



# Succinate detection using in vivo <sup>1</sup>H-MR spectroscopy identifies germline and somatic *SDHx* mutations in paragangliomas

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## Abstract

**Purpose** Germline mutations in genes encoding succinate dehydrogenase (SDH) are frequent in patients with pheochromocytoma and paraganglioma (PPGL). They lead to SDH inactivation, mediating a massive accumulation of succinate, which constitutes a highly specific biomarker of *SDHx*-mutated tumors when measured in vitro. In a recent pilot study, we showed that magnetic resonance spectroscopy (<sup>1</sup>H-MRS) optimized for succinate detection (SUCCES) could detect succinate in vivo in both allografted mouse models and PPGL patients. The objective of this study was to prospectively assess the diagnostic performances of <sup>1</sup>H-MRS SUCCES sequence for the identification of SDH deficiency in PPGL patients.

**Methods** Forty-nine patients presenting with 50 PPGLs were prospectively enrolled in our referral center for <sup>1</sup>H-MRS SUCCES. Two observers blinded to the clinical characteristics and genetic status analyzed the presence of a succinate peak and confronted the results to a composite gold standard combining PPGL genetic testing and/or in vitro protein analyses in the tumor.

**Results** A succinate peak was observed in 20 tumors, all of which had proven SDH deficiency using the gold standard (17 patients with germline *SDHx* mutations, 2 with a somatic *SDHD* mutation, and 1 with negative SDHB IHC and SDH loss of function). A false negative result was observed in 3 tumors. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of <sup>1</sup>H-MRS SUCCES were respectively 87%, 100%, 100%, 90%, and 94%.

**Conclusions** Detection of succinate using <sup>1</sup>H-MRS is a highly specific and sensitive hallmark of SDH-deficiency in PPGLs.

**Keywords** Magnetic resonance spectroscopy · Paraganglioma · Succinate · Succinate dehydrogenase

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## Introduction

Pheochromocytomas and paragangliomas (PPGL) are characterized by a very strong genetic determinism with almost 20 susceptibility genes identified so far, which mutations can impact the prognosis, clinical outcome [1], and response to treatment [2]. More than half of inherited PPGL involve mutations in genes encoding mitochondrial enzymes or transporters. Among them, *SDHx* genes (*SDHA*, *SDHB*, *SDHC*, *SDHD*, and *SDHAF2*) encode the four subunits and the assembly factor of succinate dehydrogenase (SDH), which oxidizes succinate to fumarate in the tricarboxylic acid (TCA) cycle and constitutes the complex II of the electron transport chain. *SDHx* mutations predispose to multiple PPGL and *SDHB* mutated tumors are of strongly increased risk of metastatic progression [3, 4]. While *SDHB* mutations are found in around 10% of PPGL patients, it is estimated that more than one third of patients with metastatic PPGL carry an *SDHB* germline mutation. Similarly, it is estimated that approximately 15% of PPGL patients will ultimately develop a metastatic disease, versus 50% of *SDHB*-mutation carriers. Patients with hereditary *SDHx*-related PPGL carry a germline heterozygous mutation, which is associated with the somatic inactivation of the normal allele by somatic mutation or loss of heterozygosity according to the Knudson's 2-hit model for tumor suppressor genes. This bi-allelic inactivation induces a complete loss of SDH activity in the tumor, leading to the accumulation of succinate, which acts as an oncometabolite and is suspected to mediate most tumorigenic effects related to *SDHx* mutations [5–8].

In *SDHx*-mutated PPGL, succinate concentrations increase dramatically, up to 100-fold with respect to tumors without *SDHx* mutations [9–11]. We recently adapted a magnetic resonance spectroscopy (MRS) method to detect succinate in tumors (SUCCinate Estimation by Spectroscopy (SUCCES)) relying on a monovoxel PRESS asymmetric « PROBE » sequence. This pilot study [12] performed in 9 patients with PPGL (5 with *SDHx* mutations and 4 sporadic cases) demonstrated that succinate accumulation was indeed detectable in the tumors of all *SDHx*-mutated patients but not in non-*SDHx* tumors. An unexplained choline peak was also observed in *SDHx*-mutated cases. These results supported the proof-of-concept that noninvasive detection of succinate using in vivo <sup>1</sup>H-MRS may constitute a specific biomarker of *SDHx* mutations. Following this first-in-man study, we here report the results of a prospective study aiming at evaluating the clinical relevance of <sup>1</sup>H-MRS SUCCES in the routine care of PPGL patients.

## Materials and methods

### Patients

Patients explored for PPGL in the hypertension unit of Hôpital Européen Georges Pompidou, Paris (France), were included

prospectively if they met the following criteria: (1) age  $\geq$  18 years old; (2) PPGL diagnosis in accordance with international clinical practice guidelines in a reference center, relying on biochemistry, cross-sectional, and functional imaging [13]; (3) signed informed consent for PPGL genetic testing; and (4) no contraindications to MRI (i.e., metallic implants, claustrophobia,...).

Ethical approval for the study was obtained from the institutional review board (Comité de Protection des Personnes (CPP) Ile de France II). All patients provided written informed consent for participation to the study, collection of samples, and subsequent analyses.

When a surgical procedure was performed, fresh tumor samples were frozen immediately after surgical resection and subsequently stored at  $-80^{\circ}\text{C}$  until processing following the COMETE collection procedures [14]. Histologic diagnosis was performed on paraffin-embedded formalin-fixed samples.

### Succinate detection using <sup>1</sup>H-MRS at 3 Tesla

Combined MR images and MR spectroscopic scans of patients were acquired in a 3 Tesla MRI clinical scanner (Discovery MR750w GEMSOW, GE Medical Systems, Milwaukee, WI), as previously described [12]. <sup>1</sup>H-MRS spectra were acquired using the optimized SUCCES sequence: asymmetric Point REsolved SpectroScopy (PRESS) monovoxel acquisition based on the PROBE monovoxel sequence [15], with TR: 2500 ms; TE: 144 ms; Nex: 512 (22 min acquisition) or 1024 (44 min acquisition).

Detection of tumors and VOI (Volume of Interest) positioning were performed on thin-section high-resolution T2-weighted fast spin-echo imaging in at least two orthogonal planes with the following parameters: TR: 2500 ms; TE: 85 ms; echo train length: 19; slice thickness: 2 mm; spacing: 0.3; field of view:  $14 \times 14$  cm for neck or  $42 \times 42$  cm for whole body coil; matrix:  $320 \times 320$  or on thin-section high-resolution balanced steady-state gradient echo sequence (*FIESTA*) in at least two orthogonal planes with the following parameters: TR: 4.9 ms; TE: 1.4 ms; NEX:1; Flip angle: 50; slice thickness: 3 mm; spacing: 1; field of view:  $14 \times 14$  cm for neck or  $42 \times 42$  cm for whole body coil; and matrix:  $256 \times 256$ .

### Immunohistochemistry

*SDHA* and *SDHB* protein expression were assessed on formalin-fixed paraffin embedded (FFPE) tumor samples by immunohistochemistry (IHC) as previously described [16–18] using the following antibodies and conditions: anti-*SDHA* (ab14715, Abcam; 1:1000) and anti-*SDHB* (HPA002868, Sigma-Aldrich Corp; 1:500).

## SDH activity

SDH activity was investigated, when necessary, on frozen tumor samples, homogenized using a 1-ml glass Potter-Elvehjem, using a spectrophotometric assay, as previously described [19].

## Genetic analysis

The DNA extracted from leukocytes was genotyped in all included patients, as well as the DNA extracted from frozen or FFPE-tumors when available. Genetic analyses were performed through next generation sequencing (NGS) using the “MASTR plus SDHv2” targeted panel (Multiplicom, Agilent technologies) as previously reported and in accordance with international guidelines [20, 21].

## Data analysis

Two independent investigators (CLL and ABE), blinded from clinical observations and genetic test results at the time of analyses, examined all spectra in a qualitative manner and scored for the presence or absence of a succinate peak at 2.44 ppm, lactate/lipid signal (0.9 to 1.3 ppm), and choline peak (3.2 ppm). When spectra interpretation was different between the two investigators, discordance(s) have been resolved by consensus by a panel of experts (CLL, ABE, JF, and BT). Tumor and VOI size as well as tumor heterogeneity were evaluated on the FIESTA or the T2-weighted fast spin-echo sequence.

<sup>1</sup>H-MRS data were initially compared with the genetic gold standard of SDH deficiency assessed using germline genetic testing. In cases of discrepancies between germline genetic test and <sup>1</sup>H-MRS results, a composite gold standard was applied by considering the results of tumor tissue analyses (somatic NGS, SDHB IHC, or SDH activity measurement in the tumor). Tumors were thus considered as SDH deficient when (i) an *SDHx* mutation was detected on germline and/or (ii) tumor DNAs, or (iii) when SDH inactivation was supported by both protein analyses of tissue samples (SDHB/A negative immunochemistry and loss of SDH activity).

Sensitivity (Se), specificity (Sp), positive/negative predictive values (PPV/NPV), and accuracy of the method were calculated using the SISA (Simple Interactive Statistical Analysis) procedure with continuity corrected Wilson 95% confidence intervals presentation.

## Results

### Patients

Fifty-six patients, including the 9 from the original pilot study [12], were prospectively enrolled between January 2015 and March 2019, leading to the exploration of 57 tumors with the

<sup>1</sup>H-MRS SUCCES sequence (one abdominal PGL and one head and neck PGL were explored in patient 1 (previously reported in the pilot study, Table S1) carrying an *SDHB* mutation [12]). Seven patients were excluded. One presented a surgical clip, one a non-evaluable highly necrotic tumor, and one diagnosed with a poorly differentiated neuroendocrine tumor instead of a PPGL following pathological analysis of the tumor and the “gold standard” data could not be obtained for four of them. Overall, 49 patients with 50 tumors were analyzed (Fig. 1) and their main characteristics are presented in Table 1. Median age at the time of <sup>1</sup>H-MRS examination was 44.7 years old [20–75]. The 50 tumors were located as follows: 24 head and neck PGL, 9 abdominal PGL, and 17 pheochromocytomas (PCC).

### <sup>1</sup>H-MRS SUCCES performance and added value for management of patients

A succinate peak at 2.44 ppm was observed in 20 tumors (Fig. 2), while a choline peak was detected in 27 tumors, including 17 with an associated succinate peak. Genetic status was known at the time of <sup>1</sup>H-MRS for 9 patients (18%). For the remaining 40 patients, genetic status was available for clinician after genetic counseling and NGS analyses of germline and somatic DNA in average 12.2 months ( $\pm 8.6$  months) after <sup>1</sup>H-MRS results.

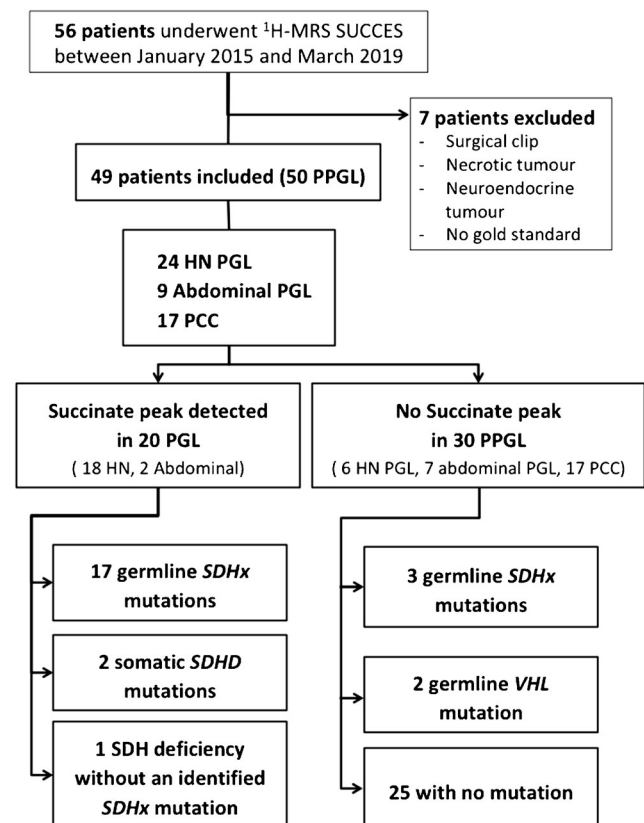


Fig. 1 Flowchart

**Table 1** Baseline characteristics of patients ( $N = 49$  patients, 50 tumors)

Age (years, median [range])	44.7 [20–75]
Gender	
Female (%)	25 (51)
Location (%)	
Head and neck PGL	24 (48)
Abdominal PGL	9 (18)
Pheochromocytoma	17 (34)
Outcome (%)	
Surgery	31 (62)
External Beam Therapy	8 (16)
Follow-up without treatment	8 (16)
Unknown	3 (6)

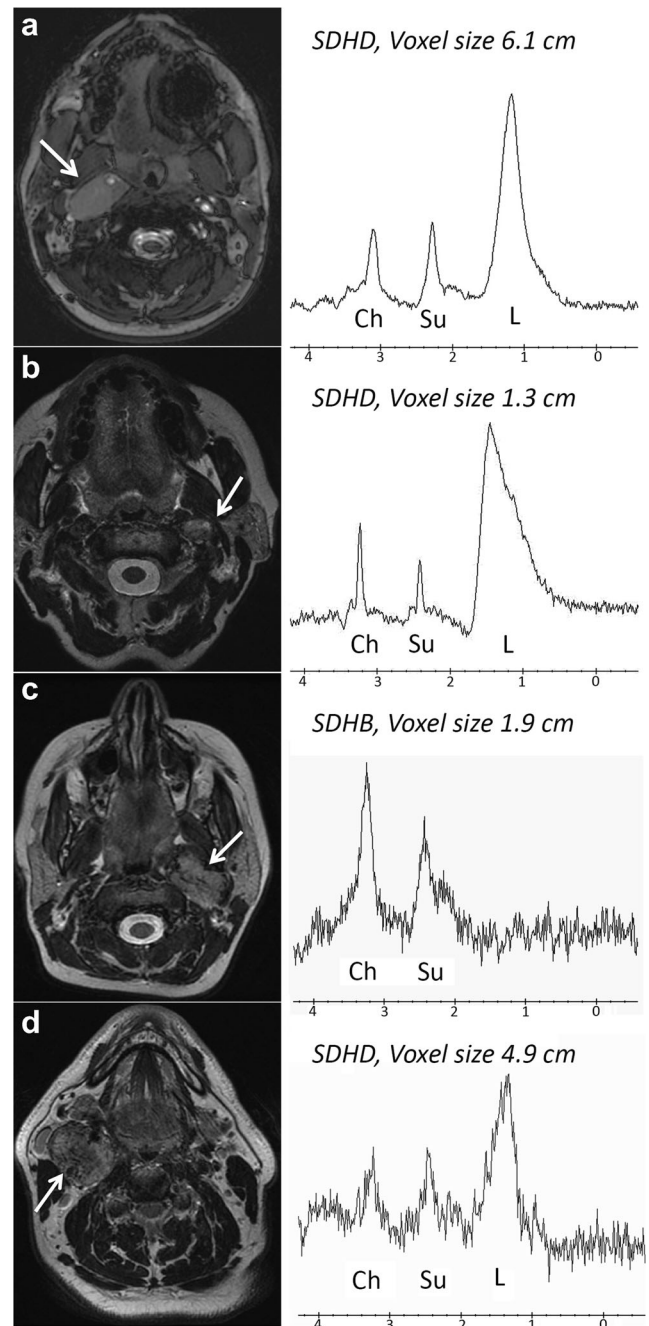
PGL, paraganglioma

Among the 19 patients (20 tumors) showing a tumor succinate accumulation, 16 (17 tumors) were confirmed to carry a germline *SDHx* mutation: 1 *SDHA*, 4 *SDHB*, 2 *SDHC*, and 9 *SDHD*.

In 3 patients,  $^1\text{H}$ -MRS SUCCES suggested SDH deficiency despite a negative genetic test. In 2 of them, NGS analysis of tumor DNA identified an *SDHD* somatic mutation (patients 20 and 21, Table S1). The third patient (patient 22, Table S1) was a 42-two year-old patient with a familial history PPGL (his father developed bilateral carotid body PGL diagnosed at 40 years old). The patient suffered from a right vagal PGL operated 10 years earlier and a left vagal PGL treated at the same period using external beam radiotherapy.  $^1\text{H}$ -MRS SUCCES was performed in a metastatic relapse of the left vagal PGL and showed a succinate peak. PPGL genetic testing was negative both in germline and in somatic DNA, but SDH deficiency was demonstrated by negative SDHB IHC and complete inhibition of SDH enzymatic activity in the tumor (Fig. 3). Hence, there was no false positive finding.

Thirty tumors (30 patients) showed no succinate accumulation detectable by  $^1\text{H}$ -MRS SUCCES. Gold standard confirmed the absence of SDH deficiency in 27 cases (Table S2). Two patients carried a germline mutation in the *VHL* gene, while germline genotyping was negative in 25 patients. In the latter cases, NGS identified 10 with somatic mutations (2 in *EPAS1*, 4 in *HRAS*, 3 in *VHL*, and 1 in *NF1* gene).

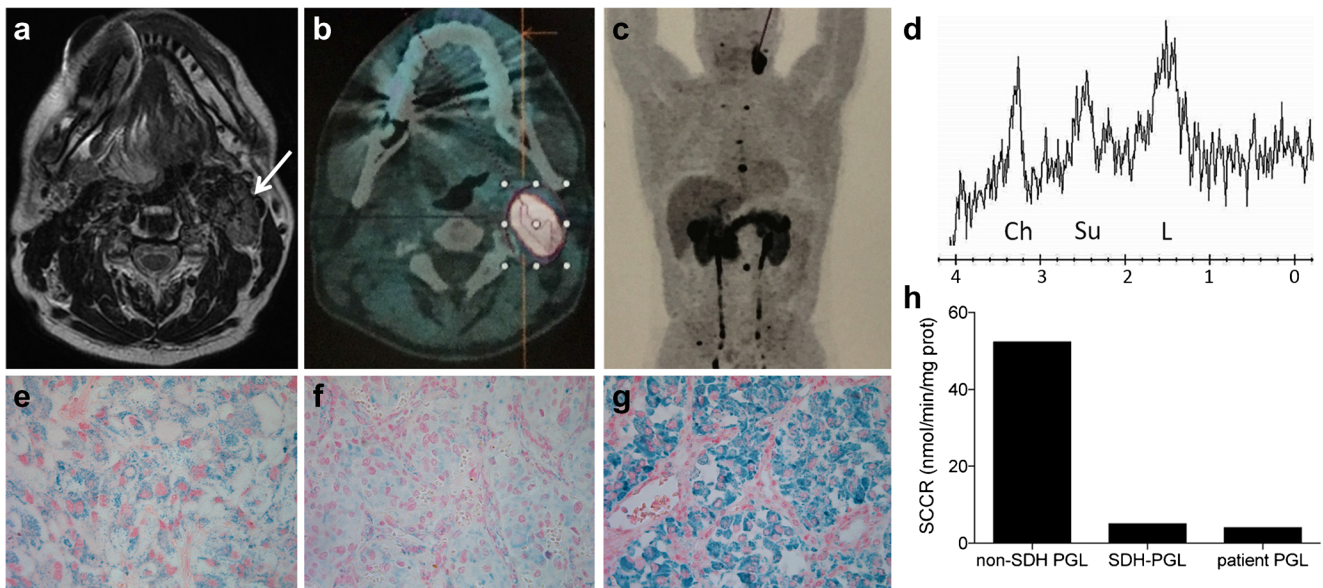
A false negative result (i.e., no succinate peak in a patient with an *SDHx* mutation) was observed in 3 tumors (Fig. 4). The first patient (*SDHB* mutated, patient 7) presented with a cervical PGL that included the carotid artery and the jugular vein, preventing the use of the  $^1\text{H}$ -MRS SUCCES which had to be performed on a very small metastatic lymph node (voxel size 0.7 cm), which may have been a limiting factor. The second patient (patient 9) carried a germline *SDHD* gene mutation and suffered from a right cervical PGL with numerous



**Fig. 2**  $^1\text{H}$ -MRS SUCCES in SDH-deficient tumors. A succinate peak is observed at 2.44 ppm in the head and neck paragangliomas from patients carrying germline *SDHD* (a: patient 11, b: patient 12, and d: patient 14) or *SDHB* (c: patient 15) mutations

hemorrhagic and necrotic spots (voxel size  $2.5\text{ cm}^3$ ). The third one was a 20-year-old patient (patient 6) followed in a familial context of germline *SDHD* gene mutation with a small left-sided PCC (voxel size  $1.5\text{ cm}^3$ ), for which respiratory motion may have compromised the result.

Discordance between the 2 investigators (CLL and ABE) were observed for 2 cases (patients 13 and 36, Tables S1 and S2) and resolved by consensus by the panel of experts.



**Fig. 3** Patient 22 with SDH deficiency demonstrated by functional studies. **a** Axial T2-weighted MRI of the left vagal PGL. **b** Maximal intensity projection of 18F-FDopa-PET/CT. **c** Axial fused maximal 18F-FDopa-PET/CT. **d**  $^1\text{H}$ -MRS spectrum showing a succinate (Su) peak at 2.44 ppm associated with a choline (Ch) and a lipids/lactate (L) peak. **e** SDHB immunohistochemistry in a non-SDH PGL shows a typical

mitochondrial granular staining while **f** the patient's tumor displays a weak diffuse SDHB staining frequently observed in *SDHD*-mutated PGL (ref) and **g** a strong SDHA positive labeling. **h** Analysis of succinate cytochrome C reductase (SCCR) activity in the patient's tumor compared with a non-SDH and an SDH-mutated PGL confirms loss of SDH activity

Altogether, at the lesion level, sensitivity, specificity, PPV, NPV, and accuracy of  $^1\text{H}$ -MRS SUCCES were respectively of 87% (Wilson CI 59.2–97.4), 100% (Wilson CI 79–99.8), 100% (Wilson CI 72.6–99.8), 90% (Wilson CI 66.8–98), and 94% (Wilson CI 78.5–98.8).

## Discussion

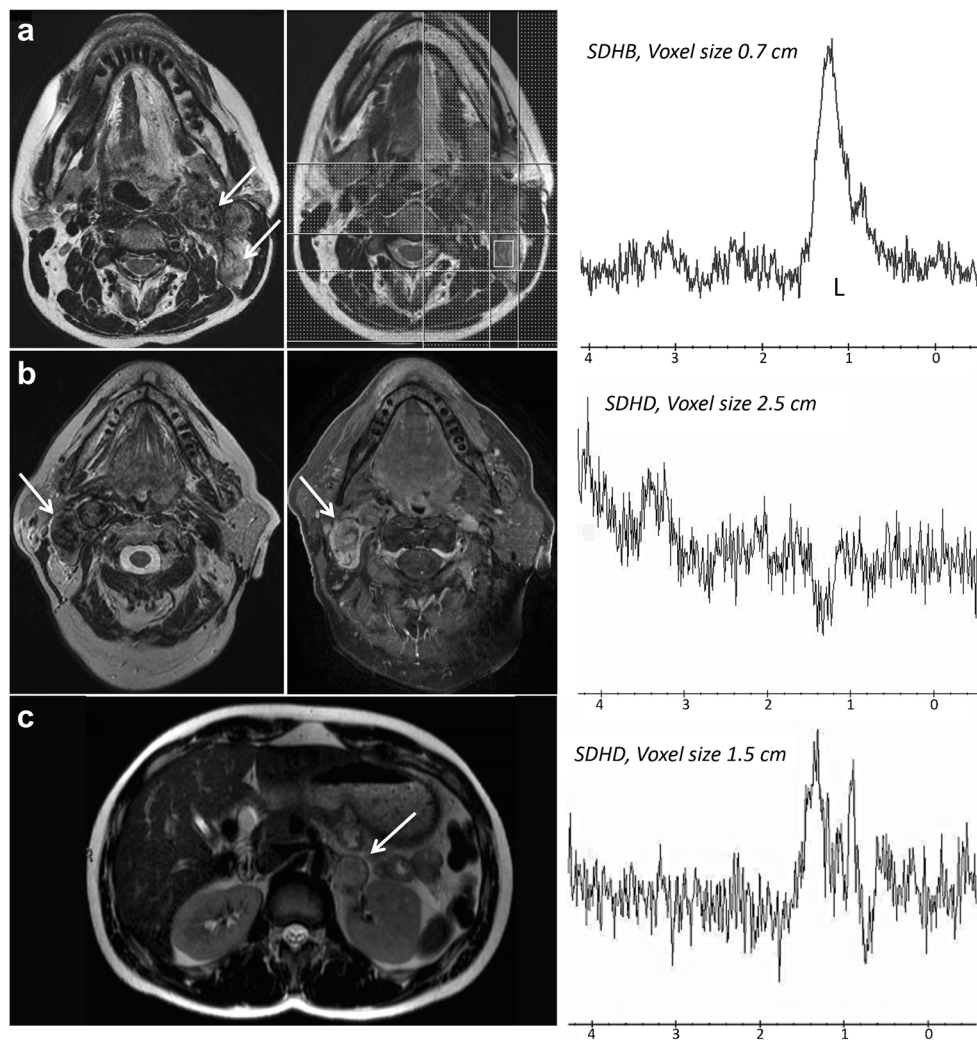
To our knowledge, this is the first prospective validation study demonstrating high sensitivity and specificity of non-invasive detection of succinate using in vivo  $^1\text{H}$ -MRS. Our results qualify the presence of a succinate peak at 2.44 ppm as a specific bona fide biomarker of SDH deficiency in patients suffering from PPGL, whatever the *SDHx* gene mutated (*SDHA*, *B*, *C*, and *D*).

We clearly show that  $^1\text{H}$ -MRS SUCCES allows early suspicion of *SDH* deficiency in routine clinical practice. Indeed, for almost 80% of patients, results of  $^1\text{H}$ -MRS SUCCES sequence were provided to clinicians before the results of genetic test. A recent multicentric retrospective study demonstrated that early knowledge of genetic status has a positive impact on the management and clinical outcome of patients with a germline *SDHx* mutation [1]. Identification of a succinate peak with  $^1\text{H}$ -MRS SUCCES sequence should therefore accelerate genetic testing in these patients, focusing and facilitating the interpretation of genetic variations in *SDHx* genes provided by NGS. In a patient with initial PPGL diagnosis, early assessment of *SDHx* mutational status may impact

patient's management, by taking into account the higher risk of metastasis of *SDHB*-related tumors, or the high rate of multiple cervical PGL for *SDHD*-mutated patients. Finally, early identification of patients with genetic predisposition will also accelerate the familial pre-symptomatic genetic screening and therefore a benefit for the relatives of index patients.

Importantly, we demonstrate that, in case of negative germline genotyping, the finding of a positive succinate peak using  $^1\text{H}$ -MRS SUCCES should definitively prompt to search for *SDHx* somatic mutations in tumor DNA. In our series, this approach led to the identification of 2 tumors with somatic *SDHD* variants, which have only been very rarely reported in the past [20, 22, 23]. It could also prompt to go deeper in the exploration of some patients like in patient 22, familial case presenting with an SDH deficiency despite the absence of germline and somatic *SDHx* variants. Several hypotheses such as an intronic mutation on germline DNA not detected by NGS analyses, epigenetic mutations in *SDHx* promoters, or mutation in a regulator gene of *SDHx* genes expression, are under evaluation to go further in this case by performing a whole genome sequencing.

Moreover, the identification of a succinate peak in a tumor is a major argument to support the pathogenicity of a variant of unknown significance (VUS) in an *SDHx* gene, which is essential to assess the risk of recurrence, to adapt the follow-up and to manage the genetic counseling to relatives [2, 20]. In our cohort, a VUS was detected in 3 patients and classified as deleterious or not (a likely benign VUS in *SDHA* was ruled out, and 2 VUS, 1 on *SDHB* (patient 1) and 1 on *SDHD* (patient 14) genes, were



**Fig. 4**  $^1\text{H}$ -MRS SUCCEs failures in SDH-deficient tumors. **a** Left panel shows both the cervical PGL including the carotid artery and the jugular vein and a small metastatic lymph node (arrows) developed by a 35-year-old patient carrying a germline *SDHB* gene mutation (patient 7). Positioning a small voxel of  $0.7\text{ cm}^3$  (middle panel) did not allow revealing a succinate peak (right). **b** T2 (left) and T1-weighted MRI

(middle) show a right cervical PGL with hemorrhagic and necrotic spots in a 68-year-old patient with a germline *SDHD* gene mutation (patient 9) and the absence of succinate peak. **c** Small left-sided PCC (voxel size  $1.5\text{ cm}^3$ ) in a 20 years old patient followed in a familial context of germline *SDHD* gene mutation (patient 6) without succinate peak

eventually classified as pathogenic), based on in silico predictions and *SDHB* IHC. In these 3 patients,  $^1\text{H}$ -MRS results were concordant with IHC results, suggesting that  $^1\text{H}$ -MRS SUCCEs is a useful contribution for patient management, especially whenever the absence of tumor tissue precludes immunohistochemical analyses. Assessing this tumor hallmark in vivo in patients with *SDHx*-related tumors will present benefits in other aspects. In a patient carrying a germline *SDHx* variant it could help: (i) to classify a suspicious lesion detected during follow-up and (ii) to confirm the metastatic development of the disease after the identification of one (or several) dubious lesion(s) in extra-paraganglionic sites on imaging follow-up. *SDHx* mutations also predispose to other types of tumors such as GIST (gastrointestinal stromal tumors), rare cases of renal cell carcinomas, and of pituitary adenomas, which are still debated [24–27]. Interestingly, in a recent study, the application of  $^1\text{H}$ -MRS was

successfully extended to metastasis of PPGL and GIST and permitted to exclude SDH deficiency in pituitary adenomas developed in an *SDHB* mutation carrier [28]. However, it is important to note that in our study, only 2 patients with an *SDHx* mutation were explored for an abdominal PGL. Additional studies should be performed in the future to evaluate  $^1\text{H}$ -MRS SUCCEs accuracy for this particular location.

Regarding clinical relevance, we suggest to perform  $^1\text{H}$ -MRS SUCCEs in all patients with a newly discovered PPGL, in patients with negative germline genetic testing in case of multiple tumors and/or a metastatic development of the disease to tailor therapeutic choices, and in patients already identified to carry a germline *SDHx* mutation in case of dubious finding on imaging follow-up. By contrast,  $^1\text{H}$ -MRS SUCCEs would not present any relevance in patients with a known mutation on a non-*SDHx* susceptibility gene.

**Table 2** Characteristics of the 23 tumors with an SDH deficiency according to <sup>1</sup>H-MRS SUCCES results

	Failures (FN)	Successes (RP)
N (%)		
✓ Patients	3/22 (14%)	19/22(86%)
✓ Tumors	3/23 (13%)	20/23 (87%)
Size (mean ± SD [range])		
✓ Tumor long axis (cm)	2.7 ± 0.4 [2.4–3.2]	4.6 ± 1.6 [2.3–8.7]
✓ Tumor volume (cm <sup>3</sup> )	6.7 ± 3.3 [3.4–10]	23.3 ± 23.5 [1.6–110]
✓ Voxel volume (cm <sup>3</sup> )	1.7 ± 0.9 [0.7–2.5]	4.6 ± 4.3 [1.2–19.2]
Location		
✓ H&N PGL	2	18
✓ Abdo PGL and PCC	1	2
Necrosis	2/3 (67%)	5/20 (25%)
SDHx mutation		
✓ SDHA	0	1
✓ SDHB	1	4
✓ SDHC	0	2
✓ SDHD	2	9
✓ Somatic SDHD	0	2
✓ Unknown	0	1
Averages		
✓ 512	1 (33%)	5/20 (25%)
✓ 1024	2 (67%)	15/20 (75%)

Imaging of patients with PPGL is crucial in every step of their management and functional imaging using PET tracers providing important information on tumor biology, highly connected with genetic status [29]. Recent development of PET/MR devices would allow to combine functional imaging and <sup>1</sup>H-MRS, providing simultaneously and non-invasively coregistered anatomical, metabolic (i.e., FDG uptake), and metabolomic (succinate accumulation) information on the tumor.

<sup>1</sup>H-MRS SUCCES allowed assessing for the presence (or absence) of a succinate peak in a qualitative manner. However, quantification of succinate in vivo with <sup>1</sup>H-MRS is feasible and development of a specific post-processing method allowing assessment of succinate as a quantitative biomarker over the time course of the disease would be of great interest especially for the evaluation of response to treatment.

It should also be stressed that SUCCES has the same limitations as any <sup>1</sup>H-MRS approach, in particular sensitivity in the low millimolar range. Accordingly, its capacity to detect succinate in PPGL lesions is dependent on: (i) the size of the tumor, which is directly related to detection sensitivity: a voxel size smaller than 1 cm<sup>3</sup> is associated with a high risk of failure (Table 2) and (ii) the presence of hemorrhagic or necrotic spots, which are sources of technical flaws: a large necrotic area or inclusion of blood vessel within a tumor may discourage exploration by <sup>1</sup>H-MRS SUCCES (Fig. 4). Nevertheless, there is still room for progress and in this respect, several adjustments may improve sensitivity and decrease the sequence duration. These include increasing MRI field using the new research-dedicated 7-

tesla devices, post-processing of the spectra, or respiratory gating for abdominal tumours. Indeed, respiratory gating would be a possible adjustment for abdominal tumors and in particular for PCC but with an increase in total duration of the sequence, a risk of patient discomfort, and then motion. Nevertheless, one solution could be to analyze respiratory-gated acquisition combined with an increased MRI field (7 tesla) that could allow reducing average number and thus sequence duration.

In conclusion, using <sup>1</sup>H-MRS to detect specific tumor phenotypes induced by genetic mutations is innocuous (no tissue sampling, no injection of radiopharmaceutical or contrast agent are needed) and precise. Following the initial pilot studies [12, 28, 30], this study crosses the second translational gap and demonstrates that <sup>1</sup>H-MRS SUCCES can be performed in a radiology department during routine clinical practice. <sup>1</sup>H-MRS improves genetic diagnosis, providing VUS characterization, and guiding geneticists to extend investigations to somatic NGS. <sup>1</sup>H-MRS SUCCES is ideal for assessing the presence of succinate repeatedly over the time course of the disease, for clinical surveillance, postoperative follow-up, and evaluation of treatment efficacy.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** Ethical approval for the study was obtained from the institutional review board (Comité de Protection des Personnes (CPP) Ile de France II).

**Informed consent** All patients provided written informed consent for participation to the study, collection of samples, and subsequent analyses.

## References

1. Buffet A, Ben Aim L, Leboulleux S, Drui D, Vezzosi D, Libe R, et al. Positive impact of genetic test on the management and outcome of patients with paraganglioma and/or pheochromocytoma. *J Clin Endocrinol Metab.* 2019;104(4):1109–18. <https://doi.org/10.1210/clinem.2018-02411>.

2. Favier J, Amar L, Gimenez-Roqueplo AP. Paranglioma and pheochromocytoma: from genetics to personalized medicine. *Nat Rev Endocrinol*. 2015;11(2):101–11. <https://doi.org/10.1038/nrendo.2014.188>.
3. Amar L, Baudin E, Burnichon N, Peyrard S, Silvera S, Bertherat J, et al. Succinate dehydrogenase B gene mutations predict survival in patients with malignant pheochromocytomas or paragangliomas. *J Clin Endocrinol Metab*. 2007;92(10):3822–8. <https://doi.org/10.1210/jc.2007-0709>.
4. Gimenez-Roqueplo AP, Favier J, Rustin P, Rieubland C, Crespin M, Nau V, et al. Mutations in the SDHB gene are associated with extra-adrenal and/or malignant pheochromocytomas. *Cancer Res*. 2003;63(17):5615–21.
5. Favier J, Briere JJ, Burnichon N, Riviere J, Vescovo L, Benit P, et al. The Warburg effect is genetically determined in inherited pheochromocytomas. *PLoS One*. 2009;4(9):e7094. <https://doi.org/10.1371/journal.pone.0007094>.
6. Letouze E, Martinelli C, Loriot C, Burnichon N, Abermil N, Ottolenghi C, et al. SDH mutations establish a hypermethylator phenotype in paraganglioma. *Cancer Cell*. 2013;23(6):739–52. <https://doi.org/10.1016/j.ccr.2013.04.018>.
7. Morin A, Letouze E, Gimenez-Roqueplo AP, Favier J. Oncometabolites-driven tumorigenesis: from genetics to targeted therapy. *Int J Cancer*. 2014;135(10):2237–48. <https://doi.org/10.1002/ijc.29080>.
8. Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, et al. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF- $\alpha$  prolyl hydroxylase. *Cancer Cell*. 2005;7(1):77–85. <https://doi.org/10.1016/j.ccr.2004.11.022>.
9. Pollard PJ, Briere JJ, Alam NA, Barwell J, Barclay E, Wortham NC, et al. Accumulation of Krebs cycle intermediates and over-expression of HIF1 $\alpha$  in tumours which result from germline FH and SDH mutations. *Hum Mol Genet*. 2005;14(15):2231–9. <https://doi.org/10.1093/hmg/ddi227>.
10. Richter S, Peitzsch M, Rapizzi E, Lenders JW, Qin N, de Cubas AA, et al. Krebs cycle metabolite profiling for identification and stratification of pheochromocytomas/paragangliomas due to succinate dehydrogenase deficiency. *J Clin Endocrinol Metab*. 2014;99(10):3903–11. <https://doi.org/10.1210/jc.2014-2151>.
11. Rao JU, Engelke UF, Rodenburg RJ, Wevers RA, Pacak K, Eisenhofer G, et al. Genotype-specific abnormalities in mitochondrial function associate with distinct profiles of energy metabolism and catecholamine content in pheochromocytoma and paraganglioma. *Clin Cancer Res*. 2013;19(14):3787–95. <https://doi.org/10.1158/1078-0432.CCR-12-3922>.
12. Lussey-Lepoutre C, Bellucci A, Morin A, Buffet A, Amar L, Janin M, et al. In vivo detection of succinate by magnetic resonance spectroscopy as a Hallmark of SDHx mutations in paraganglioma. *Clin Cancer Res*. 2016;22(5):1120–9. <https://doi.org/10.1158/1078-0432.CCR-15-1576>.
13. Lenders JW, Duh QY, Eisenhofer G, Gimenez-Roqueplo AP, Grebe SK, Murad MH, et al. Pheochromocytoma and paraganglioma: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2014;99(6):1915–42. <https://doi.org/10.1210/jc.2014-1498>.
14. Plouin PF, Gimenez-Roqueplo AP, Bertagna X. COMETE, a network for the study and management of hypersecreting adrenal tumors. *Bull Acad Natl Med*. 2008;192(1):73–82 discussion 3-5.
15. Webb PG, Sailasuta N, Kohler SJ, Raidy T, Moats RA, Hurd RE. Automated single-voxel proton MRS: technical development and multisite verification. *Magn Reson Med*. 1994;31(4):365–73.
16. Korpershoek E, Favier J, Gaal J, Burnichon N, van Gessel B, Oudijk L, et al. SDHA immunohistochemistry detects germline SDHA gene mutations in apparently sporadic paragangliomas and pheochromocytomas. *J Clin Endocrinol Metab*. 2011;96(9):E1472–6. <https://doi.org/10.1210/jc.2011-1043>.
17. Menara M, Oudijk L, Badoual C, Bertherat J, Lepoutre-Lussey C, Amar L, et al. SDHD immunohistochemistry: a new tool to validate SDHx mutations in pheochromocytoma/paraganglioma. *J Clin Endocrinol Metab*. 2015;100(2):E287–91. <https://doi.org/10.1210/jc.2014-1870>.
18. van Nederveen FH, Gaal J, Favier J, Korpershoek E, Oldenburg RA, de Bruyn EM, et al. An immunohistochemical procedure to detect patients with paraganglioma and pheochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. *Lancet Oncol*. 2009;10(8):764–71. [https://doi.org/10.1016/S1470-2045\(09\)70164-0](https://doi.org/10.1016/S1470-2045(09)70164-0).
19. Benit P, Goncalves S, Philippe Dassa E, Briere JJ, Martin G, Rustin P. Three spectrophotometric assays for the measurement of the five respiratory chain complexes in minuscule biological samples. *Clin Chim Acta*. 2006;374(1–2):81–6. <https://doi.org/10.1016/j.cca.2006.05.034>.
20. Ben Aim L, Pigny P, Castro-Vega LJ, Buffet A, Amar L, Bertherat J, et al. Targeted next-generation sequencing detects rare genetic events in pheochromocytoma and paraganglioma. *J Med Genet*. 2019. <https://doi.org/10.1136/jmedgenet-2018-105714>.
21. Group NGSiPS, Toledo RA, Burnichon N, Cascon A, Benn DE, Bayley JP, et al. Consensus statement on next-generation-sequencing-based diagnostic testing of hereditary pheochromocytomas and paragangliomas. *Nat Rev Endocrinol*. 2017;13(4):233–47. <https://doi.org/10.1038/nrendo.2016.185>.
22. Curras-Freixes M, Inglada-Perez L, Mancikova V, Montero-Conde C, Leton R, Comino-Mendez I, et al. Recommendations for somatic and germline genetic testing of single pheochromocytoma and paraganglioma based on findings from a series of 329 patients. *J Med Genet*. 2015;52(10):647–56. <https://doi.org/10.1136/jmedgenet-2015-103218>.
23. van Nederveen FH, Korpershoek E, Lenders JW, de Krijger RR, Dinjens WN. Somatic SDHB mutation in an extraadrenal pheochromocytoma. *N Engl J Med*. 2007;357(3):306–8. <https://doi.org/10.1056/NEJMc070010>.
24. Boikos SA, Pappo AS, Killian JK, LaQuaglia MP, Weldon CB, George S, et al. Molecular subtypes of KIT/PDGFR $\alpha$  wild-type gastrointestinal stromal tumors: a report from the National Institutes of Health Gastrointestinal Stromal Tumor Clinic. *JAMA Oncol*. 2016;2(7):922–8. <https://doi.org/10.1001/jamaoncol.2016.0256>.
25. Evenepoel L, Papatomas TG, Krol N, Korpershoek E, de Krijger RR, Persu A, et al. Toward an improved definition of the genetic and tumor spectrum associated with SDH germ-line mutations. *Genet Med*. 2015;17(8):610–20. <https://doi.org/10.1038/gim.2014.162>.
26. Mason EF, Hornick JL. Conventional risk stratification fails to predict progression of succinate dehydrogenase-deficient gastrointestinal stromal tumors: a clinicopathologic study of 76 cases. *Am J Surg Pathol*. 2016;40(12):1616–21. <https://doi.org/10.1097/PAS.0000000000000685>.
27. Ricketts C, Woodward ER, Killick P, Morris MR, Astuti D, Latif F, et al. Germline SDHB mutations and familial renal cell carcinoma. *J Natl Cancer Inst*. 2008;100(17):1260–2. <https://doi.org/10.1093/jnci/djn254>.
28. Casey RT, McLean MA, Madhu B, Challis BG, ten Hoopen R, Roberts T, et al. Translating in vivo metabolomic analysis of succinate dehydrogenase deficient tumors into clinical utility. *JCO Precis Oncol*. 2019;2:1–12. <https://doi.org/10.1200/PO.17.00191>.
29. Taieb D, Jha A, Treglia G, Pacak K. Molecular imaging and radionuclide therapy of paraganglioma and pheochromocytoma. *Endocr Relat Cancer*. 2019. <https://doi.org/10.1530/ERC-19-0165>.
30. Varoquaux A, le Fur Y, Imperiale A, Reyre A, Montava M, Fakhry N, et al. Magnetic resonance spectroscopy of paragangliomas: new insights into in vivo metabolomics. *Endocr Relat Cancer*. 2015;22(4):M1–8. <https://doi.org/10.1530/ERC-15-0246>.

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