

ELECTRONIC LETTER

Comorbid *VHL* and *SCA2* mutations in a large kindred: confounding diagnosis of neurological dysfunction caused by CNS *VHL* vascular tumours versus *SCA2* atrophic neurodegeneration

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Von Hippel-Lindau disease (VHL, MIM 193300) is an autosomal dominant heritable neoplastic disorder resulting from any one of 163¹ or more non-polymorphic mutations² found in the VHL tumour suppressor gene on chromosome 3p25.³ Inheritance of a mutated VHL gene predisposes to development of specific types of tumours, including brain and spinal cord haemangioblastoma, renal clear cell carcinoma, pheochromocytoma, retinal angioma, pancreatic neuroendocrine tumour/islet cell carcinoma, endolymphatic sac tumour, and epididymal and broad ligament papillary cystadenoma.⁴⁻⁸

The spinocerebellar ataxias, also referred to as olivopontocerebellar atrophy, are a diverse group of neurodegenerative disorders associated with atrophy and dysfunction of the cerebellum and brain stem. Varying degrees of extracerebellar manifestations including neuropathy and oculomotor disorders, such as slow saccades and/or ophthalmoplegia, have been described.⁹

We present clinical and genetic findings for members of a large kindred with a history of both von Hippel-Lindau disease (VHL) and spinocerebellar ataxia type 2 (*SCA2*, MIM 183090). It is noteworthy that the combined stresses of these two afflictions weakened a number of the nuclear family units of this kindred resulting in separations of parents, children, and sibs.

METHODS

We studied a multigenerational family with *SCA2* and VHL (fig 1). Index family members contacted the National Cancer Institute for screening. They were enrolled in an institutional review board (IRB) approved study of von Hippel-Lindau disease, after reviewing and signing informed consents as well as receiving counselling. Through efforts of the first family members screened, other family members were contacted and informed of our programme for clinical and genetic diagnostic screening.

Some members of the extended family had died before the initiation of these efforts. We gathered information on those persons who had died through histories given by living family members as well as information from death certificates.

Germline VHL mutation analysis was performed as previously described in detail² (analysis performed by Genetic Diagnostic Laboratory, University of Pennsylvania School of Medicine). Briefly, Southern blot analysis was performed initially, using random primer labelled probes specific for the VHL gene and the human β globin gene, to search for complete or partial deletion of the VHL gene. Next, genomic DNA was amplified by polymerase chain reaction and DNA sequences were analysed by automated sequencing. Ataxin-2 gene mutation analysis was determined by polymerase chain reaction (PCR) amplification of the repeat regions of both alleles and size determination of the PCR products was done by sequencing gel electrophoresis⁹⁻¹¹ (analysis performed by University of Pennsylvania, William Pepper Laboratory, Department of Pathology and Laboratory Medicine).

RESULTS

Case histories and molecular genetics

Patient I.1

Patient I.1 was thought to have had olivopontocerebellar atrophy (OPCA) for much of her adult life. Her OPCA is now explained by the detection in her kindred of spinocerebellar ataxia type 2 (*SCA2*). Her VHL was diagnosed at the age of 48 years. She had 16 children during her 54 year life span, giving birth to the oldest when she was 17 years old and the youngest when she was 45 years old. Only four of her children are

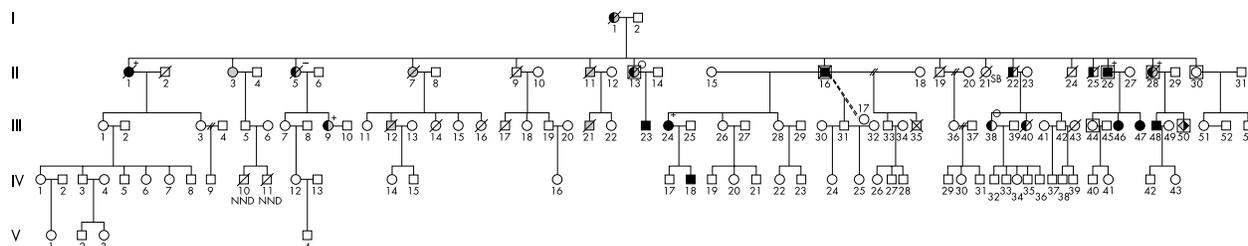


Figure 1 Family pedigree. VHL is represented by black symbols. Persons with a + superscript have both CNS (including retinal angiomas) and renal involvement. Persons with a - superscript have only renal involvement. Persons with an O superscript have only CNS involvement, including retinal involvement. Persons represented by black symbols without designation of involved site were found to have VHL by genetic testing not clinical findings. *SCA2* is represented by grey symbols. Persons with both conditions are represented by symbols that show both colours. II.16 committed suicide.



Figure 2 Magnetic resonance imaging (MRI) with gadolinium contrast on patient II.26 with VHL related haemangioblastoma (involving the cerebellum and peduncle on the right). These highly vascular tumours enhance brightly with gadolinium.

not known to have had either VHL or SCA2. Two of these children died in infancy. No medical history is available on her last born child, who was adopted shortly after birth. Two of her children were placed into foster care before her husband's death. After the death of her husband, five of her remaining children were put into foster care and later placed into adoptive homes. Patient I.1 died of pneumonia aged 54 years.

Patient II.26

Patient II.26 was seen as part of the von-Hippel Lindau screening programme at the National Institutes of Health/Clinical Center (NIH/CC). He had been discovered to have VHL at the age of 34 after detection of a haemangioblastoma in the cerebellum that was surgically resected followed by postoperative radiation treatment. The following year, he had an oesophageal abnormality that was treated with vagotomy. At the age of 44, he had bilateral clear cell renal cancer resulting in a right partial nephrectomy and left total nephrectomy. The same year he had spinal surgery to treat a syrinx associated with a spinal cord haemangioblastoma. He has baseline tachycardia thought by the cardiologist to be most likely secondary to autonomic dysfunction. The screening done at NIH/CC was notable for cystic lesions in the pancreas. On scrotal ultrasound examination, he had a multicystic mass above his left testis consistent with epididymal cystadenoma. By history, the mass had first been noted while he was in his teens. He had multiple, tiny, enhanced nodules consistent with haemangioblastomas posterior to the medulla with cysts splaying the medulla (fig 2) and haemangioblastomas were also in the cervical and thoracic spine. After genetic counselling had been given and informed consent was signed, a blood specimen was obtained for analysis of the VHL gene mutation. He was found to have a deletion of a single adenosine (A) at nucleotide 688 of the von Hippel-Lindau gene, causing a shift in the mRNA translational frame with a resultant truncated protein, which is presumed to be non-functional. This elongin binding domain mutation had not been previously reported at the time when it was detected in this kindred.

Patient III.9

Patient III.9 was first seen at the NIH/CC for von Hippel-Lindau family screening at the age of 32. She had been diag-

nosed with VHL in her early teenage years. She had retinal involvement beginning at the age of 13 treated by cryotherapy and laser therapy, presumably for retinal angiomas. She had a retinal detachment at the age of 23 requiring eye surgery with cataract removal. She had cysts of the pancreas and kidney detected at 22 years. In addition, she has carried the diagnosis of OPCA since the age of 26-28 when she was evaluated for new onset slurred speech and illegible handwriting. The screening for VHL at NIH/CC showed multiple renal cysts without renal tumours. However, a large infiltrative enhancing mass in the tail of the pancreas was suggestive of a neuroendocrine/islet cell carcinoma, and liver metastatic foci were documented by biopsy. Treatment was by distal pancreatectomy and splenectomy followed by hepatic perfusion with Melphalan. Three years later, retreatment for liver metastasis was by a combination of isolated hepatic perfusion of Melphalan with hyperthermia, radiofrequency ablation, and wedge resection of three lesions. Neuroimaging showed diffuse cerebellar and brain stem atrophy with slight atrophy of the cervical cord but no VHL brain or spinal cord haemangioblastomas were detected (fig 3) within this atrophic tissue. Her neurological examination was notable for ataxia, dysarthria, dysmetria, and bulbar signs. After genetic counselling had been given and informed consent was received, a blood specimen was sent for analysis of the VHL gene mutation. She was found to have a deletion of A at nucleotide 688 of the von Hippel-Lindau gene, identical to that found in her uncle, patient II.26. In light of the reported family and personal history of OPCA, molecular analyses were performed on genes known to cause spinocerebellar ataxia in order to delineate the genetics of her condition further. She was found to have an abnormal increase in CAG repeats in one allele of her ataxin-2 gene. This abnormal trinucleotide expansion is consistent with the diagnosis of spinocerebellar ataxia type 2. The normal number of CAG repeats ranges from 15 to 29. This patient had one normal allele with 22 repeats presumably from her unaffected father. Her second allele for the ataxin-2 gene contained 41 CAG repeats which is well into the abnormal range and consistent with her symptoms and signs on neurological examination and neuroimaging.

DISCUSSION

This kindred is notable for the inheritance of two autosomal dominant genetic mutations with the potential for confounding diagnoses and comorbid neurological effects. VHL is known to affect certain organ systems specifically. However, the diagnosis according to phenotype can be difficult owing to the variable time of onset of each of the disease manifestations. The diagnosis has traditionally been made through the use of family history data as well as through evaluation of the organ systems that are most likely to become affected. In general, the central nervous system manifestations precede the renal lesions. Renal cell carcinoma is often occult for many years and usually detected in the third decade of life, but onset at 15 years is also known.⁵ Haemangioblastomas of brain and spinal cord are often diagnosed when symptomatic, with most being detected in the second decade of life,⁴ but a diagnosis in an 11 year old has been reported.¹²

Presymptomatic screening programmes have been proposed that include neurological examinations, laboratory and imaging studies of tumour prone organ sites, and yearly eye examinations. Recommendations for the central nervous system call for MRIs of the head and spine to be done every two to three years in asymptomatic carriers.^{4,5} Molecular biology techniques can now be used in order to predict whether family members of known carriers are likely to be affected with the condition.¹⁻³

This large kindred is of interest because of the presence of a comorbid genetic mutation causing SCA2 and the potential to have its diagnosis confounded by the symptoms arising from



Figure 3 Magnetic resonance imaging, with gadolinium contrast, of patient III.9, who is affected with both SCA2 and VHL. Note the prominent atrophy of the cerebellum and brain stem showing the anatomical changes associated with the neurological signs and symptoms of SCA2 in this patient. Although this patient also has VHL, no haemangioblastomas have developed in her cerebellum, the most common site of brain tumours in VHL.

a VHL tumour of the CNS. Haemangioblastomas, which occur in over 50% of patients with VHL, commonly occur in the cerebellum, and may cause a slowly progressive ataxia as does SCA2.⁴ It is interesting that at the age of 32, patient III.9 with severe atrophy of the cerebellum, brain stem, and cervical spine, does not have the haemangioblastomas common in VHL and present in her uncle. The VHL mutation that they share obviously can cause CNS haemangioblastomas. It is possible that the severe cerebellar atrophy related to her SCA2 inhibits the angiogenesis needed for the development of highly vascular haemangioblastomas. Recent reports point out that the vascularity of VHL neoplasms is the result of overproduction of angiogenic peptides encoded by hypoxia inducible factor (HIF) responsive genes,¹³ with impaired degradation of HIF- α being required for haemangioblastoma development and renal cell carcinoma susceptibility.¹⁴ This family's VHL gene mutation in the elongin binding domain would inhibit formation of the tetramer pVHL/elongin C/elongin B/Cul2 (VCBC), thus impairing degradation of HIF- α and allowing development of haemangioblastomas and renal cell carcinoma. The hypoxia inducing gene, vascular endothelial growth factor (*VEGF*), is over-expressed in haemangioblastomas owing to mutational inactivation of the VHL gene.¹⁵ Under normoxic conditions, mutations in the VHL gene lead to increase in angiogenesis in the brain, kidney, and eye.¹⁶ When VHL deficient cell lines had reintroduction of a functional VHL gene, the instability of HIF α proteins under normoxic conditions was restored.¹⁷ It is unknown whether anti-angiogenesis or anti-vasoendothelial growth conditions and factors (*VEGF*) exist in this patient's atrophied, presumably hypovascular and hypoxic cerebellum, which may inhibit or suppress haemangioblastoma development.

Spinocerebellar ataxia 2 (SCA2) is an autosomal dominant, heritable, neurodegenerative disorder caused by a mutation of the ataxin-2 gene found on chromosome 12q24. The genetic mutation is a trinucleotide (CAG) repeat expansion known to have an inverse correlation between number of repeats and

age of disease onset. An expansion of 36 repeats was reported in association with onset at ages 37 years and 44 years, while an expansion of 52 repeats was associated with onset at 9 years.^{9, 10} Attempts are being made to distinguish among the genetically defined subtypes of spinocerebellar ataxia on the basis of phenotypic features. SCA2 is characterised by the absence of retinal degeneration and the presence, in varying degrees, of cerebellar signs, ophthalmoplegia, and peripheral neuropathies. Although specific clinical, electrophysiological, and neuroimaging findings are more commonly associated with one genetic subtype than another, giving clinicians clues as to the possible diagnosis, there is a great deal of overlap between subtypes without an exact correlation between phenotype and genotype.^{19, 20}

In her classifications of the autosomal dominant ataxias, Harding²⁰ noted that dementia and/or marked euphoria can be associated with these conditions as well as emotional lability.²⁰ While the spinocerebellar ataxias are predominantly notable for their associated movement disorders, abnormalities in attention and frontal executive function have been described.^{19, 21} Current innovations and modifications of genotypic techniques may allow researchers to associate specific types of cognitive dysfunction with OPCA subtypes.

Dementia has already been associated with SCA2 more frequently than SCA1, SCA3 (Machado-Joseph disease), SCA6, or SCA7. The dementia may be of clinical interest owing to difficulty in completing complex tasks in an unstructured environment with less marked effects on activities of daily living, such as personal grooming. Recent evaluation of a family with SCA2 showed normal range mini-mental status performance but marked abnormality of frontal-executive function on detailed questioning.²² If this type of dementia were present in the matriarch of this family (I.1), it may give insight into her difficulties keeping her family intact after the death of her husband. The day to day unpredictability of caring for seven young children may have overwhelmed her multi-tasking and prioritising abilities.

The affected members of the kindred described here had a phenotype consistent with previous descriptions of SCA2. SCA2 presents a challenge in clinical diagnosis secondary to potential intrafamilial phenotypic variation.²¹ Also some trinucleotide repeats are known to expand with subsequent generations, a phenomenon called anticipation, resulting in earlier age of disease onset.²³ It is unclear to what extent the presence of a comorbid VHL mutation impacts on the disease manifestations in this group. In the past, VHL has been associated with early death, with a median age of death in the fifth decade of life.⁵ This poor prognosis may be changing with early detection through molecular biological and radiological screening combined with timed organ sparing surgeries and other tumour ablation modalities. Distinguishing VHL central nervous system lesions is especially important because they are usually treatable. The prognosis for patients with SCA2 is unclear owing to the marked variability in phenotypic expression. This unusual kindred will bear careful monitoring as we attempt to determine the effects of the inheritance of their comorbid conditions and further describe their familial phenotype.

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