

Papillary Renal Cell Carcinoma: Analysis of Germline Mutations in the *MET* Proto-Oncogene in a Clinic-Based Population

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ABSTRACT

Approximately 10% of all renal cell carcinomas (RCCs) present a distinctive papillary histology. Familial papillary RCC (PRCC) has been described, but the majority of cases appear to be sporadic. Recently, germline mutations in the *MET* proto-oncogene on chromosome 7 have been identified in families with hereditary PRCC. We evaluated 59 patients with PRCC for the frequency of *MET* germline mutations to determine the value of genetic screening of this patient population. Between 1976 and 1997, 165 patients were identified with PRCC by retrospective chart review. Fifty-nine of 133 surviving patients agreed to provide a family history, a blood specimen, and informed consent for genetic research. DNA was isolated from peripheral blood leukocytes. Denaturing high-performance liquid chromatography (DHPLC) followed by genomic sequencing was performed on eight exons of the *MET* proto-oncogene, including exons 5–7 of the extracellular domain, exon 14, and exons 16–19 of the tyrosine kinase domain. The 59 patients in this study included 49 men and 10 women with a mean age at diagnosis of 61 years. Bilateral and/or multifocal disease was present in 13 cases (22%). No germline mutations were detected in the studied exons of the *MET* proto-oncogene (exons previously reported to contain deleterious mutations in familial PRCC). No pathological *MET* proto-oncogene germline mutations were identified in 59 patients with PRCC. The germline mutation rate in this clinic-based population of individuals with PRCC approaches 0% (CI = 0–6.18). *MET* proto-oncogene germline mutation screening does not appear to be clinically indicated in patients with PRCC without additional evidence for a genetic predisposition (positive family history, unusual age at onset, bilateral disease).

INTRODUCTION

PAPILLARY RENAL CELL CARCINOMA (PRCC) is a morphologically and clinically distinct subtype of renal cell carcinoma (RCC). Second in frequency to clear-cell carcinoma, it constitutes approximately 8–14% of all renal carcinomas (Mancilla-Jimenez *et al.*, 1976; Mydlo and Bard, 1987). PRCC also appears to be genetically distinct from clear-cell carcinoma as well. The loss of the Y chromosome, trisomies 7 and 17, and a specific translocation between chromosome X and chromosome number 1, designated t(X;1)(p11.2;q21.2), have been described in hereditary and sporadic PRCC (Kovacs *et al.*, 1990; Meloni *et al.*, 1993; Zhuang *et al.*, 1998). These cytogenetic ab-

normalities differ from those seen in clear-cell carcinomas, which typically exhibit a chromosomal loss at the 3p region (Gnarra *et al.*, 1994). However, all these findings have been identified only as somatic (acquired) abnormalities confined to tumors. They are not germline mutations, and, therefore, they do not have utility as screening tools for identifying individuals at increased risk of PRCC.

A minority of the cases of PRCC have been identified in families with an inherited predisposition for PRCC (Zbar *et al.*, 1995). Recently, germline mutations in the *MET* proto-oncogene (chromosome 7q31.1–34) were discovered in families with hereditary PRCC (Schmidt *et al.*, 1997) implicating the *MET* proto-oncogene in the pathogenesis of hereditary PRCC. The

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MET proto-oncogene encodes a receptor tyrosine kinase that is widely expressed in epithelial cells. Increased expression of *MET* proto-oncogenes has been found in a number of human cancers implicating *MET* in their pathogenesis (DiRenzo *et al.*, 1995; Jeffers *et al.*, 1996) and somatic mutations have been found in childhood hepatocellular carcinomas (Park *et al.*, 1999). The prevalence of germline mutations has not been adequately studied to date in an unselected population of patients with PRCC. Finding a germline mutation in peripheral blood would have important implications for screening family members.

Denaturing high-performance liquid chromatography (DHPLC) is a novel automated mutation technology. It involves subjecting PCR products to ion-pair reverse-phase liquid chromatography. Heteroduplexes that form have elution profiles that differ from their homozygous or wild-type counterparts, easily identifying samples harboring mutations and polymorphisms. DHPLC has been applied to mutation detection in familial hemiplegic migraine, episodic ataxia type-2 disease (Ophoff *et al.*, 1996), *PTEN* mutations in primary glioblastomas (Liu *et al.*, 1997, 1998), and identification of single nucleotide polymorphisms (SNPs) of the Y chromosome (Underhill *et al.*, 1996). In addition, its use of automated instrumentation and speed of analysis make this a unique method of screening for large population studies. We identified a cohort of patients treated for PRCC at the Mayo Clinic, in Rochester and Scottsdale, between 1976 and 1997. DHPLC and DNA sequencing were used in an attempt to identify mutations of the *MET* proto-oncogene in patients with PRCC unselected for family history, to explore the proportion of PRCC that might be attributable to this genetic mechanism, and to determine if screening all patients with PRCC would be clinically justifiable.

MATERIALS AND METHODS

Patient ascertainment

Between 1976 and 1997, 165 patients were identified by medical history review with surgically treated PRCC. Records indicated that 32 were deceased. Under the auspices of an institutional review board (IRB)-approved protocol, letters of invitation were mailed to 133 patients not known to be deceased. Fifty-nine patients returned a completed family history questionnaire, signed a written informed consent document, and sent peripheral blood specimens for analysis. The questionnaire included a detailed family cancer history. Medical records were examined to obtain information on patient and tumor characteristics. All diagnoses were based upon interpretation by a Mayo Clinic pathologist's review of the tumor tissue.

Mutation detection

Eight exons of the *MET* gene were screened for mutations, including exons 16–19 of the tyrosine kinase domain, exons 5–7 of the extracellular domain, and exon 14. These exons were screened because they have previously been reported to contain deleterious mutations (Schmidt *et al.*, 1998, 1999). DNA from peripheral blood leukocytes was isolated and purified. Samples used for mutation screening and sequencing analysis were generated in 25- μ l reaction volumes containing 50–100 ng of genomic DNA, 0.25 μ M of sense and antisense primers

for *c-MET* exons 5–7, 14, and 16–19, 0.2 mM dNTPs (Promega, Madison, WI), 1 unit of *Taq* polymerase (Ampli Taq Gold, Perkin-Elmer), and 1 \times buffer supplied by the manufacturer. Primer sequences used in this study were as described (Schmidt *et al.*, 1999). PCR amplifications were 35 cycles: 95°C for 30 sec, 58°C for 45 sec, and 72°C for 45 sec (final extension at 72°C for 10 min) following sample denaturation at 95°C for 9 min. Synthesis of appropriately sized PCR reaction products was confirmed by agarose gel electrophoresis.

DHPLC analysis was carried out as described previously (Liu *et al.*, 1998) using the WAVE DNA fragment Analysis System (Transgenomic, Santa Clara, CA). Briefly, PCR products were denatured for 4 min at 95°C and then gradually reannealed by decreasing sample temperature from 95–65°C over a period of 30 min. PCR products were then separated (flow rate 0.75 ml/min) over a period of time and through a linear acetonitrile gradient. The column mobile phase consisted of mixture of 0.1 M triethylamine acetate, pH 7.0, with (buffer A) or without (buffer B) 25% acetonitrile. The mobile-phase temperatures required for optimal resolution of homoduplex and heteroduplex DNAs were determined empirically by injecting one PCR product for each exon at increasing temperatures until a significant decrease in sample retention time was observed. Specific values for the gradient ranges (buffer A component indicated), separation times, and mobile-phase temperatures used for analyzing the amplicons are as follows: 47–38%, 6 min, and 61°C for exon 5; 46–38%, 6 min, and 57°C for exon 6; 48–40%, 6 min, and 54°C for exon 7; 56–48%, 6 min, and 58°C for exon 14-1; 53–45%, 6 min, and 55°C for exon 14-2; 51–43%, 6 min, and 57°C for exon 16; 46–38%, 6 min, and 59°C for exon 17; 49–41%, 6 min, and 58°C for exon 18; and 47–39%, 6 min, and 57°C for exon 19.

PCR products that showed heteroduplex complexes by DHPLC analysis were directly sequenced as described previously (Yokomizo *et al.* 1998). Briefly, PCR products were cleaned with exonuclease and shrimp alkaline phosphatase, mixed with 3.2 pmol of forward or reverse primer of each exon, and then sequenced at the Mayo Molecular Core Facilities at the Mayo Clinic. Sequencing reactions were done in a Thermal Cycler 9600 (Perkin-Elmer Cetus) with fluorescent terminations and analyzed on an ABI377 sequencer.

RESULTS

This study cohort (Table 1) included 49 men and 10 women (male:female ratio of 4.9:1). The mean age at diagnosis was 61 years. Mean follow-up was 5.5 years for males and 7.1 years for females. Bilateral PRCC was noted in 4 patients (7%) and multifocal PRCC noted in 12 patients (20%). Either multifocal or bilateral disease was present in 11 men (22%) and 1 female (10%). Mean age at diagnosis of multifocal disease was 63.1, and for bilateral PRCC was 62.5 years. Of these patients, only four had elevated preoperative creatinine values (normal range 0.8–1.2).

Family history was assessed first by chart review on all 165 patients with documented PRCC (included those known to be deceased). A positive family history for kidney cancer was noted in only three cases. Next, all 59 consented study participants completed a family history questionnaire that asked for

TABLE 1. CHARACTERISTICS OF THE COHORT OF PATIENTS WITH PRCC IN THIS STUDY

	<i>M:F numbers (ratio)</i>	<i>Mean age at diagnosis</i>	<i>Mean years of follow up</i>
165 PRCC patients	135:30 (4.5:1)	61.1 (M); 57.4 (F)	NA
Presumed living PRCC patients per history review	111:22	61.0 (M); 58.5 (F)	8.2 (M); 9.0 (F)
Final study participants	49:10 (4.9:1)	63.3 (M); 59.3 (F)	5.5 (M); 7.1 (F)
Known deceased PRCC patients	24:8 (3:1)	61.5 (M); 54.5 (F)	NA

See Materials and Methods for additional details. M, Males; F, Females.

cancer history on all first- and second-degree relatives and through this, two additional family histories positive for kidney cancer were found. Looking at these five cases (three of whom were participants in this study), there were 3 females and 2 males with ages at diagnosis of 34, 58, and 68 for the women, and 58 and 64 for the men. In all instances, there was only one kidney cancer reported in the family history.

One history was suggestive of hereditary nonpolyposis colon cancer syndrome (HNPCC) and the others were unremarkable. One case with a positive family history had multifocal (but unilateral) PRCC; the other four cases had only single tumors. Confirmation of the kidney cancers reported in relatives and information about the pathology of the cancers were not available. We also looked for over-representation of other site-specific cancers, and saw no definite evidence for this, although of the 6 participants whose fathers had prostate cancer, 4 occurred in the subset of patients with multifocal/bilateral disease.

Denaturing HPLC analysis followed by genomic sequencing in the eight exons of the *MET* gene including exons 16–19, exons 5–7, and exon 14 revealed no pathological mutations. Two previously reported polymorphisms were identified. One was A2138G, in exon 7 (Schmidt *et al.*, 1997), and the other was C3223T, in exon 14 (Schmidt *et al.*, 1999). The frequency of the polymorphisms was 19/59 in exon 7, and 3/59 in exon 14.

DISCUSSION

In the past decade, researchers have shown that a small but important subset of individuals develops a particular cancer because of a genetic predisposition caused by a germline mutation in important growth-regulating genes. Around 5% of women who develop breast cancer have a germline mutation in the highly penetrant *BRCA1* and *BRCA2* genes (Ford and Easton, 1995). Two-to-five percent of individuals with HNPCC have a germline mutation in one-of-five DNA mismatch repair genes (Burt and Petersen, 1996; Wijnen *et al.*, 1998). Over 35 cancer predisposing syndromes have been recognized (for review, see Lindor and Greene, 1998), and likely many more will be discovered.

Germline mutations are detectable in DNA derived from peripheral blood leukocytes and may be potentially valuable for screening populations at risk. The significance of identifying carriers of germline cancer predisposing mutations is multifold. The patient involved may have high risk of multiple primary

tumors of the primary target organ; such knowledge may affect treatment recommendations. For example, patients with hereditary PRCC are at increased risk of cancer involving both kidneys; this understanding has led to the use of partial rather than total nephrectomy for persons so affected. In addition, the cancer predisposition may include several organ systems for which prospective surveillance may be necessary. For example, patients with HNPCC are at increased risk of endometrial, gastric, ovarian, renal pelvic, and ureteral cancers, in addition to colorectal cancer. Finally, the patient's family and relatives may be at higher risk of cancers and might benefit from earlier and aggressive site-specific cancer surveillance.

PRCC accounts for approximately 15% of renal cancers. A family history of PRCC has been described, but the majority of cases appear to be sporadic. Germline mutations in the *MET* proto-oncogene have now been reported in families with autosomal dominant PRCC (Schmidt *et al.*, 1999). Schmidt *et al.* (1997) also analyzed 129 PCRRs. *MET* proto-oncogene mutations were found in 17 tumors. Of these, eight were germline, suggesting that 6.2% of "sporadic" tumors had germline mutations. This incidence, albeit low, might suggest that screening for germline mutations in all patients with PRCC may be of benefit in identifying individuals and family members at increased risk of PRCC.

We evaluated a cohort of Mayo Clinic-based individuals with pathologically proven PRCC, unselected with regard to family history, to understand further the role of the *MET* proto-oncogene in PRCC, as well as to assess further whether screening of all patients with PRCC might be clinically valuable. Our evaluation of 59 patients with PRCC included 49 men and 10 women (4.9:1 ratio). Others have reported a very similar and striking male preponderance with male:female ratios ranging from 5:1 to 8:1 (Kovacs, 1993, 1994). Whereas it is possible that this reflects some unidentified lifestyle differences, it seems more likely to suggest a true gender-specific predisposition. In this regard, little is known about the role of hormones with respect to renal tumors. Kovacs *et al.* (1994), reported that 84% of PRCC in men showed loss of Y-chromosome-specific DNA, compared to 22% with loss in nonpapillary RCC. It is also noteworthy that the male:female ratio in nonpapillary RCC is 1.5:1. On the basis of these observations, Kovacs (1994) suggested that a tumor suppressor gene may be located at one of the homologous regions of the X and Y chromosomes and that homozygous inactivation is associated with development of

PRCC. Preferential loss of the Y chromosome by nondisjunction (compared to the X chromosome, which is larger and therefore less likely to be lost) might account for some of the male predilection of PRCC.

Further evidence to support X-linked gene involvement in PRCC is the specific chromosome translocation found in a few PRCC tumor cell lines: t(X;1)(p11;q21) (Meloni *et al.*, 1993; Sidhar *et al.*, 1996). Sidhar *et al.* (1996) showed that in PRCC associated with the translocation t(X;1)(p11.2;q21.2), the *TFE3* gene, a member of the helix-loop-helix family of transcription factors, is fused with a gene on chromosome 1, designated as the *PRCC* gene. This group demonstrated the presence of a *PRCC-TFE3* hybrid transcript in three PRCCs that contained the cytogenetic translocation and, thus, concluded that the *PRCC-TFE3* fusion protein apparently represents an amino-

terminal transcriptional activation domain adjacent to a *TFE3* DNA-binding domain. Weterman *et al.* (1996) cloned the *PRCC* gene. *PRCC* is ubiquitously expressed in normal adult and fetal tissues and encodes a protein of 491 amino acids with a relatively high content of proline. No relevant homologies with known sequences at either the DNA or protein level were found and its function is unknown.

Zbar *et al.* (1995) reported on nine families with two or more individuals with PRCC, and we note that in their series the male:female ratio of affected individuals was 28:12 (2.33:1), which is much lower than the ratio reported in PRCC in general. Clearly, the gender difference in PRCC provides potential clues to tumor etiology that merit additional molecular and epidemiological exploration.

Our mutation analysis revealed no missense mutations within

TABLE 2. SUMMARY OF STUDY PARTICIPANTS WITH BILATERAL OR MULTIFOCAL PRCC

	Sex	Age of Dx	PRCC tumors	Pre-op renal status ^a	Other medical problems	Family history of cancer
1	F	58	Bilateral	Creat = 0.9; malrotation of right kidney	None	Mo: breast Fa: prostate
2	M	48	Multifocal (L)	Creat = 1.5; mild proteinuria	Hypertension for 30 years; clear cell RCC 4 years before PRCC; glomerulosclerosis with focal segmental sclerotic/proliferative lesions.	Fa: prostate Mo: breast
3	M	55	Multifocal		? Crohn's disease with sclerosing cholangitis, post liver transplant; colon polyps	Fa: colon
4	M	60	Multifocal	Creat 1.2; congenital solitary kidney (R)		Mo; throat/tongue Bro: prostate
5	M	63	Multifocal (L)	Creat = 1.2; no proteinuria	Melanoma; prostate CA	Fa, Mo, Sis: unknown cancers
6	M	67	Multifocal (L)	Creat = 1.1; no proteinuria; one simple cyst		Bro: kidney (?type) age 72 Sis: "defective" kidney
7	M	68	Multifocal (R)		Three renal cysts (L); colon polyps; cavernous hemangioma in liver	Fa: prostate
8	M	71	Multifocal (L)	Creat = 1.3; UA normal	Prior clear cell RCC.; (L) pelvicolithotomy for calcium stones	Fa: prostate
9	M	71	Bilateral (20 in right; 6 in left)	Creat 1.5	Coronary disease; diabetes; hypertension; claudication	No family history of cancer
10	M	74	Bilateral	Creat 1.6; history of renal stones	Prostate and skin cancer	Bro: testicular
11	M	47	Bilateral	History of stones; normal function	Exposure to a fluorescent chemical later removed from market due to suspected carcinogenicity	MGM: leukemia 1/2 sis: endometrial
12	M	73	Multifocal (9 grade 1 lesions)	Creat 1.1; mult small bilat renal cysts	Prior clear cell RCC; alcoholism; hypertension	Mo: breast Mat aunt: breast MatG-aunt: leukemia

^aSerum creatinine normal values: adult female = 0.6–0.9 mg/dl; adult males = 0.8–1.2 mg/dl.

eight exons of the *MET* gene, including exons 5–7, 14, and 16–19. All previously reported missense mutations have been discovered within exons 16–19 of the tyrosine kinase domain of the *MET* gene. These mutations have not been detected in chromosomes from normal individuals (Schmidt *et al.*, 1997). Additionally, the mutation detection technique used, DHPLC, has been applied to mutation detection in many syndromes (Ophoff *et al.*, 1996; Underhill *et al.*, 1996; Liu *et al.*, 1997, 1998) with a sensitivity of $\geq 98\%$ (Liu *et al.*, 1998; Arnold *et al.*, 1999). Given this, we must conclude that the germline mutations in a clinic-based population of individuals with PRCC approaches 0% (CI = 0–6.18).

Why were no germline mutations detected? In the analysis by Schmidt *et al.* (1999), 6.2% of the cases of sporadic PRCC had mutations in the *MET* proto-oncogene. According to that paper, these cases were obtained from a number of contributors who referred their subjects to a center renowned for its interest in hereditary renal cancer syndromes. Did this represent all samples available to the referring collaborators or was there a bias towards referral of younger patients with bilateral or multifocal tumors, features known to be associated with inherited predispositions? Limited information was given on patient and tumor characteristics by Schmidt and colleagues. Of their 8 patients with germline mutations, some additional data are provided for 5 patients. One patient was noted to have a positive family history and all but 1 patient had bilateral disease (80%). The average age of the patients with germline mutations was 47 years old (range 39–55) and no information is provided on the incidence of multifocality.

In contrast, our study was a clinic-based population of individuals that included 13 patients (22%) with bilateral and/or multifocal PRCC with no germline mutations detected. The average age of diagnosis was 61 years, which differs significantly from the 47 years noted by Schmidt *et al.* (1999), suggesting existence of a different mechanism for disease.

We considered whether there could be other explanations for those patients in our study that had bilateral or multifocal disease, yet had no family history of PRCC or germline mutations in the *MET* proto-oncogene that would account for this. It is known that acquired cystic disease (ACD) develops in 40% of patients with end-stage renal disease, and ACD is associated with a 40-fold increased risk for RCC, evenly divided between papillary and nonpapillary types (Kovacs, 1995). ACD is three-fold more likely to develop in males than females, and cancer is seven times more likely to develop in males than females with end-stage kidneys. A high incidence of bilateral and/or multifocal PRCC has been reported in ACD (Amin *et al.*, 1997), so this could be a confounding factor in our search for genetic etiology.

Table 2 shows the clinical features of the subset of patients in our study with bilateral or multifocal disease. Although no clear pattern is apparent, in the aggregate there does seem to be a high rate of associated renal disease, including mild renal insufficiency, renal cysts, renal stones, prior clear cell carcinomas of the kidney, and congenital anomalies of the kidney. (We have also evaluated a man not included in this study group who had bilateral and multifocal PRCC and a normal *MET* proto-oncogene analysis, and who had multiple and bilateral calcium oxalate renal calculi presumably due to a prior resection of his ileum for inflammatory bowel disease.)

All these observations support the clinical impression that local factors in the kidney unrelated to the *MET* gene are likely important in the etiology of at least some PRCCs. Larger, carefully designed epidemiological studies with matched controls will be needed to determine the magnitude of risk associated with each of these features individually and additively.

Differences in patient ascertainment are likely to explain the different results in our study that differ from previous published data. Our study reflects a clinically based patient population not preselected for characteristics that might be associated with a higher probability of hereditary predisposition. In this population, we must conclude that *MET* proto-oncogene germline testing is not clinically indicated unless there are additional findings that suggest an inherited predisposition such as a young age at diagnosis, a positive family history for PRCC, and, perhaps, bilateral disease or multifocal disease.

REFERENCES

- AMIN, M.B., CORLESS, C.L., RENSHAW, A.A., TICKOO, S.K., KUBUS, J., and SCHULTX, D.S. (1997). Papillary (chromophil) renal cell carcinoma: histomorphologic characteristics and evaluation of conventional pathologic prognostic parameters in 62 cases. *Am. J. Pathol.* **21**, 621.
- ARNOLD, N., GROSS, E., SCHWARZ-BOEGER, U., PFISTERER, J., JONAT, W., and KIECHLE, M. (1999). A highly sensitive, fast and economical technique for mutation analysis in hereditary breast and ovarian cancers. *Hum. Mutat.* **14**, 333–339.
- BURT, R., and PETERSEN, G. (1996). Familial colorectal cancer. Diagnosis and management. In *Prevention and Early Detection of Colorectal Cancer*. G. Young, P. Rozen, and B. Levin (eds.). (W.B. Saunders, London) pp. 171–194.
- DI RENZO, M.F., OLIVERO, M., GIACOMINI, A., PORTE, H., CHASTRE, E., MIROSSAY, L., NORDLINGER, B., BRETTI, S., BOTTARDI, S., GIORDANO, S., *et al.* (1995). Overexpression and amplification of the *MET/HGF* receptor gene during the progression of colorectal cancer. *Clin. Cancer Res.* **1**, 147–154.
- FORD, D., and EASTON, D.F. (1995). The genetics of breast and ovarian cancer. *Br. J. Cancer* **72**, 805–812.
- GNARRA, J.R., TORY, K., WENG, Y., SCHMIDT, L., WEI, M.H., LI, H., LAFIT, F., *et al.* (1994). Mutations in the VHL tumor suppressor gene in renal cell carcinoma. *Nature Genet.* **7**, 85–90.
- JEFFERS, M., RONG, S., and WOUDE, G.F. (1996). Hapatocyte growth factor/scatter factor-*MET* signaling in tumorigenicity and invasion/metastasis. *J. Mol. Med.* **74**, 505–513.
- KOVACS, G. (1993). Molecular differential pathology of renal cell tumours. *Histopathology* **22**, 1.
- KOVACS, G. (1994). The value of molecular genetic analysis in the diagnosis and prognosis of renal cell tumours. *World J. Urol.* **12**, 64.
- KOVACS, G. (1995). *Nephrol Dial Transplant*, Ed. Comments, pp. 593–596.
- KOVACS, G., FUZESI, L., EMANUAL, A., and KUNG, H.F. (1990). Cytogenetics of papillary renal cell tumors. *Genes Chromosomes Cancer* **3**, 249–255.
- KOVACS, G., TORY, K., and KOVACS, A. (1994). Development of papillary renal cell tumours is associated with loss of Y-chromosome-specific DNA sequences. *J. Pathol.* **173**, 39–44.
- LINDOR, N., and GREENE, M.H. (1998). The concise handbook of family cancer syndromes. *J. Natl. Cancer Inst.* **90**, 1040–1071.
- LIU, W., JAMES, C.D., FREDERICK, L., ALDERETE, B.E., and JENKINS, R.B. (1997). *P TEN/MMAC1* mutations and EGFR amplification in glioblastomas. *Cancer Res.* **57**, 5254–5257.
- LIU, W., SMITH, D.I., RECHTZIGEL, K.J., THIDOBEAU, S.N., and

- JAMES, C.D. (1998). Denaturing high performance liquid chromatography (DHPLC) used in the detection of germline and somatic mutations. *Nucleic Acids Res.* **26**, 1396–1400.
- MANCILLA-JIMENEZ, R., STANLEY, R.J., and BLATH, R.A. (1976). Papillary renal cell carcinoma. A clinical, radiologic and pathologic study of 34 cases. *Cancer* **38**, 2469–2480.
- MELONI, A.M., DOBBS, R.M., PONTES, J.E., and SANDBERG, A.A. (1993). Translocation (X:1) in papillary renal cell carcinoma. A new cytogenetic subtype. *Cancer Genet. Cytogenet.* **65**, 1–6.
- MYDLO, J.H., and BARD, R.H. (1987). Analysis of papillary renal adenocarcinoma. *Urology* **30**, 529–534.
- OPHOFF, R.A., TERWINDT, G.M., VERGOUWE, M.N., VAN EIJK, R., OEFNER, P.J., HOFFMAN, S.M.G., LAMERDIN, J.E., MOHRENWEISER, H.W., BULMAN, D.E., FERRARI, M., HAAN, J., LINDHOUT, D., VAN OMMEN, G.-J.B., HOFKER, M.H., FERRARI, M.D., and FRANTS, R.R. (1996). Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca(2+) channel gene *CACNL1A4*. *Cell* **87**, 543–552.
- PARK, W.S., DONG, S.M., KIM, S.Y., NA, E.Y., SHIN, M.S., PI, J.H., KIM, B.J., BAE, J.H., HONG, Y.K., LEE, K.S., LEE, S.H., YOO, N.J., JANG, J.J., PACK, S., ZHUANG, Z., SCHMIDT, L., ZBAR, B., and LEE, J.Y. (1999). Somatic mutations in the kinase domain of the *MET* hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas. *Cancer Res.* **59**, 307–310.
- SCHMIDT, L., DUH, F.M., CHEN, F., KISHIDA, T., GLENN, G., CHOYKE, P., SCHERER, S.W., ZHUANG, Z., LUBENSKY, I., DEAN, M., ALLIKMETS, R., CHIDAMBARAM, A., BERGERHEIM, U.R., FELTIS, J.T., CASADEVALL, C., ZAMARRON, A., BERNUES, M., RICHARD, S., LIPS, C.J., WALTHER, M.M., TSUI, L.C., GEIL, L., ORCUTT, M.L., STACKHOUSE, T., and ZBAR, B. *et al.* (1997). Germline and somatic mutations in the tyrosine kinase domain of the *MET* proto-oncogene in papillary renal carcinomas. *Nature Genet.* **16**, 68–73.
- SCHMIDT, L., JUNKER, K., WEIRICH, G., GLENN, G., CHOYKE, P., LUBENSKY, I., ZHUANG, Z., JEFFERS, M., VANDE WOUDE, G., NEUMANN, H., WALTHER, M., LINEHAN, W.M., and ZBAR, B. (1998). Two North American families with hereditary papillary renal cell carcinoma and identical novel mutations in the *MET* proto-oncogene. *Cancer Res.* **58**, 1719–1722.
- SCHMIDT, L., JUNKER, K., NAKAIGAWA, N., KINJERSKI, T., WEIRICH, G., MILLER, M., LUBENSKY, I., NEUMANN, H.P.H., BRAUCH, H., DECKER, J., VOCKE, C., BROWN, J.A., JENKINS, R., RICHARD, S., BERGERHEIM, U., GERRARD, B., DEAN, M., LINEHAN, W.M., and ZBAR, B. (1999). Novel mutations of the *MET* proto-oncogene in papillary renal carcinomas. *Oncogene* **18**, 2343–2350.
- SIDHAR, S.K., CLARK, J., GILL, S., HAMOUDI, R., CREW, A.J., GWILLIAM, R., ROSS, M., LINEHAN, W.M., BIRDSALL, S., SHIPLEY, J., and COOPER, C.S. (1996). The t(X;1)(p11.2;q21.2) translocation in papillary renal cell carcinoma fuses a novel gene PRCC to the *TFE3* transcription factor gene. *Hum. Mol. Genet.* **5**, 1333–1338.
- UNDERHILL, P.A., JIN, L., ZEMANS, R., OEFNER, P.J., and CAVALLI-SFORZA, L.L. (1996). A pre-Columbian Y chromosome-specific transition and its implications for human evolutionary history. *Proc. Natl. Acad. Sci. USA* **93**, 196–200.
- WETERMAN, M.A.J., WILBRINK, M., and GEURTS VAN KESSEL, A. (1996). Fusion of the transcription factor *TFE3* gene to a novel gene, PRCC, in t(X;1)(p11;q21)-positive papillary renal cell carcinomas. *Proc. Natl. Acad. Sci. USA* **93**, 15294–15298.
- WIJNEN, J., VASEN, H., KHAN, P., ZWINDERMAN, A.H., VAN DER KLIFT, H., MULDER, A., POPS, C., MOLLER, P., and FODDE, R. (1998). Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. *N. Engl. J. Med.* **339**, 511–518.
- YOKOMIZO, A., TINDALL, D.J., DRABKIN, H., GEMMILL, R., FRANKLIN, W., YANG, P., SUGIO, K., SMITH, D.I., and LIU, W. (1998). *PTEN/MMAC1* mutations identified in small cell but not in non-small cell lung cancers. *Oncogene* **17**, 475–479.
- ZBAR, B., TORY, K., MERINO, M., SCHMIDT, L., GLENN, G., CHOYKE, P., WALTHER, M.M., LERMAN, M., and LINEHAN, W.M. (1994). Hereditary papillary renal cell carcinoma. *J. Urol.* **151**, 561–566.
- ZBAR, B., GLENN, G., LUBENSKY, I., CHOYKE, P., WALTHER, M.M., MAGNUSSON, G., BERGERHEIM, U.S.R., PETTERSSON, S., AMIN, M., HURLEY, K., and LINEHAN, W.M. (1995). Hereditary papillary renal cell carcinoma: Clinical studies in 10 families. *J. Urol.* **153**, 907–912.
- ZHUANG, Z., PARK, W.S., PACK, S., SCHMIDT, L., VORTMEYER, A.O., PAK, E., PHAM, T., WEIL, R.J., CANDIDUS, S., LUBENSKY, I.A., LINEHAN, W.M., ZBAR, B., and WEIRICH, G. (1998). Trisomy 7-harboring non-random duplication of the mutant *MET* allele in hereditary papillary renal carcinomas. *Nature Genet.* **20**, 66–69.

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