase FISH on a tumor cell touch preparation from hereditary PRC patient 4599 with H1112R mutation (family 150). Three copies of *MET* (trisomy 7; rhodamine, red signal) are seen in the tumor cell, using c182b3 combined with a centromeric α -satellite probe (FITC, green signal) specific for chromosome 17¹⁹; D: DNA sequence of hereditary PRC patient 5946 (family 5946) showing germline mutation C to T transition (antisense, arrow) at nucleotide 3522, resulting in a valine to isoleucine change at codon 1110.

PRC patients (T42-76 and 0690-68 from family 152, 3740 from family 150, and patient 5153 from family 160) had metastases to lymph nodes, skeletal muscle and/or lungs (Figure 4, Table 2).

Mucicarmin stain was negative for intracytoplasmic mucin in all 8 PRC with *c-met* mutations examined. Vimentin immunohistochemistry stain was positive and *U. europaeus* lectin was uniformly negative in all 8 tumors stained. Electron microscopy revealed intracytoplasmic lipid and glycogen in clear cells of 3 tumors evaluated.

Histological examination of sporadic PRC from 5 patients without *c-met* mutations (patients/DNA 6056, 6058, 6080, 6083 and 6091¹⁷) revealed PRC type 1 histology identical to the histology seen in PRC with *c-met* mutations. Tumors with papillary/tubulopapillary architecture and no detectable *c-met* mutations were histologically evaluated and classified as PRC type 2 (5 cases), collecting duct carcinoma (4 cases), renal cell carcinoma with proven 1;X translocation (3 cases), metanephric adenoma (5 cases) and conventional (clear) cell carcinoma (3 cases).

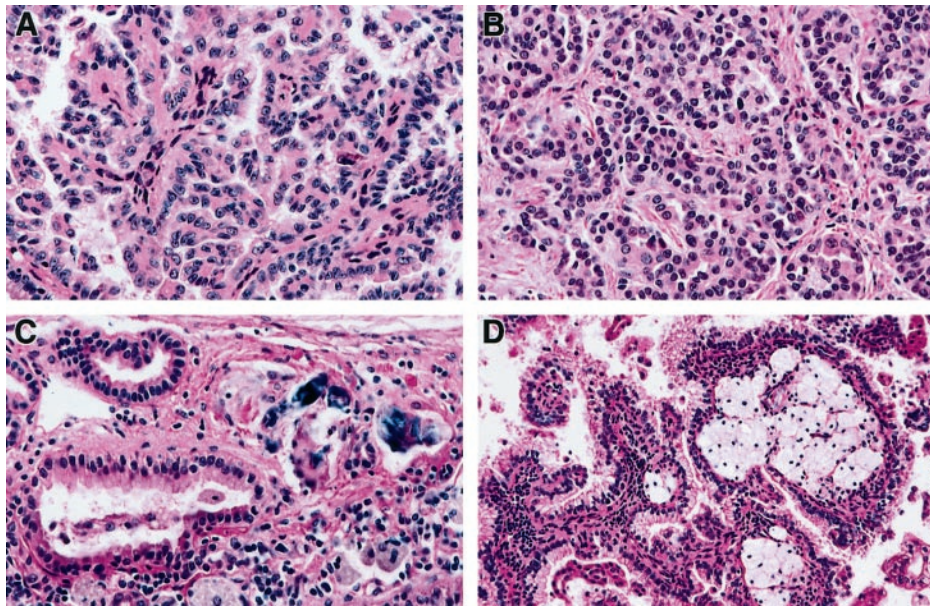


Figure 2. Representative PRC from patient 4599 (family 150) with H1112R mutation. The tumors show type 1 PRC (chromophil basophilic) histology and Fuhrman nuclear grade 1–2. **A:** Papillary architecture characterized by delicate fibrovascular cores lined by small cells with basophilic nuclei and scant amphophilic cytoplasm (H&E, $\times 400$). **B:** Tubulopapillary architecture and small cells with basophilic nuclei and amphophilic cytoplasm (H&E, $\times 400$). **C:** Psammoma bodies and foamy hemosiderin-laden macrophages are prominent microscopic features (H&E, $\times 400$). **D:** Papillary architecture, foamy macrophages in fibrovascular cores and cells with small basophilic nuclei and scant amphophilic and focal clear cytoplasm (H&E, $\times 200$).

Discussion

The Union Internationale Contre le Cancer (UICC) and American Joint Committee on Cancer (AJCC) and Heidelberg classifications of renal neoplasms recognize PRC as a distinct histopathological entity.^{1, 8} Recent cy-

togenetic and molecular genetic studies demonstrate a genetic heterogeneity of PRC,¹⁰ and therefore, the presence of the “papillary” architecture alone is no longer sufficient for the diagnosis of PRC. Papillary/tubulopapillary architecture may be seen and predominate in several types of renal tumors such as PRC type 1 and type 2,

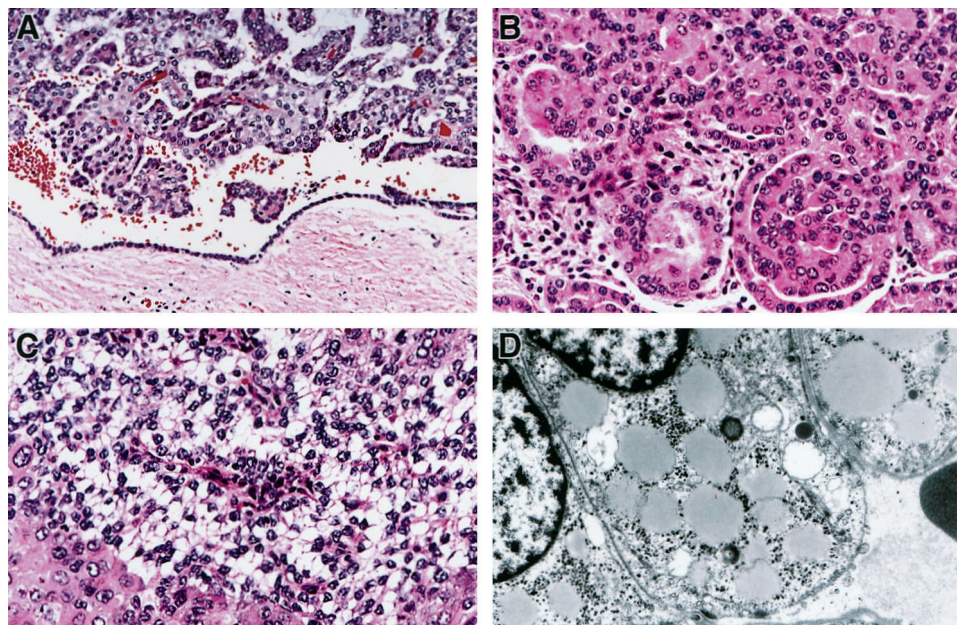


Figure 3. Histopathology of representative PRC and an ultrastructure of clear cells in hereditary PRC patients 5946 with V1110I mutation and 4599 with H1112R mutation. **A:** Fibrous pseudocapsule surrounds a 4 cm tumor from patient 5946; a retraction artifact gives an impression of a “cyst wall lined by a layer of tumor cells” (H&E, $\times 200$). **B:** A tumor from patient 4599 shows a “metanephric adenoma-like” architecture and contains cells with low grade basophilic nuclei and amphophilic and eosinophilic cytoplasm (H&E, $\times 400$). **C:** A tumor from patient 5946 demonstrates an area composed of sheets of cells with basophilic Fuhrman nuclear grade 1 nuclei and clear cytoplasm and adjacent cells with Fuhrman nuclear grade 3 nuclei and eosinophilic cytoplasm. Thin fibrovascular papillary cores are seen in cross-section in the area of clear cells (H&E, $\times 400$). **D:** Electron micrograph of clear cells from a hereditary PRC patient 5946 shows prominent intracytoplasmic lipid droplets and glycogen (magnification, $\times 6600$).

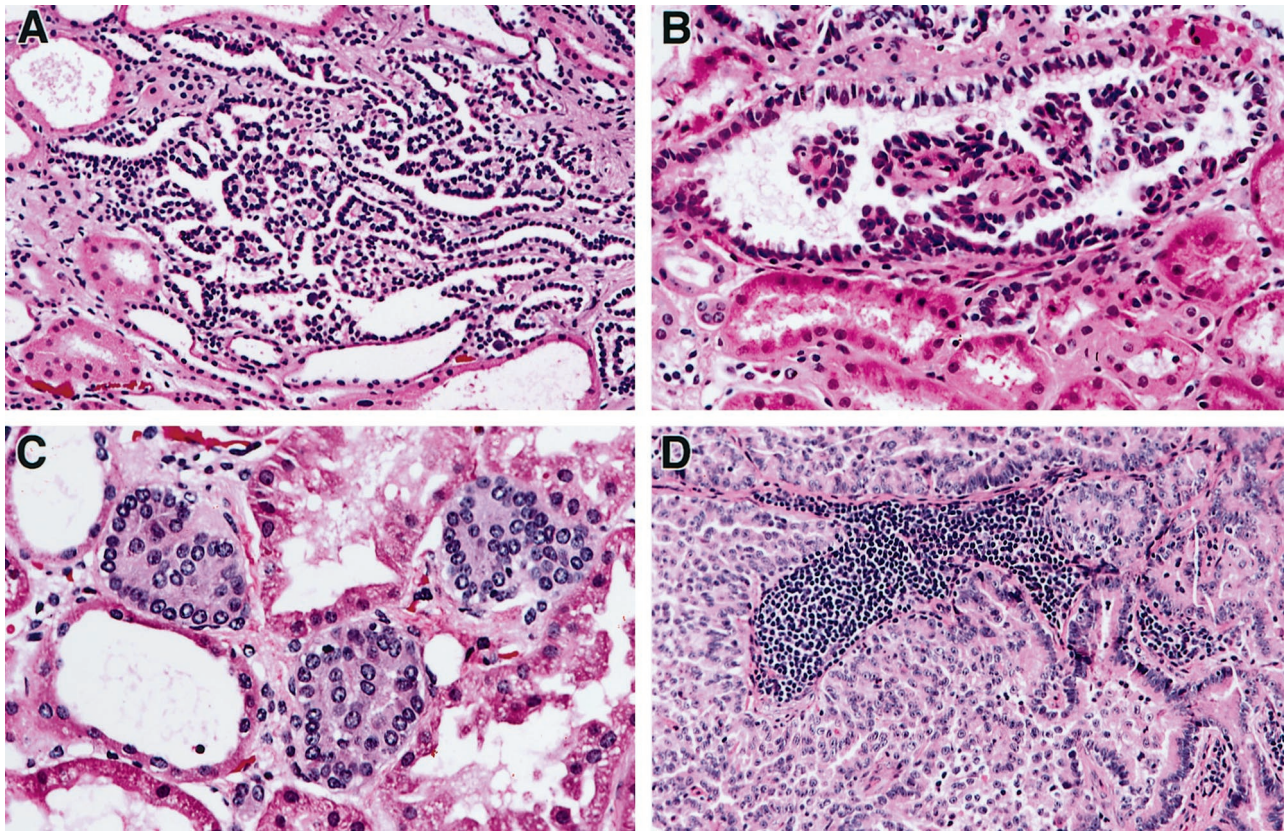


Figure 4. Multiple adenomas and microscopic papillary lesions in the surrounding kidney parenchyma and PRC metastases in hereditary PRC patients with *c-met* mutations have the architecture and histology which is similar to the architecture and histology of PRC. **A:** Papillary adenoma in patient 5946 with V1110I mutation (H&E, ×200). **B:** A microscopic papillary lesion in patient 4599 with H1112R mutation (H&E, ×400). **C:** Microscopic papillary lesions in patient 5946 with V1110I mutation (H&E, ×400). **D:** A lymph node metastasis in patient 3740 with H1112R mutation (family 150) (H&E, ×200).

collecting duct carcinoma, conventional (clear) cell carcinoma, renal cell carcinoma with 1;X translocation and metanephric adenoma.^{9, 34–36}

Hereditary PRC is a recently described entity and is characterized by multiple, bilateral renal neoplasms which are hypovascular and enhance poorly after contrast on CT/MRI.^{12, 13, 31} The disease is indolent; most patients are asymptomatic and are only found to have renal tumors during radiological screening. The genetic defect in hereditary PRC was mapped to the long arm of chromosome 7, and germline mutations of *c-met* oncogene at 7q31 were detected in affected patients.^{15, 16} Trisomies 7 and 17 are common in hereditary PRC,¹⁴ and trisomy 7 harboring non-random duplication of the mutant *c-met* proto-oncogene is thought to play a causative role in the development of multiple renal tumors.^{19, 20} Multiple bilateral renal tumors of hereditary PRC were shown to arise as different clones in the renal parenchyma.¹⁹

We report here the genotype-phenotype correlation in PRC with *c-met* mutations. We show that papillary renal neoplasms from the members of different PRC families and from patients with PRC who have no family history of renal tumors but have a mutation in the *c-met* oncogene (*c-met* genotype) share the same histological features of chromophil basophilic, type 1 PRC (PRC Type 1 phenotype). The neoplasms were multiple in kidney parenchyma and ranged in size from microscopic (less than a size of a single kidney tubule) to papillary adenomas

(less than 0.5 cm) to PRC (more than 0.5 cm). Tumors of different sizes were histologically similar to one another, suggesting that the histopathological classification based on size into microscopic renal lesion, adenoma and carcinoma is most likely arbitrary.

Papillary and/or tubulopapillary architecture was seen in all tumors but areas with solid and “metanephric adenoma-like” architectural pattern were also seen. Delicate fibrovascular cores lined by small cells with low grade (Fuhrman nuclear grade 1-2) basophilic nuclei and scant amphophilic cytoplasm predominated in all lesions regardless of size. However, tumors in 8 patients showed focal areas with Fuhrman nuclear grade 3 cells and prominent eosinophilic cytoplasm intermixed with Fuhrman nuclear grade 1–2 basophilic cells. Foamy macrophages and psammoma bodies were common in PRC with *c-met* mutations.

Clear cells were common in PRC tumors with *c-met* mutations regardless of size and constituted as much as 70% of some tumors. The cytoplasmic clearing was most prominent in tumors with necrosis and hemorrhage. In such areas clear cells showed foamy cytoplasm identical to the cytoplasm of foamy macrophages and could be secondary to phagocytosis of cellular debris by neoplastic epithelial cells. Clear cells contained intracytoplasmic lipid and glycogen and their predominance in some PRC raised the differential diagnosis of conventional (clear) cell carcinoma. Clear cells of PRC had small basophilic

nuclei and the tumor areas composed of clear cells lacked a fine vascular network characteristic of clear cell carcinoma. Delicate papillae cut in cross-section were consistently seen in clear cell areas of PRC. Predominance of other architectural patterns in clear cell carcinoma also helped to distinguish clear cell carcinoma from *c-met* positive PRC with clear cell areas.

The differential diagnosis of type 1 PRC with *c-met* mutations includes other renal tumors with papillary architecture such as PRC type 2, collecting duct carcinoma, renal cell carcinoma with 1;X translocation, conventional (clear) cell carcinoma, and metanephric adenoma.^{9, 34–36} Most of these neoplasms present as a solitary primary renal tumor, and all tumors histologically evaluated in this study for comparison lacked *c-met* mutations by molecular genetic analysis. PRC type 1 with *c-met* mutations are composed of small cells with basophilic, Fuhrman grade 1–2 nuclei, while both PRC type 2 and collecting duct carcinoma of the kidney are composed of larger cells with prominent eosinophilic cytoplasm and high grade nuclei (Fuhrman nuclear grade 3–4). Collecting duct carcinoma cells demonstrate focal intracytoplasmic mucin on mucicarmine stain which is negative in both type 1 and type 2 PRC. *U. europaeus* lectin, the stain commonly positive in collecting duct carcinoma of the duct of Bellini,⁹ was uniformly negative in PRC type 1 with *c-met* mutations.

Renal cell carcinomas of children and young adults with chromosome 1;X translocations may have prominent papillary architecture and often contain a mixture of large eosinophilic and clear cells with high grade nuclei and, in our experience, can be widely metastatic and have poor prognosis. The TFE3 gene was identified at the translocation breakpoint PRCC with 1;X translocation supporting a separate genetic mechanism for this type of renal cell carcinoma.^{37, 38}

Metanephric adenoma, in our experience, is the most common tumor type confused with PRC type 1 on histological examination. “Metanephric adenoma-like” architecture was focally observed in tumors from three hereditary PRC patients, however, nuclear and cytoplasmic features of cells in such areas were similar to the classic features of cells of type 1 PRC seen elsewhere in the tumor. True metanephric adenoma has predominately tubulopapillary architecture with solid areas and “glomeruloid-like” areas and lacks fibrovascular cores. The cells of metanephric adenoma show small densely basophilic nuclei which lack nucleoli, have a very scant cytoplasm³⁵ and have distinct ultrastructural and immunohistochemical characteristics.³⁶ Although one study suggested that metanephric adenoma may be related to PRC,³⁹ no *c-met* mutations were found in several metanephric adenomas studied by Schmidt et al.¹⁷

PRC type 1 with *c-met* mutations have metastatic potential. Documented metastases to the lymph nodes, lungs, and skeletal muscle were present in 4 patients with hereditary PRC. The metastases retained the architecture and histology of the primary tumors. The presence of papillae, psammoma bodies, basophilic nuclei with nuclear overlap and “grooves” should raise a differential diagnosis of papillary thyroid carcinoma if the tumor pre-

sents as a lymph node or lung metastasis. Negative thyroglobulin immunohistochemistry stain in metastatic cells will exclude the diagnosis of thyroid carcinoma.

c-met mutations were recently shown to play a role in 13% of patients with PRC and no family history of renal tumors.¹⁷ Interestingly, only half of these patients were found to harbor somatic *c-met* mutations and whereas another half harbored germline *c-met* mutations. Therefore, the rate of *c-met* mutations in truly sporadic PRC patients may be as low as 7%. We evaluated and report here histopathology of 6 PRC in five of the patients with *c-met* mutations and no family history of renal tumors: three with germline *c-met* mutations and two with somatic *c-met* mutations (Tables 1 and 2). All 6 PRC showed type 1 PRC histology identical to the histology of hereditary PRC patients.

A detailed pathological examination of the both kidneys of a 21-year-old man (patient 4967) from a PRC family 160 who was a H1112R mutation carrier and died of a gunshot wound revealed no renal lesions, which indicates that in hereditary PRC patients multiple PRC may not develop until later in life. Furthermore, affected individuals in PRC families are commonly asymptomatic. The tumors may not be detected until the intensive radiological screening of the family members is done and, therefore, the patient may report a negative family history for renal tumors. These reasons could contribute to the detection of the “germline” *c-met* mutations in reportedly “sporadic” PRC cases.¹⁷

In summary, patients with PRC with *c-met* mutations develop a spectrum of multiple bilateral renal lesions with papillary histology: PRC, papillary adenomas, and microscopic papillary lesions. Hereditary and sporadic PRC with a *c-met* genotype show a distinctive PRC type 1 phenotype. *c-met* positive PRC are genetically and histologically different from renal tumors seen in other hereditary renal syndromes (von Hippel-Lindau and familial renal oncocyoma)^{40, 41} and from most sporadic renal tumors with prominent papillary architecture (PRC type 2, RCC with 1;X translocation, collecting duct carcinoma, conventional (clear) RCC and metanephric adenoma). Although all hereditary and sporadic PRC with *c-met* mutations show a distinctive PRC type 1 histology, not all type 1 sporadic PRC harbor *c-met* mutations. The study supports the notion of the genetic heterogeneity of renal cell carcinomas in general and of PRC in particular and stresses the importance of the classification of renal neoplasms based on combined pathological and molecular genetic criteria.

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