Abstract. The autosomal dominant hereditary disorder von Hippel-Lindau (VHL) disease is caused by a germline mutation in the VHL gene. The symptoms of VHL include hemangioblastoma of the central nervous system, retinal angiomas, visceral tumors and multiple visceral cysts. However, advanced renal cell carcinoma (RCC) occurs in few VHL patients at initial diagnosis; in addition, sporadic VHL disease with de novo germline mutation is rare. The current study reports the clinical case of a 33-year-old Asian male patient diagnosed with advanced bilateral multicentric renal carcinomas. The patient underwent radical nephrectomy with embolectomy of the right kidney for treatment of T3b-stage RCC and laparoscopic nephron-sparing surgery of the left kidney. Sunitinib was administered following histological diagnosis and during follow-up. Genetic analysis revealed a missense mutation, c.194C>G (p.Ser65Trp). In addition, genetic analysis of the patient’s parents and brothers, who were unaffected, confirmed a diagnosis of de novo VHL disease. To the best of our knowledge, the present study reports the first known case of a sporadic de novo germline mutation of VHL at c.194C>G. Current understanding of the molecular genetics and pathophysiology of VHL disease, as well as developments in surgical and target therapies for RCC have advanced in recent years; however, early detection through genetic screening and regular clinical surveillance of VHL disease patients and their families continues to be the primary basis for managing the disease.

Introduction

The autosomal dominant hereditary disorder von Hippel-Lindau (VHL) disease is caused by a germline mutation in the VHL gene. VHL is characterized by abundantly vascularized tumors in multiple organs; such VHL tumors may include hemangioblastoma of the retina and central nervous system (CNS), renal cell carcinoma (RCC), pancreatic tumors, pheochromocytoma, endolymphatic sac tumors, epididymal cystadenoma as well as cysts and cystadenoma in the kidney, pancreas, epididymis and broad ligament (1). The incidence of VHL disease has been estimated to be 1 in 36,000 live births in the Caucasian population, with gradually more apparent clinical manifestations occurring between ~18 and 30 years of age (2). Clinical diagnostic criteria include a positive family history and at least one typical VHL tumor. For isolated cases, at least two typical manifestations, including one hemangioblastoma, are required for diagnosis (3). Cases of sporadic VHL disease are rare. Approximately 20% of VHL patients demonstrate negative family history, with disease resulting from a de novo mutation (4). The VHL gene has been mapped to chromosome 3 (3p25-26) (5). Identification of pathogenic germline VHL mutations has been reported to provide a reliable method for the confirmation of diagnosis (6). The current study reports a case of de novo VHL disease in a male patient with advanced bilateral multifocal RCC involvement. Written informed consent was obtained from the patient. The relevant literature is also reviewed.

Case report

History and examination. A 33-year-old man was admitted to Zhongda Hospital, Southeast University (Nanjing, China) with a two-month history of progressive right flank pain, accompanied by a one-month history of recurrent painless gross hematuria. The patient's previous medical history included urological surgery at the age of 23 years due to an epididymal cyst and an ophthalmologic surgery at 26 years of age for retinal detachment resulting from bilateral retinal angiomatosis, which was performed at Shuyang People's Hospital (Suqian, China). At the time of the present study, the
patient had two children, a son and a daughter, who were in good health; his father, aged 65 years, and his mother, aged 62 years, were also in good health. The patient’s two brothers, aged 40 and 37 years old, with one child each were all in good health.

Contrast-enhanced computed tomography (CT) scanning and angiography were used to detect the presence of quasi-circular enhancing lesions in the middle of the left kidney and large irregular enhancing lesions in the right kidney, extending into the vena cava, that formed an embolus (Fig. 1A-C). In addition, multiple cysts in the pancreas and kidney were detected radiologically (Fig. 1A).

**Surgery and post-surgical course.** The patient underwent a radical nephrectomy with an embolectomy of the right kidney for treatment of T3b-stage RCC (Fig. 1D). After three months, he successfully underwent laparoscopic nephron-sparing surgery (NSS) of the left kidney (Fig. 1E). Histological analysis of the renal tissue revealed clear cell carcinomas (Fig. 1F). Sunitinib was administered (50 mg every day for four weeks) following histological diagnosis and during follow-up.

**Genetic study.** Peripheral blood samples from nine genetic relatives of the patient were subjected to VHL mutation analysis. Genomic DNA was isolated from peripheral blood leukocytes using the CW0541 Blood Gen Midi kit (CW Biotech Co., Ltd., Beijing, China). The entire coding sequence of the VHL gene, including exon/intron boundaries, was amplified by polymerase chain reaction (PCR) using primers (7) manufactured by Generay Biotech Co., Ltd. (Shanghai, China). The PCR products were directly sequenced by Nanjing Springen Biotech Co., Ltd. (Nanjing, China) using the Eppendorf 5331 MasterCycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany) under the following conditions: Denaturation at 95°C for 10 min, followed by 35 cycles of 95°C for 30 sec, 56°C for 30 sec and 72°C for 30 sec, and a final extension step at 72°C for 10 min. The mutations were confirmed by two-way DNA strand sequencing and manually compared. A germline mutation was detected in exon 1 of the VHL gene [c.194C>G (p.Ser65Trp)] in genomic DNA from the peripheral blood sample of the patient, while no mutation was detected in the peripheral blood of the patient’s relatives (Fig. 2).

**Discussion**

In adults, RCC accounts for ~3% of all cancers and ~85% of all primary malignant kidney tumors (8). In addition, ~5% of RCCs have a hereditary basis, of which VHL is the most common cause. Furthermore, ~75% of RCCs have clear cell histology, which is hereditarily caused by VHL (9). RCC and cysts in VHL disease are characterized by multicentricity and bilateral location in >75% of patients (10,11). The mean age at presentation is 10-20 years earlier than the age reported for sporadic renal disease (12). Transition from a cyst to a solid lesion is rare; however, the lining epithelium may be dysplastic or may have characteristics of carcinoma-in-situ, resulting in RCC (11). The case reported in the current study presented with a typical triad of symptoms, including hematuria, flank pain and flank mass. However, cases with this ‘triad syndrome’ are more advanced and rare; RCC and cysts often remain asymptomatic for long intervals (13,14). Thus, serial imaging of the kidneys for monitoring and early diagnosis has the potential to enhance overall patient outcome. The standard method for the detection of renal involvement in patients with VHL is contrast-enhanced abdominal CT imaging (15).

At present, no definitive guidelines are available with regard to the timing and surgical approach for these multicentric bilateral tumors. However, the primary aim of treatment should be cancer control, rather than cure, as well as the preservation of functional parenchyma in order to avoid the morbidity associated with renal or adrenal loss. A number of studies have reported that the majority of RCCs in VHL exhibit a low pathological grade (16), gradual growth (17) and do not metastasize at diameters of <3 cm (18). Therefore, an individual renal lesion may be maintained under regular surveillance until it reaches 3 cm in diameter, at which point NSS is performed to balance the prevention of metastatic progression against the reduction of the number of interventions (19). However, studies have revealed that the majority of patients with VHL-associated RCC eventually develop local recurrence with the concomitant requirement for repeated renal surgery (16). Certain clinicians recommend open NSS as the standard of care for treating RCC in the setting of VHL disease due to the inability of laparoscopy to achieve reliable hypothermia, in addition to the prolonged ischemia and increased complication rates associated with this approach. However, based on our experiences, the large incision of the abdominal wall may be problematic, resulting in muscle injury, the development of hernias and cosmetic damage. Laparoscopic NSS, performed by experienced surgeons on selected patients, is an alternative to open surgery (20); the oncological outcome, based on available series with limited follow-up data, appears to be similar to the outcome achieved with open NSS (21). Furthermore, an increasing trend towards the use of percutaneous radiofrequency ablation or cryoablation for small RCCs has been observed as this method is less invasive compared with other therapies (22,23). However, in rare cases with an advanced clinical stage of RCC in which the kidney cannot be preserved, such as that of the present study, or probable end-stage renal disease from the reiterated surgical treatments of localized tumors, total nephrectomy may be the only option due to a lack of alternative therapies. For patients who have undergone bilateral nephrectomy, renal replacement therapy may not be avoided (24,25).

VHL disease occurs due to the inheritance of a mutated germline copy of the VHL gene from an affected parent. The wild type copy of the gene is then inactivated by a somatic event. However, ~20% of patients with VHL develop the disease due to a de novo mutation. Inactivation of the wild type allele observed in tumors from VHL patients is consistent with the classic ‘two hit’ hypothesis of tumorigenesis (26,27). Currently, >400 different mutations have been identified, including deletion, missense, nonsense, frameshift and insertion mutations (http://www.umd.be/VHL/W_VHL). In the present study, the germline mutation c.194C>G of the VHL gene was detected in the patient; it was predicted that this mutation may induce a serine-to-tryptophan substitution at amino acid position 65 (p.Ser65Trp) within the VHL protein (pVHL). Serine at amino acid position 65 is highly conserved among various species and a number of studies have demonstrated the
presence of mutations at this position (6,28-30). However, in previous cases involving this mutation, patients were reported to have a positive family history of VHL disease. In addition, no relatives of the patient in the current study were found to

Figure 1. Clinical information collected from the patient. Axial computed tomography images showing: (A) Multiple cysts in the pancreas and kidney (black arrows) and embolus in the right kidney vein and vena cava (white arrows); and (B) quasi-circular enhancing lesions in the left kidney and huge irregular enhancing lesions in the right kidney (white arrows). (C) Angiography showing tortuosity of vessels in the right renal hilus and filling defect in vena cava. (D) Successful removal of the right renal tumor and embolus (white arrow). (E) Tumor and cysts in left kidney are revealed under laparoscopic vision. (F) Renal clear cell carcinoma was confirmed histologically (magnification, x20; hematoxylin and eosin stain).

Figure 2. Resultant chromatogram profile following direct DNA sequencing of von Hippel-Lindau (VHL). (A) VHL c.194C>G mutation was identified in peripheral blood sample of the patient and (B) normal VHL sequences were identified in peripheral blood samples from the patient's family members. (C) Pedigree displaying the sporadic development of VHL disease.
carry this mutation or had any clinical indications of VHL disease. Therefore, for the patient discussed in the present study, a de novo mutation may have occurred in a germ cell of either parent or at the embryo stage.

In conclusion, the current study presents a rare case of a de novo VHL patient with advanced RCC at first diagnosis; to the best of our knowledge, the patient discussed in the present study is the first known case of a sporadic de novo germline mutation of VHL at c.194C>G. Current understanding of the molecular genetics and pathophysiology of VHL disease, as well as developments in surgical and target therapies for RCC have advanced in recent years; however, early detection through genetic screening and regular clinical surveillance of VHL disease patients and their families continues to be the predominant mode of disease management. The enhanced sensitivity of imaging techniques and advancements in therapeutic techniques, including radiofrequency ablation and NSS allow for the identification and management of tumors at earlier stages, thereby attenuating the morbidity and mortality rates associated with VHL disease.

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