15 YEARS OF PARAGANGLIOMA

Genetics and mechanism of pheochromocytoma–paraganglioma syndromes characterized by germline *SDHB* and *SDHD* mutations

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Abstract

Pheochromocytomas and paragangliomas (PPGL) are rare neuroendocrine neoplasms that derive from small paraganglionic tissues which are located from skull base to the pelvic floor. Genetic predisposition plays an important role in development of PPGLs. Since the discovery of first mutations in the succinate dehydrogenase D (*SDHD*) gene, which encodes the smallest subunit of mitochondrial complex II (SDH), genetic studies have revealed a major role for mutations in SDH subunit genes, primarily in *SDHB* and *SDHD*, in predisposition to both familial and non-familial PPGLs. SDH-mutated PPGLs show robust expression of hypoxia induced genes, and genomic and histone hypermethylation. These effects occur in part through succinate-mediated inhibition of α-ketoglutarate-dependent dioxygenases. However, details of mechanisms by which SDH mutations activate hypoxic pathways and trigger subsequent neoplastic transformation remain poorly understood. Here, we present a brief review of the genetic and mechanistic aspects of SDH-mutated PPGLs.

Key Words
- pheochromocytoma
- neuroendocrine tumors
- neoplasia
- molecular genetics
- molecular biology

Endocrine-Related Cancer (2015) **22**, T71–T82

Introduction

Paragangliomas are neuroendocrine neoplasms that may arise from parasympathetic or sympathetic paraganglia. In general, those arising from parasympathetic paraganglia are non-secretory, associated with head and neck paraganglia and are usually referred to as head and neck paragangliomas (HNPGL), or more specifically 'carotid paraganglioma', 'jugulotympanic paraganglioma', etc., rather than by older terms such as 'chemodectoma' or 'glomus jugulare'. Paragangliomas arising from sympathetic nervous system paraganglia usually arise in the abdomen and thorax, and secrete catecholamines (Tischler 2008). Thus they are functionally and histologically similar to pheochromocytomas (in older literature they were often described as extra-adrenal pheochromocytomas). Occasionally HNPGL can also secrete catecholamines (Erickson *et al.* 2001).

Familial HNPGL was first described more than 80 years ago (Chase 1933), and 25 years ago evidence of autosomal dominant inheritance with parent of origin effects (tumors were only manifest after paternal transmission, Fig. 1) was reported (van der Mey *et al.* 1989). Ten years later, Baysal *et al.* (2000) reported the seminal finding that

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DOI: 10.1530/ERC-15-0226

This paper is part of a thematic review section on 15th Anniversary of Paraganglioma and Pheochromocytoma. The Guest Editors for this section were Wouter de Herder and Hartmut Neumann.

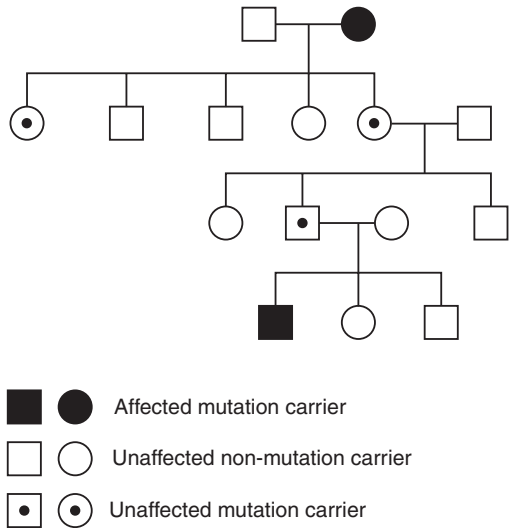

Figure 1
Sample family tree illustrating how disease can be hidden within families due to maternal imprinting of the *SDHD* gene. The susceptibility gene is carried and transmitted by females but only manifests as disease when inherited from a father.

familial HNPGL was associated with germline mutations in succinate dehydrogenase D (*SDHD*; PGL1 locus) and subsequently, *SDHD* mutations were also demonstrated to be associated with sporadic and familial pheochromocytoma (Gimm *et al*. 2000, Astuti *et al*. 2001*a*). *SDHD* encodes the D subunit of the SDH heteterotetrameric enzyme that, together with *SDHC*, anchors the SDH complex to the inner mitochondrial inner membrane. SDH has critical roles in the Krebs cycle and respiratory chain electron transport (as part of mitochondrial complex II, Fig. 2). The *SDHB* gene product, containing three iron–sulfur clusters, is part of the hydrophilic catalytic domain and binds to the *SDHA* gene product that contains a covalently attached flavin adenine dinucleotide (FAD) co-factor and the substrate binding site. *SDHB* and *SDHD* gene products bind to each other and attach the complex II holoenzyme to the mitochondrial inner membrane. Soon after the associations of *SDHD* mutations with human disease, germline *SDHC* (PGL3) mutations were reported to cause familial HNPGL (inherited as an autosomal dominant trait without parent-of-origin effects) (Niemann & Muller 2000) and germline *SDHB* (PGL4) mutations were found to cause inherited susceptibility to HNPGL and pheochromocytomas and paragangliomas (PPGL) (Astuti *et al*. 2001*b*). Subsequently germline mutations in the SDH-associated protein *SDHAF2* were found to be a rare cause of HNPGL (Hao *et al*. 2009, Bayley *et al*. 2010) and *SDHA* mutations, initially reported in the context of an autosomal recessive juvenile encephalopathy (Bourgeron *et al*. 1995), were demonstrated to be a rare cause of (dominantly inherited) predisposition to PPGL (though penetrance appears to be very low) (Burnichon *et al*. 2010). Though germline mutations in *SDHC* have been shown to be associated on rare occasions with PPGL (Mannelli *et al*. 2007), these are much less frequent than *SDHB* and *SDHD* mutations. Genetic heterogeneity of PPGLs is further highlighted by identification of germline mutations in the *VHL*, *RET*, *NF1*, *TMEM127*, and *MAX* genes (Dahia 2014). Here we review our current knowledge of *SDHB*- and *SDHD*-related disorders.

Germline *SDHB* and *SDHD* mutations

Mutation spectrum

A comprehensive database of germline *SDHB* and *SDHD* mutations is maintained at http://chromium.liacs.nl/LOVD2/SDH/home.php (Bayley *et al*. 2005). A wide variety of intragenic mutations have been described and, more recently, single or multiple exon deletions (and, occasionally, intragenic duplications; McWhinney *et al*. 2004, Cascon *et al*. 2008, Neumann *et al*. 2009). A number of frequent *SDHB* and *SDHD* mutations were observed and these may result from a high mutation rate or to founder effects. Thus the relative frequency of some mutations can vary with geographical location. In the Netherlands, two major *SDHD* founder mutations have been identified (c.274G>T (p.Asp92Tyr) and c.416T>C (p.Leu139Pro)), and these account for >90% of *SDHD* mutation carriers

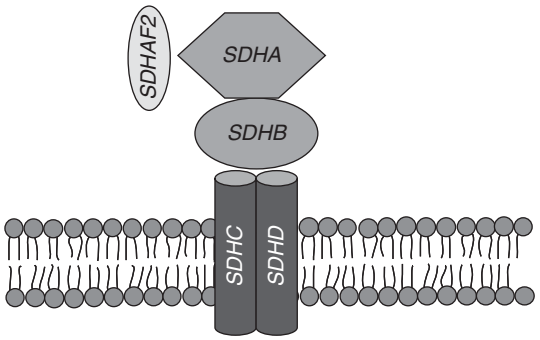

Figure 2
Schematic structure of SDH subunits is shown. SDH is comprised of four structural subunits encoded by *SDHA*, *SDHB*, *SDHC*, and *SDHD*. The *SDHC* and *SDHD* gene products are hydrophobic, sandwich a heme moiety and span the inner mitochondrial membrane. The *SDHA* and *SDHB* gene products are cytosolic and contain a covalently bound FAD and three iron–sulfur clusters respectively. Germ line mutations in *SDHAF2* (*SDH5*), which encode a non-structural assembly protein critical for flavination of the *SDHA* gene product, also predispose to PPGL.

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(van Hulsteijn *et al*. 2012). A *SDHD* c.33C→A (p.Cys11X) founder mutation has been reported in central Europe (Poland; Peczkowska *et al*. 2008). The common *SDHD* c.242C>T (p.Pro81Leu) mutation has been reported as both a recurrent and a founder mutation (Baysal *et al*. 2002). In the Dutch population, *SDHB* founder mutations are less common. The most frequent (a splice site mutation c.423+1G) intragenic mutation was about 15 times less common than the *SDHD* c.274G>T (p.Asp92Tyr) founder mutation, and a founder *SDHB* exon 3 deletion has also been reported (Bayley *et al*. 2009*a*,*b*, Hensen *et al*. 2012). In Spain, *SDHB* founder mutations (exon 1 deletion and *SDHB* c.166_170delCCT-CA) have also been reported (Cascon *et al*. 2008, 2009).

Penetrance and genotype–phenotype correlations

A major difference between the clinical presentation of germline *SDHB* and *SDHD* mutations is the parent-of-origin effect with the latter. Apart from a few exceptional cases in which clinical disease has developed after maternal transmission of a *SDHD* mutation (Yeap *et al*. 2011), the risk of clinical disease after a maternal transmission appears to be extremely remote. Notably, the paraganglioma phenotype in such cases appears mild or atypical (e.g. no multi-focal tumors) indicating functional inequality of the two parental alleles in tumor pathogenesis (reviewed in Baysal (2013)). Though to date unequivocal evidence of genomic imprinting at the *SDHD* locus has not been found, paraganglia-specific partial (quantitative) imprinting of *SDHD* cannot be excluded. Differential methylation of a minor CpG island upstream of a long non-coding RNA located at the telomeric boundary of gene-rich SDHD domain was proposed to regulate long-range enhancer-promoter interactions (Baysal *et al*. 2011).

Homozygous *SDHD* mutations have been associated with recessively inherited encephalomyopathy and mitochondrial complex II deficiency (Jackson *et al*. 2014). Tumorigenesis in SDH-mutated neoplasia appears to follow a 'two hit' (retinoblastoma-like) model and it has been proposed that the parent-of-origin effects may reflect the tendency for the 'second hit' causing inactivation of the WT allele in *SDHD*-related tumorigenesis to be loss of the whole chromosome 11. The imprinted gene cluster at 11p15.5 contains the maternally expressed growth suppressor *CDKN2B* and the paternally expressed *IGF2* growth factor (Lim & Maher 2010). In cases of a paternally inherited germline *SDHD* mutation, loss of the maternally-derived chromosome 11 would, in a single event, result in biallelic *SDHD* inactivation and loss of *CDKN1C* expression but preservation of *IGF2* expression from the paternal allele (Hensen *et al*. 2004, Margetts *et al*. 2005). In contrast, it can be hypothesized that, in individuals harboring a maternally inherited *SDHD* mutation, loss of the paternally-derived chromosome 11 would, whilst biallelically inactivating *SDHD*, result in loss of *IGF2* expression and retention of *CDKN1C* expression. Such a combination is not usually sufficient to drive tumorigenesis. In support of this hypothesis is the observation that in one case of paraganglioma after maternal transmission of a *SDHD* mutation, there was loss of the paternal *SDHD* allele and loss of the maternal 11p15.5 imprinted region (Yeap *et al*. 2011).

An alternative model to explain the parent-of-origin effects in transmission of *SDHD*-related paragangliomas suggests regulation of *SDHD* gene expression by a long-range epigenetic mechanism (Baysal *et al*. 2011). This model proposes that an imprinted small CpG island associated with a long intergenic non-coding RNA at the boundary of gene-rich *SDHD* domain regulates availability of a hypothetical distal enhancer to the *SDHD* promoter.

Particularly for germline *SDHB* mutations, the increased use of presymptomatic genetic testing in extended families has resulted in recognition that the penetrance of *SDHB* mutations is lower than initially thought. Thus initial estimates of the penetrance of germline *SDHB* mutations were in excess of 70% but have progressively fallen to 25–40% (Benn *et al*. 2006, Solis *et al*. 2009, Hes *et al*. 2010, Ricketts *et al*. 2010, Schiavi *et al*. 2010). The relatively low penetrance of *SDHB* mutations is consistent with the observation of a low *de novo* mutation rate, frequent founder mutations and the relatively high number of mutations detected in apparently isolated cases (Baysal *et al*. 2002, Neumann *et al*. 2002, Cascon *et al*. 2009, Jafri *et al*. 2013). However, the low penetrance can make the interpretation of likely pathogenicity for a novel sequence variant detected in individuals with a potentially SDH-related neoplasm complex and also raises, as yet unresolved questions, as to the type and intensity of tumor surveillance in asymptomatic gene carriers.

Though *SDHB* and *SDHD* encode components of the same protein complex, there are some differences in the relative propensities for developing different tumor types. Thus *SDHD* mutations are generally associated with a higher risk of HNPGL than non-HNPGL. For *SDHB* mutations, extra-adrenal and non-HNPGL is more often the presenting feature than HNPGL or pheochromocytoma, and there is a significantly higher risk of malignant paraganglioma and poor prognosis (~25% lifetime risk; Gimenez-Roqueplo *et al*. 2003, Amar *et al*. 2007, Ricketts *et al*. 2010). Despite the heterogeneity of *SDHB* mutations,

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there are no clear genotype–phenotype correlations but for *SDHD*, though it has been suggested that the common p.Pro81Leu mutation is associated with a very low risk of PPGL (in contrast to truncating *SDHD* mutations for which the risk is closer to that seen with germline *SDHB* mutations; Ricketts *et al*. 2010).

A small number of additional tumor types have been reported in individuals with germline *SDHB* and *SDHD* mutations. Gastrointestinal tumors (GIST) are the best defined association. Carney–Stratakis syndrome is characterized by the association of GIST with paraganglioma and, in most cases is caused by mutations in SDHX genes (McWhinney *et al*. 2007, Janeway *et al*. 2011). Germline SDHX mutation may also be detected in patients with familial or sporadic nonsyndromic WT GIST (Janeway *et al*. 2011).

Renal tumors have been reported, predominantly with *SDHB* mutations, but also with *SDHD*/*SDHC* mutations and may be the presenting feature in patients without a personal or family history of HNPGL/PPGL (Vanharanta *et al*. 2004, Ricketts *et al*. 2008, 2010). A variety of histopathologies may occur (e.g. conventional (clear cell), papillary and oncocytoma) and the lifetime risk of renal tumors in *SDHB* mutation carriers has been estimated to be up to 15% (Ricketts *et al*. 2010).

Recently, a clinical association between pituitary adenoma and PPGL has been recognized. Molecular genetic studies have shown that this association may be caused by a variety of germline mutations in known PPGL predisposition genes (e.g. *SDHB*, *SDHD*, *SDHC*, *VHL*, and MEN1), or may be sporadic, but the most frequently implicated genes are SDH-subunit genes (Xekouki *et al*. 2011, Papathomas *et al*. 2013, Dénes *et al*. 2015).

Application of *SDHB* and *SDHD* mutation testing in clinical practice

The recognition that a substantial proportion (approximately one-quarter of apparently sporadic cases (Neumann *et al*. 2002)) might harbor a germline mutation in *SDHB*, *SDHD*, *VHL*, or *RET* led to suggestions that all PPGL patients might be offered genetic testing. However, such an approach, particularly prior to the application of next generation sequencing techniques, was expensive and did not take into account the how clinical indicators can be used. In particular, family history of HNPGL/PPGL, multiple tumors, extra-adrenal location, or early age at diagnosis (mean age at diagnosis in *SDHB*/*SDHD*-related tumors is ~10 years earlier than in sporadic cases) can be used to stratify the likelihood of a germline mutation being detected and so increase cost-effectiveness by targeting higher risk subgroups (Erlic *et al*. 2009). The application of such testing protocols was assessed in an audit of a referral-based testing series of *SDHB*, *SDHD*, and *VHL* (Jafri *et al*. 2013), and it was demonstrated that though widening the testing criteria for testing sporadic pheochromocytoma cases (e.g. from only those aged <45 years at diagnosis to those aged <60 years) increased the numbers of mutation carriers tested but the cost of detecting each mutation carrier increased. A complementary approach is to undertake immunohistochemical analysis for *SDHB* protein expression in the tumors of patients who fall outside the selection criteria. Though loss of *SDHB* expression is a sensitive and specific indicator of germline SDHX mutations (van Nederveen *et al*. 2009), tumor material may not always be available to evaluate protein loss. However, as additional inherited HNPGL/PPGL genes have been identified, there has been increasing interest in the application of next generation sequencing strategies to allow comprehensive and less expensive genetic analysis. Thus specific targeted resequencing panels and exome analysis strategies have been described (Rattenberry *et al*. 2013, McInerney-Leo *et al*. 2014). As the cost of genetic analysis falls, it seems likely that there will be a move towards more extensive analysis.

Pathogenesis of SDH-mutated PPGLs

Pathogenesis of PPGLs caused by SDH mutations remains poorly understood. SDH catalyzes the oxidation of succinate to fumarate in the Krebs cycle and functions as mitochondrial complex II by transferring the extracted electrons to ubiquinone in the electron transport chain. Loss of SDH activity leads to increased succinate and reactive oxygen species (ROS). Thus, succinate and ROS are considered as the signaling molecules that ultimately trigger tumor formation upon SDH mutations. Since discovery of the first mutations in familial PPGLs in 2000–2001 (Baysal *et al*. 2000, Niemann & Muller 2000, Astuti *et al*. 2001*b*), alternative models for tumor development have been advanced using different observations and experimental models that studied the consequences of SDH genetic loss. These models can be broadly classified as constitutive hypoxic drive, inhibition of developmental neuronal culling and histone/genome hypermethylation (Fig. 3).

Constitutive hypoxic drive

The most common phenotypic manifestation of germline SDH mutations is the development of PPGL tumors

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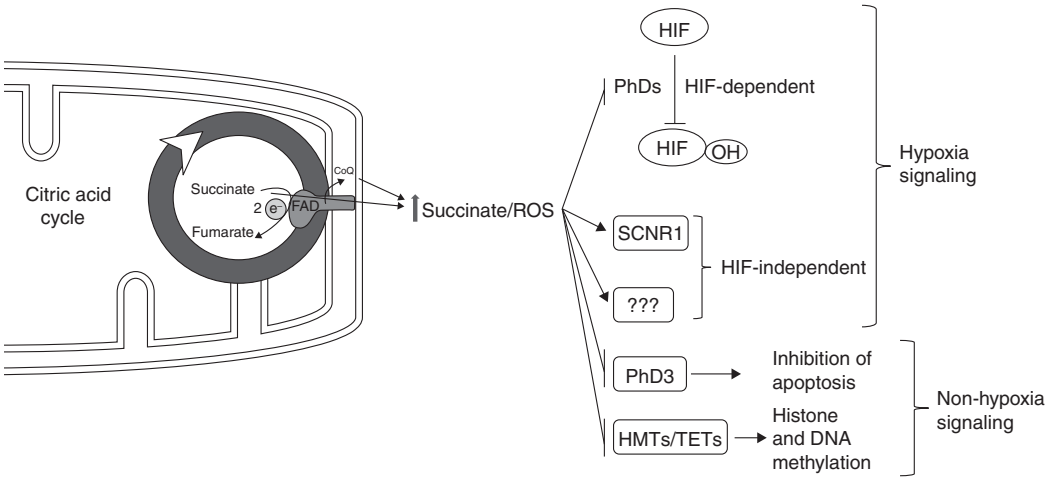

Figure 3

Overview of the proposed mechanisms of SDH-mutated paragangliomas. Mutations in SDH subunits (mainly in *SDHB* and *SDHD*) result in loss of complex II activity and drives paraganglioma formation through accumulation of ROS or succinate. Evidence favors constitutive hypoxia signaling as the initiating mechanism of paraganglioma formation. Role of HIF1α/HIF2α in mediating this hypoxic signaling remains to be confirmed in relevant cell culture and animal models. Succinate may separately stimulate certain biological pathways regulated by succinate receptors (e.g. SCNR1). Alternatively, succinate-mediated inhibition of certain α-KG-dependent enzymes such as PhD3, histone methyl transferases (HMTs), or TETs may lead to inhibition of neural apoptosis, histone, and DNA hypermethylation.

(Neumann *et al*. 2004, Dahia 2014). Gastrointestinal stromal tumors (Janeway *et al*. 2011, Pantaleo *et al*. 2011) and renal carcinoma (Neumann *et al*. 2004, Ricketts *et al*. 2008) also develop in a small minority of subjects who carry germ line SDH mutations. HNPGL, especially the carotid body (CB) paraganglioma, are characteristically associated with germ line mutations in structural subunit genes *SDHD*, *SDHC*, *SDHB*, and in regulatory subunit genes *SDHAF2* (Boedeker *et al*. 2014). The CB is an acute oxygen-sensing organ that responds to hypoxia by increasing heart and ventilation rate (Lopez-Barneo *et al*. 2008). It has been recognized that the incidence of CB paragangliomas increase among high altitude dwellers (Saldana *et al*. 1973) and those with chronic cyanotic heart diseases (Lack 1978, Opotowsky *et al*. 2015). These observations suggested early on that the SDH mutations disrupt oxygen sensing of the CB by causing an inability to register presence of normal oxygen levels (Baysal *et al*. 2000). The paraganglioma tumor formation may thus follow chronic hypoxic stimulation of the CB oxygen-sensing (chief) cells, either by environmental hypoxia or by SDH mutations that inhibit oxygen sensing. The hypothesis that chronic (pseudo)hypoxic stimulation may lead to hereditary paragangliomas is also supported by evidence that links increased altitudes to increased severity of SDH-mutated paraganglioma tumors (Astrom *et al*. 2003, Cerecer-Gil *et al*. 2010).

Gene expression profiles of SDH-mutated PPGLs

Recent genome-wide expression profiling studies show strong induction of hypoxia and angiogenesis pathways in SDH- and *VHL*-related PPGL (Dahia *et al*. 2005, Lopez-Jimenez *et al*. 2010, Shankavaram *et al*. 2013). SDH and *VHL* mutations induce both protein encoding mRNAs and miRNAs (miR-210; Tsang *et al*. 2014) that are implicated in cellular adaptation to hypoxia. Although certain differences in the induced genes were observed, the broad overlap amongst the hypoxia induced genes between SDH- and *VHL*-related paragangliomas strongly suggest that pathogenesis of SDH tumors involves constitutive hypoxic stimulation.

The *VHL* gene product (p*VHL*) is a component of the protein complex that possesses ubiquitin ligase activity which mediates the proteosomal degradation of hypoxia-inducible factors (HIFs) under normoxia (Gossage *et al*. 2015). HIFs (HIF1, HIF2, and HIF3) are transcription factors that mediate cellular adaptation to hypoxia (Semenza 2012). HIFα subunits are hydroxylated by prolyl or asparaginyl hydroxylase enzymes (PhD1, PhD2, PhD3, and FIH) in normoxia and subsequently degraded by ubiquitination (Kaelin & Ratcliffe 2008). Hypoxia inhibits the hydroxylase enzymes and leads to stabilization of HIFαs. It is thought that mutations in *VHL* lead to tumor formation through constitutive stabilization of HIFs.

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Role of HIFs in SDH-mutated PPGLs

Broad transcriptional overlap between SDH and *VHL*-related PPGL, and constitutive activation of the HIFs in *VHL* suggested that HIFs may also mediate tumor formation in SDH-mutated paragangliomas. HIF1α and/or HIF2α were detected by immunohistochemistry in both SDH-mutated and sporadic HNPGL (Pollard *et al.* 2006, Favier *et al.* 2009, Merlo *et al.* 2012). *In vitro* studies using cell lines showed that siRNA-mediated knockdown of SDH subunits led to stabilization of HIF1α (Selak *et al.* 2005, Cervera *et al.* 2008, Guzy *et al.* 2008). These studies linked increased succinate or ROS levels to the stabilization of HIFαs. Despite these *in vitro* studies, discordant results are obtained from gene expression analyses on the role of HIFs in SDH-mutated PGL tumors. Although significant overlap in gene expression patterns of SDH- and *VHL*-related PGL tumors was observed including increased HIF2α, VEGF and reduced electron transport chain genes by transcriptome-wide studies, HIF target-gene overexpression and increased glycolysis, as assessed by such genes as hexokinase II (*HK2*), lactate dehydrogenase, *MIR210*, *PHD3* (*EGLN3*), *ENO1*, and *SLC2A1* were primarily observed in *VHL*-mutated paragangliomas (Favier *et al.* 2009, Lopez-Jimenez *et al.* 2010). In fact, overexpression of 67 HIF target genes was sufficient to distinguish *VHL*- from *SDHB*-mutated pheochromocytomas (Lopez-Jimenez *et al.* 2010). Conversely, HNPGLs that overexpress HIF1α and its target genes were found to have WT SDH sequences and a subset of them was indeed found to carry somatic *VHL* mutations (Merlo *et al.* 2012, 2013).

In addition, recent sequence and functional studies identified somatic mutations in *EPAS1* which encodes the HIF2α subunit in sporadic (mostly non-head and neck) paragangliomas, a subset of which was accompanied by polycythemia (Zhuang *et al.* 2012, Comino-Mendez *et al.* 2013, Toledo *et al.* 2013). Missense mutations in *VHL*, *EPAS1* (*HIF2A*), and *PHD2* are associated with erythrocytosis. For example, endemic Chuvash polycythemia is caused by certain *VHL* missense germ line mutations (Lee & Percy 2011). In contrast, *SDHX* mutations have yet to be associated with erythrocytosis. Gene expression profiling shows that *EPAS1*-mutated paragangliomas cluster with SDH and *VHL*-related paragangliomas, and strengthens the role of constitutive hypoxic signaling in pathogenesis of SDH-mutated paragangliomas (Comino-Mendez *et al.* 2013). However, the association with erythrocytosis suggests that pathogenesis of *EPAS1*-mutated paragangliomas is more closely associated with the *VHL*-mutated paragangliomas rather than with the SDH-mutated ones.

It thus appears that gene expression studies do not provide an unequivocal evidence for involvement of HIFs in pathogenesis of SDH-mutated paragangliomas. Whether HIFs play a role in SDH–paraganglioma formation, however, remains an important question which may not be conclusively answered by gene/protein expression studies alone. It is important to note that hereditary renal tumors that result from fumarate hydratase (FH) germline mutations were initially shown to stabilize HIF1α (Pollard *et al.* 2005). Accordingly, the knockdown of FH in cell lines led to robust stabilization of HIF1α through fumarate mediated inhibition of the PhD enzymes (Isaacs *et al.* 2005). These findings are similar to the observations previously described for SDH-mutated pathology: i) HIFαs are variably detected in SDH-mutated paragangliomas by gene expression and immunohistochemical studies and ii) succinate inhibition of PhD enzymes stabilizes HIF1α upon SDH knockdown in certain cell lines. Although such observations initially suggested a role for HIF1 in tumor predisposition caused by FH mutations, deletion of the *Hif1α* gene in the *Fh1*-deficient mice, which develops renal cysts, worsened the cystic phenotype (Adam *et al.* 2011). Thus, Hif1 may not mediate the cystic renal pathology in the *Fh1*-mice. These results imply that HIF stabilization observed in SDH-mutated tumors may not necessarily indicate its causative role in tumor pathogenesis. Ultimately, animal or cell culture models that link inactivation of SDH to PPGL development or to a hypoxia-related physiological response will be required to evaluate the role of HIFs in SDH-mutated tumor pathogenesis or SDH-regulated hypoxia response.

Heterozygous inactivation of *Sdhb* or *Sdhd* genes in mice does not lead to tumor development, in particular while homozygous inactivation is embryonic lethal (Bayley *et al.* 2009*a*,*b*, Piruat & Millán-Uclés 2014). *Sdhd* conditional constitutional or paraganglia-confined homozygous deletions also show no evidence of tumor development (Diaz-Castro *et al.* 2012). These findings highlight species-specific differences in tumor susceptibility between human and mouse that follows the inactivation of mitochondrial complex II subunits. While gene knockout studies in mice did not recapitulate the paraganglioma tumor phenotype, they provide some information on activation of hypoxia-related pathways. Heterozygous *Sdhd* deletion increases sensitivity of the CB chief cells to hypoxia (Piruat *et al.* 2004), which is consistent with the hypothesis that inactivation of Sdh hampers the ability of CB chief cells to register normal oxygen levels and triggers normoxic activation of hypoxia sensing pathways. Gene expression analyses of $Sdhd^{-/-}$ tissues show mixed evidence of Hif activation. While $Sdhd^{-/-}$ MEFs showed Hif1α

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stabilization, two other tissues did not show any evidence of hypoxic pathway activation (Millán-Uclés *et al.* 2014).

In summary, mimicry of the hypoxia-associated CB paragangliomas and gene expression profiling studies provide strong evidence of constitutive hypoxic pathway activation in pathogenesis SDH-mutated paraganglioma tumors. However, determining the role of HIFs in mediating this hypoxia-driven pathogenesis requires further studies.

Inhibition of α-ketoglutarate dependent dioxygenases

The PhD enzymes are members of a large family of Fe(II)/α-ketoglutarate (KG)-dependent dioxygenases (Hausinger 2004). Inhibition of PhDs by succinate on genetic and pharmacologic inhibition of SDH raised the possibility that other dioxygenases may also contribute to paraganglioma development. Succinate accumulation by SDH inhibition has been shown to inhibit jumonji-domain histone demethylases (JmjC), leading to histone H3 hypermethylation (Smith *et al.* 2007). Succinate is also shown to inhibit other α-KG-dependent dioxygenases, including collagen prolyl-4-hydroxylases and the ten-eleven translocation (TET) family of 5-methlycytosine (5mC) hydroxylases, which leads to hypermethylation of CpG islands (Xiao *et al.* 2012). Both histone and DNA hypermethylation have the potential to alter gene expression levels. Examination of SDH-mutated paragangliomas showed downregulation of gene expression for 191 genes that acquired promoter methylation as a result of inhibition of the TET family of 5mC hydroxylases (Letouzé *et al.* 2013). Certain methylated genes including *PNMT* and *KRT19* were linked to neuroendocrine differentiation and epithelial-to-mesenchymal differentiation, respectively, raising the possibility that succinate-mediated inhibition of TET family 5mC hydroxylases may play a role in SDH-mutated paraganglioma development. Whether suppression of *PNMT*, *KRT19*, or other genes by CpG island or histone methylation provides an advantage in SDH-mutated tumor progression, however, remains to be directly demonstrated. As previously discussed, broad overlap in hypoxia-related gene expression patterns between SDH and *VHL* paragangliomas and between genetic and sporadic HNPGL suggests that the role of histone and DNA methylation in influencing global gene expression profiles may be limited. It is conceivable that whereas initiation of SDH-mutated paragangliomas may involve constitutive hypoxia-signaling, succinate-inhibition of α-KG-dependent dioxygenases may contribute to tumor progression.

Germ line mutations in FH in hereditary leiomyomatosis and renal cell cancer, somatic gain-of-function point mutations in isocitrate dehydrogenase 1 (IDH1) and IDH2 in low-grade gliomas, secondary glioblastomas, various sarcomas, and acute myeloid leukemia cause increased fumarate and D-2-hydroxyglutarate, respectively, and lead to DNA and histone methylation by inhibiting α-KG-dependent dioxygenases (Morin *et al.* 2014). α-KG-dependent dioxygenases comprise a large family of enzymes that perform diverse and important biological functions including protein modification, repair of alkylated DNA/RNA, and lipid metabolism. It is notable that there is no major overlap amongst the tumor spectra associated with *SDH*, *FH*, and *IDH1/2* mutations, although rare familial paraganglioma cases carrying *FH* germ line mutations have been recently described (Letouzé *et al.* 2013, Clark *et al.* 2014). Thus, succinate, fumarate, and D-2-hydroxyglutare, the oncometabolites generated by *SDH*, *FH*, and *IDH1/2* mutations, respectively, may inhibit not only PHD, TET, and histone demethylating enzymes but also other α-KG-dependent dioxygenases that provide an advantage for cancer cell survival.

Whether succinate receptors (SUCNR) mediate signaling in pathogenesis of SDH-mutated paragangliomas remains an underexplored area. Succinate is a ligand for GPR91 (also known as SUCNR1), a G-protein coupled receptor (He *et al.* 2004). *SUCNR1* is expressed in kidney, liver, spleen and white adipose tissue (Ariza *et al.* 2012). SUCNR1 stimulates angiogenesis in retina. Extracellular succinate activates the SUCNR and may increase the VEGF levels through HIF1α-independent mechanisms (Sapieha *et al.* 2008). SUCNR1 may mediate certain effects of hypoxia which increases the succinate levels. These results suggest that succinate can act as a physiological signaling molecule and raise the possibility that some aspects of paragangliomas, such as increased vascularity may be mediated by increased succinate signaling through SUCNR1.

Inhibition of neuronal apoptosis linked to PhD3

It has been suggested that succinate accumulation following SDH inactivation inhibits PHD3 activity, which is required for neuronal apoptosis after NGF withdrawal (Lee *et al.* 2005). According to this model, abnormal NGF signaling leading to reduced apoptosis and enhanced survival of sympathetic neurons provides a unifying model for the mechanism of pheochromocytoma formation following mutations in *NF1*, *RET*, *SDH*, and *VHL*. Notably, Lee *et al.* (2005) did not observe HIF stabilization in PC12 pheochromocytoma cell lines after SDH knockdown and suggested that succinate inhibition of PhD3, rather PhD1 which controls HIF stability,

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provides a mechanistic link between SDH inactivation and pheochromocytoma susceptibility (Lee *et al*. 2005). It is conceivable that inhibition of neuronal apoptosis and epigenetic inactivation of genes important for neuronal differentiation by TET inactivation may collaborate to promote development of paraganglioma tumors. However, it is unclear whether this common model based on inhibition of apoptosis can explain the distinct expression profiles conferred by mutations in *VHL*/*SDHX* vs *RET*/*NF1* in PPGLs and the activation of hypoxia-related pathways specifically in the SDH-mutated paragangliomas.

Other aspects of pathogenesis in SDH-mutated paragangliomas

Role of ROS in SDH-mutated pathogenesis

Mitochondrial complex II generates significant quantities of ROS (Quinlan *et al*. 2012), which is further enhanced by certain mutations (Ishii *et al*. 2005). Whether ROS contributes to pathogenesis of SDH-mutated paragangliomas is the subject of ongoing investigations. In addition to stabilizing HIF1α (Guzy *et al*. 2008), ROS generated by *SDHC* mutations has also been implicated in mutating nuclear DNA and therefore contributing to tumorigenesis (Ishii *et al*. 2005). Role of somatic mutations in SDH-mutated paragangliomas, however, remains unconfirmed. SDH-mutated paragangliomas does not frequently acquire point mutations in the non-mutated allele, which is often lost by large deletions (Dahia 2014). Recent tumor sequencing studies also show very low levels of overall mutations in SDH-mutated paragangliomas (Castro-Vega *et al*. 2015).

Malignancy among *SDHB* mutation carriers

Prevalence of malignant paragangliomas as defined by metastasis among *SDHB* mutation carriers is substantially higher than among *SDHD* carriers (13% vs 4%; van Hulsteijn *et al*. 2012). The association of *SDHB* mutations with malignancy appears to hold both for HNPGL and non-HNPGL (Boedeker *et al*. 2007). Metastasis is thought to occur through a process called epithelial–mesenchymal transition (EMT; Scheel & Weinberg 2012). EMT confers cancer cells with stem-cell like properties including the ability to migrate and grow in distant anatomic sites. Gene expression analyses of *SDHB*-related metastatic paragangliomas show differential alterations in genes implicated in EMT, such as those encoding metalloproteinases and cellular junction proteins (Loriot *et al*. 2012). Whole exome-sequencing identified *ATRX2* mutations in subset of clinically aggressive paragangliomas, including in two *SDHB*-mutated tumors (Fishbein *et al*. 2015). While such findings may help to explain the mechanism by which the metastatic behavior is acquired in *SDHB*–paragangliomas, the question remains as to why the loss of SDH through different subunit gene mutations have such distinct consequences on the metastatic potential.

Because heterozygous SDH mutations predispose to tumor formation, haploinsufficiency of an SDH subunit initiates the tumorigenic process. Hereditary paraganglioma formation usually follows loss of the unmutated *SDHB* or *SDHD* allele, which abolishes the whole mitochondrial complex II activity. It is conceivable that *SDHB* haploinsufficiency occurs in developmentally more immature paraganglionic cells that are prone to develop stem-cell like properties during tumorigenesis enabling them to migrate and proliferate in distant sites. However, *SDHD* or *SDHC* haploinsufficiency may occur in more mature paraganglionic cells that are less likely to dedifferentiate and metastasize.

Conclusion

Mutations in SDH subunits account for most familial and sporadic HNPGLs and PPGLs, and have also been linked to other neoplasms including GISTs, renal cancer, and pituitary adenomas. Abundant evidence suggests that constitutive hypoxic stimulation plays an important role in development of SDH-mutated paraganglioma tumors. However, mechanisms by which SDH regulates oxygen sensing and signaling are poorly understood. Progress would be facilitated by development of relevant animal or cell culture models that link SDH dysfunction to tumor formation and/or to altered physiological responses to hypoxia. Whether HIF1α/HIF2α is involved in the hypoxic signaling pathway suspected to drive SDH-mutated paragangliomas can be rigorously addressed only through such models. Recent studies on *Fh1* mouse model suggest that the stabilization of HIFα in tumor samples does not necessarily indicate its involvement in tumor pathogenesis. Although the association of activating *EPAS1/HIF2A* mutations with PPGL would support a role for HIFs, the co-occurrence of erythrocytosis in certain carriers suggests that pathogenesis of PPGL tumors with *EPAS1/HIF2A* mutations may be more closely associated with *VHL*-mutated than *SDHX*-mutated tumors. Succinate accumulation in SDH-mutated paragangliomas inhibits certain α-KG-dependent dioxygenases and leads to histone

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and DNA hypermethylation. Significance of these hypermethylation events in driving PPGL tumor formation and whether they influence hypoxic pathway activation requires further studies. The discovery of SDH mutations in PPGLs in early the 2000s confirmed Warburg's suspicion that defective mitochondria is the root cause of the neoplastic process (Warburg 1956) at least in certain tumor types, and heralded an era of metabolic studies that aim to understand the role of mitochondria in cancer (Wallace 2012).

Declaration of interest

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

Funding

This review did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

References

Adam J, Hatipoglu E, O'Flaherty L, Ternette N, Sahgal N, Lockstone H, Baban D, Nye E, Stamp GW & Wolhuter K 2011 Renal cyst formation in Fh1-deficient mice is independent of the Hif/Phd pathway: roles for fumarate in KEAP1 succination and Nrf2 signaling. *Cancer Cell* **20** 524–537. (doi:10.1016/j.ccr.2011.09.006)

Amar L, Baudin E, Burnichon N, Peyrard S, Silvera S, Bertherat J, Bertagna X, Schlumberger M, Jeunemaitre X & Gimenez-Roqueplo A 2007 Succinate dehydrogenase B gene mutations predict survival in patients with malignant pheochromocytomas or paragangliomas. *Journal of Clinical Endocrinology and Metabolism* **92** 3822–3828. (doi:10.1210/jc.2007-0709)

Ariza AC, Deen PM & Robben JH 2012 The succinate receptor as a novel therapeutic target for oxidative and metabolic stress-related conditions. *Frontiers in Endocrinology* **3** 22. (doi:10.3389/fendo.2012.00022)

Astrom K, Cohen JE, Willett-Brozick JE, Aston CE & Baysal BE 2003 Altitude is a phenotypic modifier in hereditary paraganglioma type 1: evidence for an oxygen-sensing defect. *Human Genetics* **113** 228–237. (doi:10.1007/s00439-003-0969-6)

Astuti D, Douglas F, Lennard TW, Aligianis IA, Woodward ER, Evans DG, Eng C, Latif F & Maher ER 2001*a* Germline SDHD mutation in familial phaeochromocytoma. *Lancet* **357** 1181–1182. (doi:10.1016/S0140-6736(00)04378-6)

Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, Skoldberg F, Husebye ES, Eng C & Maher ER 2001*b* Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *American Journal of Human Genetics* **69** 49–54. (doi:10.1086/321282)

Bayley JP, Devilee P & Taschner PE 2005 The SDH mutation database: an online resource for succinate dehydrogenase sequence variants involved in pheochromocytoma, paraganglioma and mitochondrial complex II deficiency. *BMC Medical Genetics* **6** 39. (doi:10.1186/1471-2350-6-39)

Bayley JP, Grimbergen AE, van Bunderen PA, van der Wielen M, Kunst HP, Lenders JW, Jansen JC, Dullaart RP, Devilee P, Corssmit EP *et al.* 2009*a* The first Dutch SDHB founder deletion in paraganglioma–pheochromocytoma patients. *BMC Medical Genetics* **10** 34. (doi:10.1186/1471-2350-10-34)

Bayley J, van Minderhout I, Hogendoorn PC, Cornelisse CJ, van der Wal A, Prins FA, Teppema L, Dahan A, Devilee P & Taschner PE 2009*b* Sdhd and SDHD/H19 knockout mice do not develop paraganglioma or pheochromocytoma. *PLoS ONE* **4** e7987. (doi:10.1371/journal.pone.0007987)

Bayley J, Kunst HP, Cascon A, Sampietro ML, Gaal J, Korpershoek E, Hinojar-Gutierrez A, Timmers HJ, Hoefsloot LH & Hermsen MA 2010 SDHAF2 mutations in familial and sporadic paraganglioma and phaeochromocytoma. *Lancet. Oncology* **11** 366–372. (doi:10.1016/S1470-2045(10)70007-3)

Baysal BE 2013 Mitochondrial complex II and genomic imprinting in inheritance of paraganglioma tumors. *Biochimica et Biophysica Acta* **1827** 573–577. (doi:10.1016/j.bbabio.2012.12.005)

Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PE, Rubinstein WS, Myers EN *et al.* 2000 Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* **287** 848–851. (doi:10.1126/science.287.5454.848)

Baysal BE, Willett-Brozick JE, Lawrence EC, Drovdlic CM, Savul SA, McLeod DR, Yee HA, Brackmann DE, Slattery WH III, Myers EN *et al.* 2002 Prevalence of SDHB, SDHC, and SDHD germline mutations in clinic patients with head and neck paragangliomas. *Journal of Medical Genetics* **39** 178–183. (doi:10.1136/jmg.39.3.178)

Baysal BE, McKay SE, Kim YJ, Zhang Z, Alila L, Willett-Brozick JE, Pacak K, Kim TH & Shadel GS 2011 Genomic imprinting at a boundary element flanking the SDHD locus. *Human Molecular Genetics* **20** 4452–4461. (doi:10.1093/hmg/ddr376)

Benn DE, Gimenez-Roqueplo A, Reilly JR, Bertherat J, Burgess J, Byth K, Croxson M, Dahia PL, Elston M & Gimm O 2006 Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. *Journal of Clinical Endocrinology and Metabolism* **91** 827–836. (doi:10.1210/jc.2005-1862)

Boedeker CC, Neumann HP, Maier W, Bausch B, Schipper J & Ridder GJ 2007 Malignant head and neck paragangliomas in SDHB mutation carriers. *Otolaryngology – Head and Neck Surgery* **137** 126–129. (doi:10.1016/j.otohns.2007.01.015)

Boedeker CC, Hensen EF, Neumann HP, Maier W, Nederveen FH, Suárez C, Kunst HP, Rodrigo JP, Takes RP & Pellitteri PK 2014 Genetics of hereditary head and neck paragangliomas. *Head & Neck* **36** 907–916. (doi:10.1002/hed.23436)

Bourgeron T, Rustin P, Chretien D, Birch-Machin M, Bourgeois M, Viegas-Péquignot E, Munnich A & Rötig A 1995 Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. *Nature Genetics* **11** 144–149. (doi:10.1038/ng1095-144)

Burnichon N, Briere JJ, Libe R, Vescovo L, Riviere J, Tissier F, Jouanno E, Jeunemaitre X, Benit P, Tzagoloff A *et al.* 2010 SDHA is a tumor suppressor gene causing paraganglioma. *Human Molecular Genetics* **19** 3011–3020. (doi:10.1093/hmg/ddq206)

Cascon A, Landa I, Lopez-Jimenez E, Diez-Hernandez A, Buchta M, Montero-Conde C, Leskela S, Leandro-Garcia LJ, Leton R, Rodriguez-Antona C *et al.* 2008 Molecular characterisation of a common SDHB deletion in paraganglioma patients. *Journal of Medical Genetics* **45** 233–238. (doi:10.1136/jmg.2007.054965)

Cascon A, Pita G, Burnichon N, Landa I, Lopez-Jiménez E, Montero-Conde C, Leskelä S, Leandro-García LJ, Leton R & Rodríguez-Antona C 2009 Genetics of pheochromocytoma and paraganglioma in Spanish patients. *Journal of Clinical Endocrinology and Metabolism* **94** 1701–1705. (doi:10.1210/jc.2008-2756)

Castro-Vega LJ, Letouzé E, Burnichon N, Buffet A, Disderot P, Khalifa E, Loriot C, Elarouci N, Morin A, Menara M *et al.* 2015 Multi-omics analysis defines core genomic alterations in pheochromocytomas and paragangliomas. *Nature Communications* **6** article 6044. (doi:10.1038/ncomms7044)

Cerecer-Gil NY, Figuera LE, Llamas FJ, Lara M, Escamilla JG, Ramos R, Estrada G, Hussain AK, Gaal J, Korpershoek E *et al.* 2010 Mutation of

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SDHB is a cause of hypoxia-related high-altitude paraganglioma. *Clinical Cancer Research* **16** 4148–4154. (doi:10.1158/1078-0432.CCR-10-0637)

Cervera AM, Apostolova N, Crespo FL, Mata M & McCreath KJ 2008 Cells silenced for SDHB expression display characteristic features of the tumor phenotype. *Cancer Research* **68** 4058–4067. (doi:10.1158/0008-5472.CAN-07-5580)

Chase W 1933 Familial and bilateral tumours of the carotid body. *Journal of Pathology and Bacteriology* **36** 1–12. (doi:10.1002/path.1700360102)

Clark GR, Sciacovelli M, Gaude E, Walsh DM, Kirby G, Simpson MA, Trembath RC, Berg JN, Woodward ER, Kinning E *et al.* 2014 Germline FH mutations presenting with pheochromocytoma. *Journal of Clinical Endocrinology and Metabolism* **99** E2046–E2050. (doi:10.1210/jc.2014-1659)

Comino-Mendez I, de Cubas AA, Bernal C, Alvarez-Escola C, Sanchez-Malo C, Ramirez-Tortosa CL, Pedrinaci S, Rapizzi E, Ercolino T, Bernini G *et al.* 2013 Tumoral EPAS1 (HIF2A) mutations explain sporadic pheochromocytoma and paraganglioma in the absence of erythrocytosis. *Human Molecular Genetics* **22** 2169–2176. (doi:10.1093/hmg/ddt069)

Dahia PL 2014 Pheochromocytoma and paraganglioma pathogenesis: learning from genetic heterogeneity. *Nature Reviews. Cancer* **14** 108–119. (doi:10.1038/nrc3648)

Dahia PL, Ross KN, Wright ME, Hayashida CY, Santagata S, Barontini M, Kung AL, Sanso G, Powers JF, Tischler AS *et al.* 2005 A HIF1α regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. *PLoS Genetics* **1** 72–80. (doi:10.1371/journal.pgen.0010008)

Dénes J, Swords F, Rattenberry E, Stals K, Owens M, Cranston T, Xekouki P, Moran L, Kumar A & Wassif C 2015 Heterogeneous genetic background of the association of pheochromocytoma/paraganglioma and pituitary adenoma – results from a large patient cohort. *Journal of Clinical Endocrinology and Metabolism* **100** E531–E541. (doi:10.1210/jc.2014-33)

Diaz-Castro B, Pintado CO, Garcia-Flores P, Lopez-Barneo J & Piruat JI 2012 Differential impairment of catecholaminergic cell maturation and survival by genetic mitochondrial complex II dysfunction. *Molecular and Cellular Biology* **32** 3347–3357. (doi:10.1128/MCB.00128-12)

Erickson D, Kudva YC, Ebersold MJ, Thompson GB, Grant CS, van Heerden JA & Young WF Jr 2001 Benign paragangliomas: clinical presentation and treatment outcomes in 236 patients. *Journal of Clinical Endocrinology and Metabolism* **86** 5210–5216. (doi:10.1210/jcem.86.11.8034)

Erlic Z, Rybicki L, Peczkowska M, Golcher H, Kann PH, Brauckhoff M, Mussig K, Muresan M, Schaffler A, Reisch N *et al.* 2009 Clinical predictors and algorithm for the genetic diagnosis of pheochromocytoma patients. *Clinical Cancer Research* **15** 6378–6385. (doi:10.1158/1078-0432.CCR-09-1237)

Favier J, Brière J, Burnichon N, Rivière J, Vescovo L, Benit P, Giscos-Douriez I, De Reyniès A, Bertherat J, Badoual C *et al.* 2009 The Warburg effect is genetically determined in inherited pheochromocytomas. *PLoS ONE* **4** e7094. (doi:10.1371/journal.pone.0007094)

Fishbein L, Khare S, Wubbenhorst B, DeSloover D, D'Andrea K, Merrill S, Cho NW, Greenberg RA, Else T & Montone K 2015 Whole-exome sequencing identifies somatic ATRX mutations in pheochromocytomas and paragangliomas. *Nature Communications* **6** 6140. (doi:10.1038/ncomms7140)

Gimenez-Roqueplo AP, Favier J, Rustin P, Rieubland C, Crespin M, Nau V, Khau Van Kien P, Corvol P, Plouin PF, Jeunemaitre X *et al.* 2003 Mutations in the SDHB gene are associated with extra-adrenal and/or malignant phaeochromocytomas. *Cancer Research* **63** 5615–5621.

Gimm O, Armanios M, Dziema H, Neumann HP & Eng C 2000 Somatic and occult germ-line mutations in SDHD, a mitochondrial complex II gene, in nonfamilial pheochromocytoma. *Cancer Research* **60** 6822–6825.

Gossage L, Eisen T & Maher ER 2015 VHL, the story of a tumour suppressor gene. *Nature Reviews. Cancer* **15** 55–64. (doi:10.1038/nrc3844)

Guzy RD, Sharma B, Bell E, Chandel NS & Schumacker PT 2008 Loss of the SdhB, but not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. *Molecular and Cellular Biology* **28** 718–731. (doi:10.1128/MCB.01338-07)

Hao HX, Khalimonchuk O, Schraders M, Dephoure N, Bayley JP, Kunst H, Devilee P, Cremers CW, Schiffman JD, Bentz BG *et al.* 2009 SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science* **325** 1139–1142. (doi:10.1126/science.1175689)

Hausinger RP 2004 Fe (II)/α-ketoglutarate-dependent hydroxylases and related enzymes. *Critical Reviews in Biochemistry and Molecular Biology* **39** 21–68. (doi:10.1080/10409230490440541)

He W, Miao FJ, Lin DC, Schwandner RT, Wang Z, Gao J, Chen J, Tian H & Ling L 2004 Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* **429** 188–193. (doi:10.1038/nature02488)

Hensen EF, Jordanova ES, van Minderhout IJ, Hogendoorn PC, Taschner PE, van der Mey AG, Devilee P & Cornelisse CJ 2004 Somatic loss of maternal chromosome 11 causes parent-of-origin-dependent inheritance in SDHD-linked paraganglioma and phaeochromocytoma families. *Oncogene* **23** 4076–4083. (doi:10.1038/sj.onc.1207591)

Hensen EF, van Duinen N, Jansen JC, Corssmit EP, Tops C, Romijn J, Vriends A, van der Mey A, Cornelisse C & Devilee P 2012 High prevalence of founder mutations of the succinate dehydrogenase genes in the Netherlands. *Clinical Genetics* **81** 284–288. (doi:10.1111/j.1399-0004.2011.01653.x)

Hes FJ, Weiss MM, Woortman SA, de Miranda NF, van Bunderen PA, Bonsing BA, Stokkel MP, Morreau H, Romijn JA, Jansen JC *et al.* 2010 Low penetrance of a SDHB mutation in a large Dutch paraganglioma family. *BMC Medical Genetics* **11** 92-2350-11-92. (doi:10.1186/1471-2350-11-92)

van Hulsteijn LT, Dekkers OM, Hes FJ, Smit JW & Corssmit EP 2012 Risk of malignant paraganglioma in SDHB-mutation and SDHD-mutation carriers: a systematic review and meta-analysis. *Journal of Medical Genetics* **49** 768–776. (doi:10.1136/jmedgenet-2012-101192)

Isaacs JS, Jung YJ, Mole DR, Lee S, Torres-Cabala C, Chung YL, Merino M, Trepel J, Zbar B, Toro J *et al.* 2005 HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. *Cancer Cell* **8** 143–153. (doi:10.1016/j.ccr.2005.06.017)

Ishii T, Yasuda K, Akatsuka A, Hino O, Hartman PS & Ishii N 2005 A mutation in the SDHC gene of complex II increases oxidative stress, resulting in apoptosis and tumorigenesis. *Cancer Research* **65** 203–209.

Jackson CB, Nuoffer JM, Hahn D, Prokisch H, Haberberger B, Gautschi M, Haberli A, Gallati S & Schaller A 2014 Mutations in SDHD lead to autosomal recessive encephalomyopathy and isolated mitochondrial complex II deficiency. *Journal of Medical Genetics* **51** 170–175. (doi:10.1136/jmedgenet-2013-101932)

Jafri M, Whitworth J, Rattenberry E, Vialard L, Kilby G, Kumar AV, Izatt L, Lalloo F, Brennan P, Cook J *et al.* 2013 Evaluation of SDHB, SDHD and VHL gene susceptibility testing in the assessment of individuals with non-syndromic phaeochromocytoma, paraganglioma and head and neck paraganglioma. *Clinical Endocrinology* **78** 898–906. (doi:10.1111/cen.12074)

Janeway KA, Kim SY, Lodish M, Nose V, Rustin P, Gaal J, Dahia PL, Liegl B, Ball ER, Raygada M *et al.* 2011 Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. *PNAS* **108** 314–318. (doi:10.1073/pnas.1009199108)

Kaelin WG Jr & Ratcliffe PJ 2008 Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Molecular Cell* **30** 393–402. (doi:10.1016/j.molcel.2008.04.009)

Lack EE 1978 Hyperplasia of vagal and carotid body paraganglia in patients with chronic hypoxemia. *American Journal of Pathology* **91** 497–516.

Lee FS & Percy MJ 2011 The HIF pathway and erythrocytosis. *Annual Review of Pathology* **6** 165–192. (doi:10.1146/annurev-pathol-011110-130321)

Lee S, Nakamura E, Yang H, Wei W, Linggi MS, Sajan MP, Farese RV, Freeman RS, Carter BD, Kaelin WG *et al.* 2005 Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: developmental culling and cancer. *Cancer Cell* **8** 155–167. (doi:10.1016/j.ccr.2005.06.015)

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Letouzé E, Martinelli C, Loriot C, Burnichon N, Abermil N, Ottolenghi C, Janin M, Menara M, Nguyen AT, Benit P *et al.* 2013 SDH mutations establish a hypermethylator phenotype in paraganglioma. *Cancer Cell* **23** 739–752. (doi:10.1016/j.ccr.2013.04.018)

Lim D & Maher ER 2010 Genomic imprinting syndromes and cancer. *Advances in Genetics* **70** 145–175. (doi:10.1016/B978-0-12-380866-0.60006-X)

Lopez-Barneo J, Ortega-Saenz P, Pardal R, Pascual A & Piruat JI 2008 Carotid body oxygen sensing. *European Respiratory Journal* **32** 1386–1398. (doi:10.1183/09031936.00056408)

Lopez-Jimenez E, Gomez-Lopez G, Leandro-Garcia LJ, Munoz I, Schiavi F, Montero-Conde C, de Cubas AA, Ramires R, Landa I, Leskela S *et al.* 2010 Research resource: Transcriptional profiling reveals different pseudohypoxic signatures in SDHB and VHL-related pheochromocytomas. *Molecular Endocrinology* **24** 2382–2391. (doi:10.1210/me.2010-0256)

Loriot C, Burnichon N, Gadessaud N, Vescovo L, Amar L, Libé R, Bertherat J, Plouin P, Jeunemaitre X & Gimenez-Roqueplo A 2012 Epithelial to mesenchymal transition is activated in metastatic pheochromocytomas and paragangliomas caused by SDHB gene mutations. *Journal of Clinical Endocrinology and Metabolism* **97** E954–E962. (doi:10.1210/jc.2011-3437)

Mannelli M, Ercolino T, Giache V, Simi L, Cirami C & Parenti G 2007 Genetic screening for pheochromocytoma: should SDHC gene analysis be included? *Journal of Medical Genetics* **44** 586–587. (doi:10.1136/jmg.2007.051045)

Margetts CD, Astuti D, Gentle DC, Cooper WN, Cascon A, Catchpoole D, Robledo M, Neumann HP, Latif F & Maher ER 2005 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. *Endocrine-Related Cancer* **12** 161–172. (doi:10.1677/erc.1.00865)

McInerney-Leo AM, Marshall MS, Gardiner B, Benn DE, McFarlane J, Robinson BG, Brown MA, Leo PJ, Clifton-Bligh RJ & Duncan EL 2014 Whole exome sequencing is an efficient and sensitive method for detection of germline mutations in patients with phaeochromcytomas and paragangliomas. *Clinical Endocrinology* **80** 25–33. (doi:10.1111/cen.12331)

McWhinney SR, Pilarski RT, Forrester SR, Schneider MC, Sarquis MM, Dias EP & Eng C 2004 Large germline deletions of mitochondrial complex II subunits SDHB and SDHD in hereditary paraganglioma. *Journal of Clinical Endocrinology and Metabolism* **89** 5694–5699. (doi:10.1210/jc.2004-0769)

McWhinney SR, Pasini B & Stratakis CA 2007 Familial gastrointestinal stromal tumors and germ-line mutations. *New England Journal of Medicine* **357** 1054–1056. (doi:10.1056/NEJMc071191)

Merlo A, de Quiros SB, Secades P, Zambrano I, Balbín M, Astudillo A, Scola B, Arístegui M, Suarez C & Chiara M 2012 Identification of a signaling axis HIF-1α/microRNA-210/ISCU independent of SDH mutation that defines a subgroup of head and neck paragangliomas. *Journal of Clinical Endocrinology and Metabolism* **97** E2194–E2200. (doi:10.1210/jc.2012-2410)

Merlo A, de Quirós SB, de Santa-María IS, Pitiot AS, Balbín M, Astudillo A, Scola B, Arístegui M, Quer M & Suarez C 2013 Identification of somatic VHL gene mutations in sporadic head and neck paragangliomas in association with activation of the HIF-1α/miR-210 signaling pathway. *Journal of Clinical Endocrinology and Metabolism* **98** E1661–E1666. (doi:10.1210/jc.2013-1636)

van der Mey AG, Maaswinkel-Mooy PD, Cornelisse CJ, Schmidt PH & van de Kamp JJ 1989 Genomic imprinting in hereditary glomus tumours: evidence for new genetic theory. *Lancet* **2** 1291–1294. (doi:10.1016/S0140-6736(89)91908-9)

Millán-Uclés Á, Díaz-Castro B, García-Flores P, Báez A, Pérez-Simón JA, López-Barneo J & Piruat JI 2014 A conditional mouse mutant in the tumor suppressor SdhD gene unveils a link between p21WAF1/Cip1 induction and mitochondrial dysfunction. *PLoS ONE* **9** e85528. (doi:10.1371/journal.pone.0085528)

Morin A, Letouzé E, Gimenez-Roqueplo A & Favier J 2014 Oncometabolites-driven tumorigenesis: from genetics to targeted therapy. *International Journal of Cancer* **135** 2237–2248. (doi:10.1002/ijc.29080)

van Nederveen FH, Gaal J, Favier J, Korpershoek E, Oldenburg RA, de Bruyn EM, Sleddens HF, Derkx P, Rivière J & Dannenberg H 2009 An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. *Lancet. Oncology* **10** 764–771. (doi:10.1016/S1470-2045(09)70164-0)

Neumann HP, Bausch B, McWhinney SR, Bender BU, Gimm O, Franke G, Schipper J, Klisch J, Altehoefer C, Zerres K *et al.* 2002 Germ-line mutations in nonsyndromic pheochromocytoma. *New England Journal of Medicine* **346** 1459–1466. (doi:10.1056/NEJMoa020152)

Neumann HP, Pawlu C, Peczkowska M, Bausch B, McWhinney SR, Muresan M, Buchta M, Franke G, Klisch J, Bley TA *et al.* 2004 Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. *Journal of the American Medical Association* **292** 943–951. (doi:10.1001/jama.292.8.943)

Neumann HP, Erlic Z, Boedeker CC, Rybicki LA, Robledo M, Hermsen M, Schiavi F, Falcioni M, Kwok P, Bauters C *et al.* 2009 Clinical predictors for germline mutations in head and neck paraganglioma patients: cost reduction strategy in genetic diagnostic process as fall-out. *Cancer Research* **69** 3650–3656. (doi:10.1158/0008-5472.CAN-08-4057)

Niemann S & Muller U 2000 Mutations in SDHC cause autosomal dominant paraganglioma, type 3. *Nature Genetics* **26** 268–270. (doi:10.1038/81551)

Opotowsky AR, Moko L, Ginns J, Rosenbaum M, Greutmann M, Aboulhosn J, Hageman A, Kim Y, Deng LX & Grewal J 2015 Pheochromocytoma and paraganglioma in cyanotic congenital heart disease. *Journal of Clinical Endocrinology and Metabolism* **100** 1325–1334. (doi:10.1210/jc.2014-3863)

Pantaleo MA, Astolfi A, Indio V, Moore R, Thiessen N, Heinrich MC, Gnocchi C, Santini D, Catena F, Formica S *et al.* 2011 SDHA loss-of-function mutations in KIT-PDGFRA wild-type gastrointestinal stromal tumors identified by massively parallel sequencing. *Journal of the National Cancer Institute* **103** 983–987. (doi:10.1093/jnci/djr130)

Papathomas TG, Gaal J, Corssmit EP, Oudijk L, Korpershoek E, Heimdal K, Bayley JP, Morreau H, van Dooren M, Papaspyrou K *et al.* 2013 Non-pheochromocytoma (PCC)/paraganglioma (PGL) tumors in patients with succinate dehydrogenase-related PCC–PGL syndromes: a clinicopathological and molecular analysis. *European Journal of Endocrinology* **170** 1–12. (doi:10.1530/EJE-13-0623)

Peczkowska M, Erlic Z, Hoffmann MM, Furmanek M, Cwikła J, Kubaszek A, Prejbisz A, Szutkowski Z, Kawecki A & Chojnowski K 2008 Impact of screening kindreds for SDHD p.Cys11X as a common mutation associated with paraganglioma syndrome type 1. *Journal of Clinical Endocrinology and Metabolism* **93** 4818–4825. (doi:10.1210/jc.2008-1290)

Piruat JI & Millán-Uclés Á 2014 Genetically modeled mice with mutations in mitochondrial metabolic enzymes for the study of cancer. *Frontiers in Oncology* **4** 200. (doi:10.3389/fonc.2014.00200)

Piruat JI, Pintado CO, Ortega-Saenz P, Roche M & Lopez-Barneo J 2004 The mitochondrial SDHD gene is required for early embryogenesis, and its partial deficiency results in persistent carotid body glomus cell activation with full responsiveness to hypoxia. *Molecular and Cellular Biology* **24** 10933–10940. (doi:10.1128/MCB.24.24.10933-10940.2004)

Pollard PJ, Briere JJ, Alam NA, Barwell J, Barclay E, Wortham NC, Hunt T, Mitchell M, Olpin S, Moat SJ *et al.* 2005 Accumulation of Krebs cycle intermediates and over-expression of HIF1α in tumours which result from germline FH and SDH mutations. *Human Molecular Genetics* **14** 2231–2239. (doi:10.1093/hmg/ddi227)

Pollard PJ, El-Bahrawy M, Poulsom R, Elia G, Killick P, Kelly G, Hunt T, Jeffery R, Seedhar P & Barwell J 2006 Expression of HIF-1α, HIF-2α (EPAS1), and their target genes in paraganglioma and pheochromocytoma with VHL and SDH mutations. *Journal of Clinical Endocrinology and Metabolism* **91** 4593–4598. (doi:10.1210/jc.2006-0920)

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-15-0226

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Quinlan CL, Orr AL, Perevoshchikova IV, Treberg JR, Ackrell BA & Brand MD 2012 Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions. *Journal of Biological Chemistry* **287** 27255–27264. (doi:10.1074/jbc.M112.374629)

Rattenberry E, Vialard L, Yeung A, Bair H, McKay K, Jafri M, Canham N, Cole TR, Denes J & Hodgson SV 2013 A comprehensive next generation sequencing-based genetic testing strategy to improve diagnosis of inherited pheochromocytoma and paraganglioma. *Journal of Clinical Endocrinology and Metabolism* **98** E1248–E1256. (doi:10.1210/jc.2013-1319)

Ricketts C, Woodward ER, Killick P, Morris MR, Astuti D, Latif F & Maher ER 2008 Germline SDHB mutations and familial renal cell carcinoma. *Journal of the National Cancer Institute* **100** 1260–1262. (doi:10.1093/jnci/djn254)

Ricketts CJ, Forman JR, Rattenberry E, Bradshaw N, Lalloo F, Izatt L, Cole TR, Armstrong R, Kumar V, Morrison PJ *et al.* 2010 Tumor risks and genotype–phenotype–proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. *Human Mutation* **31** 41–51. (doi:10.1002/humu.21136)

Saldana MJ, Salem LE & Travezan R 1973 High altitude hypoxia and chemodectomas. *Human Pathology* **4** 251–263. (doi:10.1016/S0046-8177(73)80012-7)

Sapieha P, Sirinyan M, Hamel D, Zaniolo K, Joyal J, Cho J, Honoré J, Kermorvant-Duchemin E, Varma DR & Tremblay S 2008 The succinate receptor GPR91 in neurons has a major role in retinal angiogenesis. *Nature Medicine* **14** 1067–1076. (doi:10.1038/nm.1873)

Scheel C & Weinberg RA 2012 Cancer stem cells and epithelial–mesenchymal transition: concepts and molecular links. *Seminars in Cancer Biology* **22** 396–403. (doi:10.1016/j.semcancer.2012.04.001)

Schiavi F, Milne RL, Anda E, Blay P, Castellano M, Opocher G, Robledo M & Cascón A 2010 Are we overestimating the penetrance of mutations in SDHB? *Human Mutation* **31** 761–762. (doi:10.1002/humu.21269)

Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB & Gottlieb E 2005 Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-α prolyl hydroxylase. *Cancer Cell* **7** 77–85. (doi:10.1016/j.ccr.2004.11.022)

Semenza GL 2012 Hypoxia-inducible factors in physiology and medicine. *Cell* **148** 399–408. (doi:10.1016/j.cell.2012.01.021)

Shankavaram U, Fliedner SM, Elkahloun AG, Barb JJ, Munson PJ, Huynh TT, Matro JC, Turkova H, Linehan WM & Timmers HJ 2013 Genotype and tumor locus determine expression profile of pseudohypoxic pheochromocytomas and paragangliomas. *Neoplasia* **15** 435–437. (doi:10.1593/neo.122132)

Smith EH, Janknecht R & Maher LJ III 2007 Succinate inhibition of α-ketoglutarate-dependent enzymes in a yeast model of paraganglioma. *Human Molecular Genetics* **16** 3136–3148. (doi:10.1093/hmg/ddm275)

Solis D, Burnichon N, Timmers H, Raygada M, Kozupa A, Merino M, Makey D, Adams K, Venisse A & Gimenez-Roqueplo A 2009 Penetrance and clinical consequences of a gross SDHB deletion in a large family. *Clinical Genetics* **75** 354–363. (doi:10.1111/j.1399-0004.2009.01157.x)

Tischler AS 2008 Pheochromocytoma and extra-adrenal paraganglioma: updates. *Archives of Pathology & Laboratory Medicine* **132** 1272–1284.

Toledo RA, Qin Y, Srikantan S, Morales NP, Li Q, Deng Y, Kim SW, Pereira MA, Toledo SP, Su X *et al.* 2013 *In vivo* and *in vitro* oncogenic effects of HIF2A mutations in pheochromocytomas and paragangliomas. *Endocrine-Related Cancer* **20** 349–359. (doi:10.1530/ERC-13-0101)

Tsang VH, Dwight T, Benn DE, Meyer-Rochow GY, Gill AJ, Sywak M, Sidhu S, Veivers D, Sue CM, Robinson BG *et al.* 2014 Overexpression of miR-210 is associated with SDH-related pheochromocytomas, paragangliomas, and gastrointestinal stromal tumours. *Endocrine-Related Cancer* **21** 415–426. (doi:10.1530/ERC-13-0519)

Vanharanta S, Buchta M, McWhinney SR, Virta SK, Peçzkowska M, Morrison CD, Lehtonen R, Januszewicz A, Järvinen H & Juhola M 2004 Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *American Journal of Human Genetics* **74** 153–159. (doi:10.1086/381054)

Wallace DC 2012 Mitochondria and cancer. *Nature Reviews. Cancer* **12** 685–698. (doi:10.1038/nrc3365)

Warburg O 1956 On the origin of cancer cells. *Science* **123** 309–314. (doi:10.1126/science.123.3191.309)

Xekouki P, Pacak K, Almeida M, Wassif CA, Rustin P, Nesterova M, de la Luz Sierra M, Matro J, Ball E, Azevedo M *et al.* 2011 Succinate dehydrogenase (SDH) D subunit (SDHD) inactivation in a growth-hormone-producing pituitary tumor: a new association for SDH? *Journal of Clinical Endocrinology and Metabolism* **97** E357–E366. (doi:10.1210/jc.2011-1179)

Xiao M, Yang H, Xu W, Ma S, Lin H, Zhu H, Liu L, Liu Y, Yang C, Xu Y *et al.* 2012 Inhibition of α-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. *Genes and Development* **26** 1326–1338. (doi:10.1101/gad.191056.112)

Yeap PM, Tobias ES, Mavraki E, Fletcher A, Bradshaw N, Freel EM, Cooke A, Murday VA, Davidson HR, Perry CG *et al.* 2011 Molecular analysis of pheochromocytoma after maternal transmission of SDHD mutation elucidates mechanism of parent-of-origin effect. *Journal of Clinical Endocrinology and Metabolism* **96** E2009–E2013. (doi:10.1210/jc.2011-1244)

Zhuang Z, Yang C, Lorenzo F, Merino M, Fojo T, Kebebew E, Popovic V, Stratakis CA, Prchal JT & Pacak K 2012 Somatic HIF2A gain-of-function mutations in paraganglioma with polycythemia. *New England Journal of Medicine* **367** 922–930. (doi:10.1056/NEJMoa1205119)

Received in final form 24 June 2015
Accepted 25 June 2015
Made available online as an Accepted Preprint
25 June 2015

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