# *SDHA* mutated paragangliomas may be at high risk of metastasis

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**Dear Editor**

We report the clinical outcomes of eleven patients with succinate dehydrogenase subunit A (*SDHA*) germline mutations from three UK tertiary referral centres to highlight a more diverse and expanding clinical spectrum of associated phenotypes. We suggest that *SDHA* paraganglioma related disease is not a low risk condition as first described. Of our six index cases, two developed metastatic disease and a further one had local vascular invasion. One patient developed multiple metachronous disease. Therefore, we believe these patients, like those with *SDHB* and *SDHD* mutations, should be part of a surveillance programme.

Paraganglioma (PGL) associated mutations in *SDHA* have only been reported in a small number of patients worldwide. There is controversy over the necessity for surveillance screening in these patients, compared to *SDHB* and *SDHD*, as penetrance is thought to be lower (Benn, et al. 2015) and variants exist with uncertain pathogenicity. Initial reports associated *SDHA* with autosomal recessive causes of juvenile encephalopathy (Leigh syndrome) (Bourgeron, et al. 1995) and homozygous mutations in *SDHA* cause severe neurological dysfunction and cardiomyopathy (Renkema, et al. 2015). *SDHA* mutations have now been associated with phaeochromocytoma and paraganglioma (PPGL) formation in an autosomal dominant manner. *SDHA* mutations account for only 3% of cases of familial PGL cases, with presumed low penetrance (Korpershoek, et al. 2011) and therefore very little data on clinical features of *SDHA*-related PPGL exist.

Six index cases were originally diagnosed between 1973 and 2011 and had histologically proven PPGL, who subsequently underwent genetic testing during the course of their follow up and were confirmed to have an underlying *SDHA* germline mutation. We performed a retrospective analysis of their notes and describe their clinical outcomes. From these six index cases, cascade genetic testing occurred and identified a further five asymptomatic carriers of *SDHA* mutations. All patients are now being followed up in specialised endocrine clinics and are undergoing annual screening, including annual clinical and biochemical assessment and cross-sectional imaging, although the frequency and modality of imaging differs between centres. To predict the pathogenicity of the DNA variants, the missense variants were investigated *in silico* using PloyPhen2 and SIFT.

Table 1 provides a detailed summary of the patients described.

The six index patients originally presented aged 18, 34, 36, 46, 47 and 68 years. Five patients presented with a single lesion at diagnosis: intra-thyroidal PGL, mediastinal PGL, phaeochromocytoma and two extra adrenal PGLs. One patient (patient 3) presented with two synchronous lesions: she had a 3-methoxytyramine (3MT) secreting carotid body tumour and a

noradrenaline secreting thoracic PGL. All patients underwent surgical resection of the primary tumours. Two patients developed recurrence in the surgical bed and both patients went on to develop metastatic disease 16 and 37 years later (patients 8 and 9). One of these two patients (patient 8) also developed an additional five metachronous lesions 7-10 years after original diagnosis. These two cases are described in more detail.

Patient 8 presented aged 46 years with headaches and malignant hypertension (210/130mHg). Urinary noradrenaline was very raised (table 1) and imaging confirmed a 5cm para-adrenal PGL, which was subsequently resected. He developed a symptomatic recurrence one year later, which was surgically resected. Eight years after his original diagnosis he presented with symptoms of catecholamine excess and four new lesions were identified and resected. On surveillance imaging three years later a new non-secretory lesion was identified. Surgical resection was undertaken one year subsequently due to increasing PGL size and plasma catecholamine levels. He remained well with no evidence of further disease on imaging until five years later when rising noradrenaline levels were noted and uptake in the left adrenal bed and in the vertebral body of L4 was demonstrated on FDG PET. A bone biopsy confirmed a metastatic deposit and he underwent external beam radiotherapy (50Gy 25#), followed by cyber-knife radiotherapy (14Gy 1#) when this lesion doubled in size. His metanephrines have remained normal since his radiotherapy six years ago, and the bone lesions have stabilised in size.

Patient 9 presented aged 18 years with a symptomatic phaeochromocytoma in 1973. She underwent an adrenalectomy and nephrectomy, and remained asymptomatic for 36 years until experiencing episodes of hot flushes, hypertension and haematuria. Imaging revealed a lesion in the para-aortic region and metanephrine levels were raised (table 1). $^{68}$Ga DOTATATE PET scan identified metastatic disease in the sacrum and T10 vertebral body with lymph node involvement and she was commenced on Octreotide LAR 30mg monthly. Most recent surveillance imaging (MRI and MIBG) shows no disease progression and she has normal biochemistry.

The five asymptomatic carriers identified through cascade screening have not had any tumours identified in surveillance imaging and all have negative biochemistry. They have had a total of 17 years' surveillance. Additionally, patient 8 has one adult son who has been undergoing clinical and radiological screening with no tumours identified to date, but has not yet undergone genetic testing. Patient 11 has three children and one sister (who has breast carcinoma) that are awaiting genetic testing, but have no history of PPGL.

The six index cases described here presented with a variety of clinical manifestations extending the known phenotypic spectrum in *SDHA* disease.

Little is known about PGL associated disease in carriers of an *SDHA* mutation. Table 2 shows the 26 cases reported in the literature with 14 different *SDHA* mutations (Burnichon, et al. 2010; Burnichon, et al. 2012; Casey, et al. 2017; Dwight, et al. 2013; Korpershoek et al. 2011; Papathomas, et al. 2015; von Dobschuetz, et al. 2015; Welander, et al. 2013). These patients presented aged 12-62 years. Unlike in *SDHB* and *SDHD*, in the described combined cases there is no obvious predilection to any specific body site.

PGLs occurring in the thyroid gland are extremely rare. A recent evaluation of the ENSAT registry identified only five cases of thyroid PGL (prevalence 0.5%). Four of these patients were subsequently found to have *SDH* germline mutations (von Dobschuetz et al. 2015). Interestingly two of these four patients carried a *SDHA* mutation, but both mutations were different to the one our patient carried. Similarly to our patient both were female and presented at similar ages (36 & 37 years), with no family history of PGL.

Recognised associations of *SDHA* mutations include gastrointestinal stromal tumours (GIST) (Papathomas, et al. 2014) and *SDHA* variants have been described in three patients with pituitary adenomas, although SDHA deficiency was only demonstrated in one tumour by immunohistochemistry (IHC) and loss of heterozygosity (LoH) was not demonstrated (Dwight et al. 2013; O'Toole, et al. 2015). To date only one case of *SDHA* deficient renal carcinoma has been reported (Yakirevich, et al. 2015). Additional findings in our cohort included: bilateral breast carcinoma 14 years after PGL diagnosis (patient 1). Patient 11's PGL was discovered incidentally during staging imaging for his prostate carcinoma and he was subsequently found to have a macroprolactinoma. His daughter also has a microprolactinoma, although is awaiting genetic testing.

Two out of six of our index patients have developed distant metastatic PPGLs. Time to disseminated disease was 16 and 37 years, and occurred following development of recurrent disease, suggesting a long duration of disease before onset of metastases.

A cautious approach must be used before ascribing definite pathogenicity to newly identified mutations. Table 3 combines the evidence that suggests pathogenicity for each of the described mutations. SDHA immunohistochemistry (IHC) was performed on tissue that was available. One sample (patient 8) demonstrated positive SDHA IHC. It has previously been described that SDHA IHC maybe positive in the presence of a definitive mutation but on rare occasions there is disparity between molecular genetic aberrations of a tumour suppressor gene and retention of protein

expression (Evenepoel, et al. 2015; Miettinen, et al. 2013; Papathomas et al. 2015). It has been hypothesised that this may be due to the second hit in the *SDHx* gene in the tumour tissue resulting in an inactive SDH complex with preservation of antigenicity (Papathomas et al. 2015).

The mutation carried by this patient (patient 8) had the most aggressive phenotype in our cohort. A recently reported metastatic case carried the same *SDHA* mutation (Casey et al. 2017). Casey *et al* went on to perform structural analysis of the effects of this mutation (using DUET scoring) and predicted it would cause mild destabilisation of the protein promoter region and part of the substrate binding region and therefore likely to affect protein stability. This may explain the positive protein expression seen in patient 8.

The patients we report highlight a more diverse and expanding clinical spectrum of *SDHA* associated phenotypes. Of our six index cases, two developed metastatic disease and a further one had local vascular invasion. There are three previous reported metastatic cases in the literature (see table 2) with three different *SDHA* mutations. Interestingly the two metastatic cases we report each carry one of these mutations (Casey et al. 2017; Korpershoek et al. 2011; Papathomas et al. 2015). With five of the 32 reported cases developing metastatic disease, we suggest that *SDHA* related disease is therefore not seen as a low risk condition.

We believe, with the current uncertainty about pathogenicity and penetrance, these patients should be part of a surveillance programme to monitor for metachronous and metastatic disease. Very few familial cases have been reported and none of our asymptomatic carriers have developed tumours. This raises questions about cascade genetic screening and subsequent clinical surveillance. However, given the recognition of aggressive behaviour in *SDHA* we believe these relatives should be monitored in surveillance programmes until the full phenotype and penetrance are established.

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This study was carried out in accordance with the Declaration of Helsinki and all the applicable local regulations. As this is an analysis on subjects' data taken during normal clinical practice, no specific authorization by ethic committee was sought.

*References:*

Benn DE, Robinson BG & Clifton-Bligh RJ 2015 15 YEARS OF PARAGANGLIOMA: Clinical manifestations of paraganglioma syndromes types 1-5. *Endocr Relat Cancer* **22** T91-103.

Bourgeron T, Rustin P, Chretien D, Birch-Machin M, Bourgeois M, Viegas-Pequignot E, Munnich A & Rotig A 1995 Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. *Nat Genet* **11** 144-149.
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. *Hum Mol Genet* **19** 3011-3020.
Burnichon N, Cascon A, Schiavi F, Morales NP, Comino-Mendez I, Abermil N, Inglada-Perez L, de Cubas AA, Amar L, Barontini M, et al. 2012 MAX mutations cause hereditary and sporadic pheochromocytoma and paraganglioma. *Clin Cancer Res* **18** 2828-2837.
Casey RT, Ascher DB, Rattenberry E, Izatt L, Andrews KA, Simpson HL, Challis B, Park S-M, Bulusu VR, Lalloo F, et al. 2017 SDHA related tumorigenesis: a new case series and literature review for variant interpretation and pathogenicity. *Molecular Genetics & Genomic Medicine*.
Dwight T, Mann K, Benn DE, Robinson BG, McKelvie P, Gill AJ, Winship I & Clifton-Bligh RJ 2013 Familial SDHA mutation associated with pituitary adenoma and pheochromocytoma/paraganglioma. *J Clin Endocrinol Metab* **98** E1103-1108.
Evenepoel L, Papathomas TG, Krol N, Korpershoek E, de Krijger RR, Persu A & Dinjens WN 2015 Toward an improved definition of the genetic and tumor spectrum associated with SDH germ-line mutations. *Genet Med* **17** 610-620.
Korpershoek E, Favier J, Gaal J, Burnichon N, van Gessel B, Oudijk L, Badoual C, Gadessaud N, Venisse A, Bayley JP, et al. 2011 SDHA immunohistochemistry detects germline SDHA gene mutations in apparently sporadic paragangliomas and pheochromocytomas. *J Clin Endocrinol Metab* **96** E1472-1476.
Miettinen M, Killian JK, Wang ZF, Lasota J, Lau C, Jones L, Walker R, Pineda M, Zhu YJ, Kim SY, et al. 2013 Immunohistochemical loss of succinate dehydrogenase subunit A (SDHA) in gastrointestinal stromal tumors (GISTs) signals SDHA germline mutation. *Am J Surg Pathol* **37** 234-240.
O'Toole SM, Denes J, Robledo M, Stratakis CA & Korbonits M 2015 15 YEARS OF PARAGANGLIOMA: The association of pituitary adenomas and phaeochromocytomas or paragangliomas. *Endocr Relat Cancer* **22** T105-122.
Papathomas TG, Gaal J, Corssmit EP, Oudijk L, Korpershoek E, Heimdal K, Bayley JP, Morreau H, van Dooren M, Papaspyrou K, et al. 2014 Non-pheochromocytoma (PCC)/paraganglioma (PGL) tumors in patients with succinate dehydrogenase-related PCC-PGL syndromes: a clinicopathological and molecular analysis. *Eur J Endocrinol* **170** 1-12.
Papathomas TG, Oudijk L, Persu A, Gill AJ, van Nederveen F, Tischler AS, Tissier F, Volante M, Matias-Guiu X, Smid M, et al. 2015 SDHB/SDHA immunohistochemistry in pheochromocytomas and paragangliomas: a multicenter interobserver variation analysis using virtual microscopy: a Multinational Study of the European Network for the Study of Adrenal Tumors (ENS@T). *Mod Pathol* **28** 807-821.
Renkema GH, Wortmann SB, Smeets RJ, Venselaar H, Antoine M, Visser G, Ben-Omran T, van den Heuvel LP, Timmers HJ, Smeitink JA, et al. 2015 SDHA mutations causing a multisystem mitochondrial disease: novel mutations and genetic overlap with hereditary tumors. *Eur J Hum Genet* **23** 202-209.
von Dobschuetz E, Leijon H, Schalin-Jantti C, Schiavi F, Brauckhoff M, Peczkowska M, Spiazzi G, Dematte S, Cecchini ME, Sartorato P, et al. 2015 A registry-based study of thyroid paraganglioma: histological and genetic characteristics. *Endocr Relat Cancer* **22** 191-204.
Welander J, Garvin S, Bohnmark R, Isaksson L, Wiseman RW, Soderkvist P & Gimm O 2013 Germline SDHA mutation detected by next-generation sequencing in a young index patient with large paraganglioma. *J Clin Endocrinol Metab* **98** E1379-1380.
Yakirevich E, Ali SM, Mega A, McMahon C, Brodsky AS, Ross JS, Allen J, Elvin JA, Safran H & Resnick MB 2015 A Novel SDHA-deficient Renal Cell Carcinoma Revealed by Comprehensive Genomic Profiling. *Am J Surg Pathol* **39** 858-863.