Case Report

A Family with Co-existing SDHB and SDHD Mutations Causing Hereditary Paraganglioma Syndrome

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Abstract

Introduction: We report the co-occurrence of a *SDHD* and a *SDHB* mutation in a family with hereditary paraganglioma syndrome. We compare this finding to simultaneous haploinsufficiency of *BRCA1* and *BRCA2*.

Presentation of Case: The 28 year old proband presented as an isolated case in the family with a malignant phaeochromocytoma. Sequencing and MLPA of *SDHD* and *SDHB* were performed. A *SDHD* splice site mutation, c.169+5G>A, was identified in the proband, his sister and their father. In addition, a *SDHB* exon 1 deletion was identified by MLPA in the proband, his sister and their mother. Both mutations have been described previously and considered to be pathogenic. An appropriate screening programme was instituted for carrier relatives.

Conclusions: To our knowledge, this is the first report of two SDH subunit mutations in a single family. Though there was no family history to suggest inherited disorder, the simultaneous testing of both genes was diagnostic. The family history is consistent with suggestions that the penetrance of *SDHB*/*SDHD* mutations is lower than initially thought.

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Introduction

The genetic predisposition to develop head and neck paragangliomas (HNPGL) has been recognised since 1982 and the association of HNPGL with phaeochromocytomas since 1974 [1, 2]. Several genes have since been identified that are associated with the hereditary paraganglioma/phaeochromocytoma syndromes. These include the succinate dehydrogenase genes of the complex II respiratory chain in the mitochondria, namely *SDHA*, *SDHB*, *SDHD*, *SDHC* and *SDHAF2* [3-6]. Other genes involved in phaeochromocytoma/paraganglioma include *NF1*, *VHL*, *RET*, *TMEM127* and *MAX* [7-10].

Familial phaeochromocytoma/paraganglioma syndromes caused by succinate dehydrogenase subunit mutations are inherited in an autosomal dominant manner, though *SDHD* mutations usually only cause disease if inherited from the father [11, 12]. Patients with *SDHD* or *SDHB* mutations are at risk of adrenal and extra-adrenal phaeochromocytomas and HNPGL as well as a smaller risk of additional tumours, for example, renal, thyroid and gastrointestinal stromal cell tumours. The mean age of diagnosis in patients with *SDHD* mutations is 31 years old and with *SDHB* mutations is 34 years [13].

The penetrance of tumours in *SDHD* mutations has been estimated at 50% by 31 years and 86% by 50 years [14]. The site-related penetrance of *SDHD* mutations has been reported as 71% at 60 years for head and neck paragangliomas and as 29% at 60 years for phaeochromocytomas [13].

The penetrance of *SDHB* mutations was described initially as 50% for paragangliomas by 35 years and 77% by 50 years [14]. Some studies, however, suggest that, at least for some mutations, the penetrance may be lower than this estimate [13, 15]. The site-related penetrance has been estimated at 29% at 60 years for head and neck paragangliomas and 52% at 60 years for phaeochromocytomas. The risk of renal tumours is 14% at 70 years with an *SDHB* mutation [11]. Up to 30% of patients with an isolated paraganglioma or phaeochromocytoma have a germline mutation, and it has been suggested that comprehensive mutation testing should be considered to identify these patients

[16, 17]. *SDHB* mutations are the most commonly identified. *SDHB* mutations convey a higher risk of malignant transformation of these tumours [14,18].

Biochemical and radiological surveillance is generally offered to all *SDHB* /*SDHD* mutation carriers and a recommended screening programme includes an annual MRI of the pelvis and abdomen, 3 yearly MRI screening from the head and neck to the base of spine and annual 24-hour urine metadrenaline analysis.

Materials and methods

The *SDHB* gene contains 8 exons and *SDHD* contains 4 exons. Direct sequencing of the coding exons and flanking intronic regions of the exons in both genes was performed using standard PCR and sequencing reactions (BigDye v3.1;Applied Biosystems Inc., Foster city, CA). Sequencing reactions were analyzed using an ABI 3730 DNA Analyzer (Applied Biosystems Inc.) for *SDHB* and on an ABI 3170 DNA Analyzer (Applied Biosystems Inc.) for *SDHD*. The data was analysed by Mutation Surveyor variant analysis software V3.30 (primer sequences on request). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (SDHB–NM_003000.2; SDHD–NM_003002.2), according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Screening for germline *SDHB*, *SDHD* and *SDHC* exon deletions was performed by MLPA dosage analysis using MRC-Holland Kit P226 and SoftGenetics GeneMarker analysis software. Further investigation of the common *SDHB* exon 1 deletion was undertaken by PCR amplifications from regions either side of the deletion (primer sequences on request). This was performed to check for any sequence variant which may prevent probe hybridisation and cause a false copy number result by MLPA.

Case Presentation and Results

The proband presented with a metastatic

paraganglioma at age 28 years. He reported a one year history of increasing lower back pain and sciatica of the left leg with altered sensation in the anterior aspect of his upper thigh, together with significant weight loss and night sweats. He did not complain of headaches, palpitations, or exertional symptoms.

When this was investigated, a CT scan and MRI scan identified a large adrenal mass with disease extending retroperitoneally around the lower thoracic/lumbar vertebrae at T12/L1, encroaching onto the spinal cord and cauda equina. The para-oesophageal nodes were also enlarged and the presence of lung deposits was detected. There were multiple bone metastases throughout the skeleton seen on an MIGB scan, including uptake in the skull, right shoulder, spine, lower ribs, left ileum, and left thoracolumbar region, in keeping with the paravertebral mass. Biopsy from one of the bone lesions confirmed paraganglioma.

Plasma catecholamines and metanephrines and 24-hour urine catecholamines were normal, confirming non-secretory disease. Plasma calcium and calcitonin levels were also normal making Multiple Endocrine Neoplasia type 2 (MEN2) unlikely.

In view of the widespread metastases and poor prognosis he was given initial palliative radiotherapy to the spine and MIBG radio-isotope therapy. The avidity of the uptake in the latter treatment was poor and it was therefore discontinued after 1 cycle. He was subsequently given palliative chemotherapy with six cycles of cyclophosphamide, vincristine, and dacarbazine (CVD) with additional bisphosphonate therapy.

He presented as an isolated case in the family, as can be seen in the pedigree (Figure 1). After the identification of germline *SDHB* and *SDHD* mutations in the proband (see later), his first degree relatives were offered presymptomatic genetic testing.
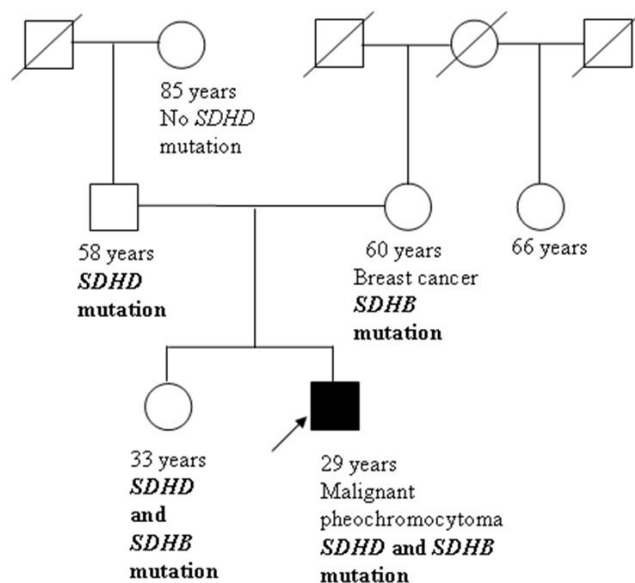

Figure 1 Pedigree of the family with double heterozygosity of a *SDHD* and a *SDHB* mutation.

The proband's sister was 33 years old when she presented for predictive testing. Although she worked in the nuclear industry, she has not been exposed to ionising radiation and has no recorded radiation dose in the workplace. On examination, she had several neck masses which later proved to be enlarged lymph nodes due to infection caused by eczema. Her MRI scan from the base of skull to pelvis and urine

metadrenaline screening showed no abnormalities.

Their father was well at 58 years old and had no clinic signs of hereditary paraganglioma syndrome. His MRI scan from base of skull to pelvis and urine metadrenaline screening demonstrated no abnormalities. The paternal grandfather had died at 51 years of an illness unrelated to hereditary paraganglioma syndrome and the paternal

grandmother was alive at 85 years with no signs of hereditary paraganglioma syndrome.

The proband's mother (aged 60 years) had been diagnosed with unilateral breast cancer at 53 years and had recovered following surgery. Her examination was normal and her MRI scan from the base of skull to pelvis and urine metadrenaline screening were normal. Recently she presented with symptoms of gastritis which did not improve with medication. A CT scan of the thorax identified a mass surrounding the lower oesophagus. Histological examination confirmed metastatic breast cancer. The maternal grandparents were deceased, the grandfather from lung cancer. A half-sister had no signs of hereditary paraganglioma syndrome.

Molecular genetic studies in the proband identified a *SDHD* and a *SDHB* mutation. A heterozygous *SDHD* splice site mutation, c.169+5G>A, was identified in exon 2. A heterozygous deletion of exon 1 of *SDHB* was also identified in the proband.

Predictive testing was offered to the family. Both mutations were identified in the proband's sister. The proband's mother carried the exon 1 deletion in *SDHB* and the proband's father carried the *SDHD* mutation, c.169+5G>A in exon 2. The paternal grandmother was tested for the familial *SDHD* mutation and she did not carry the mutation.

Discussion

To our knowledge, this is the first report of a family in which two likely pathogenic mutations in genes associated with hereditary paraganglioma syndrome have been identified.

The *SDHD* c.169+5G>A splice site mutation has been previously described as a likely pathogenic mutation in a single report. Thus Timmers et al., described an isolated case of a 43 year old male presenting with a bilateral tympanojugular and carotid tumour with this mutation [19]. cDNA analysis suggested that this mutation resulted in the skipping of exon 2. Skipping of exon 2 in *SDHD* would delete a large part of the first transmembrane helix, the loop joining the first and 2nd transmembrane helix and the N-terminal portion of the 2nd transmembrane helix, with the likelihood of this reducing the ability to bind

with the other proteins in the SDH complex [20]. This mutation is therefore likely to be pathogenic. A similar *SDHD* mutation, c.169+1G>T, has also been described as likely to be pathogenic in two unrelated cases [21].

Exon 1 *SDHB* mutations have been reported on several occasions and are thought to be pathogenic. A *SDHB* deletion that involved exon 1 has been reported as an Iberian founder mutation, having being mostly been identified in Spanish families. The penetrance of this mutation was estimated in a large family and was found to be lower than previously reported for *SDHB* mutations at 35% at 40 years [22, 23]. Exon 1 deletions of varying sizes have been described and all have been considered to be pathogenic, although the exact size of our patient's deletion has not been determined. The presence of deletions may be related to abdominal presentation and a younger age at onset [13, 23].

The implications for clinical management of this family were complex. The proband's sister is at risk of head and neck as well as extra-adrenal tumours, but the risk cannot be accurately quantified with current knowledge.

Following the detection of the *SDHD* mutation in the proband's father, the tumour risk was unclear initially, but further testing demonstrated that he had not inherited the mutation from his mother and so it is most likely that it was paternally inherited (de novo *SDHD* mutations appear to be very rare) and hence he is predicted to be at risk of developing HNPGL and/or phaeochromocytoma. The mother of the proband was asymptomatic at presentation. She previously had breast cancer and recently developed metastatic disease. *SDHB* mutations are not associated with an increased risk of breast cancer and not known to increase the risk of metastatic disease.

We suggested a similar screening programme for all of the family members, including the sister with two mutations. The reason for this was because the screening programme is designed specifically to identify the tumours caused by *SDHB* mutations at an early stage. These tumours are most likely to undergo malignant transformation and require regular monitoring. The screening programme would also identify the non-secretory head and neck tumours if they should occur, although these are more

slow-growing and less likely to undergo malignant transformation.

Simultaneous haploinsufficiency has been reported previously in cancer genes, most notably in breast cancer. Double heterozygosity in *BRCA1* and *BRCA2* genes has been reported, particularly in the Ashkenazi Jewish population. Double heterozygosity is rare and estimated at 2.2% of all gene carriers and in 0.3% of all breast cancer cases in this population group. The phenotype in these patients suggests an earlier age at diagnosis of the associated cancers, but there was no increase in the rate of developing cancer. No patients have been identified with two mutations in the same gene as this is thought to be incompatible with life [24, 25].

Conclusion

As more genes are shown to be associated with hereditary paraganglioma syndrome, in future more cases may be identified with a mutation in more than one gene, and the accompanying tumour risks for these patients may become more apparent.

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