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Succinate Dehydrogenase Kidney Cancer (SDH-RCC): An Aggressive Example of the Warburg Effect in Cancer

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Abstract

Purpose—Recently, a new renal cell cancer (RCC) syndrome has been linked to germline mutation of multiple subunits (*SDHB/C/D*) of the Krebs cycle enzyme, succinate dehydrogenase. We report our experience with diagnosis, evaluation and treatment of this novel form of hereditary kidney cancer.

Materials and Methods—Patients with suspected hereditary kidney cancer were enrolled on an NCI-IRB approved protocol to study inherited forms of kidney cancer. Individuals from families with germline *SDHB*, *SDHC* and *SDHD* mutations and kidney cancer underwent comprehensive clinical and genetic evaluation.

Results—Fourteen patients from twelve *SDHB* mutation families were evaluated. Patients presented with RCC at an early age, 33 yrs (range 15–62 yrs), four developed metastatic kidney cancer and some families were found to have no manifestations other than kidney tumors. An additional family with six individuals found to have clear cell RCC that presented at a young average age, 47 yrs (range 40–53yrs), was identified with a germline *SDHC* mutation (R133X), two of which developed metastatic disease. A patient with a history of carotid body paragangliomas and a very aggressive form of kidney cancer was evaluated from a family with germline *SDHD* mutation.

Conclusions—SDH-RCC can be an aggressive type of kidney cancer, especially in younger individuals. Although detection and management of early tumors is most often associated with good outcome, based on our initial experience with these patients and our long term experience

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with HLRCC, we recommend careful surveillance of patients at risk for SDH-RCC and wide surgical excision of renal tumors.

Keywords

renal cell cancer (RCC); hereditary kidney cancer; Krebs cycle; Succinate dehydrogenase

Introduction

Kidney cancer is fundamentally a metabolic disease; each of the genes known to cause kidney cancer, *VHL*, *MET*, *FLCN*, *fumarate hydratase*, *succinate dehydrogenase*, *TSC1*, *TSC2*, *TFE3*, *TFEB*, *MITF*, and *PTEN* is involved in the fundamental cellular processes regulating the cell's response to sensing oxygen, iron, nutrients and energy.¹ Understanding the fundamental metabolic basis of kidney cancer should provide the foundation for the development of novel approaches to therapy for this disease.

Germline mutation of the Krebs cycle (tricarboxylic acid cycle) enzyme, *fumarate hydratase*, is associated with an aggressive form of type II papillary kidney cancer in patients affected with Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC).^{2–4} In fumarate hydratase-deficient kidney cancer, a prototypic example of the Warburg effect in cancer, oxidative phosphorylation is severely compromised and the cells undergo a metabolic shift to aerobic glycolysis to generate ATP and other metabolites required for rapid growth and cell division.^{5–8} The result is an extremely aggressive, lethal form of kidney cancer with a very high metabolic rate that has a propensity to metastasize when the primary tumor is very small (less than 1 centimeter).³

A second form of inherited kidney cancer characterized by a Krebs cycle gene mutation was initially reported by Vanharanta, et al., who described three patients with kidney cancer in families with a germline mutation of succinate dehydrogenase B (*SDHB*) also associated with hereditary paraganglioma (PGL), a group of diseases associated with head and neck PGL that can include a history of pheochromocytoma (PCC).⁹ While long recognized as having a hereditary component, the genes associated with this condition were difficult to identify as mapping studies linked suspected genes to different chromosomal regions (Figure 1A). Over the past decade the genetic basis of this syndrome has been identified and four specific syndromes are linked to gene mutations in a multi-complex Krebs cycle/electron transport chain enzyme, succinate dehydrogenase (SDH).^{10–13} This enzyme is made up of four subunits (*SDHA*, *SDHB*, *SDHC*, and *SDHD*) and catalyzes the oxidation of succinate to fumarate (Figure 1B). Additionally, germline mutations of *SDHB*, *SDHC* and *SDHD* have been identified in patients with a combination of gastrointestinal stromal tumors (GISTs) and paraganglioma (PGL) and patients presenting solely with GIST.^{14,15} Multiple reports identified the presence of RCC in patients with *SDHB* mutations either with or without a personal or family history of PGL and/or PCC.^{9,16–22} Furthermore, a recent investigation describing 2 individuals with Cowden or Cowden-like syndromic features including RCC, but no *PTEN* mutation, reported *SDHD* gene variants that affected the AKT and/or MAPK pathways.²³ After this work was initially submitted for publication, Malinoc et al. reported an individual patient with a germline *SDHC* gene mutation with personal and family history of PGL who was found to have RCC tumors with loss of the wild-type *SDHC* allele.²⁴ Little is known about the clinical features and management of patients with *SDH* mutation-associated RCC (SDH-RCC). In this report we describe the National Cancer Institute (NCI) experience with patients with SDH-RCC, including management and screening recommendations.

Materials and Methods

Patients at risk for familial kidney cancer were evaluated on an NCI-IRB approved clinical protocol that included genetic counselling and evaluation. *SDH* genetic testing was recommended if patients had early onset kidney cancer or suspected hereditary PGL or PCC. Genetic testing was performed on young patients with RCC (those under 45 years of age), those with bilateral/multifocal kidney tumors, and in families with suspected hereditary RCC. As part of the protocol, patients underwent genetic counselling prior to germline mutation testing. DNA was extracted from whole blood and mutation analyses were performed by direct sequencing on all coding exons of the *SDHB* (NM_003000.2), *SDHC* (NM_003001.3) and *SDHD* (NM_003002.2) genes. Testing was performed at a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory. Members of affected kindreds with *SDHB*, *SDHC* or *SDHD* mutations that had clinical manifestations such as RCC or pheochromocytoma/paraganglioma were offered evaluation.

Patients and families affected with *SDHB*, *SDHC* and *SDHD* germline mutations and RCC were identified. All available records, imaging, and pathologic slides were reviewed. Family members who were not evaluated at the NCI were included if both the clinical and pathologic information was available. Patient information was evaluated in addition to individual and familial manifestations of *SDH* mutation. Radiologic characteristics from contrast enhanced CT or MRI imaging were reviewed for laterality, tumor characteristics, the presence of multifocality, the degree of contrast-enhancement by Hounsfield units (HU), associated renal cysts, and the presence of necrosis, fat, or calcifications. The method of diagnosis (incidental, *SDH* germline mutation screening, or local, systemic symptoms), surgical management, 2010 TNM stage, tumor size, and margin status were evaluated.

Results

Succinate Dehydrogenase B (SDHB) Kidney Cancer

Fourteen patients from 12 families with germline *SDHB* mutations were evaluated. The family histories of 11 *SDHB* mutation-associated families were reviewed (one patient was adopted). In these kindreds, five (45.5%) only had known family members affected with RCC (Figure 2A) and six (54.5%) additionally had known family members with a chromaffin (PCC/PGL) tumor (Figure 2B). Two patients had a history of PGL or PCC and one patient had a metachronous, contralateral kidney cancer recurrence 17 years after initial renal tumor resection (Table 1). Four patients presented with local symptoms; three had systemic symptoms (one with a brain metastasis, and two with back pain from osseous metastases) and the remaining were diagnosed incidentally or by family surveillance screening. The mean and median age at *SDHB*-associated RCC presentation was 33 and 30 years old, respectively (range 15–61 years).

Preoperative imaging was available for ten *SDHB* mutation patients. No patient was found with synchronous bilateral or multifocal renal masses at diagnosis. Solid tumors were observed in seven (70%) patients while three (30%) had mixed solid/cystic lesions, which were classified as Bosniak IV cysts. All tumors demonstrated a brisk enhancement pattern typical of other solid renal neoplasms.

SDHB-RCC renal tumors were managed with partial or radical nephrectomy either at the NCI or outside institution. One patient (UOB-1 III:3) with kidney cancer who presented with widely metastatic disease, including brain metastases, was not a surgical candidate and received a renal biopsy prior to hospice care. All patients treated with partial nephrectomy had a final negative margin; however, one patient undergoing a robotic partial nephrectomy was converted to an open surgical procedure after an initial positive margin was found on

the intraoperative frozen section. *SDHB*-associated renal tumor histologies were variable; however most tumors shared common “oncocytic neoplastic” features (M. Merino et. al, In Preparation, Figure 3D). Four *SDHB* mutation patients were found to have metastatic disease, three (UOB-1 III:1 and III:3, UOB-4 III:2) at the time of initial presentation and one patient (UOB-10 III:1) with T1a disease that recurred in the liver three years post-operatively (Figure 3E and 3F). The pathologic tumor stage for available cases was T1/T2 (Figure 3A and 3B) in 12/13 cases (92.3%) and T3a in the remaining case (UOB-4 III:2, Figure 3C). No patients were found to have clinical or pathologic nodal involvement.

All the *SDHB* mutations, except *SDHB* p.Ile127Leu, have been previously shown to be associated with PGL and/or PCC, and thus were considered pathogenic (LOVD v2.0 TCA Cycle Gene Mutation Database)²⁵. The novel p.Ile127Leu alters an amino acid that has alternative variants (p.Ile127Asn, p.Ile127Ser) that have been shown to be associated with PGL and/or PCC, and was thus considered pathogenic. Alterations were observed throughout the length of the *SDHB* gene consisting of 1 nonsense (c.268C>T p.Arg90X), 4 missense (c.137G>A p.Arg46Gln, c.286G>A, p.Gly96Ser, c.379A>C p.Ile127Leu, c.689G>A p.Arg230His), 2 splice site altering mutations (c.286+2T>A, c.541-2A>G) and three complete deletions of exon 1. Mutations occurred along the length of the *SDHB* protein including all three critical regulatory sites (Figure 4A and 4B).

Succinate Dehydrogenase C (*SDHC*) Kidney Cancer

Family UOB-13 (Figure 5) has been followed at NCI for nearly 10 years. Five members of this family were found to have kidney cancer. While no germline mutations were detected in known familial RCC genes such as *VHL*, *FLCN*, *FH*, *SDHB* and *SDHD*, mutation analysis of *SDHC* revealed a c.397C>T germline mutation in the proband, her affected cousin and two of her offspring. Subsequent investigation of the family’s medical history revealed that one member of this kindred had a history of a neck paraganglioma (UOB-13 II:7). Abdominal imaging of a mutation positive family member (UOB-13 III:3) identified the presence of a small renal tumor, which was removed surgically (Figures 6C and 6D). The mean and median ages of SHDC-RCC presentation were 47 and 48 years old, respectively (range 40–53 years old).

Preoperative imaging for four patients from family UOB-13 with *SDHC* renal tumors was available for evaluation. No patient was found with multifocal renal involvement; however, one patient (UOB-13 II:9) subsequently developed metachronous, contralateral disease in the presence of widespread metastases (treated with cholecystectomy, brain stereotactic radiosurgery, and partial nephrectomy) (Figure 6F and 6H). All renal lesions were solid and demonstrated a brisk enhancement pattern. No patient was found with renal cystic disease and renal tumors had no evidence of fat or calcification.

Strikingly, all seven renal tumors from this family (UOB-13) with germline *SDHC* mutation were found to be classic clear cell RCC (Figure 6B and 6H). Fuhrman grading demonstrated that all were low-grade lesions (one grade 1 and six grade 2). Of the five tumors with known tumor stage, all were T1/2 (Figure 6A and 6E). Two patients developed metastatic disease, one of which presented with and died from metastatic disease (UOB-13 II:14), while the other (UOB-13 II:9) remains alive on systemic treatment.

The germline heterozygous *SDHC* nonsense mutation (c.397C>T p.Arg133X) identified in UOB-13 was previously associated with PGL/PCC.^{25–27} This mutation would be predicted to result in the loss of full-length protein production, which is also the case of the single *SDHC* mutation previously associated with RCC, pMet1Ile.²⁴

Succinate Dehydrogenase D (SDHD) Kidney Cancer

Patient UOB-14 III:3 came from a family with an extensive history of carotid body tumors (CBT) (father, two aunts, an uncle and paternal grandfather) and had a brother with a PCC (Figure 7A). This patient presented with bilateral CBT at the ages of 17 (left side) and 21 (right side), both of which were removed surgically. He later was found with hypertension and panic attacks that led to the discovery and resection of a retroperitoneal, extra-adrenal pheochromocytoma (PGL) at age 28 and a recurrent extra-adrenal PGL at age 31. At age 45, an 8 cm renal mass was detected, resulting in a left radical nephrectomy at an outside institution, which was found to be T3aNxM1 clear cell renal carcinoma. He was subsequently evaluated at NCI where staging revealed retroperitoneal, pulmonary, and osseous metastases (Figure 7B and 7C) as well as recurrent bilateral CBTs and he expired nineteen months after the diagnosis of RCC. Both he and his son were found to have an *SDHD* mutation (c.239G>T p.Leu80Arg) that has been previously shown to associate with PGL/PCC.²⁶

Discussion

We report the NCI experience with *SDHB*-, *SDHC*- and *SDHD*-RCC with a focus on clinical and genetic features and the management of this newly described hereditary RCC syndrome. Patients with early onset kidney cancer, those with bilateral, multifocal renal tumors or those with a family history of RCC or other cancers should be considered for *SDH* germline mutation testing. When considering germline *SDH* testing, a personal or family history of PGL, PCC, or a GIST should raise awareness of possible *SDHB*, *SDHC* or *SDHD* alterations, however RCC can present as the sole finding in these families.

Early age of onset of RCC has been previously observed in patients with *SDHB* mutations.^{22,28,29} Here we confirm this observation, reporting a mean and median age of onset as 33 and 30 years old respectively for *SDHB*-RCC. In *SDHC*-RCC the age of onset of kidney cancer also appears younger than the general RCC population but older than those with *SDHB* mutations (mean and median age 47 and 48, respectively, range = 40–53 years old). Based on our experience, patients with early onset RCC should raise suspicion for *SDH* kidney cancer.

Our early clinical experience with this disorder and the understanding of its metabolic basis has shaped our management in this patient population. Several of our patients died of metastatic kidney cancer and several developed metastatic disease from small primary tumors. Knowledge of HLRCC, the other Krebs cycle-associated kidney cancer, has influenced our management of *SDH*-RCC. HLRCC kidney cancers behave aggressively and may spread when the primary tumors are small.³ The metabolic basis of HLRCC and *SDH*-RCC share common features which may contribute to a similarly aggressive form of RCC. The FH protein lies one enzyme downstream from *SDH* and the metabolic basis of enzymatic loss of function may have metabolic similarity with the impairment of Krebs cycle function, reliance on glycolysis, and a metabolic shift to aerobic glycolysis (the Warburg effect).³⁰

We believe *SDH*-RCC should be managed in a manner similar to HLRCC renal tumors.³ As *SDH*-RCC tumors have the potential to spread when the tumors are small, early identification and prompt surgical intervention is recommended. While active surveillance is often recommended for the management of small (less than 3 cm) VHL, HPRC and BHD renal tumors, surveillance is not recommended for *SDH*-RCC. In the surgical management of *SDH*-RCC renal tumors, the potential for the development of metachronous, bilateral RCC (UOB-9 III:1, UOB-10 IV:1) should be considered. As with other hereditary cancer syndromes, *SDH*-RCC patients may be at a lifelong risk to develop renal tumors and partial

nephrectomy should be considered when feasible. Because of the potential for the development of aggressive tumors, we recommend wide surgical excision of SDH-RCC tumors. Similar to fumarate hydratase-deficient kidney tumors, SDH-deficient kidney cancer is characterized by impaired oxidative phosphorylation and a metabolic shift to aerobic glycolysis (Wei, et al, in preparation). If an SDH-RCC patient is suspected of having advanced disease, FDG-PET scanning is recommended.

Recommendations for SDH-RCC Evaluation, Management and Surveillance

SDHB/C/D germline mutation testing is recommended for patients with early onset kidney cancer (under age 45), for those with a family history of PCC or PGL and for those with a family history of kidney cancer. When a renal mass lesion is detected in a *SDHB/C/D* germline carrier, surgical resection with a wide surgical margin is recommended. Annual screening for the presence of kidney cancer and PCC/PGL is recommended. The PET scan has, in our limited experience, also been helpful in identifying foci of metastatic disease (Figure 3G and 3H).

Conclusions

Succinate dehydrogenase is a critical enzyme in the Krebs cycle and electron transport chain. Germline mutations in *SDHB*, *SDHC* and *SDHD* are not only associated with hereditary paraganglioma syndromes but also with hereditary kidney cancer. We have characterized the clinical manifestations and management of *SDHB*-, *SDHC*- and *SDHD* mutation-associated RCC. SDH-RCC can present at an early age and individuals under age 45 should be considered for this hereditary form of kidney cancer even in the absence of a family history. When SDH-RCC kidney tumors are detected at an early stage, patients can have a good outcome. However, patients with SDH-RCC can be at risk for the early development of metastatic disease if the tumor is not detected and treated early.

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Figure 1. The Chromosomal Location of the Succinate Dehydrogenase Enzyme Genes and the Enzyme Assembly

A) Chromosomal mapping of *SDHAF1* (19q13.12), *SDHA* (5p15.33), *SDHB* (1p36.13), *SDHC* (1q23.3), *SDHAF2* (11q12.2), *SDHD* (11q23.1). B) The succinate dehydrogenase enzyme subunits (SDHA/B/C/D) are assembled with the aid of the SDHAF1 protein and localized with the mitochondrial membrane, in association with the SDHAF2 protein, to form both Complex II of the Electron Transport Chain and a critical component of the Krebs's Cycle. (adapted from <http://en.wikipedia.org/wiki/File:SDHBfunction.svg>)

Succinate Dehydrogenase B (*SDHB*)-Associated Kidney Cancer Families

Figure 2. *SDHB*-Associated SDH-RCC Family Pedigrees

Pedigrees for *SDHB*-associated SDH-RCC families (except the adopted patient UOB-6, who was adopted) are shown in two groups: A) families presenting with kidney cancer only and B) families with RCC and pheochromocytoma/paraganglioma. Red denotes RCC and blue denotes pheochromocytoma/paraganglioma (PCC/PGL). *SDHB* germline mutation positive patients are designated by a black dot and assessed *SDHB* germline mutation negative patients are designated by an empty black circle.

Figure 3. Imaging and Histology of *SDHB*-Associated Kidney Cancer

A) A left sided 2.2 cm T1a “oncocytic renal cancer” was detected incidentally in a 19 year old female (UOB-8 III:1). B) A 17 year old male (UOB-12 II:1), who presented with back pain and gross hematuria, underwent a right radical nephrectomy and was found to have a 7.2 cm (T2a) right “oncocytic renal cancer” with sarcomatoid features. C) A 32 year old female (UOB-4 III:2) presented with a 3 month history of back pain and a bone marrow biopsy that revealed ‘metastatic cancer consistent with renal primary’. She underwent a radical nephrectomy for this T3aN0M1 kidney cancer that was diagnosed as an “oncocytic cancer” (D). She died with widespread disease 7 months after surgery. E) Patient UOB-10 III:1 presented as a 55 year old female with a 2.8 cm T1aN0M0 renal mass, which was resected at an outside hospital with clean surgical margins. F) Two and a half years later she was found to have hepatic metastases. G) Patient UOB-5 III:1 came to NCI for routine screening for his family history of *SDHB* mutation associated disease. On screening he was incidentally found to have a left lower pole 2.8 cm T1aN0M0 renal mass and a 1 cm periaortic paraganglioma. H) Preoperative [18]fluoro-deoxyglucose PET scan was positive for both the renal mass and the paraganglioma. A left partial nephrectomy and excision of the periaortic paraganglioma was performed.

Figure 4. Mutation Map of *SDHB* mutations associated with SDH-RCC Families

A) Map of *SDHB* gene mutation in *SDHB*-associated families with RCC. Exons are shown in blue and introns shown in black lines. *SDHB* mutations in NCI-UOB families are demonstrated in grey boxes. Previously published mutations are unshaded. Deletions in exon 1 are shown in red. B) *SDHB* protein mutation map demonstrates missense mutations located throughout the protein with amino acid changes in the mitochondrial targeting sequence as well as both iron-sulfur binding domains.

Succinate Dehydrogenase C (*SDHC*)-Associated Kidney Cancer Family

Figure 5. *SDHC*-Associated SDH-RCC

The pedigree for the *SDHC*-associated SDH-RCC family NCI-UOB-13 is shown with the occurrence of RCC (red) and/or pheochromocytoma/paraganglioma (Pheo/PGL) (blue). *SDHC* germline mutation positive patients are designated by a black dot and assessed *SDHC* germline mutation negative patients are designated by an empty black circle.

Figure 6. *SDHC*-Associated Kidney Cancer

A) Patient UOB-13 II:13 was a 49 year old female who presented for screening with a germline *SDHC* mutation and with 3 sisters affected with kidney cancer. On screening she was found to have a 3.3 cm enhancing left renal lesion. She underwent a partial nephrectomy with a wide surgical margin for removal of a T1aN0M0 clear cell kidney cancer (B).

C) Patient UOB-13 III:3 was a 38 year old male who was found to have a germline *SDHC* mutation. On screening evaluation a 1.4 cm enhancing, exophytic left renal mass was identified. He underwent a retroperitoneal laparoscopic partial nephrectomy with wide surgical margins for a clear cell kidney cancer (D).

E) Patient UOB-13 II:8 was a 44 year old female who presented to the NCI for an evaluation of familial renal cancer. She was found to have a 2 cm right renal mass which was removed by right laparoscopic partial nephrectomy and found to be a T1aN0M0 clear cell kidney cancer. She remains free of disease 8 years after surgery.

Patient UOB-13 II:9 presented at age 53 with a 10 cm renal tumor (clear cell), for which she had had a left radical nephrectomy. She subsequently underwent a cholecystectomy for a gallbladder recurrence and was found to have a 2.5 cm right renal tumor (F), for which she underwent a partial nephrectomy for a clear cell kidney cancer (H). She was subsequently found to have pulmonary metastases (G) for which she has received systemic therapy.

Succinate Dehydrogenase D (*SDHD*)-Associated Kidney Cancer Family

Figure 7. *SDHD*-Associated SDH-RCC

A) The pedigree for the *SDHD*-associated SDH-RCC family (NCI-UOB-14) is shown with the occurrence of RCC (red) and/or pheochromocytoma/paraganglioma (PCC/PGL) (blue). The mutation positive patients are designated by a black dot. Patient III:1 had multiple carotid body tumors and retroperitoneal, extra-adrenal pheochromocytomas (PGL). At age 45 he was found to have an 8 cm left renal mass, which was removed surgically and found to be a T3aNxM1 clear cell renal carcinoma. He was subsequently found to have retroperitoneal (B) and pulmonary (C) recurrence and he died 18 months after the diagnosis of kidney cancer.

Clinicopathologic and mutation analysis for SDHB, SDHC and SDHD RCC patients.

Table 1

SDH Subunit	Family	Patient	Mutation	Age	Chromaffin Tumor History		Metastatic Disease (site)	Sex	Presentation	Stage (TMN)	Size	Surgery	Status at Follow-Up (months)
					Personal	Family							
B	UOB-1	III:3	Exon 1 Deletion	36	No	No	Yes (OS)	M	Systemic	TxNxM1	N/A	Biopsy	DOD (N/A)
B	UOB-1	III:1	N/S	25	No	No	Yes (OS)	M	Systemic	T1aN0M1	3.8	RN	DOD (N/A)
B	UOB-2	III:1	Exon 1 Deletion	27	No	No	No	F	Incidental	T1aN0M0	2.7	PN	NED (17.0)
B	UOB-3	II:1	Exon 1 Deletion	42	Yes	Yes	No	F	Incidental	T1aN0M0	2.2	PN	NED (3.3)
B	UOB-4	III:2	c.137G>A (p.Arg46Gln)	32	No	Yes	Yes (OS)	F	Systemic	T3aN0M1	5	RN	DOD (6.1)
B	UOB-5	III:1	c.268C>T (p.Arg90X)	61	Yes	Yes	No	M	Incidental	T1aN0M0	2	PN	NED (50.4)
B	UOB-6	-	c.286-2T>A (Splice)	37	NA	NA	No	M	Local	Unknown	11	RN	NED (30.0)
B	UOB-7	III:1	c.379A>C (p.Ile127Leu)	28	No	No	No	M	Local	T1bN0M0	5	PN	NED (36.2)
B	UOB-8	III:1	c.541-2A>G (Splice)	19	No	Yes	No	F	Incidental	T1aN0M0	2	PN	NED (40.6)
B	UOB-9	III:1	c.689G>A (p.Arg230His)	52	No	Yes	No	F	Incidental	T1aN0M0	5	RN	NED (35.5)
B	UOB-10	IV:1	c.286G>A (p.Gly96Ser)	17	No	No	No	F	Local	T2aN0M0	9	RN	NED (212.4)
				34					Incidental	T1aN0M0	1.5	PN	
B	UOB-10	III:1	c.286G>A (p.Gly96Ser)	55	No	No	Yes (LI,OS)	F	Incidental	T1aN0M0	1.8	PN	AWD (65.8)
B	UOB-11	V:1	c.268C>T (p.Arg90X)	15	No	Yes	No	M	Incidental	T1aN0M0	2	PN	NED (10.8)
B	UOB-12	II:1	c.137G>A (p.Arg46Gln)	17	No	No	No	M	Local	T2aN0M0	7.2	RN	NED (6.0)
C	UOB-13	II:14	N/S	46	No	Yes	Yes (N/A)	M	Unknown	TxNxM1	N/A	RN	DOD (133.5)
C	UOB-13	II:11	N/S	52	No	Yes	No	F	Unknown	Unknown	N/A	RN	DOC (182.7)
C	UOB-13	II:9	c.397C>T (p.Arg133X)	53	No	Yes	Yes (LI,LU,BR)	F	Local	T2aN0M0	10	RN	AWD (54.8)
				68					Incidental	T1aN0M1	2.5	PN	
C	UOB-13	II:13	c.397C>T (p.Arg133X)	49	No	Yes	No	F	Incidental	T1aN0M0	3	PN	NED (240.8)
C	UOB-13	II:8	N/S	44	No	Yes	No	F	Incidental	T1aN0M0	1.5	PN	NED (62.0)
C	UOB-13	III:3	c.397C>T (p.Arg133X)	40	No	Yes	No	M	Screened	T1aN0M0	2	PN	NED (12.3)
D	UOB-14	III:1	c.239G>T (p.Leu80Arg)	45	Yes	Yes	Yes (OS,LU,R)	M	Unknown	T3aNxM1	8	RN	DOD (18.7)

N/S – Not Screened for Mutation N/A – Not Available RN – Radical Nephrectomy PN – Partial Nephrectomy Incidental – Detected while Asymptomatic, Local – Localized Symptoms, Systemic – Metastatic site/Constitutional Symptoms, Screened – Due to Germline Mutation Site of Metastases: LI – Liver, LU – Lung, OS – Osseous, BR – Brain, R - Retroperitoneal DOD – Dead of Disease, AWD – Alive with Disease, NED – No Evidence of Disease, DOC – Died of Other Cause