CHAPTER 3

Phenotype of *SDHB* Mutation Carriers in the Netherlands

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Abstract

Background: SDHB mutation carriers are predisposed to developing paragangliomas. The objective of this study was to assess genotype-phenotype correlations of a Dutch cohort of *SDHB* mutation carriers and assess potential differences in clinical phenotypes related to specific *SDHB* founder mutations.

Methods: Forty-seven consecutive *SDHB* mutation carriers were included. Initial screening consisted of measurement of 24-hour urinary excretion of catecholamines and their metabolites in duplicate, repeated annually if initial biochemical screening was negative. Whole-body imaging studies with MRI or CT and/or ^{123}I-MIBG scintigraphy were performed in case of catecholamine excess, and MRI or CT scans of thorax, abdomen and pelvis were performed every two years regardless of catecholamine levels. Repetitive head-and-neck MRI was performed at 2-year intervals.

Results: Mean follow-up was 3.6 ± 3.6 years. Twenty-seven persons (57%) carried the *SDHB* c.423+1 G>A mutation and seven persons (15%) the *SDHB* c.201-4429_287-933del (exon 3 deletion) mutation. No differences were found in the clinical phenotype of carriers of these two specific *SDHB* mutations. By end of follow-up, 49% of *SDHB* mutation carriers displayed no biochemical or radiological evidence of manifest disease. Three persons (6%) had been diagnosed with a pheochromocytoma (PCC), four with a sympathetic PGL (sPGL) (9%), 18 with a HNPGL (38%), and two persons (4%) had developed a malignant paraganglioma, i.e. metastatic disease.

Conclusions: The two main Dutch *SDHB* founder mutations do not differ in clinical expression and result in a relatively mild phenotype. Over one-third of *SDHB* mutation carriers develop HNPGL, with sPGL/PCC in only 15% and malignancy in only 4%.

Introduction

Paragangliomas (PGLs) are rare neuroendocrine tumors of paraganglia, which are neural-crest derived chromaffin tissues associated with the autonomic nervous system. In general, paragangliomas of the head and neck region (HNPGLs) are neoplasms of the parasympathetic part of the autonomous nervous system, while adrenal paragangliomas (also termed pheochromocytomas or PCC) and extra-adrenal paragangliomas (sympathetic paragangliomas or sPGLs) are neoplasms of the sympathetic nervous system.

PGLs can occur sporadically or as part of a hereditary syndrome (i.e. hereditary paraganglioma syndrome, von Hippel-Lindau disease, multiple endocrine neoplasia (MEN) type 2 or neurofibromatosis type 1).[1,2] Hereditary paraganglioma syndrome is associated with germline mutations in genes encoding subunits A, B, C, D or assembly factor 2 of the mitochondrial complex II-succinate dehydrogenase (SDH),[3-7] *TMEM127* or *MAX*.[8,9] These various germline mutations have distinct phenotypic effects. *SDHB* mutations have an age-dependent and incomplete penetrance.[10,11] They are more frequently associated with PCC, sPGLS and malignant disease than mutations in the other subunits of the *SDH* gene.[10-12] Although it was previously reported that up to 71% of PGLs in *SDHB* mutation carriers are malignant,[1,11,13] we recently showed that the pooled prevalence of malignant PGL in populations comprising both asymptomatic *SDHB* mutation carriers and mutation carriers with manifest non-malignant PGL can be as low as 13%.[14]

Mutations are distributed across the entire *SDHB* gene, with no particular mutation hot spots.[15] Two founder mutations in *SDHB* have been identified in Dutch PGL families, the c.423+1 G>A mutation and the c.201-4429_287-933del, p.Cys68fs mutation.[16,17] The high prevalence of founder mutations in the Netherlands gives us the unique opportunity to evaluate a substantial number of carriers of specific mutations.

In order to investigate possible genotype-phenotype correlations of specific *SDHB* mutations, the objective of this study was to determine the clinical, biochemical and radiological characteristics of a Dutch cohort of *SDHB* mutation carriers.

Subjects and Methods

We evaluated the clinical, biochemical and radiological data of 47 consecutive heterozygous *SDHB* mutation carriers using a retrospective study design. The Leiden University Medical Center (LUMC) is a tertiary referral center for patients with PGLs and subjects were followed in the outpatient clinic of the Department of Endocrinology of the LUMC.

All *SDHB* mutation carriers were investigated according to structured protocols used for standard care and including questions focused at tumor- and catecholamine related signs and symptoms. In order to detect sPGLs/PCCs and HNPGLs in (presymptomatic) mutation

carriers, biochemical screening for catecholamine excess was performed annually, magnetic resonance imaging (MRI) or computed tomography (CT) scans of thorax, abdomen and pelvis every two years, ENT examination including otoscopy and laryngoscopy annually and repetitive head-and-neck MRI every three years (annually if HNPGLs were present). Biochemical screening included the annual measurement of (nor)epinephrine, vanillylmandelic acid (VMA) and dopamine in two 24-h urinary samples. From 2005 onwards, (nor)metanephrine and 3-methoxytyramine (3-MT) were added to these measurements. Urine was collected over a 24 hour period, in duplicate, under strict dietary regulations (patients abstained from bananas, nuts, alcohol, coffee, tea and other caffeine containing beverages in the two days preceding and during urine collection), and after an at least one week withdrawal of medication that might interfere with catecholamine secretion or after changing antihypertensive medication to doxazosin. In order to ascertain adequacy of collection, urinary creatinine secretion was also measured. In case of excessive catecholamine secretion (i.e. any value above the upper reference limit), radiological assessment by MRI or CT scans of thorax, abdomen and pelvis and/or ^{123}I metaiodobenzylguanidine (MIBG)-scans were performed to identify potential sources of excessive catecholamine production outside the head and neck region.

Since there are no reliable histologic features to distinguish benign from malignant PGLs, malignant disease was defined as the presence of metastases, i.e. the presence of chromaffin tissue in nonchromaffin organs or tissues distant from the primary tumor.[18-20]

In all surgically resected PGLs, diagnosis was confirmed by pathological investigation.

Testing for *SDH* mutations was performed in persons who gave informed consent. In index cases, the *SDHB* gene was scanned for the presence of mutations at the Laboratory for Diagnostic Genome Analysis at the LUMC. All exonic and adjacent intronic regions of these genes were tested by direct sequencing using the Sanger method on an ABI 377 Genetic Analyser (Applied Biosystems, Carlsbad, CA, USA) and multiplex ligation-dependent probe amplification (MLPA) was carried out with the P226 MLPA kit (MRC Holland, Amsterdam, the Netherlands).[21] Family members of index patients were tested for the family-specific *SDHB* mutation. Mutations were classified as pathogenic, as variants of unknown clinical significance (VUS; variant of uncertain significance), or as likely pathogenic. Missense variants were considered likely pathogenic when fulfilling several of the following criteria: strong evolutionary conservation of the amino acid; SIFT ('sorting intolerant from tolerant'), PolyPhen ('polymorphism phenotyping') and Align-GVGD (Grantham variation and Grantham deviation) scores as possibly pathogenic; the Grantham distance between the original and mutated amino acid larger than 100; presence in multiple independent patients; linkage in several families; absence in the general population (http://evs.gs.washington.edu/EVS/, accessed March 22, 2013).

Follow-up ended February 1st 2013 or, when lost to follow-up, date of the last contact with the endocrinologist.

Assays

Urinary excretion rates of (nor)epinephrine and dopamine in 24-h urine collections were quantified using reversed high-performance liquid chromatography (HPLC) and an electrochemical detector. Inter- and intra-assay coefficients of variations (CV's) for epinephrine were 4.3-9.0%, ranging from high to low levels. For norepinephrine, these data were 2.7-3.6% and for dopamine, 3.1-4.8%. Urinary excretion of VMA was measured using HPLC with fluorometric detection, with inter- and intra-assay CV's of 7.4-8.1% and 2.4-9.1%, respectively. (Nor)metanephrine and 3-MT were determined by stable isotope mass fragmentography. The CV's of the 3-O-methylated catecholamine metabolites ((nor)metanephrine and 3-MT) ranged from 1.7 to 4.2.[22]

Reference ranges were obtained in healthy volunteers. These were: norepinephrine 0.06-0.47 μmol/24h, epinephrine ≤ 0.16 μmol/24h, dopamine 0.46-3.40 μmol/24h, VMA ≤30 μmol/24h, metanephrine 33-99 μmol/mol creatinine, normetanephrine 64-260 μmol/mol creatinine and 3-MT 45-197 μmol/mol creatinine.[23]

Data analysis

IBM SPSS Statistics version 20.0 (SPSS inc., Chicago, IL) was used for data analysis. Results were expressed as means ± standard deviation (SD). Fisher's exact test was used to test whether proportions differed significantly. Differences were considered statistically significant at $p \leq 0.05$.

Results

Forty-seven *SDHB* mutation carriers were included: 18 men (38%) and 29 women (62%). The mean duration of follow-up was 3.6 ± 3.6 years (range 0-18). Three persons (6%) were lost to follow-up: two persons moved away and one person was lost to follow-up for unknown reasons.

Genetics

Details of *SDHB* mutations are outlined in Table 1. Twenty-seven persons (57%) carried the *SDHB* c.423+1 G>A mutation. The *SDHB* c.201-4429_287-933del (exon 3 deletion) mutation was present in seven persons and the *SDHB* c.590C>G (p.Pro197Arg) mutation and *SDHB* c.328A>C (p.Thr110Pro) mutation in three persons each. The family history was positive for PGL in 30 persons (64%).

Table 1: Germline mutations in *SDHB* mutation carriers

cDNA	*SDHB* predicted protein change	Number of subjects
c.423+1G>A	Abberant splicing (pathogenic)	27
c.201-4429_287-933del	Deletion of exon 3 (pathogenic)	7
c.590C>G	p.Pro197Arg (likely pathogenic)	3
c.328A>C	p.Thr110Pro (VUS)	3
c.574T>C	p.Cys192Arg (likely pathogenic)	1
c.649C>T	p.Arg217Cys (likely pathogenic)	1
c.653G>C	p.Trp218Ser (likely pathogenic)	1
del promotor and exon 1	p.0 (pathogenic)	1
c.725G>A	p.Arg242His (VUS)	1
c.343C>T	p.Arg115*[a] (pathogenic)	1
c.1A>G	p.? (likely pathogenic)	1

VUS = variant of uncertain significance

a New nomenclature; formerly known as 'X'.

Initial presentation

The mean age at presentation at the outpatient clinic of the Department of Endocrinology was 48.6 ± 14.6 years (range 19-75). Twenty-eight persons were referred to the outpatient clinic of the Department of Endocrinology by a clinical geneticist following a positive *SDHB* mutation test, and eighteen persons were referred by an ear, nose and throat (ENT) specialist, a vascular surgeon or a general internal medicine specialist after they had been diagnosed with one or multiple PGLs. One person was referred by her general practitioner because of a suspected pheochromocytoma.

Radiological imaging had been performed in 23 persons before presentation at the Department of Endocrinology. Nineteen persons had been diagnosed with PGLs: 14 with HNPGLs, 3 with sPGL (of which 2 had been resected), 1 with PCC and 1 with PCC and HNPGL (both PCCs had been resected). Four persons displayed no sign of manifest disease. In 24 persons, radiological imaging to detect PGLs had not yet been performed at the time of presentation.

Follow-up

Clinical characteristics at the end of follow-up of the cohort as a whole and for the specific *SDHB* mutations with multiple carriers are outlined in Table 2. To explore potential differences in clinical phenotypes related to the specific mutations within the *SDHB* gene, carriers of the two most common *SDHB* mutations, the c.423+1 G>A mutation and the c. 201-4429_287-933del mutation, were compared. The number of carriers of the other mutations within the *SDHB* gene was small, and they were not included in our analyses due to a lack of statistical power. Statistical analyses showed no significant differences in number and location of HNPGLs, sPGLs or PCCs, nor in the occurrence of malignant disease, other

tumors or the number of asymptomatic mutation carriers between carriers of the *SDHB* c.423+1 G>A mutation and carriers of the *SDHB* c. 201-4429_287-933del mutation.

Table 2: Clinical phenotypes of specific mutations within the *SDHB* gene

	Total cohort (*n* = 47)	**c.423+1 G>A (*n* = 27)**	**c.201-4429_287-933del (*n* = 7)**	**c.590C>G (*n* = 3)**	**c.328A>C (*n* = 3)**
HNPGL (%)	18 (38)	6 (22)	4 (57)	1 (33)	2 (67)
- 1 HNPGL	14	5	4	0	1
- 2 HNPGLs	4	1	0	1	1
CBT (%)	5 (11)	1 (4)	1 (14)	1 (33)	1 (33)
- left	3	1	1	0	1
- right	0	0	0	0	0
- bilateral	2	0	0	1	0
VBT (%)	4 (9)	3 (11)	0	0	1 (33)
- left	0	0			0
- right	4	3			1
- bilateral	0	0			0
JTT (%)	11 (23)	3 (11)	3 (43)	0	1 (33)
- left	5	1	2		0
- right	6	2	1		1
- bilateral	0	0	0		0
sPGL (%)	4 (9)	2 (7)	0	0	0
PCC (%)	3 (6)	0	1 (14)	0	0
- left	2		1		
- right	1		0		
- bilateral	0		0		
Malignant PGL (%)	2 (4)	0	0	0	0
Other tumors	7	5	0	0	1
- breast	1	1			0
- hemato-oncological	1	0			0
- gastro-intestinal	1	1			0
- nervous system	1	1			0
- skin	2	2			0
- other	1	0			1
Asymptomatic (%)	23 (49)	18 (67)	2 (29)	2 (67)	1 (33)

HNPGL = head and neck paraganglioma, CBT = carotid body tumor, VBT = vagal body tumor, JTT = jugulotympanic tumor

sPGL = sympathetic paraganglioma, PCC = pheochromocytoma, PGL = paraganglioma

In the whole cohort, thirty-eight percent of *SDHB* mutation carriers had developed one or multiple HNPGLs, with jugulotympanic tumors being the most prevalent (in 23%). Of the 7 persons operated for their HNPGLs, carotid body tumors were resected in three and jugulotympanic tumors in four persons. One person had received radiotherapy and one person radiosurgery, both for a jugulotympanic tumor.

During follow-up at the outpatient clinic of the Department of Endocrinology, two persons were analyzed for retroperitoneal localized sPGL and one for a PCC. Biochemical phenotypes are presented in Table 3. All lesions were detected via radiological imaging of thorax, abdomen and pelvis, performed due to excessive levels of urinary catecholamines. In all three persons, the lesions were resected and pathologically confirmed as PGL/PCC. Urinary levels of catecholamines normalized postoperatively.

Two persons (4%) developed a malignant paraganglioma, i.e. metastatic disease to bone and bone and lymph nodes, respectively (Table 4). The primary tumor was a sPGL in both, and both tumors were resected prior to start of follow-up at the Department of Endocrinology (LUMC). Patients are alive at 14 and 48 months after diagnosis of malignant PGL, respectively.

Seven other malignancies were reported. One *SDHB* mutation carrier had Waldenström's macroglobulinemia, one a ganglioneuroma and one showed a carcinoma of the meibomian glands. One person suffered from breast cancer and one from colorectal cancer. One person was diagnosed with a basal cell carcinoma and one with a melanoma.

At the end of follow-up, 23 *SDHB* mutation carriers (49%) displayed no radiological or biochemical evidence of PGLs, i.e. were asymptomatic mutation carriers.

Table 3: Biochemical phenotypes of sympathetic paragangliomas and pheochromocytomas

Case	Location	NE	E	DA	VMA	NMN	MN	3-MT	Catecholamine-related signs and symptoms
1	sPGL	+	-	-	+	n.a.	n.a.	n.a.	hypertension, palpitations, perspiration, pallor, nausea, vomiting
2	sPGL	+	-	-	+	+	-	+	hypertension, palpitations, perspiration, headache, collapse
3	PHEO	-	-	-	-	+	+	+	perspiration

n.a. = not assessed

sPGL = sympathetic paraganglioma, PHEO = pheochromocytoma

NE = norepinephrine, E = epinephrine, DA = dopamine, VMA = vanillylmandelic acid, NMN = normetanephrine, MN = metanephrine, 3-MT = 3-methoxytyramine

Table 4: Malignant paragangliomas

Case	Sex	*SDHB* mutation	Location PGL	Years since first diagnosed PGL	Location metastases	Treatment	Outcome
1	M	c.343C>T	Pelvic retroperitoneal PGL	1	Bone	^{131}I-MIBG therapy, radiotherapy, lutetium octreotate therapy	Alive at age 32 (14 months after diagnosis of malignant PGL)
2	F	c.725G>A	Right para-adrenal PGL	6	Bone, lymph nodes	Resection para-aortic lymph nodes, lutetium octreotate therapy	Alive at age 49 (48 months after diagnosis of malignant PGL)

M = male, F = female, PGL = paraganglioma

Discussion

The aim of the present study was to assess possible genotype-phenotype relations in a Dutch cohort of *SDHB* mutation carriers. More than half of the mutation carriers in our cohort carried the *SDHB* c.423+1 G>A mutation. Comparison of the clinical phenotype of this specific mutation with the second most frequent specific *SDHB* mutation within our cohort, the c. 201-4429_287-933del mutation, revealed no significant differences.

Although *SDHB* mutation carriers often present as non-familial cases,[10,12,16,24] 64% of *SDHB* mutation carriers in our cohort had a positive family history for PGL. At the LUMC, the larger part of *SDHB* mutation carriers are family members of index patients who were actively invited for molecular genetic testing at the Department of Clinical Genetics following identification of the index case.

By the end of follow-up, three mutation carriers (6%) had been diagnosed with a PCC and four with a sPGL (9%). These percentages are substantially lower than in previous studies assessing clinical characteristics in *SDHB* mutation carriers, which reported figures of 18-28% for PCCs and 38-84% for sPGLs.[10,11,24] These differences may be attributable to the fact that these previous studies comprised more index cases; hence referral bias may have overestimated the prevalence of PCCs and sPGL. Furthermore, two of these studies included subjects from international registries[10,11] and the variation in frequency of founder mutations and corresponding penetrance in different countries prohibits simple generalizations of study results.

In contrast, the number of *SDHB* mutation carriers diagnosed with one or multiple HNPGLs (38%), was higher in our cohort than reported in other studies (3-31%).[10,11,24] Since the

LUMC is a national referral center for patients with HNPGLs, a referral bias may also be operating in our study, but now for HNPGLs.

Forty-nine percent of included *SDHB* mutation carriers displayed no signs of manifest disease by the end of follow-up. *SDHB* mutations have an age-dependent penetrance; the c.423+1 G>A mutation has a reported penetrance of 26% at 48 years[25] and comprised the larger part of our cohort. The number of asymptomatic mutation carriers in our cohort is probably an underestimation of the actual percentage of asymptomatic *SDHB* mutation carriers, since we were only able to include *SDHB* mutation carriers engaged in clinical investigations. Unaffected mutation carriers may be less likely to be inclined to undergo regular investigations and may therefore remain under the clinical radar.

Four percent of the mutation carriers in our cohort developed malignant PGL. This figure differs from that of a recent systematic review, in which the pooled risk for developing malignant PGL in prevalence studies comprising both asymptomatic *SDHB* mutation carriers and *SDHB* mutation carriers with manifest non-malignant PGL was 13%[14] This discrepancy might suggest that the Dutch *SDHB* mutations show a mild phenotype or could be due to the previously mentioned possible acquisition bias operating in the LUMC.

Several malignancies of the extraparaganglial system have been reported in *SDHB* mutation carriers, such as renal clear or papillary cell carcinoma, gastrointestinal stromal tumor (GIST) and papillary thyroid carcinoma.[10,24,26] Although our study could not confirm the association of *SDHB* mutations with any of these malignancies, the occurrence of a melanoma in a *SDHB* mutation carrier has been reported previously.[12]

In conclusion, the two main Dutch *SDHB* founder mutations do not differ in clinical expression and display a relatively mild phenotype. In the Netherlands, over one-third of *SDHB* mutation carriers develop HNPGL, with sPGL/PCC in 15%; only 4% being malignant.

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