CHAPTER 7

Mortality in *SDHD* Mutation Carriers Is Not Increased Compared to the General Population

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Abstract

Background: Germline mutations in subunit D of the succinate dehydrogenase gene (*SDHD* mutations) are associated with an increased risk of developing head and neck paragangliomas, which are associated with severe treatment-related morbidity, and pheochromocytomas, which can prove fatal when left untreated. Therefore, mortality of *SDHD* mutation carriers is of interest.
The objective of this study was to estimate mortality rates of a Dutch cohort of *SDHD* mutation carriers compared with those of the general population.

Methods: Cohort study. All subjects who tested positive for *SDHD* mutations at the Leiden University Medical Center (LUMC) before July 1st 2012 and who visited the Departments of Otorhinolaryngology or Endocrinology at least once were included, as well as persons with a paraganglioma (PGL) diagnosis and a positive family history with a proven *SDHD* mutation, i.e. obligate *SDHD* mutation carriers.
The standardized mortality ratio (SMR) was calculated. *SDHD* mutation carriers were followed from the date of the mutation carrier's first *SDHD* mutation related contact at the LUMC until death, date of emigration or December 12th 2012. Clinical data were retrieved from medical records. Information on mortality was obtained from the Municipal Personal Records Database. Mortality rates for the Dutch population were obtained from the Dutch Central Bureau of Statistics, stratified by sex, age and calendar time.

Results: 275 *SDHD* mutation carriers with a mean duration of follow-up of 7.6 years were included in the study, of which 89% were diagnosed with HNPGL. Seven percent were diagnosed with sympathetic PGL and another seven percent with pheochromocytoma. Four percent of carriers were diagnosed with malignant PGL. Seven percent did not show any signs of manifest disease, i.e. they were unaffected *SDHD* mutation carriers.
None of the participants were lost to follow up. This resulted in 2242 person-years of observation. The SMR for the whole cohort was 1.07 [95% Confidence Interval (CI) 0.67 to 1.73]. The SMR for female *SDHD* mutation carriers was 1.24 [95% CI 0.56 to 2.76] and for male *SDHD* mutation carriers 1.00 [95% CI 0.55 to 1.81].

Conclusions:
Mortality in *SDHD* mutation carriers is not increased. The results of this study enable us to provide (newly identified) mutation carriers with prognostic information concerning the effect of harboring *SDHD* mutations on mortality.

Introduction

Background

Germline mutations in subunit D of the succinate dehydrogenase (SDH) gene predispose carriers to the development of paragangliomas (PGLs).[1] *SDHD* mutations are mainly associated with multifocal PGLs in the head and neck region (HNPGLs), although sympathetic PGLs (sPGLs; extra-adrenal PGLs) and adrenal PGLs (i.e. pheochromocytomas) also occur.[2-4] Even though the majority of HNPGLs are benign, indolent tumors,[5] their location in close proximity to important neurovascular structures may lead to serious morbidity and cranial nerve impairment in case of tumor growth.[6] Surgical treatment however, leads to neurovascular complications in up to 60% of cases; especially cranial nerve injury and, less frequently, carotid artery lesions.[7-8] Therefore, whether or not to treat HNPGLs has to be carefully thought over and a "wait and scan" policy should be considered.[5]

Due to their ability to hypersecrete catecholamines, pheochromocytomas can give rise to severe cardiovascular complications such as shock, myocardial infarction, a dissected aortic aneurysm or heart failure due to toxic cardiomyopathy.[9-12] In order to prevent these potentially lethal consequences, adrenalectomy is indicated when a pheochromocytoma is detected.[13] After appropriate preoperative care to modulate the effects of catecholamine release, perioperative mortality is nil.[14-16]

The pooled incidence of malignant PGL, defined as the presence of metastases,[17-19] in populations comprising both asymptomatic *SDHD* mutation carriers and *SDHD* mutation carriers with manifest non-malignant PGL is about 8%.[20] Prognosis in malignant PGL is poor: five-year survival rates of 20-55% for malignant sPGL and pheochromocytoma[21-22] and 60% for malignant HNPGL are reported,[23] although a few cases with patients living more than 20 years after diagnosis have been described.[24-25]

To date, an increasing number of *SDHD* mutation carriers are identified through (presymptomatic) testing of family members of *SDHD* mutation carriers with manifest disease, i.e. index cases. It is important to be able to provide newly identified mutation carriers with prognostic information, including the impact of the *SDHD* mutation on mortality. Since *SDHD* mutations are associated with an increased risk for HNPGLs and fatal cases of (untreated) pheochromocytomas have been described it is of interest to know whether this translates into an increased mortality risk. However, this has never been studied in *SDHD* mutation carriers. Therefore, the objective of this study was to compare mortality rates of a Dutch cohort of *SDHD* mutation carriers with the general Dutch population.

Subjects and methods

Eligibility criteria

The database of the Laboratory for Diagnostic Genome Analysis (LDGA) of the Leiden University Medical Center (LUMC), a tertiary referral center for patients with PGLs, was used to identify carriers of *SDHD* mutations. Screening for SDH mutations was performed in all persons diagnosed with PGL who agreed to genetic testing. For persons aged between 12 and 16 years, the informed consent of both parents also was required.

In index patients, all exonic and adjacent intronic regions of the SDH genes were tested for the presence of mutations 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).[3] Family members of index patients were tested for the family-specific mutation.

Consecutive *SDHD* mutation carriers who tested positive before July 1st 2012 and who visited the Departments of Otorhinolaryngology or Endocrinology at least once, in order to retrieve clinical data, were included in this study. In addition, persons with a PGL diagnosis and a positive family history with a proven *SDHD* mutation known in the outpatient clinics of the Departments of Endocrinology and Otorhinolaryngology of the LUMC were also included, since they were considered to be obligate *SDHD* mutation carriers. Persons of which no information could be retrieved at the Municipal Personal Records Database (see below) were excluded.

Clinical characteristics

Clinical data were retrieved from medical records. Since 2002, a standard evaluation protocol was implemented at the departments of Endocrinology and Otorhinolaryngology. In order to detect (hormonally active) PGLs, biochemical screening and repetitive head-and-neck magnetic resonance imaging (MRI) were performed at intervals of 2 years (in unaffected mutation carriers with intervals of 3 years). The initial diagnostic protocol in those patients in the period before 2002 was identical to that of 2002 and onwards, with the exception of protocolized follow-up every 2 years.

Biochemical screening included the 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.[4] In case of excessive catecholamine secretion (i.e. any value above the upper reference limit), radiological assessment by MRI or computed tomography (CT) scans of thorax, abdomen and pelvis was performed to identify potential sources of excessive catecholamine production outside the head and neck region, followed by whole-body ^{123}I metaiodobenzylguanidine (MIBG)-scans

when a suspected lesion was found. At the LUMC patients with pheochromocytomas or sPGLs are preferably operated on after adequate preoperative α- and β-adrenergic blockade. In all surgically resected PGLs, diagnosis was confirmed by pathological investigation.

Mortality

For this study, *SDHD* mutation carriers were observed from the date of genetic testing. It might well be that patients have been followed already before genetic *SDHD* testing, but including this follow-up time in the mortality analysis would have introduced immortal time bias with underestimation of mortality rates.[26] Obligate *SDHD* mutation carriers were observed from their first PGL-related contact at the LUMC. Because our aim was to determine the relation between mortality and carrying the *SDHD* mutation and not between mortality and diagnosis of PGL, we also included these patients in our mortality analyses.

Follow-up ended December 12th 2012, or at date of death or, in case of moving abroad, at date of emigration. Ten patients are currently being followed-up at another hospital.

For all included (obligate) *SDHD* mutation carriers an inquiry was sent to the Municipal Personal Records Database (GBA) on December 12th 2012. The GBA registers all deaths for Dutch inhabitants. To compare mortality between (obligate) *SDHD* mutation carriers and the general population, the standardized mortality ratio (SMR) was calculated. Mortality rates for the Dutch population were obtained from the Dutch Central Bureau of Statistics (The Netherlands), using rates stratified by sex, age (per one year) and calendar period (one year periods). The SMR was calculated by dividing the observed number of deaths in the *SDHD* cohort, and the expected number of deaths calculated as the sum of the stratified number of expected deaths (stratum specific mortality rates from the general population times follow-up time at risk). STATA 12.0 (Stata Corp, Texas, USA) was used for statistical analysis.

Results

Clinical characteristics

275 *SDHD* mutation carriers were included, of which 131 (48%) were female. Clinical characteristics are displayed in Table 1. Of all included persons, 193 (70%) were molecular genetically tested. The remaining 82 individuals were considered to be obligate *SDHD* mutation carriers. In our cohort, 80.4% carried the *SDHD* c.274G>T (p.Asp92Tyr) mutation, 11.2% the *SDHD* c. 416T>C (p.Leu139Pro) mutation and 2.9% the *SDHD* c284T>C (p.Leu95Pro) mutation. Six other specific *SDHD* mutations were found in the remaining 5.4% of included subjects.

Mean age at first *SDHD* mutation- or PGL-related contact at the LUMC was 43.5 ± 14.4 years. At the end of follow-up, a total of 620 HNPGLs were found in 246 patients (89%), of which 200 patients had multiple HNPGLs. One hundred forty-three patients (52%) were

treated for their HNPGLs, in the other cases a wait and scan policy was adopted. The majority of patients who received therapy were operated on; only seven patients were treated exclusively with radiotherapy or embolization. Five patients (2%) declined radiologic imaging of the head and neck region, because of the absence of symptoms. Therefore the disease status of these patients was not known.

In total 231 (84%) patients underwent biochemical screening at least once, leading to the diagnosis of 20 sPGLs and 18 pheochromocytomas. A watchful waiting policy was adopted in four sPGL patients: the lesions were surgically difficult to assess and considering the small risk of malignant transformation and the lack of catecholamine excess-related symptoms, it was decided not to operate. One patient with a nonproducing pheochromocytoma did not want to have an operation. Fifteen patients with sPGL and 17 patients with pheochromocytoma were surgically treated and the resection of one sPGL is scheduled. Ten persons (4%) were diagnosed with malignant PGL. In seven cases the primary tumor was a HNPGL. Finally, twenty individuals (7%) did not show any signs of manifest disease during follow-up, i.e. they were unaffected *SDHD* mutation carriers.

Table 1: Clinical Characteristics

	Number of patients (%)
Male/female	144 (52) /131 (48)
Mean age at the first *SDHD* mutation- or PGL-related contact	43.5 ± 14.4 years
Mean duration of follow-up	7.6 years (range 0-45)
HNPGL (%)	246 (89)
treated % of tumors	35
Carotid body PGL (%)	229 (83)
treated % of tumors	38
Vagal body PGL (%)	126 (46)
treated % of tumors	13
Jugulotympanic PGL (%)	74 (27)
treated % of tumors	56
Other HNPGL (%)	5 (2)
treated % of tumors	40
sPGL (%)	20 (7)
treated % of tumors	80
Pheochromocytoma (%)	18 (7)
treated % of tumors	94
Malignant PGL (%)	10 (4)
Unaffected (%)	20 (7)

PGL = paraganglioma, HNPGL = head and neck paraganglioma, sPGL = sympathetic paraganglioma

Mortality and SMR

Mortality data were available for all 275 included *SDHD* mutation carriers at the GBA, so there was no loss to follow-up. During a mean follow-up of 7.6 years (range 0-45 years), 18 *SDHD* mutation carriers with a mean age of 65.2 ± 15.5 years died. A total of 2242 person-years were available for comparison with normative data of the Dutch population. The SMR was 1.07 [95% Confidence Interval (CI) 0.67 to 1.73] for the whole cohort, indicating no increased mortality in SDHD-mutation carriers compared to the general population. The SMR for female *SDHD* mutation carriers was 1.24 [95% CI 0.56 to 2.76] and for male *SDHD* mutation carriers 1.00 [95% CI 0.55 to 1.81].

Discussion

The aim of the present study was to compare mortality rates in *SDHD* mutation carriers with the general population. Our results show that mortality in *SDHD* mutation carriers is not increased, even despite the presence of HNPGL in a majority of mutation carriers. Since the prevalence of *SDHD* related morbidity is high, the results of the study involve an important message for (newly identified) *SDHD* mutation carriers. However, whether the normal mortality risk is a consequence of protocolized screening for catecholamine overproduction and eventually surgical treatment of pheochromocytomas cannot be answered from this study.

To our knowledge, this is the first study that investigated mortality in *SDHD* mutation carriers. The high prevalence of *SDHD* founder mutations in the Netherlands and the historical interest in research of PGLs at the LUMC gave us the unique opportunity to perform this study in a large cohort of *SDHD* mutation carriers with a long duration of follow-up. Since mortality is affected by sex, calendar period and age and, in addition, *SDHD* mutations show an age-related penetrance,[2,27] we stratified mortality rates for these parameters. The use of small strata (1 year) for age and calendar time, rule out the presence of residual confounding for these parameters. Although our results point towards no increased mortality risk in *SDHD* mutation carriers, some uncertainty remains given the fact that the upper limit of the confidence interval is as high as 1.73. Therefore other cohort studies are needed to replicate our results.

The high prevalence of founder mutations in the Netherlands is probably due to the fact that Dutch society was segregated based on socioeconomic and religious differences until the second half of the 20th century, leading to endogamy in isolated populations. This allowed proliferation of Dutch founder mutations.[28] The p.Asp92Tyr and p.Leu139Pro founder mutations in *SDHD* are the most prevalent cause of hereditary PGLs in the Netherlands,[3-4,29] however these specific mutations are very rare in other series. It must be noted that this prohibits simple generalizations of our study results to other cohorts of *SDHD* mutation carriers.

Considering the poor prognosis in malignant PGL, an increased mortality in *SDHD* mutation carriers could be expected if many *SDHD* mutation carriers would have developed malignant PGL. In our study, mortality rates were not influenced largely by malignant PGL because of the low prevalence of malignant PGL in Dutch *SDHD* mutation carriers.[4] Furthermore, an increased mortality in *SDHD* mutation carriers may be expected because of the morbidity which can result from (treatment of) HNPGLs and the potential fatal course of (untreated) pheochromocytomas.[6,8,30-31] That our results proved this to be untrue, could partly be due to the follow-up policy at the LUMC. Firstly, although 90% of *SDHD* mutation carriers in our cohort developed a HNPGL, only 35% of these HNPGLs have been treated. In the other cases, a "wait and scan" policy was adopted. This may have resulted in a decreased treatment-related morbidity and possibly mortality. Secondly, the mutation carriers included in our cohort are screened for the presence of pheochromocytomas on a regular basis. Since pheochromocytomas detected by screening of patients with a hereditary predisposition have a much lower prevalence of signs and symptoms, lower catecholamine excess, and smaller tumors compared to sporadic pheochromocytomas detected by signs and symptoms,[32] this may have led to a decrease in both disease related and treatment related morbidity and possibly mortality.

Our center previously investigated the survival of Dutch patients diagnosed with a HNPGL between 1945 and 1960. A significant difference in survival compared to the general population was not found.[33] That study did not assess mutation status, however since the greater part of HNPGL patients in the Netherlands are carriers of *SDHD* mutations,[3] most of the included patients in that study must have been *SDHD* mutation carriers. This previous study reported results of a period of time in which HNPGL patients were not screened for the presence of pheochromocytomas, although the prevalence of pheochromocytomas in Dutch *SDHD* mutation carriers is reported to be 9%.[4] That survival was anyhow not affected in these patients implies that the presence of pheochromocytomas may not have a major impact on mortality, even if left undetected and untreated.

Because included subjects in our study are under medical surveillance, other health hazards may also be revealed in an earlier stage and therefore the expected deaths in the study cohort may be lower than those in the general population. In our study, seven percent of the study population did not display any signs of manifest disease, i.e. they remained unaffected mutation carriers. The number of unaffected mutation carriers in our cohort is probably an underestimation of the actual percentage of unaffected *SDHD* mutation carriers, since we were only able to include *SDHD* mutation carriers under regular follow-up. Unaffected mutation carriers may be less likely to be inclined to undergo regular investigations and may therefore remain under the clinical radar. Although this may have resulted in a more "diseased cohort", this only strengthens our results that the presence of *SDHD* mutations does not increase mortality.

In conclusion, mortality in *SDHD* mutation carriers is not increased. The results of this study enable us to provide (newly identified) mutation carriers with prognostic information concerning the effect of harboring *SDHD* mutations on mortality.

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