Clinical aspects of SDHx-related pheochromocytoma and paraganglioma

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Abstract

Paragangliomas (PGLs) derive from either sympathetic chromaffin tissue in adrenal and extra-adrenal abdominal or thoracic locations, or from parasympathetic tissue of the head and neck. Mutations of nuclear genes encoding subunits B, C, and D of the mitochondrial enzyme succinate dehydrogenase (*SDHB* 1p35-p36.1, *SDHC* 1q21, *SDHD* 11q23) give rise to hereditary PGL syndromes PGL4, PGL3, and PGL1 respectively. The susceptibility gene for PGL2 on 11q13.1 remains unidentified. Mitochondrial dysfunction due to *SDHx* mutations have been linked to tumorigenesis by upregulation of hypoxic and angiogenesis pathways, apoptosis resistance and developmental culling of neuronal precursor cells. *SDHB*-, *SDHC*-, and *SDHD*-associated PGLs give rise to more or less distinct clinical phenotypes. *SDHB* mutations mainly predispose to extra-adrenal, and to a lesser extent, adrenal PGLs, with a high malignant potential, but also head and neck paragangliomas (HNPG). *SDHD* mutations are typically associated with multifocal HNPG and usually benign adrenal and extra-adrenal PGLs. *SDHC* mutations are a rare cause of mainly HNPG. Most abdominal and thoracic *SDHB*-PGLs hypersecrete either norepinephrine or norepinephrine and dopamine. However, only some hypersecrete dopamine, are biochemically silent. The biochemical phenotype of *SDHD*-PGL has not been systematically studied. For the localization of PGL, several positron emission tomography (PET) tracers are available. Metastatic *SDHB*-PGL is the best localized by [¹⁸F]-fluorodeoxyglucose PET. The identification of *SDHx* mutations in patients with PGL is warranted for a tailor-made approach to the biochemical diagnosis, imaging, treatment, follow-up, and family screening.

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Introduction

Paragangliomas (PGLs) derive from either sympathetic tissue in adrenal and extra-adrenal locations or from parasympathetic tissue of the head and neck (DeLellis *et al.* 2004). A PGL arising from chromaffin cells of the adrenal medulla is also referred to as pheochromocytoma (Lenders *et al.* 2005, Pacak *et al.* 2005). Typical locations for extra-adrenal sympathetic tissue-derived PGLs are: (1) the Zuckerkandl body, a sympathetic ganglion located at the root of the inferior mesenteric artery, (2) the sympathetic plexus of the

urinary bladder, the kidneys, and the heart, and (3) the sympathetic ganglia in the mediastinum. Throughout the manuscript, the abbreviation ‘sPGL’ refers to sympathetic paraganglioma, i.e., pheochromocytoma and extra-adrenal sympathetic PGL. When referring to tumors of parasympathetic origin, the term ‘head and neck PGL’ (HNPG) will be used. The vast majority of sPGLs produce catecholamines, whereas only 5% of the HNPG do (Erickson *et al.* 2001). Typical symptoms and signs of catecholamine excess include headache, palpitations, diaphoresis, and hypertension.

sPGLs are rare tumors (incidence 2–8 per million) that occur either sporadically or as part of a hereditary syndrome. The ‘classical’ familial syndromes with which sPGL is associated are multiple endocrine neoplasia type 2 (*RET* mutations), von Hippel-Lindau disease (*VHL* mutations), hereditary PGL/pheochromocytoma syndromes (*SDHx* mutations), and rarely neurofibromatosis type 1 (*NF1* mutations). The hereditary PGL/pheochromocytoma syndromes have been more recently identified. These familial PGL syndromes are associated with three different genes that encode subunits D, C, and B of the mitochondrial enzyme succinate dehydrogenase (SDH): SDHD (locus PGL1 on 11q23), SDHC (locus PGL3 on 1q21), and SDHB (locus PGL4 on 1p35-p36.1; Baysal *et al.* 2000, Niemann & Muller 2000, Astuti *et al.* 2001). An up-to-date overview of all reported *SDHx* allelic variants that give rise to familial PGL syndromes is available online at http://chromium.liacs.nl/lovd_sdh/ (Fokkema *et al.* 2005). In addition to these established hereditary PGL syndromes, novel susceptibility loci for pheochromocytoma are being identified (Dahia *et al.* 2005).

Within a European–American registry of patients with PGL, the prevalence of underlying *SDHx* mutations was 10% among 371 patients with apparently sporadic sPGL (6% SDHB, 4% SDHD) and 28% among 121 patients with HNPGL (7% SDHB, 4% SDHC, and 17% SDHD; Schiavi *et al.* 2005). Important information on the clinical characteristics of different *SDHx*-related PGL syndromes was gathered by large multinational PGL study consortia (Neumann *et al.* 2004, Amar *et al.* 2005a, Schiavi *et al.* 2005, Benn *et al.* 2006). *SDHB* mutations mainly predispose to extra-adrenal, and to a lesser extent, adrenal sPGLs, with a high malignant potential (Young *et al.* 2002, Neumann *et al.* 2004, Benn *et al.* 2006, Brouwers *et al.* 2006, Pham *et al.* 2006, Timmers *et al.* 2007a). *SDHD* mutations are typically associated with multifocal HNPGL and usually benign adrenal and extra-adrenal sPGL (Neumann *et al.* 2004, Benn *et al.* 2006). *SDHC* mutations appear to be a rare cause of mainly HNPGL (Schiavi *et al.* 2005) and sPGL (Mannelli *et al.* 2007). More recently, germ line *SDHB*, *SDHC*, and *SDHD* mutations have implicated in a new clinical syndrome, the Stratakis–Carney dyad associating gastrointestinal stromal tumor and PGL (Pasini *et al.* 2008).

In this review, distinct clinical expressions associated with mutations in different subunits of the *SDHx* gene will be discussed, as well as present insights in to optimal strategies for establishing the biochemical diagnosis, tumor localization, and therapy of PGL. We will present our recommendations regarding genetic

testing and tumor screening among relatives of patients with *SDHx*-related PGL.

SDH and tumorigenesis

SDH is part of the mitochondrial electron transport chain (complex II, succinate-ubiquinone oxidoreductase) and catalyses the oxidation of succinate to fumarate in the Krebs cycle (Scheffler 1998). Subunits A and B of this complex (SDHA, SDHB) constitute the catalytic core of the enzyme, while SDHC with SDHD anchor the complex to the matrix face of the mitochondrial inner membrane. Abnormal SDH function due to mutations of nuclear DNA encoding for one of its subunits results in two completely different phenotypes. Defects in SDHA cause metabolic neurodegenerative disorders like congenital Leigh syndrome (Bourgeron *et al.* 1995) and late-onset optic atrophy, ataxia and myopathy (Birch-Machin *et al.* 2000), whereas *SDHB*, *SDHC*, and *SDHD* mutations predispose to familial PGL. The molecular and cellular mechanisms linking these latter *SDHx* mutations and tumorigenesis have not been fully elucidated. Also, the pathophysiology of distinct clinical phenotypes associated with abnormalities in different SDH subunits remains to be unraveled.

Consistent with Knudson’s two-hit hypothesis for tumorigenesis involving in a tumor suppressor gene, a heterozygous germ line mutation in an *SDHx* gene is usually associated with somatic loss of the non-mutant allele in the tumor, i.e., loss of heterozygosity (Gimenez-Roqueplo *et al.* 2003). In *SDHB* and *SDHD*-associated PGL, this was shown to result in a complete abolition of SDH enzymatic activity and an activation of the hypoxic–angiogenic pathway via an activation of the transcription hypoxia-inducible factor (HIF1- α) and its main target genes such as *VEGF* (Gimenez-Roqueplo *et al.* 2001, 2002). Actually, the abnormal SDH function induces an accumulation of succinate. It was shown *in vivo* in tumoral tissues and *in vitro* in different cellular models that the succinate accumulation induces an inhibition of the prolyl hydroxylase activity and consequently a stabilization of HIF1 α (Lee *et al.* 2005). HIF1 α regulates the transcription of a number of genes that are known to be involved in tumorigenesis and angiogenesis (Selak *et al.* 2005). Mitochondrial dysfunction and activation of the hypoxic pathway might be interlinked with other mechanisms of tumorigenesis involving oxidative stress and apoptosis resistance (Gottlieb & Tomlinson 2005). Another important theory interlinking the pathogenesis of different hereditary forms of PGL pertains to the developmental culling of sympathetic

neuronal precursor cells. Specifically, in the case of *SDHx*-related PGL, c-Jun-dependent neuronal apoptosis following withdrawal of neuronal growth factor is decreased through inhibition of prolyl hydroxylase by succinate (Lee *et al.* 2005).

Apart from a role in the pathogenesis of PGL in patients with germ line mutations, somatic *SDHx* mutation may also be rarely involved in the development of sporadic PGL (Gimm *et al.* 2000, van Nederveen *et al.* 2007).

SDHB (PGL4)

Typically, *SDHB*-related sPGLs arise in extra-adrenal locations and have strong tendency for metastatic spread (Neumann *et al.* 2004, Amar *et al.* 2005a, Benn *et al.* 2006; Table 1). Malignant PGL is defined as the presence of metastatic lesions at sites where chromaffin tissue is normally absent, e.g., lymph nodes, bone, lung, and liver (Linnoila *et al.* 1990). Among 84 patients with an apparently sporadic sPGL, eight had an *SDHB* mutation, 62.5% of them had an extra-adrenal tumor, and 84% had a malignant disease. In this series, in the presence of an *SDHB* mutation, the odd ratio for an extra-adrenal tumor as primary site was calculated to 19.8 and for a malignant to 19 (Gimenez-Roqueplo *et al.* 2003). Among 32 patients with *SDHB*-associated PGL(s) in the German/Polish cohort (Neumann *et al.* 2004), 28% had an adrenal PHEO, 59% had an extra-adrenal abdominal or

thoracic sPGL, and 31% had a HNPGL. Twenty-eight percent had multifocal tumors. A similar percentage of extra-adrenal sPGL was reported by the International SDH Consortium (Benn *et al.* 2006). In the NIH cohort of *SDHB*-mutation carriers with mainly metastatic sPGL, as many as 96% presented with an extra-adrenal tumors (Timmers *et al.* 2007a).

The mean age at diagnosis of PGL in *SDHB* mutation carriers in previous series was ~30 years (Neumann *et al.* 2004, Timmers *et al.* 2007a). However, initial tumors may occur between age 7 and 65 (Elston *et al.* 2006, Pham *et al.* 2006, Timmers *et al.* 2007a). In the International SDH Consortium population, age-related penetrance (the proportion of gene carriers manifesting the signs or symptoms of the disease by a given age) among *SDHB* mutation carriers was 29% by age 30 and 45% by age 40 (Benn *et al.* 2006). Genetic and/or environmental factors responsible for this large variability in penetrance are unknown.

Patients with *SDHB*-associated sPGL frequently develop metastatic disease. Reported malignancy rates in patients with *SDHB*-associated sPGL vary from 34 to 70% (Neumann *et al.* 2004, Amar *et al.* 2005a, Benn *et al.* 2006, Timmers *et al.* 2007a) versus 10% for sPGL in general (Plouin *et al.* 1997, Goldstein *et al.* 1999, Amar *et al.* 2005b, Khorram-Manesh *et al.* 2005). Reversely, the prevalence of deleterious *SDHB* mutations among patients with malignant sPGL is 30%, and even higher (48%) if the tumor originates from an extra-adrenal location (Brouwers *et al.* 2006). In another NIH study, nearly one-third of patients had evidence of malignant disease at the initial diagnosis of sPGL, which is similar to the previous finding of 22% (Benn *et al.* 2006). The overall 5-year survival rate of patients with malignant sPGL varies between 34 and 60% (Mundschenk & Lehnert 1998, John *et al.* 1999). An underlying *SDHB* mutation was shown to be an independent predictor of mortality (Amar *et al.* 2007): in 54 patients with malignant sPGL, the 5-year probability of survival was 36% in *SDHB* mutation carriers and 67% in the absence of *SDHB* mutation. Median survival after the diagnosis of the first metastasis was 42 months for the *SDHB* mutation carriers and 244 months for patients with no *SDHB* mutation. Finally, in multivariate analysis, only the presence of *SDHB* mutations was significantly and independently associated with survival: the presence of *SDHB* mutations was associated with an excess mortality (relative risk 2.7).

Of note is that the diagnosis of *SDHB*-related sPGL is frequently delayed due to an atypical clinical presentation (Timmers *et al.* 2007a). A majority presents with

Table 1 Characteristics of *SDHx*-related PGL and HNPGL

	PGL1	PGL3	PGL4
PGL syndrome			
SDH subunit defect	SDHD	SDHC	SDHB
Gene locus	11q23	1q21	1p35–36.1
sPGL	Frequent	Possible	Very frequent
Adrenal	Frequent	Possible	Possible
Extra-adrenal	Possible	Possible	Very frequent
Multifocal	Possible	Not reported	Frequent
Malignant	Rare	Not reported	Very frequent
Norepinephrine secreting	Possible	Possible	Very frequent
Epinephrine secreting ^a	Possible	Possible	Possible
Dopamine secreting	Unknown	Unknown	Frequent
HNPGL	Very frequent	Very frequent	Frequent
Multifocal	Very frequent	Not reported	Possible
Malignant	Rare	Rare	Rare

SDH, succinate dehydrogenase; PGL, paraganglioma; HNPGL, head and neck paraganglioma.

^aOnly in adrenal paraganglioma.

pain and other problems related to the space occupying effects and invasive growth of the tumor, rather than to signs and symptoms related to catecholamine excess. Apart from malignant sPGL, *SDHB* mutations have been suggested to be associated with renal cell carcinomas of early onset (Neumann *et al.* 2004, Vanharanta *et al.* 2004, Ricketts *et al.* 2008).

Previously, genotype–phenotype correlations failed to distinguish differences in tumor location and malignant potency of *SDHB*-related PGL between different mutations (Benn *et al.* 2006). In addition, clinical phenotypes may largely differ between family members with the same *SDHB* mutation. In our recent study, features that are associated with aggressive tumor behavior, including young age at presentation, large tumor size, metastatic disease at presentation, and hypersecretion of dopamine were equally distributed among patients with missense versus truncating mutations and were independent of exon locations (Timmers *et al.* 2007a). Identical mutations may result in *SDHB*-related tumors of variable location and severity.

SDHC (PGL3)

SDHC mutations are mainly associated with the development of HNPGL. A limited number of *SDHC* mutation carriers have been identified worldwide (Niemann & Muller 2000, Bauters *et al.* 2003, Niemann *et al.* 2003, Baysal *et al.* 2004, Schiavi *et al.* 2005, Bayley *et al.* 2006, Mannelli *et al.* 2007, Peczkowska *et al.* 2008). Within large population-based international registries, the prevalence of *SDHC* mutations varied between 0% for patients with sPGL (Amar *et al.* 2005a, Schiavi *et al.* 2005) and 4% for patients with HNPGL (Schiavi *et al.* 2005). In general, the clinical behavior of *SDHC*-related sPGL appears to be similar to that of benign sporadic sPGL. However, a malignant catecholamine-producing PGL at the carotid bifurcation has been reported in a patient with a IVS5 + 1G > T *SDHC* mutation (Niemann *et al.* 2003). *SDHC* mutations were originally believed to be associated only with HNPGL, but recently, adrenal and extra-adrenal sPGL were reported (Mannelli *et al.* 2007, Peczkowska *et al.* 2008).

SDHD (PGL1)

SDHD mutations are mainly associated with multifocal HNPGL and benign PGL. Among 34 patients with *SDHD*-associated PGL(s) in the German/Polish cohort (Neumann *et al.* 2004), 74% had multifocal PGL. Fifty-three percent had an adrenal sPGL, 39% had an extra-adrenal abdominal or thoracic sPGL, and 79%

had a HNPGL. Predominant location in the head and neck was confirmed by the other consortium (89%; Benn *et al.* 2006).

In the German/Polish population, the mean (s.d.) ages at tumor diagnosis were 30.6 years (14.3) in *SDHD* mutation carriers (Neumann *et al.* 2004). The study by the International SDH Consortium showed that by age 40, an estimated 69% of *SDHD* mutation carriers are diagnosed with HNPGL, whereas by age 60, 35% are diagnosed with sPGL (Benn & Robinson 2006).

Rare cases of *SDHD*-associated metastatic HNPGL have been described (Neumann *et al.* 2004, Amar *et al.* 2005a, Benn *et al.* 2006, Ogawa *et al.* 2006). Among 200 carriers of the well-known Dutch founder *SDHD* mutation D92Y (Baysal *et al.* 2000), there were three patients with metastatic HNPGL and two with metastatic sPGL (Havekes *et al.* 2007). It was argued that *SDHD*-associated malignancy might be specifically linked to the D92Y mutation and/or gene–environment interactions in the Netherlands. It was later shown that *SDHD*-associated malignant sPGL is not exclusively linked to the Dutch founder mutation (Timmers *et al.* 2008a). In general, however, both parasympathetic and sPGL in *SDHD* mutation carriers are rarely malignant.

With respect to genotype–phenotype correlations in *SDHD*-related tumors, carriers of a nonsense and/or splicing mutation were suggested to develop symptoms at an earlier age than missense–mutation carriers, possibly due to the formation of a truncated protein and failure of the normal function of mitochondrial complex II (Astrom *et al.* 2003). Also there is a tendency for *SDHD* mutation carriers of truncating mutations to develop pheochromocytoma in addition to HNPGL (Astrom *et al.* 2003, Benn *et al.* 2006).

Biochemical diagnosis and imaging

Suspicion of sPGL is usually prompted by (paroxysmal) symptoms suggestive of catecholamine excess, hypertension or by the incidental finding of an adrenal tumor. To establish a biochemical diagnosis of sPGL, plasma and/or 24 h urine concentrations of the catecholamines epinephrine and norepinephrine, and their *O*-methylated metabolites metanephrine, and normetanephrine ('metanephrines') are measured. Especially, plasma-free metanephrines and urinary fractionated metanephrines both have an excellent sensitivity (>97%) for detecting sPGL (Lenders *et al.* 2002).

The majority of *SDHB*-related sPGLs, secrete either norepinephrine or both norepinephrine and dopamine

(Timmers *et al.* 2007a), a profile that is consistent with mainly extra-adrenal and metastatic PGL (Eisenhofer *et al.* 2005b). Some *SDHB*-related sPGLs exclusively overproduce dopamine, but not other catecholamines (Eisenhofer *et al.* 2005a, Bonnet *et al.* 2006). Therefore, measurement of plasma levels of dopamine or its *O*-methylated metabolite methoxytyramine should be considered in *SDHB*-related sPGL (Eisenhofer *et al.* 2005a). However, a considerable number of *SDHB*-related sPGLs do not produce or secrete any catecholamines, e.g., are ‘biochemically silent’ (Timmers *et al.* 2008b). Lack of catecholamine hypersecretion results from the absence of tyrosine hydroxylase, the enzyme for the rate-limiting step in catecholamine synthesis. In these patients, lack of typical symptoms of catecholamine excess may delay the diagnosis. With respect to *SDHD*-associated sPGL, no studies on the biochemical phenotype have been reported so far.

Once a biochemical diagnosis of sPGL has been established, the source of catecholamine hypersecretion is subsequently localized by anatomical and functional imaging studies. Adequate localization of the tumor(s) is especially important in *SDHx*-mutation carriers, since they are prone to multiple and metastatic tumors at presentation. Conventional computed tomography (CT) and magnetic resonance imaging (MRI) have an excellent sensitivity (>90%) for detecting PGL, but lack specificity (Maurea *et al.* 1996). Tumors detected by CT or MRI can subsequently be identified as sPGL by functional imaging agents that specifically target the catecholamine synthesis, storage and secretion pathway of chromaffin cells. These include ^{123}I -metaiodobenzylguanidine (MIBG) scintigraphy, ^{18}F -fluorodopamine, and ^{18}F -fluorodihydroxyphenylalanine positron emission tomography (PET; Ilias *et al.* 2005). However, for localizing metastatic lesions of *SDHB*-related sPGL, ^{18}F -fluorodeoxyglucose PET is superior to other functional imaging techniques (Timmers *et al.* 2007b). Impairment of mitochondrial function due to loss of SDH function may cause tumor cells to shift from oxidative phosphorylation to aerobic glycolysis (the ‘Warburg effect’ (Warburg 1956)) and may thereby lead to ^{18}F -fluorodeoxyglucose uptake. This possible bio-energetic signature on imaging awaits confirmation at a molecular level. Furthermore, ^{111}In -pentetreotide scintigraphy, another less specific technique was found to have an excellent sensitivity (>90%) for HNPGL (Kwekkeboom *et al.* 1993) and may be useful for screening for these tumors in carriers of *SDHx* gene mutations (Neumann *et al.* 2004, Benn *et al.* 2006).

Treatment and prognosis

The mainstay in the treatment of non-metastatic sPGL is surgical resection. If feasible, laparoscopic removal of intra- and extra-adrenal sPGL is preferred over conventional laparotomy (Janetschek *et al.* 1998). With adequate pharmacological preparation, operative mortality is down to below 1% (Niemann *et al.* 2002). Pre-operative management is aimed at the prevention of catecholamine-induced complications like hypertensive crisis, cardiac arrhythmias, pulmonary edema, and cardiac ischemia due to manipulation of the tumor, and to prevent hypotensive crises after tumor removal. Randomized controlled trials on different regimes for pre-operative drug treatment are lacking. The authors prefer to combine an α -adrenoceptor blocking agent (phenoxybenzamine, doxazosin), a β -adrenoceptor blocking agent (propranolol, atenolol), and α -methyl-paratyrosine, an inhibitor of catecholamine synthesis. According to previous cohort studies, the life-expectancy after surgery of benign sPGL, in general is either normal (Stenstrom *et al.* 1988) or mildly decreased (Khorram-Manesh *et al.* 2005) as compared with the general population. For obvious reasons, long-term results after surgery for *SDHx*-related sPGL in particular are lacking. Considering the high risk of developing additional tumors (*SDHB*, *SDHD*) and/or metastatic lesions (*SDHB*), we recommend indefinite post-operative follow-up with bi-annual measurement of blood pressure and annual plasma or urine metanephrines, as well as annual MRI of the neck, thorax, abdomen, and pelvis.

There is no effective treatment for malignant sPGL. The usefulness of surgical debulking of tumor tissue has not been established. Treatment with therapeutic doses of ^{131}I -MIBG or combination chemotherapy (cyclophosphamide, vincristine, and dacarbazine) may induce (partial) responses (Averbuch *et al.* 1988, Fitzgerald *et al.* 2006). External beam irradiation can be useful in the treatment of local tumor complications. Symptomatic treatment by decreasing catecholamine burden can be obtained with α -adrenergic blockers and α -methyl-paratyrosine. The prognosis after diagnosis of metastatic sPGL is highly variable, with an estimated 5-year survival rate of 50% (Eisenhofer *et al.* 2004). Specific data on *SDHB*-associated malignant sPGL are lacking. In our experience, survival among these patients is highly variable as well. In one case, prolonged survival exceeding 30 years has been reported (Young *et al.* 2002).

Genetic testing

The *SDHB*, *SDHC*, and *SDHD* traits are inherited in an autosomal dominant fashion. However, as stated above, the penetrance of these traits is incomplete. Furthermore, *SDHD*-related disease is characterized by maternal genomic imprinting: individuals who inherit a mutation from mother remain free of PGL, but may still pass on the mutation to their offspring. Because of incomplete penetrance and genomic imprinting, a considerable number of patients with *SDHx*-related PGL have no apparent family history of PGL. As many as 63–90% of patients with *SDHB*-related PGL had a apparently sporadic presentation (Benn et al. 2006, Timmers et al. 2007a), whereas 21% of patients with *SDHD*-related PGL do (Benn et al. 2006). These observations indicate that a negative family history by no means rules out an underlying *SDH* mutation. Reversely, among patients with apparently sporadic sPGL, the prevalences of *SDHB* and *SDHD* mutations are 4–7% and 1–4% respectively (Neumann et al. 2002, Amar et al. 2005a).

According to recent expert-based recommendations, genetic testing for an underlying mutation should be considered in all patients with sPGL, regardless of family history and age (Gimenez-Roqueplo et al. 2006). In the absence of clinical evidence of other syndromic features, testing of specific genes (*RET*, *VHL*, *NF1*, *SDHx*) should be prioritized on the basis of individual clinical characteristics. More specifically, the *SDHx* genes should be addressed in the first line of genetic testing in case of HNPGL (*SDHD*, *SDHB*, *SDHC*), extra-adrenal sPGL or concurrent sPGL/HNPGL (*SDHB*, *SDHD*), and malignant sPGL (*SDHB*). HNPGL can also occur as part of VHL syndrome (Hull et al. 1982). Apart from intragenic mutations, there are few reports on patients with PGLs relating to large deletions of the *SDHB* and *SDHC* gene (McWhinney et al. 2004, Cascon et al. 2006). These whole gene or large partial deletions remain undetected by conventional gene analysis, but can be detected using semiquantitative multiplex PCR analysis (Amar et al. 2007). Since limited clinical data on families with such deletion are available, it remains to be elucidated whether this genotype is associated with a phenotype distinct from intragenic mutations.

Tumor screening in *SDHx*-mutation carriers

Asymptomatic carriers of an *SDHx*-mutation among the relatives of affected patients may benefit from tumor screening for early detection of PGL, because

the chances of post-operative morbidity and metastatic spread rise with increasing tumor size. Tumor screening may be particularly useful in *SDHB*-mutation carriers, who are at risk for aggressive PGLs with a delayed and atypical clinical presentation. However, genetic testing of relatives and subsequent tumor screening in mutation carriers is controversial, since unnecessary investigations can cause significant psychological distress and the screening may not be of cost-effective due to a limited disease penetrance. So far, no solid information from large family screening studies on the age-related penetrance of *SDHx*-related disease and on the yield of tumor screening in asymptomatic mutation carriers are available.

Until evidence-based guidelines for *SDHx* family screening become available, we recommend to offer genetic testing, preceded by careful genetic counseling, to all first-degree family members of patients with *SDHx*-related PGL (Young & Abboud 2006), and to subsequently carry out a clinical, biochemical, and radiological tumor screening among mutation carriers. The only exceptions are the children of female *SDHD* mutation carriers, who do not require clinical surveillance because of maternal imprinting. We recommend a surveillance protocol that includes annual history and physical examination, annual biochemical testing, e.g., plasma-free metanephrines and/or 24 h urine for fractionated metanephrines, and MRI of the neck, chest, abdomen, and pelvis every 1–2 years. In the selected cases, additional functional imaging may be of use in the screening for PGL (Kwekkeboom et al. 1993, Pacak et al. 2001). In case of *SDHB* mutation carriership, we recommend to commence investigations as early as before the age of 10. The approach to screening should be adjusted according to the individual history of the patient and findings on prior investigations.

Conclusion

The identification of mutations of different subunits of the *SDH* gene in patients with PGL is important, since it guides a tailor-made approach to the biochemical diagnosis, localization, treatment, and follow-up of these tumors.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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