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Molecular and immunohistochemical characterization reveals novel *BRAF* mutations in metanephric adenoma

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Abstract

Metanephric adenoma (MA) is a rare benign renal tumor comprised of a neoplastic proliferation of primitive metanephric tubular cells. A previous study identified *BRAF* V600E mutations in approximately 90% of MA and found that similar *BRAF* exon 15 mutations are exceedingly rare in other common renal tumors, including renal cell carcinoma (RCC) and oncocytoma. A recent follow-up study has validated mutation-specific immunohistochemistry (IHC) for detection of *BRAF* V600E mutations in a small cohort of MA. Here, we extend these findings to a larger, independent cohort of MA, demonstrating an overall 88% sensitivity and 100% specificity for *BRAF* V600E IHC. In addition, we report two cases of MA with novel *BRAF* exon 15 mutations, including a V600D missense mutation and a compound V600D and K601L missense mutation. Finally, we evaluate *BRAF* V600E IHC in a large TMA cohort of common renal tumors and find no significant expression in several RCC subtypes. These data support a role for *BRAF* V600E IHC in diagnostically challenging cases of MA and expand the spectrum of *BRAF* exon 15 mutations in this uncommon but unique renal neoplasm.

Keywords

BRAF; metanephric adenoma; immunohistochemistry; V600E; Sanger sequencing

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Introduction

Metanephric adenoma (MA) is a benign, often asymptomatic and incidentally identified renal tumor comprised of primitive metanephric tubular cells¹⁻³. Patients commonly present in the fifth to sixth decade of life, and there is a slight female preponderance (F:M = approximately 2:1). Grossly, MA is usually a solid, solitary, unilateral mass that lacks a true fibrous capsule. On average, these tumors measure 5.5 cm in greatest dimension, however, they may be very small (less than 0.5 cm) or large (greater than 10 cm). Microscopically, MA usually appears solid at low magnification, although areas of cystic degeneration are sometimes present¹⁻³. On higher magnification, these tumors consist of primitive metanephric tubular epithelial cells with scant cytoplasm and monotonous round to oval nuclei with fine chromatin and without nucleoli; these cells are usually arranged in small acinar structures, although areas with papillary, tubular, and glomeruloid growth patterns may be identified. Generally, there is minimal admixed paucicellular or hyalinized stroma, but cases may also demonstrate regressive changes, including dense sclerosis, dystrophic calcification, and/or psammomatous calcification. Mitotic activity is usually very low or absent in MA, and necrosis is very rare.

In the majority of cases, MA can be diagnosed on routine hematoxylin and eosin (H&E) stain; however, the differential diagnosis includes the solid variant of papillary renal cell carcinoma (RCC), as well as epithelial predominant Wilms tumor (WT) – both of which require definitive treatment¹⁻³. In challenging cases, particularly core needle biopsy specimens, immunohistochemistry (IHC) may be helpful³⁻⁵. MA usually express WT1 and CD57 and can demonstrate focal CK7 expression in areas with elongated tubules^{3, 6}.

A recent study by *Choueiri et al.* has begun to shed light on the molecular underpinnings of this renal tumor, demonstrating that approximately 90% of MA harbor *BRAF* V600E mutations⁷. *BRAF* encodes a serine/threonine-specific protein kinase upstream of the MAPK/ERK signaling pathway, and somatic activating *BRAF* mutations have been identified in a wide variety of common human malignancies, including melanoma, papillary thyroid carcinoma, and colonic adenocarcinoma^{8, 9}. *BRAF* exon 15 mutations, including the V600E missense mutation, are frequently detected in a range of benign and malignant human tumors¹⁰⁻¹⁵, however, *BRAF* mutations in common non-MA renal tumors (i.e., RCC, oncocytoma, WT) are either very infrequent (less than 1%) or absent^{7, 16, 17}. A mutation-specific antibody against the *BRAF* V600E protein product has been recently validated for IHC detection of *BRAF* V600E mutations *in situ* and utilized successfully in a variety of human tumor types^{10, 18-26}. In a follow-up study to their work on *BRAF* V600E mutations in MA, *Pinto et al.* demonstrated that six of six (100%) MA cases, including five with confirmed *BRAF* V600E mutations, exhibited *BRAF* V600E expression by mutation-specific IHC²⁷. In contrast, less than 1% of cases from a large TMA cohort of common renal tumors demonstrated *BRAF* V600E expression.

In this study, we extend the findings reported in *Pinto et al.* to a larger, independent cohort of MA and report two cases of *BRAF* V600E-negative MA with novel *BRAF* exon 15 mutations. Finally, we evaluate *BRAF* V600E protein expression by mutation-specific IHC in a large TMA cohort of common renal tumors.

Materials and Methods

Identification of MA cases

This study was approved by the Institutional Review Board at the University of Michigan Medical School. A comprehensive retrospective search of the University of Michigan Health System (UMHS) pathology records database was performed to identify all available MA cases between 1985 and 2014, and a total of eleven such cases were available for the purposes of this study. H&E stained slides from all cases were reviewed by two study pathologists (A.M.U. and R.M), and representative formalin-fixed, paraffin-embedded (FFPE) tissue blocks were selected for BRAF V600E IHC and *BRAF* exon 15 sequencing.

Renal tumor tissue microarray (TMA) construction

A TMA representing common renal tumor types from eighty-six unique patients was constructed with FFPE tissue from partial or total nephrectomy specimens retrieved from the UMHS pathology specimen archive. This TMA included specimens from various renal tumor types, including: chromophobe RCC (n = 26); oncocytoma (n = 20); papillary RCC (n = 20); clear cell RCC (n = 16); RCC, unclassified (n = 2); clear cell papillary RCC (n = 1); and, Xp11 translocation-associated RCC (n = 1). The tumor samples were represented on this TMA in at least triplicate cores, and samples of benign renal parenchyma from ten patients served as internal controls.

BRAF V600E IHC

Whole sections were obtained from MA FFPE tissue blocks for BRAF V600E IHC (clone VE1; pre-dilute; 790-4855; Ventana Medical Systems, Tucson, AZ), which was performed using a BenchMark ULTRA automated stainer and the ultraView Universal DAB Detection Kit (Ventana Medical Systems) by the CLIA-certified clinical IHC laboratory of the Department of Pathology at UMHS. This anti-BRAF V600E antibody is a mouse monoclonal antibody generated against a synthetic peptide representing the mutated BRAF V600E amino acid sequence (from amino acid 596 to 606; GLATEKSRWSG)^{18, 20}. Whole sections from a melanoma case with a confirmed *BRAF* V600E mutation were included as batch positive controls for BRAF V600E IHC; consistent with previously published data (as well as practical experience in the UMHS clinical IHC laboratory), the batch positive control demonstrated diffuse, moderate to strong cytoplasmic staining in melanoma cells but negative or weak staining in adjacent non-neoplastic tissue^{10, 18-27}. BRAF V600E IHC results for all MA cases were reviewed and scored independently by two study pathologists (A.M.U. and R.M.), who were blinded to both the general and specific *BRAF* exon 15 genomic sequencing results; only cases with diffuse, moderate to strong cytoplasmic staining in tumor cells were recorded as BRAF V600E IHC positive. For assessment of the renal tumor TMA, only cases with cores showing diffuse, moderate to strong cytoplasmic staining in tumor cells were recorded as BRAF V600E IHC positive.

BRAF exon 15 genomic sequencing

For each MA case, tumor genomic DNA was isolated from FFPE tissue blocks using the QIAamp DNA FFPE Tissue Kit (Qiagen, Venlo, Netherlands), according to the

manufacturer's protocol. Commercially available male human genomic DNA (G1471; Promega, Madison, WI) served as a wild type *BRAF* exon 15 control, while genomic DNA isolated from SK-MEL-5 cells (HTB-70; ATCC, Manassas, VA) using the DNeasy Blood & Tissue Kit (Qiagen) served as a positive *BRAF* V600E control. *BRAF* exon 15 was PCR amplified from 50 nanograms of genomic DNA using flanking sequence-specific primers (forward: 5'-M13-TTTGTGAATACTGGGAACTATGAAA-3'; and, reverse: 5'-TCATCCTAACACATTTCAAGCC-3') and HotStarTaq DNA polymerase (Qiagen). The PCR conditions were as follows: initial denaturation at 95°C for 15 minutes; 45 cycles of 94°C for 60 seconds, 50°C for 60 seconds, and 72°C for 60 seconds; and, final extension at 72°C for 7 minutes. After confirming the expected amplicon size by standard agarose gel electrophoresis, all PCR products were incubated with ExoSAP-IT (Affymetrix, Santa Clara, CA) prior to bi-directional Sanger sequencing by the University of Michigan DNA Sequencing Core using the M13 forward and *BRAF* exon 15 PCR reverse primers. The resulting chromatograms were analyzed with Sequencher software, version 4.5 (Gene Codes, Ann Arbor, MI), and compared to reference sequence for *BRAF* exon 15 (NM_004333.4).

BRAF Exon 15 subcloning

BRAF exon 15 PCR products from samples with complex somatic aberrations were resolved by standard gel electrophoresis, and the excised DNA fragments were purified using the S.N.A.P. Gel Purification Kit (Life Technologies, Waltham, MA). The purified PCR products were subcloned into the pCR4-TOPO vector using the TOPO TA Cloning Kit (Life Technologies) and transformed into Mach1 cells (Life Technologies) under kanamycin selection. Plasmid DNA was isolated from ten independent bacterial colonies and subjected to Sanger sequencing with M13 forward or reverse primers by the University of Michigan Sequencing Core. The resulting chromatograms were analyzed as described above.

Results

Clinicopathologic features of a retrospective MA cohort

Eleven cases of MA from the UMHS pathology records database were available for this study (Table 1). These patients included six women and five men, and the age at diagnosis ranged from 16 to 84 years (median = 45 years). Six of the tumors arose in the right kidney, while four developed in the left kidney; one MA occurred within the left side of a horseshoe kidney. One patient had polycythemia at the time of presentation, consistent with prior estimates of the incidence in patients with MA¹. Eight of the cases were from partial or total nephrectomy specimens, while the other three were from core needle biopsies. The maximum tumor dimension ranged from 1.3 to 5.1 cm (median = 2.7 cm). These tumors demonstrated the typical histomorphologic spectrum of MA, including varying degrees of hyalinization, psammomatous calcification, and papillary, tubular, or glomeruloid growth patterns (see Table 1 for details).

BRAF V600E IHC in a retrospective cohort of MA

Given the reported high frequency of *BRAF* V600E mutations in MA⁷, as well as the high sensitivity and specificity of mutation-specific IHC for detecting these mutations in a wide

variety of neoplasms (including MA)^{10, 18, 19, 21, 22, 24-27}, we sought to examine BRAF V600E IHC in our retrospective MA cohort. Seven (64%) of the cases in our cohort demonstrated diffuse, moderate to strong cytoplasmic BRAF V600E IHC staining (Figure 1), while the remaining four cases showed negative to weak cytoplasmic BRAF V600E IHC staining (Figure 2). Of note, in the vast majority of cases, the adjacent benign renal parenchyma showed variable weak cytoplasmic and weak to moderate nuclear staining. Overall, the tumor areas in the IHC positive cases demonstrated unequivocal, diffuse moderate to strong cytoplasmic expression of mutated BRAF protein.

Concordance between BRAF V600E mutation status by mutation-specific IHC and Sanger sequencing of BRAF exon 15

As determined by mutation-specific IHC, the frequency of *BRAF* V600E mutations in our retrospective MA cohort was lower than previously reported (64% versus 89%)⁷. To further investigate this apparent difference, we interrogated our cohort for V600E mutations via Sanger sequencing of *BRAF* exon 15. Genomic DNA harvested from the SK-MEL-5 melanoma cell line (which carries the *BRAF* V600E mutation) and commercially available wild type male human genomic DNA were utilized as positive and negative controls, respectively (data not shown). All seven cases with diffuse, moderate to strong cytoplasmic BRAF V600E IHC staining harbored a thymidine to adenine substitution at codon 600 (i.e., GTG → GAG), which corresponds to the *BRAF* V600E missense mutation (Figure 1). In addition, for one of the remaining four cases with negative to weak cytoplasmic BRAF V600E IHC staining, Sanger sequencing demonstrated a *BRAF* V600E mutation (Figure 2J-L). All other cases were negative for *BRAF* V600E mutations, and no other *BRAF* exon 15 mutations were detected in tumors harboring *BRAF* V600E mutations. Therefore, overall eight (73%) of the cases in our retrospective MA cohort contain *BRAF* V600E mutations, and the overall sensitivity and specificity for mutation-specific IHC was 88% and 100%, respectively.

Novel BRAF exon 15 mutations in V600E-negative MA

For the three remaining cases with negative to weak cytoplasmic BRAF V600E IHC staining and without detectable *BRAF* V600E mutations by Sanger sequencing, *BRAF* exon 15 was closely examined for novel, non-V600E mutations. For one case, compound two base substitution of adenine and thymidine at codon 600 for thymidine and guanine results in the missense *BRAF* V600D mutation (Figure 2D-F), and for another case, a complex compound substitution and/or indel at codons 600 and 601 results in the compound missense *BRAF* mutations V600D and K601L (Figure 2G-I). These novel *BRAF* exon 15 mutations were subsequently confirmed by Sanger sequencing of the subcloned PCR product (data not shown). No *BRAF* exon 15 mutations were identified in the third case (Figure 2A-C).

BRAF V600E IHC in a large TMA cohort of common renal tumors

Finally, previously published data suggests that *BRAF* V600E mutations are rare in other common renal tumors^{7, 17, 27}. Investigation of the available provisional The Cancer Genome Atlas (TCGA; www.cbioportal.org) renal cancer datasets showed no *BRAF* codon 600 mutations in either chromophobe RCC (n = 66) or clear cell RCC (n = 523), and only one

case of papillary RCC (n = 168) harbored a *BRAF* V600E mutation; no other *BRAF* exon 15 mutations were identified in this large RCC sequencing cohort. In addition, a recent study examined *BRAF* V600E IHC in a large TMA cohort of renal tumors, including clear cell RCC, papillary RCC, chromophobe RCC, and oncocytoma, and found that less than 1% of these tumors showed positive staining with this mutation-specific antibody²⁷. We sought to independently extend these findings in a large TMA cohort of common renal tumors from our institution. *BRAF* V600E IHC was performed on a TMA comprising tumor samples from eighty-six unique patients (see Materials and Methods). Consistent with the rarity of documented *BRAF* V600E mutations in common renal tumors, none (0%) of the 84 tumors available for evaluation demonstrated diffuse, moderate to strong cytoplasmic staining (Figure 3). Importantly, representative cores of benign renal parenchyma from a subset of the TMA tumor cases demonstrated variable weak cytoplasmic and weak to moderate nuclear staining – similar to the aforementioned adjacent benign renal parenchyma in the MA cases. This reproducible (albeit non-specific) background staining serves as a positive internal control for *BRAF* V600E IHC and, thereby, supports the experimental validity of negative *BRAF* V600E IHC in this TMA cohort of common renal tumors.

Discussion

In this study, we examined *BRAF* V600E protein expression by mutation-specific IHC in a large cohort of MA. *BRAF* V600E IHC showed high sensitivity and specificity (88% and 100%, respectively) for the detection of *BRAF* V600E mutations (as assayed by Sanger sequencing) in this MA cohort. In addition, we report three cases of *BRAF* V600E-negative MA, including two with novel *BRAF* exon 15 mutations: one with a *BRAF* V600D mutation; and, one with compound *BRAF* V600D and K601L mutations. Finally, we showed that *BRAF* V600E protein expression by mutation-specific IHC was essentially absent in a large TMA cohort of common renal tumors.

Our results are in concert with previously published data regarding *BRAF* V600E mutations and *BRAF* V600E protein expression in MA^{7, 27}. One additional case of MA with a confirmed *BRAF* V600E mutation has been reported in the literature²⁸. Thus, together with the cases reported in this study, thirty-five of forty-one (85%) published genotyped MA harbor *BRAF* V600E. When the novel *BRAF* mutations identified in our study are included, overall thirty-seven of forty-one (90%) published genotyped MA contain *BRAF* exon 15 mutations. Similarly, of the published MA cases analyzed for *BRAF* V600E protein expression by mutation-specific IHC, thirteen of seventeen (76%) showed the diffuse, moderate to strong cytoplasmic staining characteristic of tumors with *BRAF* V600E mutations^{10, 18-27}. The diagnosis of MA is often straightforward on routine H&E stains, however, some cases present challenging diagnostic dilemmas – particularly differentiating MA from the solid variant of papillary RCC⁴. In addition, abdominal radiographic imaging has increased the number of incidentally identified small renal masses, many of which are amenable to core needle biopsy diagnosis²⁹; however, the diagnosis of MA on core needle biopsy may be challenging because of limited material available for evaluation. In these cases, ancillary studies (especially IHC) may be helpful³⁻⁵. MA characteristically express WT1 and CD57 and are frequently negative for CK7 and AMACR expression⁶. The value of *BRAF* V600E IHC in diagnostically challenging cases has not been specifically examined,

however given the relatively low frequency of documented *BRAF* V600E mutations and/or *BRAF* V600E expression in other common renal tumors^{7, 16, 17, 27}, positive IHC staining for *BRAF* V600E supports the diagnosis of MA. At the same time, however, it is also important to recognize that the absence of *BRAF* V600E staining does not exclude the possibility of MA.

This study is the first to document non-V600E *BRAF* exon 15 mutations in MA. In the only other published large MA sequencing study, a small pilot cohort of seven tumors was initially analyzed for oncogenic mutations by mass spectrometric genotyping with the OncoMap platform, which identified recurrent *BRAF* V600E mutations⁷; an additional twenty-two cases were then analyzed for specific hotspot *BRAF* mutations (including *BRAF* V600D) by multiple base extension (MBE) chemistry and secondarily validated by pyrosequencing with the *BRAF* Pyro[®] kit (Qiagen) in a CLIA-certified laboratory. Interestingly, in contrast to our study, no non-V600E *BRAF* exon 15 mutations were identified. In addition to one case without detectable *BRAF* exon 15 mutations, we identified two cases with novel *BRAF* exon 15 mutations: one with a *BRAF* V600D mutation; and, one with compound *BRAF* V600D and K601L mutations. While the sample size of our cohort is too small to draw definitive conclusions, there was no obvious correlation between *BRAF* genotype and specific clinicopathologic factors (i.e., age, histomorphologic features; see Table 1).

BRAF V600D mutations are uncommon but have been identified in variety of neoplasms, including melanoma, cholangiocarcinoma, and Langerhans cell histiocytosis^{9, 30-32}. Similar to the more common V600E mutation, a variety of *in vitro* assays have demonstrated sensitivity of cultured cells lines harboring *BRAF* V600D mutations to the *BRAF* inhibitors vemurafenib and dabrafenib³³⁻³⁵. These findings suggest that *BRAF* V600D mutations may be functionally similar to V600E mutations in the tumorigenesis of MA – probably downstream activation of the MAPK/ERK signaling pathway. The significance of the novel *BRAF* K601L mutation identified in our study is unclear. Although codon 601 is a hotspot for *BRAF* exon 15 mutations, according to the Catalogue of Somatic Mutations In Cancer (COSMIC) database, this particular mutation has only been reported in one other case – a lung adenocarcinoma³⁶. Sanger sequencing of the subcloned PCR product showed that the compound mutation (V600D/K601L) in this case likely arose as a single frame-preserving indel event, and given the concomitant V600D mutation, the K601L mutation may be of little additional functional significance. Overall, four of forty-one (10%) published genotyped MA do not harbor *BRAF* exon 15 mutations. Increasingly, recurrent somatic mutations in other signaling pathways genes have been described in *BRAF* V600E-negative neoplasms^{10, 37}. In addition, a candidate tumor suppressor gene in MA has been mapped to a small region on chromosome 2p³⁸, and deletion of a portion of chromosome 2p has been documented in a *BRAF* V600E-positive MA case²⁸. These findings suggest a need for additional comprehensive sequencing of MA – both cases with and without *BRAF* exon 15 mutations.

Finally, MA is thought to represent one end of a spectrum of benign metanephric neoplasms that includes metanephric adenofibroma (MAF) and metanephric stromal tumor (MST). Furthermore, this group of benign metanephric neoplasms has been hypothetically

postulated as the possible hyperdifferentiated, benign counterpart to WT – a morphologically heterogeneous group of tumors that may represent multiple distinct genetic entities. With this in mind, it is interesting to note the documented lack of *BRAF* mutations in WT¹⁶. Future studies should rigorously examine WT, MAF, and MST for *BRAF* exon 15 mutations. It will also be important to examine these tumors for BRAF V600E protein expression by mutation-specific IHC.

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Figure 1. BRAF V600E protein expression in MA, as detected by IHC with a mutation-specific antibody, is highly correlated with the presence of BRAF V600E mutation by Sanger sequencing (A,D,G,J) H&E, (B,E,H,K) BRAF V600E IHC, and (C,F,I,L) BRAF exon 15 genomic sequencing of four MA cases. All four cases show the typical histomorphologic features of MA and demonstrate diffuse, moderate to strong cytoplasmic BRAF V600E staining. Adjacent non-neoplastic kidney parenchyma shows negative to weak cytoplasmic staining and weak to moderate nuclear staining. In each case, Sanger sequencing of BRAF exon 15 reveals a corresponding thymidine to adenine substitution at codon 600 (arrowheads in C,F,I,L); this substitution results in the missense BRAF V600E mutation. Sanger sequencing confirmed the presence of BRAF V600E mutations in the remaining three MA cases with diffuse, moderate to strong cytoplasmic BRAF V600E staining (data not shown). No other BRAF exon 15 mutations were identified in these seven cases. Magnification: (A,D,G,J) 20X; and, (B,E,H,K) 40X.

Figure 2. Novel BRAF exon 15 mutations in BRAF V600E-negative MA
(A,D,G,J) H&E, (B,E,H,K) BRAF V600E IHC, and (C,F,I,L) BRAF exon 15 genomic sequencing of four MA cases. All four cases show the typical histomorphologic features of MA but demonstrate negative to weak cytoplasmic and/or nuclear staining. Adjacent non-neoplastic kidney parenchyma shows negative to weak cytoplasmic staining and weak to moderate nuclear staining. For one case, Sanger sequencing of BRAF exon 15 revealed no mutations (wild type sequence at codons 600 and 601 shown in panel C). Two additional cases of V600E-negative MA showed novel BRAF exon 15 mutations by Sanger sequencing. For one case, compound substitution of adenine and thymidine at codon 600 for thymidine and guanine (arrowheads in F) results in the missense BRAF V600D mutation; for the other case, a complex compound substitution and/or indel at codons 600 and 601 (arrowheads in I) results in the compound missense BRAF mutations V600D and K601L. (These novel BRAF exon 15 mutations were subsequently confirmed by Sanger sequencing

of the subcloned PCR product.) Finally, for one case, Sanger sequencing demonstrated a thymidine to adenine substitution at codon 600 (arrowhead in L), corresponding to a BRAF V600E missense mutation; this is the only instance of discordance between V600E mutation-specific immunohistochemistry and Sanger sequencing. Magnification: (A,D,G,J) 20X; and, (B,E,H,K) 40X.

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Figure 3. BRAF V600E protein is not detected by a mutation-specific antibody in a large cohort of common non-MA renal tumors

BRAF V600E IHC of representative TMA cores of (A) type I papillary RCC, (B) chromophobe RCC, (C) eosinophilic variant of chromophobe RCC, (D) oncocytoma, (E) type II papillary RCC, and (F) clear cell RCC. Variable weak cytoplasmic and/or moderate nuclear staining is present (see clear cell RCC in panel F), similar to background non-neoplastic renal parenchyma; however, no moderate or strong cytoplasmic staining is identified in any of the eighty-four non-MA renal tumors analyzed. These results are in accord with prior sequencing data and IHC studies showing only very rare BRAF V600E mutations in RCC^{7, 27}. Magnification: 20X.

Table 1

Clinicopathologic features of a retrospective MA cohort.

Gender	Age (years)	Specimen Type	Lat	Size (cm)	Histologic Features							BRAF V600E IHC	BRAF exon 15 mutation(s)
					P	T	G	C	H	PC	Ne		
M	16	Nep	L	4.4	Fo	Fo	Fo	N	N	NFo	N	Pos	V600E
F	32	Nep	R	1.3	NFo	NFo	Fo	N	Fo	NFo	Fo	Neg	V600D
M	32	Nep	L	3.2	NFo	Fo	Fo	N	NFo	E	N	Neg	Wild type
F	41	Nep	R	3.6	Fo	Fo	Fo	N	F	Fo	N	Pos	V600E
F	43	Nep	R	5.1	Fo	Fo	Fo	N	E	E	N	Neg	V600E
F	45	Nep	L	2.0	NFo	NFo	Fo	Fo	NFo	E	N	Pos	V600E
F	55	Nep	L	2.3	Fo	N	N	Fo	Fo	E	N	Pos	V600E
M	60	CNB	L	2.7	N	Fo	Fo	N	N	N	N	Pos	V600E
M	62	CNB	R	1.8	N	Fo	N	N	N	N	N	Neg	V600D, K601L
F	74	CNB	L	2.5	N	N	Fo	N	N	N	N	Pos	V600E
M	84	Nep	R	3.4	Fo	Fo	Fo	Fo	E	N	Fo	Pos	V600E

M = male, F = female, Nep = partial or total nephrectomy, CNB = core needle biopsy, Lat = laterality, L = left, R = right, P = papillary, T = tubular, G = glomeruloid, C = cystic, H = hyalinization, PC = psammomatous calcification, Ne = necrosis, S = sclerosis, N = no, Fo = focal, NFo = non-focal, E = extensive, IHC = immunohistochemistry, Pos = positive, Neg = negative