A Novel Mutation in the von Hippel-Lindau Tumor Suppressor Gene Identified in a Japanese Family with Pheochromocytoma and Hepatic Hemangioma

Kentaro Takahashi¹, Keiji Iida¹, Yasuhiro Okimura², Yutaka Takahashi¹, Junko Naito¹, Shin-ichiro Nishikawa¹, Seizo Kadowaki¹, Genzo Iguchi¹, Hidesuke Kaji⁴ and Kazuo Chihara¹

Abstract

Von Hippel-Lindau (VHL) syndrome is a neoplastic syndrome caused by a mutation in the VHL gene. There is a discrepancy between the phenotypes of human VHL syndrome and VHL gene-disrupted mouse models. A heterozygous VHL gene-disrupted model (vhl +/-) developed hepatic vascular lesions; in contrast, hepatic hemangioma is a rare manifestation of human VHL syndrome. We identified a novel mutation (P154S) in the VHL gene in a Japanese family with pheochromocytoma. One of the members demonstrated hepatic hemangiomas, suggesting that there may be a relationship between the mutation of the VHL gene and hepatic vascular lesions, even in humans.

Key words: von Hippel-Lindau syndrome, pheochromocytoma, hemangioma, mutation

(DOI: 10.2169/internalmedicine.45.1547)

Introduction

Von Hippel-Lindau (VHL) syndrome is a hereditary autosomal dominant syndrome characterized by the development of highly angiogenic tumors in the specific regions such as the retina, cerebellum, spinal cord, kidneys, adrenals, and epididymis (1). Several hundred germline mutations in the VHL gene have been reported (2) since the gene was identified by positional cloning in 1993 (3). VHL syndrome is classified into 2 categories, namely, type 1 and type 2 that have a low and high risk of pheochromocytoma (PHE), respectively. Type 2 VHL syndrome is further subclassified into a low (type 2A) and high (type 2B) risk of renal cell carcinoma (RCC) or cerebellar hemangioblastoma (HAB), and familial PHE without RCC or HAB is designated as type 2C (4). The VHL-encoded protein, pVHL, consists of 213 amino acids and possesses 2 functional subdomains, α and β (5). The α helical domain extends from codon 158 to 189 and binds elongin C, which in turn binds elongin B, Cullin2, and Rbx1 to form an ubiquitin E3 ligase complex (6). On the other hand, the β sheet corresponds to codon 63 to 157 and binds directly to hypoxia inducible factor (HIF) α subunits, thereby controlling the stability of HIF and tumor angiogenesis (6). Most of the mutations causing type 2 VHL syndrome are missense point mutations located at the region encoding the α domain of pVHL, whereas type 1 VHL syndrome is often caused by a gross alteration or the complete deletion of pVHL. Thus, the phenotype of VHL syndrome would be related to the site, size, and type of the VHL gene disruption although there continues to be a great variation in clinical manifestation.

In animal models, homozygous disruption of the VHL gene in mice results in embryonic lethality due to the lack of placental vasculogenesis (7-10). On the other hand, mice heterozygous for a VHL null mutation survive and are sus-
ceptible to the development of spontaneous vascular tumors in the liver (8-10) as well as in other organs (9, 10) without RCC. However, hepatic vascular tumors are rare in human VHL syndrome. Despite recent progress in studies on genotype-phenotype correlation in VHL syndrome, there is limited information concerning the relationship between the VHL gene mutation and human hepatic hemangioma (HH).

In this study, we report the case of a Japanese family with type 2C VHL syndrome caused by a novel mutation in the VHL gene located at the boundary of the α and β domains of pVHL. One of the family members developed HH as well as PHE.

Subjects and Methods

Case report

A 65-year-old Japanese man (patient 1) was referred to our hospital for the evaluation of hypertension. His height was 166.0 cm; weight, 57.0 kg; body mass index, 20.7 kg/mm²; and blood pressure, 170/100 mmHg. Serum epinephrine, norepinephrine, and dopamine concentrations were 0.18 ng/ml (normal range: less than 0.17 ng/ml), 4.2 ng/ml (normal range: 0.15-0.57 ng/ml), and 0.03 ng/ml (normal range: less than 0.03 ng/ml), respectively. Daily urinary excretion concentrations of epinephrine, norepinephrine, dopamine, and vanillylmandelic acid were 16.5 μg/day (normal range: 1-23 μg/day), 452 μg/day (normal range: 29-120 μg/day), 660 μg/day (normal range: 100-1,000 μg/day), and 7.0 mg/day (normal range: 1.4-4.9 mg/day), respectively. He was clinically diagnosed with PHE based on biochemical markers, bilateral adrenal tumors that were detected by abdominal computed tomography (CT) scanning, and 131I-metaiodobenzylguanidine (MIBG) scintigraphy that showed marked uptake of radioactivity into the right adrenal gland (Fig. 1). The CT scanning of patient 1 also demonstrated HH (Fig. 2). His son (patient 2) was also indicated to possess a right adrenal tumor, and was diagnosed with PHE according to the pathological examination of the tumor resected by surgery. The remaining 3 family members, namely, the wife of patient 1, another son, and daughter, were healthy and without PHE. The ages of the family members are indicated in Fig. 4. There is no clinical evidence of multiple endocrine neoplasia (MEN) type 2 in this family. Neither RCC nor HAB were detected in the family members. Subsequently, we analyzed the VHL gene in this
Figure 2. The abdominal CT findings of patient 1 demonstrating multiple hepatic hemangiomas.
a: non-enhanced CT; b: early phase after enhancement; c: late phase after enhancement.
R: right; L: left.

Figure 3. Direct sequence analysis of exon 2 of the VHL gene in patient 1. Compared with a normal subject, the patient was heterozygous for a C to T transversion at the first nucleotide of codon 154. This mutation was predicted to result in the substitution of proline for serine at codon 154. The arrow indicates the mutation site.

Genetic analysis

Informed consent for analysis of the VHL gene was obtained from all 5 family members, and the study was approved by the ethical committee of the Kobe University Graduate School of Medicine (approval number: 274).

Genomic DNA was isolated from the peripheral blood leukocytes of the members and normal controls as described previously (11). Each of the 3 exons of the VHL gene was individually amplified by polymerase chain reaction (PCR) with primer pairs described previously (12). The amplification products were purified and analyzed by direct sequencing using a DNA sequencer (model 310, Perkin-Elmer, Applied Biosystems, Foster City, CA, USA).

Restriction enzyme analysis

To confirm and screen the mutation, PCR-based restriction fragment length polymorphism (RFLP) analysis of exon 2 of the VHL gene was performed. The PCR products of exon 2 of the VHL gene of the family members were digested with BstN1 (New England Biolabs, Inc., Beverly, MA, USA). The digested fragments were separated on a 3% NuSieve agarose gel (FMC BioProducts, Rockland, ME, USA) and visualized by ethidium bromide staining.

Results

Genetic analysis

As shown in Fig. 3, sequencing of the VHL gene of patient 1 revealed a heterozygous C to T mutation at the first base of codon 154. Patient 2 demonstrated an identical alteration (data not shown). This mutation was predicted to convert codon 154 from proline to serine (P154S). No additional abnormalities were detected in the VHL genes of these patients.

Restriction enzyme analysis

The sequence of the wild-type VHL gene contains a recognition site for BstN1. The C to T transition at the first base of codon 154 found in patients 1 and 2 disrupts this
Figure 4. Restriction enzyme analysis of the PCR products obtained from the family members. The ages (years) of the members are indicated in the pedigree. A 241-bp fragment including exon 2, was amplified by PCR. The wild-type PCR products contained a BstN1 site and produced 2 fragments of length 139 and 102 bp by digestion with BstN1, whereas the C to T transition disrupted this BstN1 site and was uncut by BstN1. Patient 1 and 2 showed 3 bands consisting of 241-, 139-, and 109-bp fragments, indicating their heterozygosity for this mutation, whereas the other family members showed 2 bands consisting of 139- and 109-bp fragments.

Discussion
Here, we reported a novel missense mutation (P154S) of the VHL gene in a Japanese family with PHE. Although several hundred mutations were identified in various sites of the VHL gene in humans (12), the P154S mutation has not been reported thus far. Furthermore, HH, a rare manifestation of human VHL syndrome, was identified in one of the family members.

One of the biochemical characteristics of PHE with VHL syndrome is relatively low levels of epinephrine secretion compared to norepinephrine, whereas PHE with MEN type 2 shows a high secretion of both epinephrine and norepinephrine (13). It has been reported that relatively low levels of epinephrine in VHL syndrome is caused by the reduced activity of phenylethanolamine N-methyltransferase, the enzyme that converts norepinephrine to epinephrine (13). In this regard, our results showing normal levels of epinephrine and increased levels of norepinephrine are in agreement with the characteristics of VHL syndrome. Although patient 1 possessed bilateral adrenal tumors, only the right adrenal tumor showed marked uptake of radioactivity in MIBG scintigraphy (Fig. 1). One possible explanation of this laterality is that the activity of catecholamine production in the left adrenal tumor was markedly decreased although the size of the left adrenal tumor was larger than the right one. Another possibility is that the left adrenal tumor is a non-functioning adrenal adenoma, whereas the right adrenal tumor is a PHE. Abdominal magnetic resonance imaging was not carried out in patient 1 due to his claustrophobia. Since adrenalectomy was not performed in patient 1, we were unable to obtain a histological diagnosis in patient 1.

Recent animal studies using VHL gene-disrupted models have provided new information. First, the inactivating mutation of the VHL gene is associated with hepatic vascular lesions in mice (7-10), although there are a few case reports of VHL syndrome-associated HH in humans (14-16). Second, no RCCs were identified in the heterozygous VHL gene disrupted (vhl +/-) mice (7-10), whereas RCC is one of the most frequent manifestations in human VHL syndrome (2). Vascular lesions in various organs occur in both mice and humans. However, it is still unclear why the susceptibility to vascular lesions depending on tissues is different in mice as compared to humans. Species-specific or environmental factors may play an additional role in the development of the vascular lesion.

HH is a rare manifestation of human VHL syndrome although HABs of the retina and central nervous system are frequently associated with this syndrome (2, 14-16). In the patients with the P154S mutation of the VHL gene in our study, HAB was not observed and hepatic lesions showed a CT appearance typical of HH. McGrath et al (15) reported that the feature of the CT appearance in an unenhanced CT scan of HH is a hypodense region. Following contrast infusion, it showed intense peripheral enhancement with an incomplete centripetal fill-in. This finding was consistent with that observed in patient 1. Since HH is not rare in a normal population, it is still possible that PHE with HH in patient 1 is incidental. However, the results from the vhl +/- mice suggest that the pVHL plays a crucial role in the inhibition of hepatic vascular lesions even in humans. In this regard, this is the first report on the mutation of the VHL gene identified in VHL syndrome with HH.

Genotype-phenotype correlations have been studied in VHL syndrome (2, 17). Codon 154 is located at the boundary of the portion of the VHL gene that encodes α and β domains of pVHL. Therefore, it is possible that the mutation at codon 154 affects the function of both α and β domains of pVHL. In our study, 2 family members with the P154S mutation in the VHL gene developed PHE, whereas other members without a mutation did not (Fig. 4), suggesting
that the P154S mutation is responsible for the development of PHE. In a previous report, a mutation at codon 154 of the VHL gene (P154L), which was the same codon but converted into a different amino acid, also caused the development of PHE but not HH (18). The converted amino acid as well as the site of mutation may be critical for developing HH in humans. Recently, it has been suggested that mutant pVHL-containing E3 ubiquitin ligase fails to degrade an atypical protein kinase C (19), resulting in failure of c-jun-dependent apoptosis during normal development of neuronal precursor cells (20); in addition, this mechanism may be one of the causes of the development of PHE in VHL syndrome. Thus, it is possible that the vascular lesion in the liver, HH, occurs due to the same mechanism in patients with the mutant pVHL protein. Although hepatic vascular lesions were not observed in patient 2, the son of patient 1, it is still possible that patient 2 with the P154S mutation will develop HH later in life. Further studies are necessary to clarify the relationship between genotypes and hepatic vascular lesions in humans.

In conclusion, we identified a novel P154S mutation of the VHL gene in a Japanese family with PHE. One of the family members developed a rare phenotype of VHL syndrome, i.e., HH as well as PHE.

We thank Miss Chika Ogata, Miss Kayo Imura, and Miss Kana Takeuchi for their excellent technical assistance.

This work was supported in part by Grants-in-Aid for Scientific Research from the Japanese Ministry of Education, Science, Sports and Culture and grants from the Japanese Ministry of Health, Labor and Welfare, Novo Nordisk A/S Growth, and Growth Science Foundation, 2002 and 2003.

References


© 2006 The Japanese Society of Internal Medicine
http://www.naika.or.jp/imindex.html