# Head and neck paragangliomas : genetics, heredity and clinical characteristics

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Chapter 3

# Mutation spectrum of the succinate dehydrogenase genes in the Netherlands

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## Abstract

Mutations in four genes encoding subunits or cofactors of succinate dehydrogenase (*SDH*) cause hereditary paraganglioma and pheochromocytoma syndromes. Mutations in SDHB and SDHD are generally the most common, whereas mutations in SDHC and SDHAF2 are far less frequently observed. A total of 1045 DNA samples from Dutch paraganglioma and pheochromocytoma patients and their relatives were analyzed for mutations of *SDHB, SDHC, SDHD or SDHAF2*. Mutations in these genes were identified in 690 cases, 239 of which were index cases. The vast majority of mutation carriers had a mutation in *SDHD* (87.1%). The second most commonly affected gene was *SDHAF2* (6.7%). Mutations in *SDHB* were found in only 5.9% of samples, whereas *SDHC* mutations were found in 0.3% of samples. Remarkably, 69.1% of all carriers of a mutation in an SDH gene in the Netherlands can be attributed to a single founder mutation in *SDHD*, c.274G>T (p.Asp92Tyr). Moreover, 88.8% of all *SDH* mutation carriers carry one of just six Dutch founder mutations in *SDHB*, *SDHD* and *SDHAF2*. The dominance of *SDHD* mutations is unique to the Netherlands, contrasting with the higher prevalence of *SDHB* mutations found elsewhere. In addition, we found that most SDH mutation-related paragangliomas-pheochromocytomas in the Netherlands can be explained by only six founder mutations in *SDHAF2*, *SDHB* and *SDHD*. The findings underline the regional differences in the SDH mutation spectrum, differences that should be taken into account in the development of effective screening protocols. The results show the crucial role that demographic factors play in the frequency of gene mutations.

## Introduction

Mutations in genes encoding subunits or cofactors of succinate dehydrogenase (SDH), an enzyme complex bound to the inner membrane of the mitochondria, are an important cause of hereditary paraganglioma syndrome[1-3]. SDH plays an important dual role as complex II in the electron transport chain and as an enzyme of the tricarboxylic acid (TCA) cycle, catalyzing the oxidation of succinate to fumarate. It consists of four subunits: a flavoprotein (SDHA) and iron-sulphur protein (SDHB), which together make up the catalytic domain, and SDHC and SDHD, both transmembrane proteins. In addition to the *SDHB*, *SDHC* and *SDHD* genes, an additional SDH-related paraganglioma tumor suppressor was recently identified[4]. An important cofactor in SDH stability and functionality, *SDHAF2* resides as a soluble protein within the mitochondrial matrix and plays a role in the attachment of the flavin adenine dinucleotide (FAD) cofactor to SDH[5].

Hereditary paragangliomas in the Netherlands are frequently caused by mutations in the *SDHD* gene, but mutations in *SDHAF2*, *SDHB* and *SDHC* are also found[4,6-9]. Founder mutations in SDHD including the c.274G>T, p.Asp92Tyr mutation and the c.416T>C, p.Leu139Pro mutation play a major role in the prevalence of hereditary paraganglioma in the Netherlands[8]. More recently, two founder mutations in *SDHB* were identified in Dutch paraganglioma and pheochromocytoma families[6,10]. The c.232G>A, p.Gly78Arg mutation is the only *SDHAF2* mutation found in Dutch paraganglioma patients, and all patients share a common ancestor[4]. To date, no *SDHC*-linked paraganglioma families have been described in the Netherlands.

In this study, we describe the frequency of mutations in *SDHB*, *SDHC*, *SDHD* or SDHAF2 in 1045 paraganglioma and pheochromocytoma patients and their relatives. The results were obtained from the Leiden University Medical Center (LUMC), a dedicated referral center for paragangliomas and the primary referral laboratory for SDH mutation analysis in the Netherlands. As almost all Dutch paraganglioma patient samples are analyzed here, the results represent the actual prevalence of mutations in genes encoding subunits of the SDH complex in the Netherlands.

## Materials and methods

### Patients

Peripheral blood leukocyte DNA samples were collected from patients with paraganglioma and pheochromocytoma, and their relatives, from 1990 to 2009, at the Department of Human Genetics and the Laboratory for DNA Diagnostics of the LUMC, the primary national referral center for SDH mutation scanning. The majority of DNA samples from patients and their relatives were sent for genetic analysis only, with only summary clinical data. The reason for referral was diagnosis of 'paraganglioma', 'pheochromocytoma', 'chemodectoma' or 'glomus tumor' in all cases. As the nomenclature of paragangliomas is not unequivocal and has changed over time, exact data regarding tumor location (i.e. head-and-neck region, adrenal medulla or extra-adrenal) are therefore unavailable for many patients, and are not further discussed. Cases were considered to be familial if two or more affected individuals were identified within the same kindred. An index case is defined as the initial patient who presented with paraganglioma.

### Mutation and deletion screening

The *SDHB*, *SDHC*, and *SDHD* genes were scanned for the presence of mutations from 2000 to 2009. All exonic regions of these genes were tested by direct sequencing using the Sanger method on an ABI 377 Genetic Analyzer, starting with the exons containing the known Dutch founder mutations in *SDHD* followed by exons that had previously been found to contain pathogenic mutations in *SDHB*, *SDHC*, and *SDHD* (in that order) in the Dutch population. If this analysis was negative, scanning was completed by analyzing the remaining exons of these genes. After the identification of large founder mutation-related families in the Netherlands, the current strategy is to scan the *SDHB*, *SDHC*, and *SDHD* genes as indicated by the clinical phenotype or as requested by the submitting clinician. In 2007, mutation-negative cases were retrospectively analyzed with multiplex ligation dependent probe amplification (MLPA) for the presence of large deletions in *SDHB*, *SDHC*, and *SDHD*. MLPA has been carried out on all mutation-negative cases since then, using the P226 MLPA kit (MRC Holland, Amsterdam) containing probes for all exons and the promoter of each of these genes (27 different probes), according to the manufacturer's protocol. In cases with a negative *SDHB*, *SDHC* and *SDHD* mutation analysis, *SDHAF2* was tested, as recently described[5]. Informed consent was obtained for DNA testing according to protocols approved by LUMC Ethics Review Board.

## Results

A total of 1045 samples from paraganglioma and/or pheochromocytoma patients and their relatives were analyzed for mutations in SDH-related genes. Mutations in *SDHB*, *SDHC*, *SDHD* or *SDHAF2* were found in 690 cases, 239 of whom were index cases (Table 1). No mutations in SDH genes were found in 101 index cases and 254 family members of SDH mutation carriers (Table 1). The majority of SDH mutation carriers in the Netherlands carry a mutation in SDHD (87.1%), followed by mutations in *SDHAF2* (6.7%), *SDHB*(5.9%) and *SDHC* (0.3%). By far the most prevalent mutation is the c.274G>T, p.Asp92Tyr mutation in *SDHD*, accounting for 69.1% of all SDH mutation carriers (Figure 1). Altogether, 88.8% of the SDH mutation carriers in the Netherlands carried one of six Dutch founder mutations in the *SDHD*, *SDHB* or *SDHAF2* genes (Table 2 and Figure 1).

A total of 340/1045 cases tested for SDH mutations were index cases. Mutations in *SDHAF2*, *SDHB*, *SDHC* or *SDHD* were identified in 239/ 340 (70.2%) index cases, most frequently a mutation in *SDHD* (62.1%; Table 1). The most prevalent mutation among index cases was also the c.274G>T, p.Asp92Tyr mutation in *SDHD*, accounting for 157/239 index mutation carriers (65.6%).

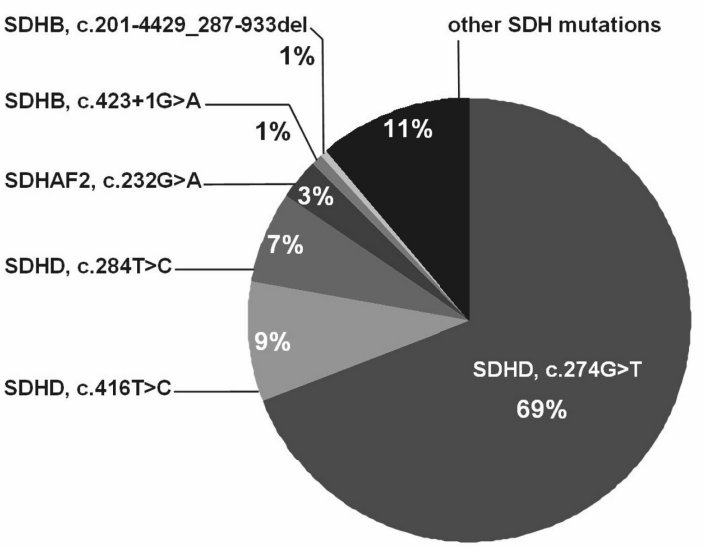


**Figure 1.** Relative frequencies of Dutch succinate dehydrogenase (SDH) founder mutation carriers. The graph includes a total of 613 carriers of a founder mutation in an SDH-related gene.

**Table 1.** Mutations in *SDHAF2, SDHB, SDHC*, and *SDHD*. In total, 690 Dutch paraganglioma and pheochromocytoma patients tested positive for a mutation in *SDHAF2, SDHB, SDHC* or *SDHD*, of which 239 are index cases. One mutation was identified in *SDHAF2*, 9 different mutations in *SDHB*, 2 mutations in *SDHC* and 16 mutations in *SDHD*. No SDH mutations were identified in 254 family members of SDH mutation carriers and 101 index cases.

| Gene | Exon | DNA mutation | Protein mutation | Cases (n) | Index cases(n) |
|---|---|---|---|---|---|
| *SDHAF2* | 4 | c.232G>A | p.Gly78Arg | 46 | 4 |
| *SDHB* | 2 | c.136C>T | p.Arg46X | 3 | 2 |
| | 2 | c.141G>A | p.Trp47X | 1 | 1 |
| | 3 | c.201-4429_287-933del | exon 3 deletion | 5 | 5 |
| | 3 | c.268C>T | p.Arg90X | 5 | 1 |
| | 4 | c.343C>T | p.Arg115X | 2 | 1 |
| | 4 | c.423+1G>A | Splicesite | 22 | 9 |
| | 6 | c.574T>C | p.Cys192Arg | 1 | 1 |
| | 6 | c.590C>G | p.Pro197Arg | 1 | 1 |
| | 7 | c.653G>C | p.Trp218Ser | 1 | 1 |
| *SDHC* | 4 | c.214C>T | p.Arg72Cys | 1 | 1 |
| | 5 and 6 | c.242-241_510+1978del | exon 5 and 6 deletion | 1 | 1 |
| *SDHD* | 1 and 2 | c.1-8828_169+442 del | exon 1 and 2 deletion | 1 | 1 |
| | 2 | c.112C>T | p.Arg38X | 8 | 1 |
| | 2 | c.120_121insC | p.Glu42ArgfsX27 | 6 | 3 |
| | 2 | c.169_169+9 del TGTATGTTCT | unknown | 4 | 1 |
| | 2 | c.54_55dupC | p.Leu19ProfsX50 | 8 | 1 |
| | 2 | c.64C>T | p.Arg22X | 3 | 1 |
| | 3 | c.181delG | p.Ala61LeufsX25 | 2 | 1 |
| | 3 | c.208A>G | p.Arg70Gly | 1 | 1 |
| | 3 | c.209G>T | p.Arg70Met | 1 | 1 |
| | 3 | c.242C>T | p.Pro81Leu | 16 | 8 |
| | 3 | c.274G>T | p.Asp92Tyr | 477 | 157 |
| | 3 | c.279T>G | p.Tyr93X | 7 | 1 |
| | 3 | c.284T>C | p.Leu95Pro | 4 | 2 |
| | 3 | c.287dupC | p.Ala97fs | 1 | 1 |
| | 4 | c.337_340 del GACT | p.Asp113MetfsX21 | 3 | 1 |
| | 4 | c.416T>C | p.Leu139Pro | 59 | 30 |

SDH, succinate dehydrogenase.

## Discussion

The majority of SDH mutation carriers in the Netherlands harbor the c.274G>T, p.Asp92Tyr mutation in *SDHD*. Several very large families residing in the western part of the Netherlands are known to carry this mutation, all linked by a strong founder effect[12]. The second most widespread SDH mutation in the Netherlands is the c.416T>C, p.Leu139Pro founder mutation in *SDHD*, but this mutation accounts for hardly more than 10% of the number of p.Asp92Tyr mutation carriers, emphasizing the dominant role of the latter mutation. Compared with the high prevalence of *SDHD* mutations, *SDHB* mutations are far less common (87.1% vs. 5.9%), but the majority of *SDHB* mutation carriers also harbor known founder mutations, specifically the intron 4 splice site mutation, c.423+1G>A or the exon 3 deletion, c.201-4429_287-933del (6,9) (Tables 1 and 2).

**Table 2.** Dutch founder mutations of the *SDHAF2*, *SDHB* and *SDHD* genes.

| Gene | DNA mutation | Protein mutation | References |
|---|---|---|---|
| *SDHAF2* | c.232G>A | p.Gly78Arg | Hao et al.[5] and Bayley et al.[4] |
| *SDHB* | c.423+1G>A | Intron4 splicesite | Hes et al.[10] |
| | c.201-4429_287-933del | exon 3 deletion | Bayley et al.[6] |
| *SDHD* | c.274G>T | p.Asp92Tyr | Baysal et al.[2] and Taschner et al.[8] |
| | c.284T>C | p.Leu95Pro | Dannenberg et al.[11] and Taschner et al.[8] |
| | c.416T>C | p.Leu139Pro | Cremers et al.[7], Dannenberg et al.[11] and Taschner et al.[8] |

SDH, succinate dehydrogenase.

The difference in prevalence between *SDHB* and *SDHD* mutation carriers may in part be attributable to the lower penetrance of *SDHB* mutations[10,13-15]. Despite their common forebears, most patients with a Dutch founder mutation in *SDHB* present without a family history of paraganglioma, suggesting that many more *SDHB* mutation carriers await discovery[13-17].

We noted a remarkable 14-fold difference in the number of *SDHD* and *SDHB* mutation carriers, and even taking only index cases into account, *SDHD* mutation carriers still predominate with a ratio of around 10:1 (Table 1). None of the international studies that have reported variation in the relative frequencies of *SDHB* and *SDHD* mutations in head-and-neck paraganglioma cases have described such a large difference. A recent large Italian study identified a twofold higher prevalence of *SDHD* mutations[18], whereas

a broader European study showed an approximate 1:1 distribution of *SDHB* and *SDHD* mutation carriers[14]. Other studies have shown a 2.7- to 4.5-fold higher frequency of *SDHB* mutation carriers[13,15]. In general, *SDHB* mutations are more common than *SDHD* mutations, indicating that SDHB mutation carriers in the Netherlands only appear to be scarce because of the higher prevalence of *SDHD* founder mutations.

Haplotype studies of the most prevalent founder mutations have shown unequivocally that mutation carriers share a common haplotype surrounding the mutations, and therefore share a common ancestor. The Dutch SDHD mutation, p.Asp92Tyr, is estimated to be 200-960 years old based on coalescence time calculations, and all known Dutch carriers of the SDHAF2 mutation, p.Gly78Arg, share a common haplotype and have also been linked to a common ancestor[12,19].

In addition to mutations of *SDHB* and *SDHD*, we identified 46 carriers of the c.232G>A, p.Gly78Arg mutation in *SDHAF2*. Four large *SDHAF2*-linked paraganglioma families from the south-east Netherlands are now known to share a common ancestor, a male, born in 1771 and who married three times[20]. These families have remained largely in the same area and the p.Gly78Arg mutation is a founder mutation in the south-east Netherlands, accounting for a significant proportion of the paraganglioma cases seen in the region.

Paraganglioma syndrome due to mutations in *SDHC* is extremely rare in the Netherlands. We have identified only two *SDHC* mutations, c.242- 241_510+1978del and c.214C>T, in two patients (0.3%). Like *SDHB*, *SDHC* mutations may have been under-reported because of the often sporadic-like presentation of *SDHC*-linked paraganglioma syndrome[3,21].

The remarkable prevalence of Dutch SDH founder mutations is most probably because of the unusual social and demographical history of the Netherlands. Until only a generation ago, Dutch society was highly segregated, primarily on the basis of religious differences. This segregation affected social, political and cultural life, and was further aided by socio-economic, geographic, and linguistic factors. These factors limited intermarriage until well into the twentieth century, and led to the creation of genetically isolated populations, facilitating the proliferation of Dutch founder mutations, both in SDHD and other disease genes[22]. The p.Asp92Tyr founder mutation in SDHD shows a strong geographic focus even today[8,12].

Mutations of *SDHB*, *SDHC*, *SDHD* and *SDHAF2* each result in distinct hereditary paraganglioma syndromes, with differing modes of inheritance, penetrance, risk

of pheochromocytoma, and risk of malignant paraganglioma, meaning that prior identification of the affected gene is essential to provision of effective genetic counseling to the individual patient[14,15,21]. Several algorithms prioritizing gene-specific mutation testing in paraganglioma patients have been proposed, based on phenotypic characteristics, and with the dual objectives of minimizing mutation screening and cost reduction[23,24]. Although these algorithms represent a useful starting point for genetic analysis, it is doubtful whether the effectiveness and outcome of such algorithms are universally applicable, as the a priori chance of finding a mutation in a specific gene differs from country to country. Recognition of regional differences in the prevalence of mutations will allow the tailoring of genetic screening on the basis of local knowledge.

This study shows that the majority of mutations in SDH subunits or cofactors in the Netherlands involve SDHD, followed by SDHAF2, SDHB and SDHC, and the majority of mutation carriers harbor the Dutch SDHD founder mutation, p.Asp92Tyr. This finding is in stark contrast with the extensive genetic heterogeneity found elsewhere and underlines the importance of regional differences in the mutation spectrum of genes associated with hereditary paraganglioma syndrome.

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