Chapter 2

Recent advances in paraganglioma genetics

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Abstract

The last 10 years have seen enormous progress in the field of paraganglioma and pheochromocytoma genetics. The identification of the first gene related to paraganglioma, *SDHD*, encoding a subunit of mitochondrial succinate dehydrogenase (SDH), was quickly followed by the identification of mutations in *SDHC* and *SDHB*. Very recently several new SDH-related genes have been discovered. The *SDHAF2* gene encodes an SDH co-factor related to the function of the SDHA subunit, and is currently exclusively associated with head and neck paragangliomas. *SDHA* itself has now also been identified as a paraganglioma gene, with the recent identification of the first mutation in a patient with extra-adrenal paraganglioma. Another SDH-related co-factor, SDHAF1, is not currently known to be a tumor suppressor, but may shed some light on the mechanisms of tumorigenesis. An entirely novel gene associated with adrenal pheochromocytoma, *TMEM127*, suggests that other new paraganglioma susceptibility genes may await discovery. In addition to these recent discoveries, new techniques related to mutation analysis, including genetic analysis algorithms, SDHB immunohistochemistry, and deletion analysis by MLPA have improved the efficiency and accuracy of genetic analysis. However, many intriguing questions remain, such as the striking differences in the clinical phenotype of genes that encode proteins with an apparently very close functional relationship, and the lack of expression of *SDHD* and *SDHAF2* mutations when inherited via the maternal line. Little is still known of the origins and causes of truly sporadic tumors, and the role of oxygen in the relationships between high altitude, familial and truly sporadic paragangliomas remains to be elucidated.

Introduction

Prior to the year 2000, knowledge of the genetics of paraganglioma and pheochromocytoma was confined to mutations of the *VHL*, *RET* and *NF1* genes. The identification of mutations in the succinate dehydrogenase subunit D gene (*SDHD*) in patients with head and neck paraganglioma was therefore a major breakthrough[1]. The association of paraganglioma with mutations in SDHD, and later with mutations in other SDH subunits, has helped elucidate both the role of the mitochondrial SDH complex and intermediary metabolism in tumorigenesis. The subsequent discovery of SDH mutations in patients with pheochromocytomas and extra-adrenal paragangliomas led to a recognition that paragangliomas and pheochromocytomas share not only similar cellular origins, but can also have a comparable genetic basis[2].

Paragangliomas of the head and neck are generally benign tumors that arise in the paraganglion tissue associated with the autonomic nervous system. Paragangliomas most frequently arise in the head and neck region, as carotid body tumors in the carotid bifurcation (approximately 80%). Other frequently seen locations within the head and neck region are along the jugular bulb or tympanic nerve (17.5%), or the paraganglia along the vagal nerve (4.5%)[3].

Pheochromocytomas and extra-adrenal paragangliomas are tumors associated with the sympathetic nervous system, are commonly described as sympathetic paragangliomas (sPGLs), and show a close embryological and physiological relationship to head and neck paragangliomas. They are most commonly derived from the chromaffin cells of the adrenal medulla (pheochromocytoma). Approximately 10-20% occur elsewhere in the abdomen, but they can occur in any of the sympathetic paraganglia from the neck to the pelvic floor[4]. Extra-adrenal sympathetic paragangliomas show a greater degree of malignancy than either pheochromocytomas or head and neck paragangliomas[5].

Here we discuss recent advances in the understanding of the genetic basis of both head and neck paragangliomas and pheochromocytomas, and further developments relevant to the genetic diagnosis of these tumors.

Genetics

Presently, causative gene mutations can be identified in around 32% of paraganglioma pheochromocytomas[6]. Hereditary tumor syndromes which have pheochromocytoma within their spectrum include the multiple endocrine neoplasia syndromes, MEN2A and MEN2B, caused by mutations of the *RET* (Rearranged in Transfection) proto-oncogene,

subtypes of von Hippel-Lindau (VHL) disease, caused by mutations of the *VHL* tumor suppressor gene, and neurofibromatosis type 1 (NF1) resulting from mutations of the *NF1* tumor suppressor gene[7]. These syndromes account for around 17% of cases but are rarely associated with head and neck or extra-adrenal paragangliomas[6].

More recently, mutations in genes associated with the mitochondrial succinate dehydrogenase (SDH) complex (*SDHA*, *SDHB*, *SDHC*, *SDHD* and *SDHAF2*) have been shown to cause head and neck paragangliomas, extra-adrenal paragangliomas, and pheochromocytomas (Table 1)[1,2,8-11]. These genes account for the remaining 15% of cases[6]. All of these genes are tumor suppressors, showing loss of heterozygosity (LOH), the loss of the normal allele in the tumor, in conjunction with the germline mutation. This results in loss of a protein subunit, which in turn destabilizes the SDH complex and abolishes its enzymatic activity[12].

Succinate dehydrogenase is an enzyme of the mitochondrial tricarboxylic acid cycle, and also plays an important role as the complex II component of the electron transport chain, contributing to the generation of ATP by oxidative phosphorylation. These combined roles place SDH at the center of two of the essential energy producing processes of the cell. SDHA, a flavoprotein, and SDHB, an iron-sulfur protein, together form the main catalytic domain, while SDHC and SDHD are the membrane-anchoring subunits of SDH and play a role in passing electrons through the electron transport chain. Despite the fact that SDH proteins are all components of the same protein complex, mutations lead to clear differences in clinical phenotype. The molecular basis for this clinical divergence is not currently known.

SDHD

Researchers in the Netherlands were the first to successfully tackle the genetics of head and neck paraganglioma and they were greatly assisted by the unusual social and demographic history of the country[13-18]. Until relatively recently, the Netherlands was characterized by significant religious, social, and geographic obstacles to intermarriage, leading to the creation of many genetically isolated populations[19]. Such populations facilitate the proliferation of founder mutations, one of them being the well-known Dutch SDHD founder mutation, p.Asp92Tyr[20]. The increased prevalence of this and other SDHD founder mutations, relative to SDHB mutations, facilitated the initial mapping of the SDHD locus[13,14].

The subsequent identification of the gene in 2000 represented a significant discovery as it was the first time that a mitochondrial protein was shown to be a tumor suppressor[1].

It was also the first protein with a role in intermediary metabolism to be directly linked to tumorigenesis. Mutations in *SDHD* most frequently result in benign head and neck paragangliomas and are much less commonly associated with sympathetic paragangliomas and adrenal pheochromocytomas[21]. The proportion of *SDHD* mutation carriers that will develop a tumor (penetrance) is high (87-100%), although not all carriers with a tumor will develop additional tumor-related symptoms[22,23].

SDHB

The identification of mutations in SDHD as a cause of hereditary paraganglioma syndrome quickly led to the discovery of the role of other SDH subunits. SDHB plays a major role in hereditary paraganglioma syndrome, and is now known to be a significant cause of adrenal pheochromocytomas, but is chiefly associated with extra-adrenal paragangliomas[2,6]. Since its discovery, *SDHB* has been found to be the dominant gene in hereditary paraganglioma syndrome in many parts of the world, despite a relatively low penetrance of *SDHB* mutations of 25-40%[24-26]. Due to their lower penetrance, *SDHB* mutations are often found in apparently sporadic patients[27]. *SDHB* mutations primarily predispose to sPGLs, and around 20% of *SDHB* mutation carriers will develop metastatic disease[5,6].

SDHC

SDHC was the second SDH subunit gene identified as a cause of paragangliomas[11]. Paragangliomas due to mutations in SDHC are much rarer than SDHB- and SDHD-related paragangliomas, accounting for less than 1% of all patients in a recent study[6]. SDHC mutations result primarily in head and neck paragangliomas, but have also been identified in patients with sympathetic paragangliomas[28,29].

SDHAF2

While the role of the *SDHB*, *SDHC* and *SDHD* genes in paraganglioma/pheochromocytoma has been known for a number of years, several novel SDH-related genes have only been identified very recently. The first was a gene encoding a novel protein involved in the addition of the flavin-adenine dinucleotide (FAD) prosthetic group to form the active SDHA flavoprotein[10]. While the approximate location of this paraganglioma-associated gene had been known for over a decade, referred to as PGL2 locus, a yeast screen of respiration deficient mutants facilitated the fortuitous discovery of a conserved mitochondrial protein of unknown function that physically associated with the SDHA flavoprotein[15,16]. Initially named SDH5, the succinate dehydrogenase complex assembly factor 2 (SDHAF2) was shown to be essential for the correct flavination of SDHA and function of the SDH complex. The c.232G>A (p.Gly78Arg) missense mutation in *SDHAF2*, identified in a large

Dutch head and neck paraganglioma kindred, results in the loss of SDHA flavination and activity of the SDH complex[10].

In a follow-up study with the joint aims of identifying new mutation carriers and assessing the frequency of *SDHAF2* mutations amongst 443 paraganglioma and pheochromocytoma patients, it became clear that mutations in this gene make a very modest contribution to the overall genetic burden in these syndromes[8]. No mutations of *SDHAF2* were identified in any patient with a pheochromocytoma, and all currently affected mutation carriers have head and neck paraganglioma exclusively. Only one additional SDHAF2 related family was identified, which interestingly carried the exact mutation, p.Gly78Arg, previously found in the Netherlands, but without evidence of a familial relationship to the Dutch kindred[8]. Although apparently a simple loss of function mutation in yeast, the recurrence of this mutation and absence of other mutations may suggest that the SDHAF2 protein with the specific p.Gly78Arg mutation retains residual activity, allowing the protein to participate in other, currently unknown, cellular activities, most feasibly the addition of FAD prosthetic groups to other flavoproteins[8,10,30].

A striking aspect of *SDHAF2* mutations, and the probable explanation for the rapid identification of all mutation carriers, is the very high penetrance. Of the 42 identified mutation carriers thought to be at risk, 37 are known to have developed a tumor. All currently unaffected mutation carriers are under the age of 45. This level of penetrance will usually lead to a familial presentation and such families will have already come to the attention of clinicians. Seven mutation carriers are known to have inherited the mutation via the maternal line, and are not thought to be at risk of tumor development (see ‘‘Inheritance’’ below).

The studies above suggest that *SDHAF2* mutation screening should only be considered in patients who suffer exclusively from head and neck paragangliomas, who have familial antecedents, multiple tumors, or a very young age of onset, and in whom the *SDHB*, *SDHC* and *SDHD* genes have been shown to be negative for mutations and deletions by sequencing and multiplex ligation-dependent probe amplification (MLPA).

SDHA

The identification of SDHAF2 as a paraganglioma-related tumor suppressor that interacts with SDHA was unexpected, as SDHA itself was the only SDH subunit not known to be mutated in paraganglioma cases. *SDHA* is the largest gene and protein of the SDH complex and is the major catalytic subunit of the enzyme. For 10 years following the discovery of *SDHD*, it remained a mystery why no mutations of *SDHA* could be found in

paraganglioma patients, a mystery which deepened with the identification of *SDHAF2* as a paraganglioma related tumor suppressor gene. Recently the first SDHA mutation was reported, (c.1765C>T, p.Arg589Trp-exon 13) in a patient with a catecholamine secreting extra-adrenal paraganglioma[9]. This patient had no family history of paraganglioma or any related endocrine syndrome.

It remains unclear why *SDHA* mutations in paragangliomas are so rare, but the patient above may suggest that *SDHA* mutations show reduced penetrance and most mutation carriers escape the development of clinical symptoms. Equally, and as suggested above for *SDHAF2*, the scarcity of *SDHA* mutations could be attributable to a secondary cellular function of SDHA, leading to intolerance for missense and truncating mutations that eliminate all enzyme activity.

The most stable of the SDH proteins when soluble, SDHA has been reported to be a component of a mitochondrial ATP-sensitive potassium channel[31]. While SDHB also seemed to be involved in this complex, the main protein interaction was between SDHA and the mitochondrial ATP-binding cassette protein 1 (mABC1), and the complex could be inhibited by 3-nitropropionate (NPA), a specific inhibitor of SDHA[32]. Whether the maintenance of this complex is essential to cell viability remains to be determined.

Alternatively, if we assume that an LOH event which deletes the remaining normal allele is required for tumorigenesis, loss of essential genes in the proximity of *SDHA* may not be tolerated, or other local genomic factors may be preventing the secondary LOH event. An exact molecular description of the LOH event in the case described by Burnichon et al. and in any subsequent cases may provide useful insights[9].

A few rare cases of congenital SDHA deficiency due to homozygous recessive mutations are known[33-35]. While the patients themselves tend to be severely affected by developmental abnormalities or cardiomyopathy early in life, due to mitochondrial deficiency, the heterozygous parents of these patients have never been reported to develop paraganglioma, perhaps suggesting that LOH events are indeed rare in conjunction with mutations of *SDHA*.

Mutations seen in these patients are generally missense and the only known truncating mutation in a patient was found together with a missense mutation on the opposing allele suggesting that complete loss of SDHA function may not be compatible with life[36].
Whether the patient described by Burnichon et al. will prove to be first of many paraganglioma cases related to *SDHA* mutations is presently unclear[9]. The current

significance of SDHA in the clinical management of paraganglioma-pheochromocytoma is minimal, but this may change if future studies identify additional mutation carriers.

SDHAF1

The identification of *SDHAF2* as a paraganglioma gene underlines the curious fact that another recently identified gene is not currently known to be involved in paraganglioma, but may nevertheless further our understanding of the role of SDH in paraganglioma formation. Succinate dehydrogenase complex assembly factor 1 (SDHAF1) is a novel LYR-motif protein; the first SDH assembly factor identified in any organism, and is located within the mitochondrial matrix[37]. Identified in consanguineous families of Turkish and Italian origin, homozygous mutations of the *SDHAF1* gene result in infantile leukoencephalopathy in affected children, and symptoms include rapidly progressive psychomotor regression beginning in the first year of life, reminiscent of the clinical symptoms seen in homozygous *SDHA* mutations carriers[38]. Patients show defective succinate dehydrogenase (complex II), with only 20-30% residual activity in muscle and fibroblasts, and the accumulation of lactate and succinate in the brain white matter. Disruption of the homologous gene or expression of the mutated gene in yeast caused SDH deficiency and failure of oxidative phosphorylation-dependent growth. Because the LYR tripeptide motif found in SDHAF1 is also seen in several iron-related proteins and may be a signature for proteins involved in Fe-S metabolism, this protein may well be associated with the SDHB subunit.

Loss of SDHB is currently thought to be central to tumorigenesis in paragangliomas, but none of the parents in *SDHAF1* families, who are heterozygous mutation carriers, have been reported to develop paragangliomas[39]. The explanation for the lack of tumor development in these mutation carriers and heterozygous *SDHA* mutation carriers may lie in the biochemical activity of SDH-complex II. *SDHA* homozygous mutation carriers generally show retention of complex II activity of at least 20% (range 20-61%), and likewise, homozygous *SDHAF1* mutation carriers show 20-30% residual activity[33,35]. In contrast, SDH related tumors, including those related to SDHD, SDHB, SDHA and SDHAF2 carry an inactivating mutation which, combined with the loss of the wild type allele (LOH), results in almost complete loss of activity[9,12,40]. As *SDHAF1* and most *SDHA* mutations do not eliminate all enzyme function, even allowing for LOH in a specific cell, a residual activity of 10-30% is apparently sufficient to prevent the development of paragangliomas.

A further interesting aspect of the biochemical profile of *SDHAF1* and *SDHA* mutation carriers is the accumulation of succinate. In both cases succinate will accumulate and can lead to the nuclear translocation of hypoxia-inducible factor 1 (HIF-1)[37,41]. The nuclear

translocation of HIF-1 may be an important mechanism in triggering tumorigenesis in paraganglioma progenitor cells, but its occurrence in *SDHAF1* and *SDHA* mutation carriers may suggest that complete loss of SDH activity is required to achieve levels of succinate accumulation sufficient to drive HIF-1 translocation to the extent needed to initiate tumorigenesis[42,43]. For a detailed discussion of these and other recent developments in the understanding of the molecular basis of tumorigenesis, we refer readers to a recent review[43].

Although none of the heterozygous mutation carriers in *SDHAF1* families currently seem susceptible to the development of paragangliomas-pheochromocytomas, the recent example of *SDHA* emphasizes that no SDH-related gene can be entirely excluded when one is considering the genetics of these tumors[9].

TMEM127

In addition to the recently reported genes related to succinate dehydrogenase, a novel tumor suppressor gene associated with a clinical phenotype of exclusively adrenal pheochromocytoma has also been described[44]. The gene encodes a putative transmembrane protein, TMEM127, and is found on chromosome 2q11. TMEM127 is a highly conserved and broadly expressed protein with three transmembrane regions, but has no known functional domains. Transfection experiments showed that the protein is found in both the plasma membrane and the cytoplasm, and suggested that TMEM127 may participate in protein trafficking between the plasma membrane, golgi and lysosomes.

Previous gene expression studies have indicated that pheochromocytomas fall into two broad categories based on the transcriptional profile, which may translate to the molecular pathways leading to tumorigenesis[45]. SDH and VHL associated tumors show a signature of angiogenesis, hypoxia, enhanced expression of the extracellular matrix, and reduced expression of components of the oxidative response and tricarboxylic cycle. Tumors linked to *NF1* or *RET* mutations show an upregulation of biological pathways including genes that mediate translation initiation, protein synthesis, and kinase signaling, and are both associated with the RAS/RAF/MAP kinase signaling pathway[45].

TMEM127-related pheochromocytomas show a transcriptional profile similar to NF1 and RET related tumors[44]. However, neither RAS activation nor AKT phosphorylation was seen, indicating that TMEM127 loss is not identical to either NF1 or RET. The authors focused on the mammalian target of rapamycin (mTOR), which is deregulated on loss of NF1, and could show that the C1 mTOR complex is specifically affected by *TMEM127* knockdown, leading to increased phosphorylation of targets of mTORC1. Knockdown of *TMEM127* also

resulted in larger cells with higher rates of proliferation. Pheochromocytomas carrying a *TMEM127* mutation showed hyperphosphorylation of mTOR effector proteins, all these data together indicating that TMEM127 is a negative regulator of mTOR.

The authors were able to identify mutations in 4 out of 12 families without known mutations in other susceptibility genes, and in 3 of 83 apparently sporadic patients. Of the seven distinct germline mutations identified, six were truncating, and the deletion of the wild-type allele in tumor DNA indicates that this is a bone fide tumor suppressor gene.

The identification of TMEM127 underlines that there are several pathways that can lead to adrenal, extra-adrenal, and head and neck paragangliomas. Whether there are important links between the essential molecular pathways of NF1, RET, and TMEM127 on the one hand and the VHL and SDH-related proteins on the other, is presently unclear, but hypoxia can regulate both HIF-1 and mTORC1, perhaps related to expression of BCL2/Adenovirus E1B 19-KD protein-interacting protein 3 (BNIP3)[46,47]. As each of these genes is associated with patterns of biological and clinical expression that are not yet understood, it is clear that we are only at the beginnings of our knowledge of these syndromes.

Inheritance

Inheritance of paraganglioma syndrome differs significantly dependent on the gene involved. While *SDHB*- and *SDHC*-linked paraganglioma families show normal autosomal dominant inheritance, *SDHD*- and *SDHAF2*-linked families show an exclusively paternal transmission of tumor susceptibility[10,18]. The recognition of this phenomenon was made possible by the same social and demographic factors in the Netherlands that facilitated the initial mapping of the *SDHD* locus, and specifically by the increased prevalence of *SDHD* mutations, relative to *SDHB* mutations. Although mutations in *SDHD* and *SDHAF2* can be inherited via the maternal and paternal lines, tumor formation following maternal transmission of a mutation is extremely rare[18,48].

The failure of maternally transmitted mutations to initiate tumorigenesis initially suggested that an imprinted gene expressed only from the paternal allele could be the underlying cause of the tumor[18]. The subsequent identification of SDHD, with its central role in cell biology, called this assumption into question. It was also established that the gene does not show mono-allelic expression, at least in the tissues analyzed to date[1,49]. The concept of gene expression of *SDHD* exclusively from the paternal allele is also contradicted by the normal development of mutation carriers with a paternally inherited mutation.

Table 1. Summary of genes, known protein functions and related syndromes.

	Locus	Protein function	Gene mechanism	No. of unique mutations[a]	Syndrome	Primary locations
SDHD	11q23	One of the two transmembrane subunits of Complex II of the respiratory chain	Autosomal dominant with LOH + imprinting	110	Hereditary paraganglioma/ pheochromocytoma	Head and neck; parasympathetic trunk
SDHB	1p36.-p35	The iron-sulfur protein catalytic subunit of Complex II	Autosomal dominant with LOH	175	Hereditary paraganglioma/ pheochromocytoma	Abdominal/ thoracic paraganglia, adrenal; sympathetic trunk
SDHC	1q23.3	One of the two transmembrane subunits of Complex II of the respiratory chain	Autosomal dominant with LOH	34	Hereditary paraganglioma/ pheochromocytoma	Head and neck; parasympathetic trunk
SDHAF2	11q12.2	Mitochondrial assembly factor for Complex II – interacts directly with SDHA	Autosomal dominant with LOH + imprinting	1	Hereditary paraganglioma/ pheochromocytoma	Head and neck; parasympathetic trunk
SDHA	5p15	The flavoprotein catalytic subunit of Complex II	Autosomal dominant with LOH	1	Hereditary paraganglioma/ pheochromocytoma	Abdominal paraganglia, sympathetic trunk
SDHA	5p15	The flavoprotein catalytic subunit of Complex II	Autosomal recessive	7	Mitochondrial encephalopathy/ Leigh Syndrome	Systemic
SDHAF1	19q13.12	Mitochondrial assembly factor for Complex II – interacts directly with SDHB?	Autosomal recessive	2	Infantile leukoencephalopathy	Systemic
TMEM127	2q11.2	Transmembrane protein involved in protein trafficking	Autosomal dominant with LOH	8	Hereditary pheochromocytoma	Adrenal; sympathetic trunk

[a] Number of mutations derived from the literature or the TCAC gene mutation database [34].

The additional occurrence of this phenomenon in paraganglioma families linked to *SDHAF2*, (like *SDHD*, located on chromosome 11), while it is absent in *SDHB*- and *SDHC*-related tumors (both genes located on chromosome 1), suggested that chromosomal location could be a factor in *SDHD*- and *SDHAF2*-related tumors.

It is known that the entire maternal copy of chromosome 11 is lost in many paragangliomas[49-51]. Although *SDHD* and *SDHAF2* themselves seem not to be imprinted, the main cluster of imprinted genes in the human genome is located on the same chromosome, at 11p15.5. This suggests a model in which a maternally expressed, paternally imprinted gene is an essential initiator or modifier of tumor development in these syndromes[48,49]. Indeed, the only report to date that has claimed to show the maternal transmission of tumor susceptibly together with an *SDHD* mutation showed that the patient had also acquired an altered methylation profile and therefore probably an altered imprinted status of H19, a known paternally imprinted tumor suppressor gene on 11p15[48,52]. In addition, it is known that VHL-related pheochromocytoma also show loss of the maternal copy of the chromosome 11p15.5 region specifically, indicating that this model may have wider importance[53,54].

High altitude paraganglioma

Long before the identification of any of the genes now known to play a role in paraganglioma, it was recognized that living at high altitude can have a profound influence on the development of carotid body hyperplasia and carotid body tumors[55-57]. A number of mammalian species are known to develop pronounced hyperplasia or tumors with a prevalence of up to 10% in humans and up to 40% in bovines, in contrast to an estimated low altitude prevalence of head and neck paraganglioma of 1 in 500,000 or less[58,59].

This increased prevalence and the central role of the carotid body in oxygen sensing suggested a role for oxygen sensing in the tumorigenesis of paragangliomas. The identification of succinate dehydrogenase and subsequent molecular studies has affirmed this link. A number of studies have linked the central mediator of cellular hypoxia, HIF-1, to defects in succinate dehydrogenase[60]. These studies postulate that a so-called ‘pseudohypoxia’ results from the inhibition of succinate dehydrogenase, leading to the accumulation of succinate, resulting in the activation of HIF-1 through the inhibition of prolyl hydroxylase-mediated degradation[42,61]. The HIF-1 transcription factor complex initiates the transcription of a range of genes that mediate an adaptive response to reduced oxygen[62]. How the activation of the HIF-1 protein may lead to the initiation of tumorigenesis in the carotid body and the exact relation of physiological hypoxia to molecular ‘pseudo-hypoxia’ awaits further investigation. Despite this suggestive link,

the possible role of succinate dehydrogenase mutations in high altitude paraganglioma cases has received little attention and the first genetic analysis failed to identify any mutations[63]. Recently, Cerecer-Gil et al. identified a family with two SDHB-linked cases of high altitude paraganglioma, residing at elevations of up to 2,200 m[64]. These are the first cases to link high altitude paraganglioma to mutations of the succinate dehydrogenase genes. While the occurrence of paraganglioma in this family could be purely coincidental to their place of residence, two factors indicated that elevation may be playing a role in the expression off these tumors. One of the patients showed a remarkably aggressive recurrent tumor, which achieved a volume almost equivalent to the original tumor within 2 months of excision. This behavior is in sharp contrast with the indolent growth pattern normally seen in head and neck paragangliomas, with a mean doubling rate of 4.2 years[65]. In addition, both patients developed head and neck tumors, while abdominal tumors occur much more frequently in SDHB mutation carriers. The identification of SDHB mutations in high altitude paraganglioma may serve to renew interest in this fascinating but underappreciated field of paraganglioma research, and refocus attention on the role of oxygen levels in the initiation and development of these tumors.

New strategies in mutation analysis

The importance of the SDH-related genes in paraganglioma-pheochromocytoma has led to extensive genetic screening of patients, even in the absence of clear familial antecedents. In patients with pheochromocytomas, in addition to the SDH genes, the *RET* and *VHL* genes should also be screened. The costs involved in analyzing all of these genes can be considerable, and are increasing with each new gene identified. Efforts have been made to use clinical data to derive algorithms to guide rational genetic testing, with the aims of efficiency and cost reduction[6,21,66]. Perhaps the most comprehensive of these is that proposed by Mannelli et al., but even this is now in need of updating[6]. Such algorithms are now widely used and assist the rapid identification of mutation carriers, but many patients may provide few useful clinical parameters, or may not conform to the rather broad criteria of these algorithms.

Mutation analysis is generally carried out using DNA sequencing, but this technique can rarely detect large deletions. Both MLPA and similar multiplex PCR methods have been applied in SDH deletion analysis, and have led to the recognition that deletions can represent up to 10% of all mutations[67-69].

While algorithms have improved the efficiency of genetic testing, recently a supplementary approach has been developed with the use of SDHB immunohistochemistry. As originally noted by Douwes Dekker et al., paragangliomas show loss of staining for the

iron protein component of SDH, encoded by *SDHB*[12]. This finding was subsequently explored by van Nederveen et al. who showed that in a series of 220 paragangliomas and pheochromocytomas, 102 tumors with known mutation of one of the SDH genes were negative for SDHB staining while *RET*, *VHL* and *NF1* cases were uniformly positive[39]. Only 6 cases were found to be negative and not explained by a known mutation in one of the SDH genes. This translates to a sensitivity of 95% (C.I. 87-100%) and specificity of 84% (C.I. 60-97%).

The utility of this approach was subsequently confirmed in an independent series of tumors by Gill et al. and was also shown to be useful in identifying the gastrointestinal stromal tumor (GIST) component of the Carney triad (CT)[70,71]. Showing that a GIST is a legitimate constituent of this tumor syndrome would potentially allow earlier diagnosis, when compared to current methods which focus on clinical criteria and require the co-occurrence of paraganglioma and pulmonary chondroma. These authors also showed that some cases of apparently sporadic GISTs also show loss of SDHB staining and propose that these represent a new subtype of GISTs.

The development of a reliable SDHB immunohistochemical procedure and the demonstration that SDHB staining can accurately distinguish SDH-related cases from other groups represents an important advance, where tumor material is available. As head and neck paragangliomas are often not operated for a considerable period after initial diagnosis, while most pheochromocytomas will be removed upon diagnosis, phaeochromocytomas represent the most useful group of tumors for the application of this technique.

Conclusion

The last 10 years have seen enormous progress in the field of head and neck paraganglioma and pheochromocytoma genetics. Six new genes have been added to a list that previously included only *VHL*, *RET* and *NF1*, and the number of patients in whom a gene mutation can be identified has doubled, and now stands at around 30-35%. New techniques related to mutation analysis, including analysis algorithms, MLPA and SDHB immunohistochemistry, have improved the efficiency and accuracy of genetic analysis.

The identification of mutations in *SDHAF2* has revealed that proteins ancillary to succinate dehydrogenase can also be tumorigenic, and the belated identification of a mutation in *SDHA* in a paraganglioma patient has demonstrated that no SDH-related gene can be

entirely excluded from consideration when thinking about the genetics of these tumor syndromes.

Finally, the recent identification of *TMEM127* by Dahia et al. has shown that entirely novel genes may be related to these tumor syndromes and suggests that others may await discovery[44].

References

1. Baysal BE, Ferrell RE, Willett-Brozick JE et al., Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science 287 (2000) 848-851.
2. Astuti D, Latif F, Dallol A, et al., Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet. 69 (2001) 49-54.
3. Lack EE, Tumors of the adrenal gland and extra-adrenal paraganglia. In: Atlas of tumor pathology. AFIP (1997) fascicle 19.
4. Petri BJ, Van Eijck CH, de Herder WW, et al., Phaeochromocytomas and sympathetic paragangliomas. Br J Surg. 96 (2009) 1381-1392.
5. Benn DE, Gimenez-Roqueplo AP, Reilly JR et al., Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. J Clin Endocrinol Metab. 91 (2006) 827-836.
6. Mannelli M, Castellano M, Schiavi F et al., Clinically guided genetic screening in a large cohort of Italian patients with pheochromocytomas and/or functional or nonfunctional paragangliomas. J Clin Endocrinol Metab. 94 (2009) 1541-1547.
7. Tischler AS, Pheochromocytoma and extra-adrenal paraganglioma: updates. Arch Pathol Lab Med. 132 (2008) 1272-1284.
8. Bayley JP, Kunst HP, Cascon A et al., SDHAF2 mutations in familial and sporadic paraganglioma and phaeochromocytoma. Lancet Oncol. 11 (2010) 366-372.
9. Burnichon N, Briere JJ, Libe R et al., *SDHA* is a tumor suppressor gene causing paraganglioma. Hum Mol Genet. 19 (2010) 3011-3020.
10. Hao HX, Khalimonchuk O, Schraders M et al., SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. Science 325 (2009) 1139-1142.
11. Niemann S, Muller U, Mutations in SDHC cause autosomal dominant paraganglioma, type 3. Nat Genet. 26 (2000) 268-270.
12. Douwes Dekker PB, Hogendoorn PCW, Kuipers-Dijkshoorn N et al., SDHD mutations in head and neck paragangliomas result in destabilization of complex II in the mitochondrial respiratory chain with loss of enzymatic activity and abnormal mitochondrial morphology. J Pathol. 201 (2003) 480-486.
13. Heutink P, Van der Mey AGL, Sandkuijl LA et al., A gene subject to genomic imprinting and responsible for hereditary paragangliomas maps to chromosome 11q23-qter. Hum Mol Genet. 1 (1992) 7-10.
14. Heutink P, Van Schothorst EM, Van der Mey AGL et al., Further localization of the gene for hereditary paragangliomas and evidence for linkage in unrelated families. Eur J Hum Genet. 2 (1994) 148-158.
15. Mariman EC, Van Beersum SE, Cremers CWRJ et al., Analysis of a second family with hereditary non-chromaffin paragangliomas locates the underlying gene at the proximal region of chromosome 11q. Hum Genet. 91 (1993) 357-361.
16. Mariman EC, Van Beersum SE, Cremers CWRJ et al., Fine mapping of a putatively imprinted gene for familial non-chromaffin paragangliomas to chromosome 11q13.1: evidence for genetic heterogeneity. Hum Genet. 95 (1995) 56-62.
17. Van Baars FM, Cremers CW, Van den Broek P, Veldman JE, Familiar non-chromaffinic paragangliomas (glomus tumors). Clinical and genetic aspects (abridged). Acta Otolaryngol. 91 (1981) 589-593.
18. Van der Mey AGL, Maaswinkel-Mooy PD, Cornelisse CJ, et al., Genomic imprinting in hereditary glomus tumours: evidence for new genetic theory. Lancet 2 (1989) 1291-1294.
19. Zeegers MP, Van Poppel F, Vlietinck R et al., Founder mutations among the Dutch. Eur J Hum Genet. 12 (2004) 591-600.
20. Taschner PE, Jansen JC, Baysal BE et al., Nearly all hereditary paragangliomas in The Netherlands are caused by two founder mutations in the SDHD gene. Genes Chromosom Cancer 31 (2001) 274-281.
21. Erlic Z, Rybicki L, Peczkowska M et al., Clinical predictors and algorithm for the genetic diagnosis of pheochromocytoma patients. Clin Cancer Res. 15 (2009) 6378-6385.

22. Hensen EF, Jansen JC, Siemers MD et al., The Dutch founder mutation SDHD.D92Y shows a reduced penetrance for the development of paragangliomas in a large multigenerational family. Eur J Hum Genet. 18 (2010) 62-66
23. Neumann HPH, Pawlu C, Peczkowska M et al., Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA 292 (2004) 943-951.
24. Hes FJ, Weiss MM, Woortman SA et al., Low penetrance of a SDHB mutation in a large Dutch paraganglioma family. BMC Med Genet. 11 (2010) 92
25. Solis DC, Burnichon N, Timmers HJ et al., Penetrance and clinical consequences of a gross SDHB deletion in a large family. Clin Genet. 75 (2009) 354-363.
26. Schiavi F, Milne RL, Anda E et al., Are we overestimating the penetrance of mutations in SDHB? Hum Mutat. 31 (2010) 761-762.
27. Bayley JP, Grimbergen AR, Van Bunderen PA et al., The first Dutch SDHB founder deletion in paraganglioma-pheochromocytoma patients. BMC Med Genet. 10 (2009) 34.
28. Mannelli M, Ercolino T, Giache V et al., Genetic screening for pheochromocytoma: should SDHC gene analysis be included? J Med Genet. 44 (2007) 586-587.
29. Peczkowska M, Cascon A, Prejbisz A et al., Extra-adrenal and adrenal pheochromocytomas associated with a germline SDHC mutation. Nat Clin Pract Endocrinol Metab. 4 (2008) 111-115.
30. Yao L, Barontini M, Niederle B et al., Mutations of the metabolic genes IDH1, IDH2, and SDHAF2 are not major determinants of the pseudohypoxic phenotype of sporadic pheochromocytomas and paragangliomas. J Clin Endocrinol Metab. 95 (2010) 1469-1472.
31. Ardehali H, Chen Z, Ko Y et al., Multiprotein complex containing succinate dehydrogenase confers mitochondrial ATP-sensitive K^+- channel activity. Proc Natl Acad Sci USA 101 (2004) 11880-11885.
32. Coles CJ, Edmondson DE, Singer TP, Inactivation of succinate dehydrogenase by 3 nitropropionate. J Biol Chem. 254 (1979) 5161-5167.
33. Pagnamenta AT, Hargreaves IP, Duncan AJ et al., Phenotypic variability of mitochondrial disease caused by a nuclear mutation in complex II. Mol Genet Metab. 89 (2006) 214-221.
34. Bayley JP, Devilee P, Taschner PE, The SDH mutation database: an online resource for succinate dehydrogenase sequence variants involved in pheochromocytoma, paraganglioma and mitochondrial complex II deficiency. BMC Med Genet. 6 (2005) 39.
35. Levitas A, Muhammad E, Harel G et al., Familial neonatal isolated cardiomyopathy caused by a mutation in the flavoprotein subunit of succinate dehydrogenase. Eur J Hum Genet. 18 (2010) 1160-1165.
36. Horvath R, Abicht A, Holinski-Feder E et al., Leigh syndrome caused by mutations in the flavoprotein (Fp) subunit of succinate dehydrogenase (SDHA). J Neurol Neurosurg Psychiatry 77 (2006) 74-76.
37. Ghezzi D, Goffrini P, Uziel G et al., SDHAF1, encoding a LYR complex-II specific assembly factor, is mutated in SDH-defective infantile leukoencephalopathy. Nat Genet. 41 (2009) 654-656.
38. Parfait B, Chretien D, Rotig A et al., Compound heterozygous mutations in the flavoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome. Hum Genet. 106 (2000) 236-243.
39. Van Nederveen FH, Gaal J, Favier J et al., An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. Lancet Oncol. 10 (2009) 764-771.
40. Gimenez-Roqueplo AP, Favier J, Rustin P et al., Functional consequences of a SDHB gene mutation in an apparently sporadic pheochromocytoma. J Clin Endocrinol Metab. 87 (2002) 4771-4774.
41. Briere JJ, Favier J, Benit P et al., Mitochondrial succinate is instrumental for HIF1alpha nuclear translocation in *SDHA*-mutant fibroblasts under normoxic conditions. Hum Mol Genet. 14 (2005) 3263-3269.
42. Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG et al., Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell 7 (2005) 77-85.
43. Bayley JP, Devilee P, Warburg tumours and the mechanisms of mitochondrial tumour suppressor genes. Barking up the right tree? Curr Opin Genet Dev. 20 (2010) 324-329.
44. Qin Y, Yao L, King EE et al., Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. Nat Genet. 42 (2010) 229-233.

45. Dahia PL, Ross KN, Wright ME et al., A HIF1alpha regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. PLoS Genet. 1 (2005) 72-80.
46. Wouters BG, Koritzinsky M, Hypoxia signalling through mTOR and the unfolded protein response in cancer. Nat Rev Cancer 8 (2008) 851-864.
47. Pollard PJ, El Bahrawy M, Poulsom R et al., Expression of HIF-1 alpha, HIF-2 alpha (EPAS1), and their target genes in paraganglioma and pheochromocytoma with VHL and SDH mutations. J Clin Endocrinol Metab. 91 (2006) 4593-4598.
48. Pigny P, Vincent A, Cardot BC et al., Paraganglioma after maternal transmission of a succinate dehydrogenase gene mutation. J Clin Endocrinol Metab. 93 (2008) 1609-1615.
49. Hensen EF, Jordanova ES, Van Minderhout IJHM et al., Somatic loss of maternal chromosome 11 causes parent-of-origin-dependent inheritance in SDHD linked paraganglioma and phaeochromocytoma families. Oncogene 23 (2004) 4076-4083.
50. Riemann K, Sotlar K, Kupka S et al., Chromosome 11 monosomy in conjunction with a mutated SDHD initiation codon in nonfamilial paraganglioma cases. Cancer Genet Cytogenet. 150 (2004) 128-135.
51. Dannenberg H, de Krijger RR, Zhao J et al., Differential loss of chromosome 11q in familial and sporadic parasympathetic paragangliomas detected by comparative genomic hybridization. Am J Pathol. 158 (2001) 1937-1942.
52. Yoshimizu T, Miroglio A, Ripoche MA et al., The H19 locus acts in vivo as a tumor suppressor. Proc Natl Acad Sci USA 105 (2008) 12417-12422.
53. Margetts CDE, Astuti D, Gentle DC et al., Epigenetic analysis of HIC1, CASP8, FLIP, TSP1, DCR1, DCR2, DR4, DR5, KvDMR1, H19 and preferential 11p15.5 maternal-allele loss in von Hippel-Lindau and sporadic phaeochromocytomas. Endocr Relat Cancer 12 (2005) 161-172.
54. Mircescu H, Wilkin F, Paquette J et al., Molecular characterization of a pediatric pheochromocytoma with suspected bilateral disease. J Pediatr. 138 (2001) 269-273.
55. Arias-Stella J, The carotid body at high altitudes. Meet Am Ass Pathol Bact. (1969) Abstract 150.
56. Edwards C, Heath D, Harris P et al., The carotid body in animals at high altitude. J Pathol. 104 (1971) 231-238.
57. Arias-Stella J, Valcarcel J, The human carotid body at high altitudes. Pathol Microbiol (Basel) 39 (1973) 292-297.
58. Arias-Stella J, Bustos F, Chronic hypoxia and chemodectomas in bovines at high altitudes. Arch Pathol Lab Med. 100 (1976) 636-639.
59. Saldana MJ, Salem LE, Travezan R, High altitude hypoxia and chemodectomas. Hum Pathol. 4 (1973) 251-263.
60. King A, Selak MA, Gottlieb E, Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. Oncogene 25 (2006) 4675-4682.
61. Koivunen P, Hirsila M, Remes AM et al., Inhibition of hypoxia inducible factor (HIF) hydroxylases by citric acid cycle intermediates - possible links between cell metabolism and stabilization of HIF. J Biol Chem. 282 (2007) 4524-4532.
62. Semenza GL, Regulation of physiological responses to continuous and intermittent hypoxia by hypoxia-inducible factor 1. Exp Physiol. 91 (2006) 803-806.
63. Jech M, Varado-Cabrero I, Bores-Saavedra J et al., Genetic analysis of high altitude paragangliomas. Endocr Pathol. 17 (2006) 201-202.
64. Cerecer-Gil NY, Figuera LE, Llamas FJ et al., Mutation of SDHB is a cause of hypoxia-related high-altitude paraganglioma. Clin Cancer Res. 16 (2010) 4148-4154.
65. Jansen JC, Van den Berg R, Kuiper A et al., Estimation of growth rate in patients with head and neck paragangliomas influences the treatment proposal. Cancer 88 (2000) 2811-2816.
66. Neumann HPH, Erlic Z, Boedeker CC et al., Clinical predictors for germline mutations in head and neck paraganglioma patients: cost reduction strategy in genetic diagnostic process as fall-out. Cancer Res. 69 (2009) 3650-3656.
67. Schouten JP, McElgunn CJ, Waaijer R et al., Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res. 30 (2002) e57.

68. Cascon A, Montero-Conde C, Ruiz-Llorente S et al., Gross SDHB deletions in patients with paraganglioma detected by multiplex PCR: a possible hot spot? Genes Chromosom. Cancer 45 (2006) 213-219.
69. Bayley JP, Weiss MM, Grimbergen A et al., Molecular characterization of novel germline deletions affecting SDHD and SDHC in pheochromocytoma and paraganglioma patients. Endocr Relat Cancer 16 (2009) 929-937.
70. Gill AJ, Benn DE, Chou A et al., Immunohistochemistry for SDHB triages genetic testing of SDHB, SDHC, and SDHD in paraganglioma-pheochromocytoma syndromes. Hum Pathol. 41 (2010) 805-814.
71. Gill AJ, Chou A, Vilain R et al., Immunohistochemistry for SDHB divides gastrointestinal stromal tumors (GISTs) into 2 distinct types. Am J Surg Pathol. 34 (2010) 636-644.