Succinate dehydrogenase gene mutations are strongly associated with paraganglioma of the organ of Zuckerkandl

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Abstract

Organ of Zuckerkandl paragangliomas (PGLs) are rare neuroendocrine tumors that are derived from chromaffin cells located around the origin of the inferior mesenteric artery extending to the level of the aortic bifurcation. Mutations in the genes encoding succinate dehydrogenase subunits (SDH) B, C, and D (SDHx) have been associated with PGLs, but their contribution to PGLs of the organ of Zuckerkandl PGLs is not known. We aimed to describe the clinical presentation of patients with PGLs of the organ of Zuckerkandl and investigate the prevalence of *SDHx* mutations and other genetic defects among them. The clinical characteristics of 14 patients with PGL of the organ of Zuckerkandl were analyzed retrospectively; their DNA was tested for *SDHx* mutations and deletions. Eleven out of 14 (79%) patients with PGLs of the organ of Zuckerkandl were found to have mutations in the *SDHB* (9) or *SDHD* (2) genes; one patient was found to have the Carney–Stratakis syndrome (CSS), and his PGL was discovered during surgery for gastrointestinal stromal tumor. Our results show that *SDHx* mutations are prevalent in pediatric and adult PGLs of the organ of Zuckerkandl. Patients with PGLs of the organ of Zuckerkandl should be screened for *SDHx* mutations and the CSS; in addition, asymptomatic carriers of an *SDHx* mutation among the relatives of affected patients may benefit from tumor screening for early PGL detection.

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Introduction

The organ of Zuckerkandl consists of extra-adrenal chromaffin cells located just below the origin of the inferior mesenteric artery and above the bifurcation of the aorta. This collection of paraganglia was first described by Hungarian anatomist and surgeon, Emil Zuckerkandl, who initially named them Nebenorgane (auxiliary organs; Ober 1983). The chromaffin cells that comprise the organ of Zuckerkandl originate from the same neural crest progenitor cells that give rise to the

adrenal medulla. While these structures normally degenerate and involute by adolescence, small clusters of chromaffin cells remain that can serve as sites of future tumor development. At least 135 cases of paragangliomas (PGLs) of the organ of Zuckerkandl have been reported in the literature, but their association with specific mutations and/or genetic syndromes has not been described (Subramanian & Maker 2006).

Germline mutations in the genes encoding subunits B, C, and D of succinate dehydrogenase (SDH), the

mitochondrial complex II, have been associated with the development of PGLs (Baysal *et al.* 2000, Astuti *et al.* 2001, 2003, Gimenez-Roqueplo *et al.* 2003, Brouwers *et al.* 2006, Ghayee *et al.* 2009). The *SDHB*, *SDHC*, and *SDHD* genes encode mitochondrial proteins which serve as tumor suppressors, and loss-of-function mutations in these genes are linked to formation of PGLs (Gottlieb & Tomlinson 2005). The SDH enzyme is involved in cellular energy metabolism through the tricarboxylic acid cycle, the oxidative phosphorylation, and the electron transport chain (Gottlieb & Tomlinson 2005). Although the mechanism that explains the link between mitochondrial dysfunction and tumor formation remains unknown, it is thought to be linked to an increase in reactive oxygen species and/or the activation of the hypoxia pathway or apoptosis (Eng *et al.* 2003, Gottlieb & Tomlinson 2005, Benn & Robinson 2006, Favier *et al.* 2009). The development of metastatic disease may be related to up-regulation of angiogenesis; however, the exact mechanism remains to be elucidated (Favier *et al.* 2002).

While an association with mutations in *SDHB* and *SDHD* genes has been found in patients with mediastinal PGLs, a specific analysis of *SDH* mutations in patients with Zuckerkandl organ PGLs has not been performed to date. *SDHD* gene mutations are specifically associated with head and neck PGLs and much less frequently with malignant PGLs (Baysal *et al.* 2000, Benn *et al.* 2006, Havekes *et al.* 2007, Timmers *et al.* 2008). Mutations in the *SDHB* gene are associated with a high rate of malignancy and aggressive disease; among patients with metastatic PGLs, the frequency of *SDHB* mutations is between 30 and 83% (Gimenez-Roqueplo *et al.* 2003, Neumann *et al.* 2004, Amar *et al.* 2005, Brouwers *et al.* 2006). *SDHB* mutations have also been associated with shorter survival (Amar *et al.* 2007). Tumors classified as extra-adrenal abdominal PGLs (including a grouping of paraaortic/pericaval, bladder, remnants of the organ of Zuckerkandl, perirenal, retroperitoneal, and periadrenal) are associated with mutations in both *SDHB* and *SDHD* genes (Benn *et al.* 2006). Anecdotal reports have described six patients with tumors of the organ of Zuckerkandl and *SDHD* (2) or *SDHB* (4) mutations; however, to our knowledge this is the first series with a specific focus on tumors of this anatomic site (Gimenez-Roqueplo *et al.* 2003, Donahue *et al.* 2008).

From an initial observation of two patients with Zuckerkandl organ tumors who had *SDHx* mutations, we searched our clinical database for other patients with tumors involving this organ; we then tested the

DNA of these patients retrospectively for mutations in *SDHx*. Three of the patients had mutations in *SDHx* identified prior to their evaluation at our institution. In the present study we report in detail the clinical characteristics, biochemical phenotype, and clinical course of 14 patients with Zuckerkandl organ tumors seen at the National Institutes of Health (NIH) and the University of Texas M.D. Anderson Cancer Center over the past 20 years; in these patients, we examined the frequency of *SDHx* mutations.

Materials and methods

Subjects of protocol

Fourteen patients with primary PGLs of the organ of Zuckerkandl with a median age at diagnosis of 22.5 years (range 9–71) seen at the NIH, University of Texas M.D. Anderson Cancer Center and Dana-Farber Cancer Institute between 1989 and 2009 are presented in this study. A retrospective chart analysis was performed in order to identify patients with Zuckerkandl organ PGLs via review of abdominal computed tomography (CT) scans and analysis of operative and pathology reports. We reviewed radiological scans from all the cases and included only those tumors defined by the radiologist to be consistent with the specific anatomic localization of the Zuckerkandl organ. The patients were followed up for an average of 8 years. Initial patient symptoms, biochemical phenotype, details of primary tumor, and patient outcome were recorded. The presence of disease in sites where chromaffin cells are not normally present, i.e. disease in an extra-paraganglionic site (lymph nodes, bone, liver, and lung), was used to define metastatic disease as differentiated from multiple sites of disease (Gimenez-Roqueplo *et al.* 2008). Consent was obtained from each patient after full explanation of the purpose and nature of all procedures used. The investigation was approved by the NICHD IRB and the M.D. Anderson Cancer Center IRB.

Genetic studies

Three patients were known to harbor *SDHx* mutations prior to their evaluation at the NIH. For those patients without prior studies, genetic testing of germline DNA for mutations in *SDHB*, *C*, and *D* genes was performed. Blood samples for *SDHx* mutation analysis were collected prospectively for purposes of genetic testing. Genomic DNA was extracted from whole blood as described previously. The four exons of *SDHD*, eight exons of *SDHB*, and six exons of *SDHC* were amplified and sequenced by PCR based bidirectional sequencing

by Mayo Medical Laboratories, Rochester, MN, USA or by the Division of Molecular Diagnostics at the University of Pittsburgh Medical Center, as described previously (Brouwers *et al.* 2006). Deletion testing was performed using a combination of multiplex ligation-dependant probe amplification and Luminex FlexMap Technologies (Monico *et al.* 2007).

Hormone assays

Plasma catecholamines and metanephrines, and urinary catecholamines and metanephrines were measured using standard HPLC assays, as described previously, at the NIH Clinical Center and Mayo Medical Laboratories (Eisenhofer *et al.* 1986, Lenders *et al.* 1993).

Results

Patient characteristics are presented in Table 1. The median age of the nine males and five females at diagnosis was 22.5 years (range 9–71). Eleven out of 14 (79%) patients with organ of Zuckerkandl PGLs were found to have mutations in the *SDHB* (9) or *SDHD* (2) genes. Four of these represent nonsense mutations, two missense mutations, two deletions, two frameshift and one splice site mutation. No mutations were found in three patients. Details of the mutations are presented in Table 1; two of the mutations represent novel mutations, *SDHB* p.Arg38ProfsX40 and *SDHB*

Tyr61X, the remaining six are reported previously (Baysal *et al.* 2000, Brouwers *et al.* 2006, Timmers *et al.* 2007, Pasini *et al.* 2008, Solis *et al.* 2009). Three of the 14 patients had a first degree relative with a history of pheochromocytoma or PGL. None of the 14 patients were related to one another.

Eight out of 14 (57%) patients had signs and symptoms related to catecholamine excess at the time of diagnosis. Results obtained from imaging studies used to detect tumor are presented in Table 1. In all 14 patients, CT scans were positive for visualization of Zuckerkandl organ tumor. ¹²³I-metaiodobenzylguanidine (¹²³I-MIBG) scans were able to detect tumors in eight out of nine cases, and T2 magnetic resonance imaging was positive in all eight patients in whom it was used. Four out of 14 (30%) patients presented with the organ of Zuckerkandl PGL as their only primary tumor. While eight patients presented with metastatic disease, not all of these had the organ of Zuckerkandl PGL at the time of presentation. Seven out of 14 (50%) patients who had organ of Zuckerkandl tumors as part of their initial presentation also had multiple primary tumors or metastatic disease at initial diagnosis. Two out of 14 (14%) patients were identified to have the organ of Zuckerkandl PGL as an additional site of disease on long-term follow-up. One patient was diagnosed with the Carney–Stratakis syndrome (CSS) and was found to have the organ of Zuckerkandl PGL incidentally during surgery for gastrointestinal stromal tumor (GIST). The size of the organ of Zuckerkandl

Table 1 Patient characteristics

Patient	Mutation	Biochemistry	Size of tumor (cm)	Metastatic at dx?	Patient outcome
M,49	<i>SDHB</i> p.Arg38ProfsX40 ^a	U: NE, DA, NMN	10	Yes	AWD
M,20	Negative	Not done	3.7	No	NED
F,33	Negative	P: NE, CGA	3.1	Yes	DOD
		U: NE, NMN			
M,9	<i>SDHB</i> p.Arg46X	P: NMN	14.5	No	AWD
F,9	<i>SDHB</i> first exon deletion	P: NMN, NE, DA	3	Yes	AWD
M,15	<i>SDHB</i> p.Arg46X	U: NMN, NE, DA	3.5	No	AWD
		P: NMN, NE			
M,34	<i>SDHB</i> first exon deletion	P: NE, NMN	2.4	Yes	AWD
F,18	Negative	P: NMN, CGA	1.4	Yes	AWD
F,26	<i>SDHD</i> p.Arg38X	P: NE	6	No	AWD
M,24	<i>SDHB</i> Tyr61X ^a	P: NE	2.5	No	AWD
		U: NE, NMN			
M,58	<i>SDHB</i> p.Trp200Cys	Not done	6	Yes	AWD
F,71	<i>SDHB</i> IVS1 + 1G > T	P: NMN, MN, NE, EPI	10	Yes	DOD
M,21	<i>SDHD</i> p.Leu20CysfsX66	P, U: NE, NMN	2	Yes	AWD
M,11	<i>SDHB</i> p.Val140Phe	P: NE, NMN	5.5	No	AWD
		U: NE, NMN			

P, plasma; U, urine; NE, norepinephrine; EPI, epinephrine; NMN, normetanephrine; CGA, chromogranin A; DA, dopamine; AWD, alive with disease; DOD, dead of disease; NED, no evidence of disease.
^aNovel mutation.

Figure 1 Sites of metastatic disease, $n=14$ patients.

PGLs was a mean of 5.3 ± 3.8 cm. Analysis of the biochemical data reveals that nine patients had a noradrenergic phenotype, and three had both noradrenergic and dopaminergic phenotypes (Table 1). Four patients developed metastatic disease during follow-up, the time to metastases ranged from 2 to 19 years (6 ± 8.7). Sites of metastatic disease and multiple sites of disease are presented in Fig. 1. Surgical resection of the primary tumor was performed in 11/14 (79%) of the patients, while four patients received therapeutic ^{131}I -MIBG, two received octreotide therapy, four received conventional radiation therapy, three patients with aggressive and metastatic disease additionally received chemotherapy.

Images obtained through imaging studies and surgical images demonstrating the organ of Zuckerkandl PGLs are shown in Figs 2 and 3. Pathology of a representative patient's organ of Zuckerkandl PGL that is consistent with neuroendocrine tumor is presented in Fig. 4.

Discussion

The present study including 14 patients with PGLs of the organ of Zuckerkandl establishes for the first time an association of these tumors with *SDHB* and *SDHD* mutations. Our data also show that PGLs of the organ of Zuckerkandl are strongly associated with a noradrenergic phenotype and have an aggressive behavior, likely related to the *SDHB* mutational status. The identification of *SDHx* mutations in patients with

the organ of Zuckerkandl PGLs has important implications for patient care and genetic screening of family members.

PGLs arise from sympathetic chromaffin tissue both in adrenal gland and outside of the adrenal gland in the abdomen or thorax, or alternatively originate from parasympathetic tissues in the head and neck (Benn et al. 2006). A particular grouping of PGLs, termed organ of Zuckerkandl PGLs, refer to a group of

Figure 2 Diagnostic imaging from patient F.18. (A) ^{123}I -metaiodobenzylguanidine (^{123}I -MIBG) anterior maximum intensity projection showing right adrenal mass (dashed arrow) and nodule anterior to vertebrae L5 representing the organ of Zuckerkandl tumor (closed arrow). There is a focus in the mid-portion of the left kidney (open arrow) thought to be due to activity retained in the collecting system. (B) Hybrid computed axial tomography (SPECT) and CT (SPECT/CT) ^{123}I -MIBG with contrast of the abdomen showing a 2.6-cm heterogeneously enhancing partially necrotic right adrenal mass (open arrow) and a 1.6-cm enhancing mass anterior to the spine and just inferior to the aortic bifurcation (closed arrow). (C) Surgical photo, arrow indicates the site of tumor below aortic bifurcation.

extra-adrenal chromaffin tissue situated between the origin of the inferior mesenteric artery and the bifurcation of the aorta (Ober 1983). These PGLs are remnants of the primitive sympathetic nervous system. The function of the organ of Zuckerkindl is unknown in humans; however, it is thought to act as a chemoreceptor in other species (Hollinshead 1940). Mutations in genes encoding the SDH subunits B, C, and D of mitochondrial complex II have been associated with PGL development (Baysal *et al.* 2000, Astuti *et al.* 2001, 2003, Gimenez-Roqueplo *et al.* 2003, Brouwers *et al.* 2006, Ghayee *et al.* 2009). While most head and neck PGLs are associated with *SDHD* gene mutations, PGLs derived from the sympathetic nervous system are often related to *SDHB* gene mutations. This is especially true for those deriving from extra-adrenal intra-abdominal lesions, which are frequently metastatic (Brouwers *et al.* 2006, Burnichon *et al.* 2009). Recently, mediastinal PGLs were also found to be strongly associated with mutations in *SDHB* gene (Ghayee *et al.* 2009).

While the majority of our patients had mutations in the *SDHx* genes, there are two important exceptions. One patient with primary adrenal pheochromocytoma as well as with organ of Zuckerkindl PGL who did not have a mutation in *SDHx* also had congenital polycythemia. She was also tested (data not shown) for a mutation of the gene encoding the prolyl hydroxylase domain 2 protein, but she was found to be negative (Ladroue *et al.* 2008). Another patient who presented with a GIST and was incidentally found to have an organ of Zuckerkindl PGL at the time of surgery was also not found to have *SDHx* mutation or deletion. Although most patients with CSS have *SDHx* mutations, up to 10% may harbor deletions that are not detectable by current screening methods (Carney & Stratakis 2002, Pasini *et al.* 2008).

Testing for SDH subunits is now recommended for all cases of pheochromocytomas and PGLs, and testing should be offered to first-degree relatives for cancer surveillance and early detection (Neumann *et al.* 2002, Amar *et al.* 2005, Benn & Robinson 2006, Jimenez *et al.* 2006, Prodanov *et al.* 2009). In addition, young patients with extra-adrenal pheochromocytomas should be tested for germline mutations of the von Hippel–Lindau (*VHL*) gene (Pacak *et al.* 2007, Boedeker *et al.* 2009). Germline mutations in the *SDHD* and *SDHB* genes should be considered a risk factor for PGLs of the organ of Zuckerkindl, and radiographic analysis of this area should be performed. However, exact surveillance recommendations for asymptomatic carriers of *SDHx* mutations may vary between institutions (Benn & Robinson 2006).

Figure 3 Diagnostic imaging from patient M,34. (A) [^{18}F]-2-fluoro-deoxy-D-glucose (FDG PET/CT) demonstrates an enhancing retroperitoneal mass (closed arrow). (B) Magnetic resonance imaging (MRI) anterior demonstrates two retroperitoneal masses, 4×3 and 4×2 cm (closed arrows), one at the level of the right renal hilum and the other a periaortic mass between the abdominal aorta and the inferior vena cava. (C) Surgical photo demonstrating anatomical site of tumor (closed arrow) and the common iliac artery (open arrow).

Clinical presentation of pheochromocytomas and PGLs is variable depending on functionality of tumor; 70–80% of PGLs of the organ of Zuckerkindl are clinically functional (Subramanian & Maker 2006).

Figure 4 Low 4× (A), medium 10× (B), and high 40× (C) power magnification of PGL cells in a representative patient. Immunohistochemistry for synaptophysin showing strong positivity of tumor cells (D).

Most extra-adrenal paraganglia as well as paraganglia in the setting of a *SDHB* mutation primarily secrete norepinephrine or norepinephrine and dopamine, as they lack phenylethanolamine *N*-methyltransferase, the enzyme responsible for the conversion of norepinephrine to epinephrine (Timmers *et al.* 2007). Our patient group is consistent with this finding in the predominance of noradrenergic phenotype. Interestingly, three of our patients had tumors with mixed secretion of both norepinephrine and dopamine, all of whom had *SDHB* mutations, consistent with previous studies linking this specific biochemical phenotype to the presence of *SDHB* mutations (Timmers *et al.* 2007). Surgical resection is the preferred treatment for organ of Zuckerkandl PGLs; 11/14 of our patients underwent resection; however, a subset of patients presented with metastatic disease that was not amenable to resection. Similar to what is known about other extra-adrenal pheochromocytomas, organ of Zuckerkandl PGLs have a higher rate of malignancy than adrenal pheochromocytomas (Altergott *et al.* 1985). A review of 135 cases of organ of Zuckerkandl PGLs found that 41% of these tumors were malignant based on the presence of metastases and local invasion, with the most common sites of metastatic disease including bone, liver, and lungs (Subramanian & Maker 2006). This finding is well supported by our data that shows that 86% of patients with organ of Zuckerkandl tumors had multiple sites of disease and/or metastatic disease.

We conclude that *SDHB* and *SDHD* mutations are indicators of possible organ of Zuckerkandl tumors and should lead to appropriate clinical studies and investigation of family members. This is also supported by our findings and previous findings that noradrenergic and dopaminergic phenotype in the same patient strongly suggest the presence of *SDHB* gene mutation that should be addressed in planned genetic testing. As organ of Zuckerkandl PGLs are linked to SDHx-associated disease, having a PGL in the organ of Zuckerkandl impacts the probability of an *SDHB* or *D* mutations and warrants mutation testing.

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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