A Sri Lankan family with cerebellar hemangioblastoma due to a heterozygous nonsense mutation in the von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase (VHL) gene.

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Key words: Genetics; hemangioblastoma; VHL gene; Von Hippel-Lindau disease

Abstract

Mutations in the von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase (VHL) gene cause a variety of phenotypes including von Hippel-Lindau (VHL) disease. This report describes a Sri Lankan family with three siblings with cerebellar haemangioblastoma due to a nonsense mutation in the VHL gene. A heterozygous nucleotide substitution in exon 3 was identified in all three siblings resulting in a stop codon at amino acid position 175 leading to a truncated non-functional VHL protein [NM_000551.3(VHL):c.525C>G;p.Tyr175Ter; rs5030835C>G]. Patients with rare tumours characteristic of VHL should undergo clinical and genetic evaluation for VHL.

Introduction

The von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase (VHL) gene [GenBank: NG_008212.3, OMIM# 193300], is a tumor suppressor gene which spans a 14.25-kb genomic region at 3p25.3. It encodes for two alternatively spliced transcript variants. Transcript variant 1 (NM_000551.3) which is encoded by all 3 exons is translated to a protein with 213 amino acid residues (NP_000542.1) while transcript variant 2 (NM_198156.2) is translated to a protein with 172 acid residues (NP_937799.1) [1].

Mutations in the VHL tumor suppresser gene cause a variety of phenotypes including von Hippel-Lindau disease (VHL), familial phaeochromocytoma and inherited polycythaemia [2]. VHL is an autosomal dominantly inherited familial cancer syndrome predisposing to a variety of malignant and benign tumors [3] such as haemangioblastomas of the cerebellum, spinal cord, brainstem and retina, clear cell renal carcinomas, pheochromocytomas, endolymphatic sac tumours, pancreatic islet cell tumours, haemangiomas of the adrenals, liver and lungs, epididymal and broad ligament papillary cyst adenomas as well as visceral cysts in the kidneys and pancreas [4]. A germline mutation of the VHL gene is the basis of familial inheritance of VHL syndrome. According to Knudson's ("Two Hit") hypothesis, both alleles of a tumor suppresser gene need to be mutated in order for a tumour to develop, therefore a patient who manifests a tumour, inherits one mutation from a parent, and develops the second mutation in the same gene in the affected organ as a somatic mutation, at which point the tumour begins to manifest [5].

To date, more than 300 mutations have been identified in families with VHL disease, consisting of partial and whole gene deletions, frameshift, nonsense, missense, and splice site mutations [6]. About 20% of cases are due to de novo mutations. This report describes a Sri Lankan family with 3 siblings with cerebellar haemangioblastoma due to a heterozygous nonsense mutation in the VHL gene.

The Family

A 28 year old female who was clinically diagnosed with a cerebellar hemangioblastoma was referred to the Human Genetics Unit for genetic evaluation. The patient was clinically diagnosed with cerebellar haemangioblastoma at the age of 13 years, since then, she had undergone four surgeries for removal of the recurrent tumour in the posterior cranial fossa. In addition, a tumor arising from the fourth ventricle of the brain was also surgically removed. She also developed a

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cervical spinal cord haemangioblastoma (Figure 1). Two of her male siblings were also diagnosed with cerebellar hemangioblastoma. The CT scan of one of the brothers showed dilatation and a cystic mass in the lateral third ventricle as well as a renal cyst. Figure 2 shows the pedigree of the family with VHL disease.

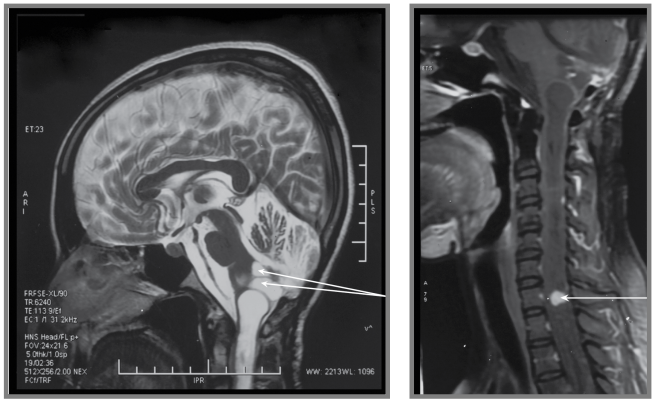

Figure 1. (a) T2 weighted sagittal MRI brain of the female proband showing the recurrent cerebellar haemangioblastoma and (b) T1 weighted post contrast sagittal MRI of the cervical spine showing spinal haemangioblastoma.

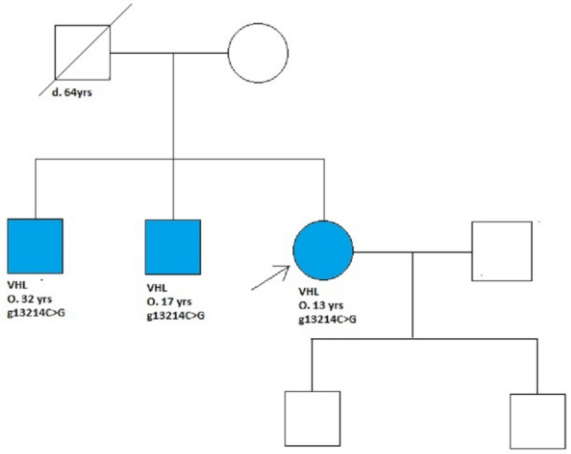

Figure 2. Pedigree of the family with VHL disease showing the familial mutation in the three siblings.

Genotyping

The VHL gene was sequenced in the patient and her 2 siblings after obtaining their written informed consent. DNA was extracted from peripheral blood using QIAamp blood DNA midi kit from Qiagen. All 3 exons and flanking intronic regions of the VHL gene were sequenced using an ABI PRISM 3130 Genetic Analyzer. The published human VHL gene Reference Sequence file obtained from GenBank (http://www.ncbi.nlm.nih.gov) was used for comparison of the nucleotide sequences generated from the patients and to confirm the presence of any mutations.

A heterozygous nonsense mutation was identified in all 3 individuals in exon 3 of the VHL gene. A single nucleotide substitution at position 13214 (NG_008212.3.g13214C>G) replaced the codon for amino acid tyrosine (UAC) in transcript variant 1 (NM_000551.3.c525C>G) to a stop codon (UAG) resulting in premature termination of the VHL protein at amino acid position 175 (NP_000542.1.pTyr175Ter). This mutation has previously been reported in other families and documented in the dbSNP database and assigned the SNPID rs5030835 (http://www.ncbi.nlm.nih.gov/projects/SNP/rs=50308 35). Figure 3 shows the partial electropherogram with the point mutation at position 13214 of the VHL gene.

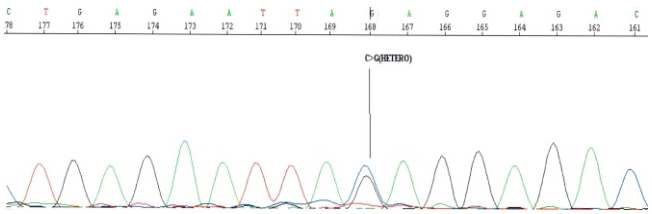

Figure 3. Partial electropherogram of the patient showing the heterozygous nonsense mutation in the VHL gene.

Discussion

This report describes a Sri Lankan family with three siblings with cerebellar haemangioblastoma due to a heterozygous nonsense mutation in the VHL gene. VHL mutations are associated with various benign and malignant tumours resulting in high morbidity and mortality rates. Mutations in the VHL gene are known to cause haemangioblastomas of the central nervous system (CNS) in 60-80% of VHL patients [6,7].

A study conducted by van der Harst et al. in 1998 reported that 8 out 68 patients with pheochromocytoma had mutations in the VHL gene. Among these patients, two were relatives and had a familial mutation [8]. Familial mutations in the VHL gene have also been reported in VHL families presenting with clear cell renal cell carcinoma. Recent advances in understanding the genetic basis of VHL disease has resulted in improved diagnosis of VHL disease and provided greater insights into the molecular pathogenesis of the disease [1]. The

prognosis can be improved through early screening, diagnosis and surveillance [9]. Molecular genetic testing coupled with genetic counseling is now considered standard for the evaluation of patients and families with suspected VHL [10].

Acknowledgments

We would like to express our thanks to Dr. Udari Liyanage, Senior Lecturer & Consultant Radiologist, Department of Anatomy, Medical Faculty, Colombo for providing radiological information and Mr. Sanka P. Bandara of the Audio-Visual Unit, Medical Faculty, Colombo for preparing the radiological photographs.

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Key Points:

- Mutations in the VHL gene are known to predispose to haemangioblastomas of the central nervous system in 60-80% of patients with VHL disease.
- Molecular genetic testing coupled with genetic counseling should be offered to patients and families with suspected VHL disease.
- The prognosis can be improved through early screening, diagnosis and surveillance.