VHL Gene Mutation Analysis of a Chinese Family with Non-Syndromic Pheochromocytomas and Patients with Apparently Sporadic Pheochromocytoma

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Abstract

Objective: The Von Hippel-Lindau syndrome (VHLD), an inherited neoplastic syndrome predisposing to central nervous system hemangioblastoma (CNS), pheochromocytoma (PCC), renal cell carcinoma (RCC), retinal hemangioma (RA) and renal cysts, is caused by mutations or deletions of the VHL tumor-suppressor gene. To assess VHL genotype-phenotype correlations with function of pVHL a gene mutation analysis of members in a Chinese family with non-syndromic PCCs and individuals with apparently sporadic pheochromocytoma (ASP) was performed. Materials and Methods: DNA samples of 20 members from the Chinese family with non-syndromic PCCs and 41 patients with ASP were analyzed by polymerase chain reaction and direct sequencing, confirmed by Taqman probe. Results: Three novel mutations (H125P, 623(TTTGTG) and R120T) were identified in the Chinese family and in 3 among 41 ASP patients. The mutations were all located in exon 2 of VHL gene encoding β-domain of pVHL. The tumor type in H125P carriers and R120T carriers was VHL type 2C. And 623(TTTGTG) carriers presented VHL type 2B or type 2C. Conclusions: VHL gene abnormalities were identified in the Chinese family with non-syndromic PCCs and patients with ASP, resulting in dysfunction of pVHL. H125P and R120T could be associated with VHL type 2C, while 623(TTTGTG) might be linked with VHL type 2B or type 2C. Not only is the genetic analysis helpful for early diagnosis and treatment of patients with VHLD, it is also beneficial for research into VHLD pathogenesis.

Keywords: von Hippel-Lindau syndrome - gene - mutation - pheochromocytomas - familial - sporadic

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Introduction

VHL gene is a tumor suppressor gene mapped to chromosome locus 3p25-26, encoding two different protein VHL (pVHL30 and pVHL19) that regulates hypoxia-inducible genes, the fibronectin matrix assembly, and angiogenesis. pVHL19 with 160 amino acid protein is located in the nucleus, pVHL30 with 213 amino acid is expressed both in the nucleus and cytoplasm. The pVHL19 is similar to the pVHL30 in biochemistry and function, affecting a series of processes including cell-cycle regulation, mRNA stability, hypoxia-inducible gene expression and transcription, etc (Lonser et al., 2003).

The pVHL19 contains two functional subdomains: a smaller, helical α-domain, which consists of three helices (H1, H2, and H3), and a larger β-domain (residues 63-154 and residues 193-204), which forms a seven-stranded β sandwich and an α helix. These two subdomains contain different protein binding sites: The α-subunit is connected to a region of HIF-α that regulates the expression of VEGF (Binderup et al., 2013). Under the conditions of hypoxic and dysfunctional of pVHL, the expression of HIF-α and VEGF may rise, which may promote tumor incidence and growth. VHL gene mutations is related to the pathogenesis of VHLD (Gao et al., 2013)

According to genotype and phenotype, VHLD can be divided into two types: Type I not with pheochromocytoma (PCC) and Type II with PC. According to whether patients have renal cysts and renal cell carcinoma (RCC), Type II patients are further divided into three sorts: type 2A with low risk of renal cysts and RCC, type 2B with high risk of renal cysts and RCC and type 2C with isolated PCC. The incidence of VHLD patients with PCC is about 10% to 34%. Most of them have typical clinical presentation, but a few are asymptomatic (Jime'nez et al., 2006). The main causes of VHL type I are correlated with missense mutations and partial deletions, which cause parts of dysfunction or a complete defect of protein function. On the other hand, the causes of VHL type II are usually...
related to missense mutations affecting pVHL to connect to the binding sites. It was suggested by a report that the estimate of hereditary disease in apparently sporadic pheochromocytoma (SPA) was as high as 24% (Jimenez et al., 2006).

Thus, some medical institutions recommend genetic testing for patients with VHLD and SPA (Ali Tooteei and Shirin Hasani-Ranjbar, 2012; Bausch et al., 2013; Andreasson et al., 2013). In order to find the relationship between VHL gene mutation type and the tumor type, we carried out VHL gene detection in a Chinese family with non-syndromic PCCs and patients with ASP. And now we present the result of VHL gene analysis.

Materials and Methods

Families with non-syndromic PCCs
The family with PCCs includes 56 members of 4 generations, and is from Gansu Province. Among them, 7 extended over 3 generations were diagnosed as PCCs. One died before admission and another died due to a cardiovascular incident. Five were initially evaluated; evaluation included medical history, physical examination, abdominal ultrasonography, computed tomography (CT) or magnetic resonance imaging (MRI). Laboratory tests included routine biochemical testing, blood catecholamines and 24h urinary catecholamine metabolite determination. The results showed that the levels of blood catecholamines and 24h urinary vanillylmandelic acid were elevated significantly (more than 3 times).

All 5 patients (probands, see Table 1) underwent surgical resection. The tumors were benign under pathological examinations. There were no recurrence and malignant transformation over the period of up to 23-year follow-up. Twenty members, including 5 live patients and 15 direct relatives, accepted genetic examination.

Patients with ASP
Forty-one unrelated patients were selected from 91 with ASP who were registered in Urology Center of Lanzhou General Hospital, China, between 2005 and 2010, with the selection criteria (age below 60 yr, undertaking surgery resection and confirmed by pathology). They all participated in preoperative evaluation, including medical history, physical examination, abdominal ultrasonography, CT or MRI, routine biochemical testing, blood catecholamines and 24h urinary catecholamine metabolite determination. There were 17 males and 24 females (42.80±9.73 years in age); 15 cases had the left adrenal tumor, 24 cases with the right adrenal tumor, and 2 cases with bilateral adrenal tumors.

Materials and PCR: An informed consent was obtained from all participants. Genomic DNA analysis from peripheral blood leukocytes was conducted at the Urology Center of Lanzhou General Hospital, China for the 41 unrelated patients who underwent surgery for ASP and 20 members of the Chinese family. DNA extraction was performed by using D9081 kit (Takara Biotechnology, Dalian, China) according to the manufacturer’s instruction. The three exons of the VHL gene were amplified and directly sequenced.

Primer sequences are available upon request. And the primers were synthesized by Takara. PCR amplification was carried out in 1×1 PCR buffer (Takara) that contained 400 mOl/liter deoxynucleotide triphosphate, 0.4 mOl/liter of each primer, 2.5 units of Taq polymerase (Takara), and 100-200ng of DNA template in a 50μl volume. PCR conditions: one cycle of 15 min at 95°C; 30 cycles of 45 s at 95°C; 45 s at 63°C; 45 s at 72°C; followed by one cycle of 10 min at 72°C (Exon 1); one cycle of 15 min at 95°C; 30 cycles of 45 s at 95°C; 45 s at 59°C; 45 s at 72°C; followed by one cycle of 10 min at 72°C (Exon 2); one cycle of 15 min at 95°C; 30 cycles of 45 s at 95°C; 45 s at 57°C; 45 s at 72°C; followed by one cycle of 10 min at 72°C (Exon 3). The 7500 PCR System (Applied Biosystems, USA) was used for PCR reactions. The PCR products were performed sequence analysis by direct cycle sequencing using the Big Dye terminator v3.1 DNA Sequencing Kit (Applied Biosystems, USA) and an automated sequencer ABI PRISM 3730 (Applied Biosystems, USA). Mutations were confirmed by Taqman probe (the primers and probes were synthesized by Takara and checked by NCBI-BLAST).

Results
VHL gene mutations, including 2 missense mutations and 1 insertion mutation, were identified in all 20 members of the Chinese family (5 patients and 15 relatives) as well as all patients with ASP. In the family, a disease-causing heterozygous H125P mutation was detected in the 5 probands with non-syndromic PCCs, whose onset age was between 8 and 20.

There were some members of this family also had H125P mutation of the VHL gene, but this proved to be a neutral variant and the levels of blood catecholamines and 24h urinary catecholamine metabolite were normal. One member (proband 1, male, 17 years old) was admitted to the hospital because of recurrent headaches, Palpitations, sweating and blurred vision of five month. Proband 1 had unilateral multiple PCCs. The carrier 1 (sister to proband 1) with bilateral PCCs, was accepted surgery, and the pathological result was benign. The presenting tumor types in probands and carriers from this family were non-symptomatic.

Out of the 41 unrelated patients with ASP, one patient had R120T missense mutation and 2 patients had 623 (‘‘TTTGTtG) insertion mutations, respectively. The proband 6 carrying R120T missense mutation had unilateral renal cyst and hypertension, whose family screening showed the presence of the same mutation in her two healthy relatives (carrier 9 and carrier 10, see Table 1), but her mother (carrier 8) also had unilateral PCC, whose outcome of pathology was benign.

There were no newly developed VHL-related tumors on the carrier 11 and carrier 12, carrying 623 (‘‘TTTGTtG) mutations. The proband 8 with unilateral PCC had bilateral renal cysts. His mother (carrier 13) with unilateral PCC was asymptomatic, refusing to accept surgery. The family pedigrees and mutation sequencings table were presented in our early study (Andreasson et al., 2013; Zinnamosca et al., 2013).
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VHL Gene Mutation and Pheochromocytoma Clinical Phenotype

Table 1. Summary of Clinical Data and Mutation Analysis Results

| Patient   | gender | PCC (age at first diagnosis, years) | Other clinical presence | VHLD type | Mutation type | Mutation |
|-----------|--------|-------------------------------------|-------------------------|-----------|--------------|----------|
| Carriers1 | Male   | Unilateral multiple, 18            |                         | IIC       | Missense     | H125P    |
| Carriers2 | Male   | Bilateral, 29                     |                         | IIC       | Missense     | H125P    |
| Carriers3 | Male   | Bilateral, 17                     |                         | IIC       | Missense     | H125P    |
| Carriers4 | Male   | Asymptomatic                      |                         | IIC       | Missense     | H125P    |
| Carriers5 | Male   | Asymptomatic                      |                         | IIC       | Missense     | H125P    |
| Carriers6 | Male   | Asymptomatic                      |                         | IIC       | Missense     | H125P    |
| Carriers7 | Male   | Asymptomatic                      |                         | IIC       | Missense     | H125P    |
| Carriers8 | Female | Bilateral, 16                     |                         | IIC       | Missense     | H125P    |
| Carriers9 | Female | Unilateral, 28                    |                         | IIC       | Missense     | R120T    |
| Carriers10 | Female | Unilateral, 54                   |                         | IIC       | Missense     | R120T    |
| Carriers11 | Male   | Unilateral, 22                    |                         | IIC       | Insertion    | 623 TTGTG |
| Carriers12 | Female | Asymptomatic                      |                         | Insertion | 623 TTGTG    |
| Carriers13 | Male   | Unilateral, 29                    | Bilateral Renal cysts  | IIB       | Insertion    | 623 TTGTG |
| Carriers14 | Female | Unilateral, 50                   |                         | IIC       | Insertion    | 623 TTGTG |

*: members from a Chinese family with Non-syndromic PCCs; #: patients with ASP

Discussion

In the present study, we reported that genetic abnormal origin of VHLD in members from the Chinese family with non-syndromic PCCs and in 3 of 41 patients with ASP (7.3%). We detected 3 disease-causing novel genetic mutations, included 2 missense mutations (H125P and R120T) and an insertion mutation (623 TTGTG), located in exon 2 encoding β-domain of pVHL. Mutations resulted in dysfunction pVHL and were associated mainly with non-syndromic PCCs, accurate genetic counseling and predictive testing, tumor resection, postoperative care, and close follow-up based on gene-specific clinical protocols. For the purposes of genetic counseling and genetic screening, we need to underscore the importance of several demographic and clinical features associated with the presence of a germline mutation, including age at diagnosis, multifocal disease, extra-adrenal location, and malignant tumors. According to the present study and reference, individuals whose age at onset were less than 50 years and had a family history, non-syndromic suspected pheochromocytoma, ganglion cell tumor syndrome group and pheochromocytoma, should accept VHL gene detecting. These individuals and their relatives who have no abnormal clinical findings limited our ability to detect genetic modifiers and environmental factors.

As the sequencing technology progresses, genetic examinations for ASP are recommended for individuals with familiar history of PCCs especially for those of which a family member was diagnosed with PCCs under the age of 20 years, the PCCs were bilateral or in case of an extra-adrenal pheochromocytoma with catecholamine synthesis. Evidence indicates. Testings play an important role in medical management, such as timely diagnosis of non-syndromic PCCs, accurate genetic counseling and predictive testing, tumor resection, postoperative care, and close follow-up based on gene-specific clinical protocols. For the purposes of genetic counseling and genetic screening, we need to underscore the importance of several demographic and clinical features associated with the presence of a germline mutation, including age at diagnosis, multifocal disease, extra-adrenal location, and malignant tumors. According to the present study and reference, individuals whose age at onset were less than 50 years and had a family history, non-syndromic suspected pheochromocytoma, ganglion cell tumor syndrome group and pheochromocytoma, should accept VHL gene detecting. These individuals and their relatives could benefit from genetic detecting technology immediately.
In conclusion, in our study we identified the VHL gene mutations (H125P, 623\(^{TTTGTtG}\) and R120T) in all individuals with PCCs who are members of a single Chinese family as well as three patients with APS in a cohort of 41. These mutations resulted in dysfunction of \(pVHL\). The genetic analysis is helpful for medical management of patients with HVLD or ASP and exploration of VHLD pathogenesis.

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