A Novel Germline Mutation in the von Hippel-Lindau Gene in Patients in Kuwait

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Key Words

von Hippel-Lindau disease · Polymerase chain reaction-single strand conformation polymorphism

Abstract

Objective: To determine the germline mutation in an extended family in which 1 member was diagnosed clinically with von Hippel-Lindau (VHL) disease and to investigate 3 generations of the family. **Subjects and Methods:** The polymerase chain reaction-single strand conformation polymorphism sequencing techniques were used to identify the germline mutation in the *VHL* gene in the patient and also to study 9 other members of the extended family over 3 generations. **Results:** The patient and 3 other members of the family were shown to have the same mutation in the splice donor site of the first intron. The mutation was identified as IVS1 + 1 G→T. **Conclusion:** The findings of this study indicate the presence of VHL mutation in a Kuwaiti family with Arab parentage. It is hoped that the study would contribute to understanding the types of mutation in VHL in the Middle East. Its early detection and diagnosis would help in genetic counseling of VHL patients.

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Introduction

Von Hippel-Lindau (VHL) disease is an autosomal dominant familial tumor syndrome. *VHL* gene is a tumor suppressor gene isolated and localized at 3p25.5 [1, 2]. The basis of familial inheritance of *VHL* is a germline mutation in this gene. This results in great risk of developing retinal and central nervous system hemangioblastoma as well as kidney cysts and clear cell carcinoma, cyst adenomas of other organs and pheochromocytomas [3–5]. The mutation spectrum of *VHL* is heterogeneous with mutations scattered throughout most of the *VHL* gene. Although some recurrent mutations have been reported, most families have their own unique germline mutation [6–8]. VHL age of onset is variable and depends on the expression of the disease within an individual [9]. After DNA analysis has identified presymptomatic affected individuals in a family, carriers of the mutated *VHL* gene can be monitored closely and given appropriate treatment.

The prevalence of VHL has been estimated in the Western population to be between 1:35,000 and 1:40,000 [2, 10]. Identifying such patients has been problematic as it involves comprehensive screening of all potentially affected family members. In addition, the prevalence of the genetic defect in the Middle Eastern population is

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Fig. 1. SSCP results for all 10 screened members of the two families simplified as P1–10. Arrows indicate in patients P1, 3, 8 and 10 the extra band in the mutated DNA due to conformation polymorphism. NC = Normal control.

uncertain [1, 7, 11–14]. This is partly due to the fact that the nature of the mutations involved in VHL in Middle Eastern Arabs has not been determined. Therefore, the genetic mutation in one extended Kuwaiti family of 3 generations was characterized using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP).

Subjects and Methods

Subjects

Ten members (7 male, 3 female) of 3 generations (2 grandparents, 2 parents and 6 children) of an Arabic-Persian family, of which 1 was clinically diagnosed with VHL disease, were recruited for the study. Five milliliters of EDTA blood was withdrawn once from this patient, 9 other members of the family and 15 controls (8 male, 7 female). Genomic DNA was extracted from the blood samples immediately.

DNA Extraction and PCR

Genomic DNA from blood samples was isolated by proteinase K digestion and phenol-chloroform extraction as previously described [15]. The cloned portion of *VHL* cDNA has three exons of 852 nucleotides [16]. Five sets of primers covering the three exons and the exon-intron boundaries were used [6]. The oligonucleotide primers were synthesized and supplied by Genelink (New York, USA, www.genelink.com). Each PCR for all exons and exon-intron boundaries was performed using 100 ng genomic DNA, 1 × PCR buffer (Perkin Elmer, USA), 10 pmol of each PCR primer, 25 μmol/l deoxynucleoside triphosphate, 2.0 mmol/l MgCl₂, and 0.25 U Taq polymerase (Perkin Elmer) in a total volume of 50 μl. The PCRs were performed on a DNA thermal cycler (9700 Perkin Elmer) using the following parameters: first one denaturation step at 95 °C for 3 min, then 35 cycles of denaturation at 95 °C for 1 min, annealing at 58 °C for 1 min, and elongation at 72 °C for 1 min. Then a final step of 10 min at 72 °C. Ten microliters of the PCR product was checked on 1% agarose.

Single Strand Conformation Polymorphism

SSCP analysis was performed by mixing 3 μl of the PCR reaction with 3 μl of stop-solution (10 mM NaOH, 95% formamide, 0.05%

xylene cyanol, 0.05% bromophenol blue), heated to 95 °C for 5 min, and chilled on ice for 6 min. The entire mixture was then loaded onto a horizontal nondenaturing 0.9 mm × 50 cm × 21 cm gel. This gel was 60 ml of 10% polyacrylamide with 5% glycerol, 48 μl of tetramethylethylenediamine and 380 μl of 20% (w/v) ammonium persulfate. The gel was run at 250 V and 45 W overnight at room temperature with 1 × Tris/borate/EDTA running buffer. The resulting bands were visualized by silver nitrate staining.

Silver Nitrate Staining

The gels were fixed in two changes of fixation solution (10% ethanol, 0.5% acetic acid) each for 5 min with gentle shaking. They were stained for 15 min in freshly prepared 0.1% silver nitrate and rinsed briefly with distilled water. Gels were then incubated in developing solution (1.5% NaOH, 0.1% formaldehyde) for 30 min. Finally the gels were treated with 0.75% Na₂CO₃ solution for 10 min, sealed in a plastic bag and photographed.

DNA Sequencing

The SSCP-positive samples were amplified by PCR in a total volume of 50 μl. The PCR product was ethanol-precipitated and dissolved in 15 μl of sterile dH₂O. Four microliters of the cleaned PCR product was mixed with 3.2 μl (1 pmol) of either the forward or the reverse primer, and 8 μl of the dye terminator ready sequence reaction mix (Prism™ Ready Reaction Big Dye™ Terminator Cycle Sequencing Kit; Perkin Elmer). The sequencing reaction was then carried out in a 9700 thermocycler programmed to 25 cycles of 96 °C for 10 min, 50 °C for 5 s, and 60 °C for 4 min. The sequencing reaction was ethanol precipitated and 25 μl of HiDi formamide was added to the pellet, mixed, heated for 2 min at 95 °C, kept on ice until the sequencing, run on an automated DNA sequencer (ABI3100; Applied Biosystem, USA).

Results

Of the 10 members of the family, SSCP showed an extra band in 4 of the children (3 male, 1 female) including the patient (fig. 1). The remaining family members and normal controls did not show any such band. Representative sequencing spherograms of normal subjects and the VHL patient are shown in figure 2.

Fig. 2. Sequencing results using the ABI3100 automated sequencer showing the G-T mutation in the first nucleotide of intron one, IVS1 + 1 G→T, where A is the wild type and B is the mutant.

The mutation in the *VHL* gene was found in nucleotide number one of the first intron, designated as IVS1 + 1 G→T mutation. This nucleotide is in the splice donor site of intron 1. The origin of the mutation was from the Arabic side of the family. The patient with VHL mutation was 39 years old, while the other 2 males were 32 and 20 years old, and the female was 24 years. The patient aged 39 years died only a few weeks before completion of the study.

Discussion

As previously noted, the age of onset of VHL is variable and depends on the expression of the disease within an individual [9]. The clinical diagnosis depends on the onset of the symptoms; therefore gene carriers cannot be identified prior to manifestation of disease complications especially if they lack a family history of the disease. Hence, late diagnosis or misdiagnosis of this disease might result in a severe outcome, and most probably premature death. Using the PCR/SSCP/sequencing method, we confirmed the clinical diagnosis of the primary patient who was diagnosed with VHL disease by the identification of the single nucleotide substitution in the exon one-

intron one boundary from G to T, which is nucleotide IVS1 + 1 G→T. This nucleotide is an intron splice donor for intron 1, which is the first such mutation to be identified not only in Kuwait, but also among the 908 screened families worldwide [6, 7, 11–14]. A similar position mutation was identified, but on the intron 2-exon 3 boundary at nucleotide 677-1, which is an intron splice acceptor for exon 3 [11, 14].

The prevalence of *VHL* has been estimated to be between 1:35,000 and 1:40,000 [2, 10]. Applying this to the Middle Eastern region, there should be between 25 and 30 patients with VHL disease in Kuwait, but the necessary comprehensive screening of all potentially affected family members has made identifying such patients problematic. The screening approach used in this study, PCR/SSCP/sequencing, is a diagnostic tool which should be adopted to improve the diagnosis of VHL disease in patients without an established disease history, as well as provide presymptomatic diagnosis in relatives of VHL patients with known mutations.

Conclusion

The findings of this study indicate the presence of VHL mutation in a Kuwaiti family with Arab parentage. It is hoped that the study would contribute to understanding the types of mutation in VHL in the Middle East. Its early detection and diagnosis would help in genetic counseling of VHL patients.

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