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ORIGINAL ARTICLE

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A novel succinate dehydrogenase subunit B germline variant associated with head and neck paraganglioma in a Dutch kindred: A family-based study

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Objective: In the Netherlands, the majority of hereditary head and neck paragangliomas (HNPGL) are caused by germline variants in the succinate dehydrogenase genes (SDHD, SDHB, SDHAF2). Here, we evaluate a four-generation family linked to a novel SDHB gene variant with the manifestation of a HNPGL.

Design: A family-based study.

Setting: The VU University Medical Center (VUmc) Amsterdam, a tertiary clinic for Otolaryngology and Head and Neck Surgery.

Participants and main outcome measures: The index patients presented with an embryonic rhabdomyosarcoma and a non-Hodgkin lymphoma. Array-based comparative genomic hybridisation (aCGH) analysis and multiplex ligation-dependent probe amplification (MLPA) revealed a novel deletion of exon 1-3 in the SDHB gene, suspected to predispose to paraganglioma (PGL)/pheochromocytoma (PHEO) syndrome type 4. Subsequently, genetic counselling and DNA testing were offered to all family members at risk. Individuals that tested positive for this novel SDHB gene variant were counselled and additional clinical evaluation was offered for the identification of HNPGL and/or PHEO.

Results: The DNA of 18 family members was tested, resulting in the identification of 10 carriers of the exon 1-3 deletion in the SDHB gene. One carrier was diagnosed with a carotid body PGL and serum catecholamine excess, which was surgically excised. Negative *SDHB* immunostaining of the carotid body tumour confirmed that it was caused by the *SDHB* variant. The remaining 9 carriers showed no evidence of PGL/PHEO.

Conclusion: Deletion of exon 1-3 in the SDHB gene is a novel germline variant associated with the formation of hereditary HNPGL.

1 | INTRODUCTION

Paragangliomas (PGLs) are rare, highly vascularised, usually benign neoplasms of paraganglia, neuroendocrine organs derived from neural crest chromaffin cells. PGLs can be found throughout the body in association with the parasympathetic or sympathetic nervous system. PGLs of the head and neck region are associated with the parasympathetic nervous system. They secrete

catecholamines in 4%-30% of the cases, which may cause elevated blood pressure, palpitations, flushes and agitation, and may ultimately result in severe cardiovascular complications.[1-3] Head and neck paragangliomas (HNPGLs) are most commonly found at the carotid bifurcation (60%), but can also arise at the jugular bulb, along the vagal nerve or the tympanic nerve.[4] The closely related pheochromocytoma (PHEO), also known as adrenal PGL, together with the thoracic and abdominal extra-adrenal PGL are paraganglion tumours associated with the sympathetic nervous system.

In about 40% of the patients with an apparently sporadic presentation of PGL or PHEO, a genetic predisposition can be identified.[4] There is considerable genetic heterogeneity in PGL and PHEO, currently over 30 different genes have been associated with PGL/PHEO formation. The majority of HNPGL, extra-adrenal PGL and PHEO are caused by germline variants in genes encoding subunits and cofactors of the succinate dehydrogenase (*SDH*) enzyme complex (*A*, *B*, *C*, *D*, *AF2*).[5-7] The associated syndromes are quite distinct. *SDHB*-linked tumour syndrome is usually characterised by single tumours, and gene variant carriers develop more frequently extra-adrenal PGLs, PHEOs and metastatic disease than carriers in the other subunits of the SDH gene.[8] Furthermore, SDHB gene variants are implicated in the development of renal cell carcinoma, papillary thyroid carcinoma and GIST tumours.[8-10]

In this study, we describe the occurrence of HNPGL in a four-generation family linked to a novel SDHB gene variant. The index patients are 2 young sisters that did not present themselves with a HNPGL or PHEO, but with a rhabdomyosarcoma and a non-Hodgkin lymphoma. Array-based comparative genomic hybridisation (aCGH) analysis and subsequent multiplex ligation-dependent probe amplification (MLPA) revealed a deletion of exon 1-3 in the SDHB gene in both patients that has not been described previously. Although *SDHB* immunostaining of the rhabdomyosarcoma and non-Hodgkin lymphoma showed that there was no causal relationship between the SDHB gene variant and these tumours, this novel exon deletion was suspected to predispose to PGL/PHEO syndrome type 4 based on the characteristics of the gene variant itself. Subsequently, genetic counselling and DNA testing were offered to all family members. Clinical evaluation of the germline variant carriers revealed an asymptomatic carotid body PGL in an aunt of the index patients.

Key Points

- A deletion of exon 1-3 in the SDHB gene is associated with the formation of hereditary HNPGL.
- Cascade screening of family members carrying this mutation is important to detect pre-symptomatic PGL. Especially in case of catecholamine-producing tumours, timely intervention may prevent cardiovascular complications.

2 | MATERIALS AND METHODS

All the participating family members gave written informed consent for the clinical study and DNA test. In case of individuals under 18 years of age, written informed consent was obtained from their parents.

Data were collected from the VU University Medical Center (VUmc), Amsterdam, the Netherlands, a tertiary referral centre for HNPGL and/or PHEO in the Netherlands. Family members at risk were offered genetic counselling and pre-symptomatic screening as part of the protocol for standard care of pathogenic *SDHB* variant carriers at risk in the Netherlands.[11] SDHB gene variant analysis was performed by the Leiden Genome Technology Center (LGTC) of the Leiden University Medical Center (LUMC, Leiden, the Netherlands), using Sanger sequencing on an ABI 377 (Applied Biosystems, Carlsbad, CA) Genetic Analyzer and multiplex ligation-dependent probe amplification (MLPA), P266 MLPA-kit (MRC Holland, Amsterdam, the Netherlands).

Germline variant carriers were offered annual clinical surveillance for PGL and PHEO at the departments of Otolaryngology/Head and Neck Surgery and Endocrinology/Metabolic diseases of the VUmc. Annual biochemical screening for excessive catecholamine excretion included the measurement of (nor)adrenaline, vanillylmandelic acid (VMA), dopamine, (nor)metanephrine and/or 3-methoxythyramine (3-MT) in two 24-hour urinary samples, and/or plasma-free (nor)metanephrine and/or 3-methoxythyramine (3-MT). All carriers were offered magnetic resonance imaging (MRI) of the thorax/abdomen/pelvis once every 2 years and head and neck region once every 3 years. Upon detection of a HNPGL (a carotid body tumour) and catecholamine excess in one carrier, a multidisciplinary team consisting of otolaryngologists, endocrinologists, geneticists, radiotherapists and vascular surgeons advised surgical resection of the PGL. The surgery was performed at the VUmc, Amsterdam, the Netherlands. *SDHB* immunostaining was performed on tumour tissue according to the protocol described elsewhere.[12]

3 | RESULTS

The index patients, 2 sisters (13 and 16 years old), were referred to the Department of Clinical Genetics of the VU University Medical Center for etiologic evaluation because of a medical history of a embryonic rhabdomyosarcoma and a non-Hodgkin lymphoma, respectively. Array-based comparative genomic hybridisation (aCGH) and multiplex ligation-dependent probe amplification (MLPA) in both sisters revealed a 16p12.2 microdeletion, a 20q12 deletion, and a novel exon 1-3 deletion in the SDHB gene.

Based on positive *SDHB* immunostaining of the two index tumours, no causal relationship could be established between the SDHB gene variant and the occurrence of these tumours. Even so, on grounds of the characteristics of the SDHB gene variant alone, it was suspected that this variant could be pathogenic. Both a deletion

in *SDHB* exon 1 and in exon 3 are known to cause hereditary PGL syndrome type 4 and predispose to HNPGL, PHEO, extra-adrenal PGL and malignant PGL/PHEO.[13-15] Despite the lack of apparent symptomatic neuroendocrine tumours in this family, cascade screening was offered to the family members of these 2 patients.

Subsequently, 18 relatives at risk belonging to a four-generation family with a total of 43 members were tested for this specific *SDHB* exon deletion. Eight individuals tested negative and were considered not to be at risk of PGL/PHEO formation. Ten family members (6 women, 4 men) were carriers of the deletion of exon 1-3 in the SDHB gene (Figure 1).

The mean age at detection of the gene variant was 34 years (range 11-57). The mean duration of follow-up was 26 months (range 12.6-32.3). None of the germline variant carriers presented with signs or symptoms suggestive of a PGL/PHEO during clinical examination. However, a slight increase in plasma-free (nor)metanephrine or 3-MT levels was observed in 5 variant carriers (50%) at the time of diagnosis (Table 1). In one of these patients,

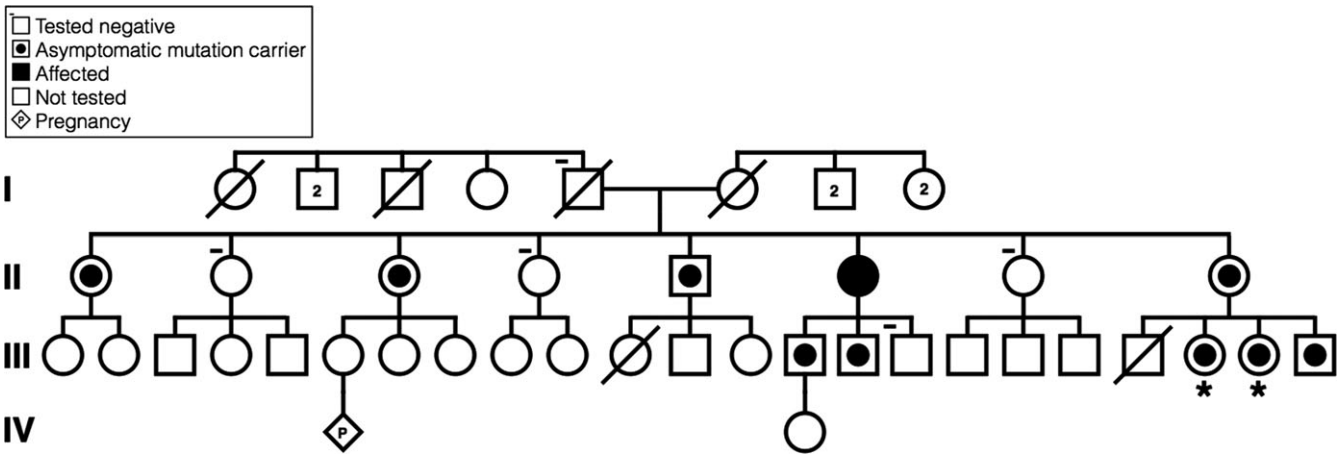

FIGURE 1 Pedigree of the SDHB-linked family. The asterisks show the index patients

TABLE 1 Phenotype of 10 relatives carrying the exon 1-3 deletion in *SDHB*

	Sex	Age at diagnosis	PGL location	Catecholamine biochemistry at diagnosis	Additional mutation	Other tumour (in history)
1	F	39	None	Normal (serum)	16p12.2 del	
2	M	29	None	Normal (serum)	None	
3	M	23	None	Elevated NM (serum) Elevated M (serum) Elevated 3-MT (serum)	None	
4	F	56	None	Elevated NM (serum) Elevated M (serum) Elevated 3-MT (serum)	None	
5	F	53	None	Elevated NM (serum) Normal M (serum) Normal 3-MT (serum)	None	
6	M	51	None	Elevated NM (serum) Elevated M (serum) Normal 3-MT (serum)	None	
7	F	46	Carotid body PGL[a]	Elevated NM (serum) Normal M (serum) Normal 3-MT (serum)	None	
8	F	16	None	Normal (urine)	None	Non-Hodgkin Lymphoma[b]
9	F	13	None	Normal (urine)	16p12.2 del 20q12 del	Embryonal rhabdomyosarcoma[b]
10	M	10	None	Normal (urine)	16p12.2 del	

F, female; M, male; PGL, paraganglioma; NM, normetanephrine; 3-MT, 3-methoxythyramine;.
[a]*SDHB*-associated;
[b]not-associated.

pre- and post-contrast enhanced 3D time-of-flight (TOF) MR angiography of the head and neck region revealed a mass at the right carotid bifurcation, suggestive of a carotid body PGL (Figure 2).

Increased plasma-free normetanephrine levels were observed at time of diagnosis and 24-hour ambulatory blood pressure monitoring revealed blood pressure peaks. MR imaging of the thorax, abdomen and pelvis showed no other localisation of a PGL or PHEO. Additional iodine-123-meta-iodobenzylguanidine (MIBG) scintigraphy did not unequivocally identify the source of the catecholamine overproduction. A multidisciplinary team consisting of otolaryngologist, endocrinologists, radiotherapists and vascular surgeons advised surgical resection on grounds of the causative SDHB gene variant and catecholamine excess. An alpha blockade protocol until the day of surgery was followed, because of increased preoperative plasma levels of catecholamine and observed peaks in blood pressure. The carotid body PGL was removed in total via a transcervical approach in an uncomplicated procedure (Figure 3). Histologic evaluation confirmed the diagnosis HNPGL, and negative *SDHB* immunostaining indicated the causal relation between the *SDHB* exon 1-3 deletion and the carotid body PGL. Biochemical evaluation 4 months after surgery showed normalisation of plasma-free normetanephrine levels.

4 | DISCUSSION

4.1 | Synopsis of new findings

In this report, we describe a novel deletion of exon 1-3 in *SDHB* causing HNPGL formation. We tested 18 relatives belonging to a four-generation family consisting of 43 members, of whom 10 were identified to carry this novel *SDHB* germline variant. One patient was diagnosed with an asymptomatic carotid body PGL and serum catecholamine excess. Negative *SDHB* immunostaining of the tumour tissue confirms the association between this novel *SDHB* variant and the carotid body PGL.[4]

4.2 | Comparisons with other studies

Interestingly, the two paediatric index patients were diagnosed with a non-Hodgkin lymphoma and an embryonal rhabdomyosarcoma. It is now known that *SDHx* germline variants do not only predispose to PGL/PHEO, but also to non-paraganglionic tumours such as gastrointestinal stromal tumours, renal cell carcinomas and pituitary adenomas.[8-10] The complete spectrum of the *SDHB*-linked phenotype has, however, not yet been fully elucidated, because the SDHx genes are not routinely evaluated in individuals with non-endocrine tumours. A recent study has highlighted a possible role for SDHx gene variants in lymphoid malignancies. One *SDHB* variant carrier has been described with a Hodgkin lymphoma and an abdominal extra-adrenal PGL, and one *SDHC* variant carrier with Hodgkin lymphoma and a positive family history of PHEO/GIST.[16] However, both the Hodgkin lymphoma and normal lymphoid tissues of the *SDHB* variant carrier displayed minimal *SDHB* staining, precluding definitive assessment of SDHB protein loss. Based on positive *SDHB* immunostaining of rhabdomyosarcoma and lymphoma tissue in our index patients, a causal relationship between the deletion of exon 1-3 in *SDHB* and the occurrence of these tumours could not be established.

Only 1 patient was affected with an apparently asymptomatic carotid body PGL, detected at the age of 47. The risk of developing a PGL or PHEO (or penetrance) in *SDHB* germline variant carriers is subject of recent debate. Initially, it was estimated to range between 50% and 70% at 50 years of age.[7,9,17] These estimates are probably

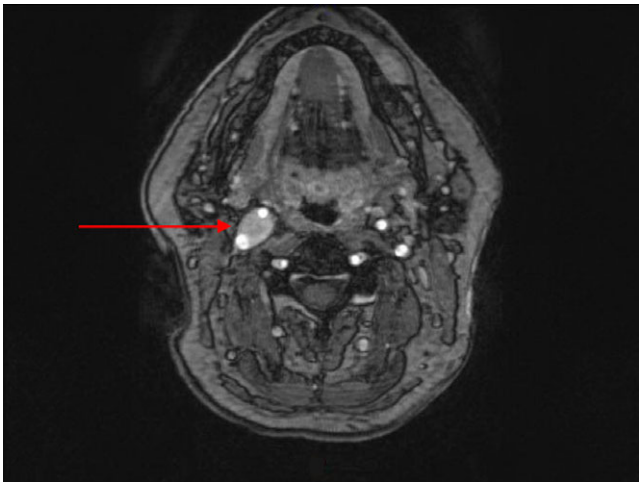

FIGURE 2 Axial magnetic resonance imaging (MRI) of the head and neck region showing a Shamblin type II carotid body paraganglioma. 3D TOF (time-of-flight angiography) sequence is used to visualise flow within vessels, without the need to administer contrast intravenously

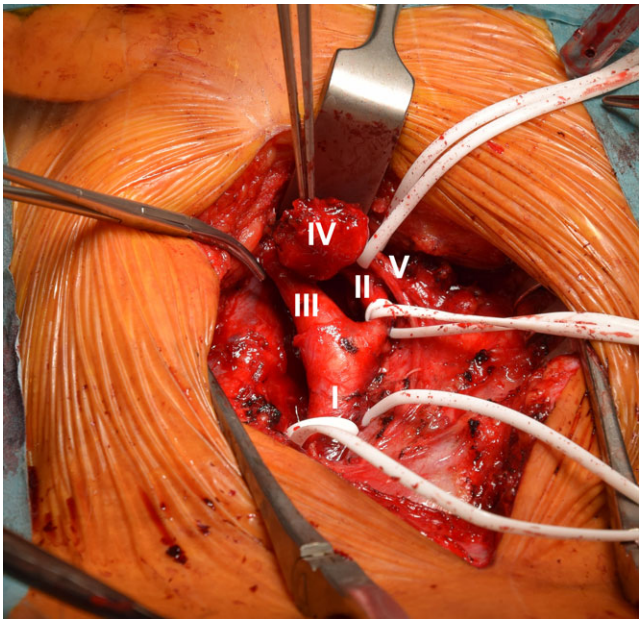

FIGURE 3 Surgical resection, via a transcervical approach, of the paraganglioma located between the right internal and external carotid arteries, and its close relationship with the hypoglossal nerve. I, common carotid artery; II, external carotid artery; III, internal carotid artery; IV, paraganglioma; V, hypoglossal nerve

inflated, as recent studies using more thorough pedigree analysis and a more robust statistical correction for the ascertainment bias show a lower age-dependent penetrance of *SDHB* variants, approximately 9%-21% at 50 years. These reports also indicate that there are no significant differences in the penetrance of different types of *SDHB* variants. The low penetrance of the *SDHB* variant found in this family is in line with these recent reports.[18-20]

4.3 | Clinical applicability of the study

The management of HNPGL is challenging and requires a tailor-made approach. The management strategy is based on several important factors, such as patient characteristics (ie age, comorbidities and patient preferences) and tumour characteristics (ie localisation, size, growth rate, biochemical activity and multicentricity). The causal gene variant plays an increasingly prominent role in the clinical decision-making, as different genes confer different risks and are associated with different clinical phenotypes. *SDHB* variants causing PGL syndrome type 4 are usually associated with single PGL/PHEO that have a higher risk of progression to metastatic disease than PGL/PHEO associated with other SDH genes.[7,17] This study identifies a novel deletion of exon 1-3 in *SDHB* causing HNPGL. Cascade testing of family members at risk identified a patient with a pre-symptomatic carotid body tumour, elevated catecholamine levels and high blood pressure, which normalised after surgical resection of the tumour.

5 | CONCLUSION

In this report, we present a novel deletion of exon 1-3 in the SDHB gene associated with the formation of hereditary HNPGL. The penetrance of this gene variant seems low. Cascade screening of family members carrying this mutation is important to detect pre-symptomatic PGL. Especially in case of catecholamine-producing tumours, timely intervention may prevent cardiovascular complications.

CONFLICT OF INTEREST

None to declare.

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