Short Report

Mosaicism in von Hippel–Lindau disease with severe renal manifestations

von Hippel–Lindau (VHL) disease is an autosomal dominant inheritable disease caused by a germline mutation in VHL tumor suppressor gene (VHL). It is characterized by highly vascular and cystic tumors, including hemangioblastomas of central nervous system (CNS) and retina, and visceral lesions such as clear renal cell carcinomas (RCC) and renal cysts, pheochromocytomas, pancreatic cysts and tumors, endolymphatic sac tumors, and papillary cystadenomas in epididymis and broad ligament (1–3). CNS hemangioblastomas and clear cell RCC are the two most common manifestations of VHL disease, occurring in up to 70–80% patients (4).

The VHL phenotype observed varies among families, though even among members of the same family. VHL can be classified into Type 1 and Type 2 based on the absence (Type 1) or presence (Type 2) of pheochromocytoma. Type 2 patients are further subdivided into Type 2A (pheochromocytoma without RCC or pancreatic cysts), 2B (pheochromocytoma with RCC or pancreatic cysts), or 2C (only pheochromocytoma) (4).

Molecular genomic analysis routinely confirms the clinical diagnosis. In some patients, however, the use of standard molecular diagnostic techniques may not be sufficient for the detection of germline VHL mutations and for the subsequent diagnosis of VHL (5). It is well documented that mosaicism is one of the obstacles to achieving a correct molecular diagnosis in hereditary diseases (6). Here, we report the case of a VHL patient with a germline mosaic VHL mutation. The patient presented with bilateral RCC, and initial polymerase chain reaction (PCR)-direct sequencing for
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VHL mutations was negative. However, the detection of a heterozygous c.194C>G (p.Ser65Trp) VHL mutation in the patient’s daughter prompted further genetic testing. A mosaic c.194C>G (p.Ser65Trp) germline VHL mutation was detected in the patient upon further analysis.

Patients and methods

Patients

At our center, all patients suspected of VHL disease based on the clinical diagnosis criteria, and their relatives, are tested for germline mutations in the VHL gene using previously described methods of PCR-direct sequencing and UPQFM-PCR (universal primer quantitative fluorescent multiplex PCR) (7). After retrospective review, we identified a family with a history of atypical VHL, and this prompted further genomic study.

VHL mosaic mutation analysis

Genomic DNA samples from peripheral blood leukocytes were used for DNA amplification. We performed allele-specific primer PCR using the primers F: 5’-GAA GAAGACGGCGGGAGGAGTC and R: 5’-GGCT CGCGCGAGTTCaC (specific for the c.194C>G mutant DNA) with a thermal cycling program of 94°C for 30 s, 63°C for 30 s, and 72°C for 30 s (35 cycles). The c.194C>G mutation creates a BseY1 restriction enzyme site. Additionally, VHL exon 1 PCR products from the patient, her daughter, and a normal control were purified, digested with the BseY1 enzyme, and separated on a 2% agarose gel.

Results

A 65-year-old female was admitted to the outpatient clinic of the Urology Department, Peking University First Hospital, with a chief complaint of blood in her urine without pain in December of 2007. An abdominal computed tomography scan revealed bilateral RCC with multiple renal cysts as well as multiple pancreatic cysts (Fig. 1). Fifteen years ago, the patient was also found to have multiple cysts in both kidneys. She had no other clinical manifestations usually associated with VHL, such as the symptoms from CNS, retinal or adrenal involvement. Because of the diffuse nature of her bilateral kidney lesions, radical nephrectomy was not possible. The patient died 1 year after initial presentation.

The patient’s son had a normal phenotype. Her daughter presented with a spinal tumor at the age of 18, which was surgically resected and confirmed to be a CNS hemangioblastoma, with subsequent recurrence and additional surgery at 36 years of age. No other VHL-associated symptoms were observed in this patient, and she died 1 year after the second surgery.

Genomic DNA from patient’s peripheral blood had initially been negative for a VHL mutation by PCR-direct sequencing and UPQFM-PCR. However, a heterozygous c.194C>G (p.S65W) mutation was found in her daughter’s genomic DNA sample (Fig. 2b). In order to explore the genetic relationship between the patient and her daughter, we subsequently reexamined the patient’s DNA. A lower G curve at the c.194 position, which had been previously thought to be a sequencing background artifact, was identified (Fig. 2a).

Allele-specific PCR detected a 137 bp product in DNA samples from the patient and her daughter, but not from the patient’s son or from healthy controls. Furthermore, the PCR product from the patient was weaker than that from her daughter (Fig. 2c), suggesting the presence of a mosaic c.194C>G VHL mutation in the patient and the inheritance of the mutation by her daughter alone.

We then digested the PCR product amplified from exon 1 of the patient and her daughter with BseY1 restriction enzyme, measured the density of the bands, and calculated the mutation rate in peripheral leukocytes (Fig. 2d). This mutation was present in 18.8 ± 3.84% of peripheral leukocytes in the patient.

Discussion

Mosaicism is defined in an individual arising from a single zygote by the presence of at least two cell lines differing in genotype (8). Mosaicism can increase the difficulty in obtaining a correct diagnosis for Mendelian diseases (6). Here, we provide a mosaic patient with

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Fig. 1. Abdominal computed tomography scanning images of the 65-year-old patient showing bilateral big renal carcinomas (a and b) with multiple renal cysts (a, thin arrows) and pancreatic cysts (a, thick arrow).
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Fig. 2. Sequencing results of VHL exon 1 (a) from the patient (mother) showing a small G curve at c.194 position, and (b) from her daughter showing a heterozygous mutation of c.194C>G. (c) Allele-specific polymerase chain reaction (PCR) indicated that the patient and her daughter harbored the mutation of c.194C>G in VHL, and the PCR product from the patient was weaker than that from her daughter. Genomic DNA samples from her son and normal controls were negative using this allele-specific primer pair for PCR. (d) PCR products of VHL exon 1 (390 bp) from the patient, her daughter and a normal control were purified, digested with BseY1 enzyme, and separated in a 2% agarose gel. The 215 and 175 bp fragments seen in the gel indicate the presence of c.195C>G mutation. After calibration of the length of the three fragments, the c.194C>G mutation rate can be calculated from the band intensities of 175 bp + 215 bp/390 bp + 175 bp + 215 bp. The c.194C>G mutation rate was 18.8 ± 3.84% in the patient (repeated for three times), and was approximately 50% in her daughter.

severe manifestations of VHL disease, but without initial evidence of a VHL mutation by routine molecular diagnostic approaches. The patient had bilateral RCC and multiple renal cysts, as well as multiple pancreatic cysts, and her daughter had recurrent VHL-associated CNS hemangioblastomas. VHL mosaicism has been previously found in asymptomatic parents of patients otherwise diagnosed with de novo germline mutations in the VHL gene, or in symptomatic patients negative for a VHL mutation by routine molecular diagnostic methods (9–11). To date, only a few cases of mosaic VHL mutations have been reported. In these asymptomatic parents, and in the symptomatic patients who initially test negative for VHL germline mutations, further examination is necessary in order to discover possible VHL mosaicism, which has important implications in clinical diagnosis and genetic counseling (11).

The actual incidence of VHL mosaicism remains unknown. Sgambati et al. showed that 42 (23%) of 181 VHL disease kindreds had no family history, and 2 of those 42 cases (4.8%) were found to have VHL mosaicism in the parents (10). This rate is similar to that of families affected by Type 2 neurofibromatosis, but is much lower than the 10% found in retinoblastoma (12, 13). Mosaicism results from the occurrence of a somatic mutation during embryogenesis. Consequently, different organs and tissues may have different ratios of genetically mutant cells. This fact makes mosaic diagnosis especially difficult, as the unavailability of appropriate and varied visceral tissues from phenotypically normal parents reduces the reliability of molecular testing. Furthermore, if parents have only one affected offspring, and are asymptomatic with or without isolated VHL-associated tumors, the possibility of VHL mosaicism is often ignored. Additionally, patients without a family history of VHL are usually treated as having a de novo VHL mutation. Presumably, therefore, the incidence of VHL mosaicism is more common than is currently estimated (11). Clinicians should be aware of the possibility of VHL mosaicism in patients without an identified family history of VHL.

Mosaicism partially provides an explanation for the clinical heterogeneity and variable disease severity observed in VHL. The time during embryogenesis at which the mosaicism occurs and the proportion of mutant cells in different cell lineages can have a significant effect on the phenotype of patients. Previously reported patients with confirmed VHL mutation mosaicism have had variable phenotypic expressivity ranging from clinically insidious changes to the full spectrum of the disease (9–11). Compared with their affected offspring, parents with mosaic VHL mutations have late onset or modest phenotypes. This phenomenon was also observed in this family. The mosaic patient died of RCC later in life, whereas her daughter died of complications related to CNS hemangioblastomas at 37 years of age. However, it should be mentioned that this mosaic VHL patient had severe bilateral kidney disease probably related to the fact that her kidney tissue contained a higher portion of mutant cells than did her peripheral lymphocytes, or the concurrent mutation of other genes such as the SWI/SNF (SWItch/sucrose non-fermentable) chromatin remodeling complex gene PBRM1 (frequently identified in RCC) (14).
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VHL mosaicism in asymptomatic parents is an important reason for the existence of affected patients without an identified family history of VHL. The incidence of VHL mosaicism may be more common than is generally estimated. Therefore, clinicians should carefully consider the possibility of mosaicism in some atypical VHL disease families, as this genetic possibility has important implications in clinical diagnosis and genetic counseling.

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References