

1 **Translating *in vivo* metabolomic analysis of succinate dehydrogenase deficient tumours**
2 **into clinical utility**

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64 **Abstract**

65 *Purpose:* Mutations in the mitochondrial enzyme succinate dehydrogenase (SDH) subunit
66 genes are associated with a wide spectrum of tumours including pheochromocytoma and
67 paraganglioma (PPGL) ^{1,2}, gastrointestinal stromal tumours (GIST) ³, renal cell carcinoma
68 (RCC) ⁴ and pituitary adenomas⁵. SDH-related tumorigenesis is believed to be secondary to
69 accumulation of the oncometabolite succinate. Our aim was to investigate the potential
70 clinical applications of MRI spectroscopy (¹H-MRS) in a range of suspected SDH-related
71 tumours.

72 *Patients and methods:* Fifteen patients were recruited to this study. Respiratory-gated single-
73 voxel ¹H-MRS was performed at 3T to quantify the content of succinate at 2.4 ppm and
74 choline at 3.22 ppm.

75 *Results:* A succinate peak was seen in six patients, all of whom had a germline *SDHx*
76 mutation or loss of SDHB by immunohistochemistry. A succinate peak was also detected in
77 two patients with a metastatic wild-type GIST (wtGIST) and no detectable germline *SDHx*
78 mutation but a somatic epimutation in *SDHC*. Three patients without a tumour succinate peak
79 retained SDHB expression, consistent with SDH functionality. In six cases with a borderline
80 or absent peak, technical difficulties such as motion artefact rendered ¹H-MRS difficult to
81 interpret. Sequential imaging in a patient with a metastatic abdominal paraganglioma
82 demonstrated loss of the succinate peak after four cycles of [¹⁷⁷Lu]-DOTATATE, with a
83 corresponding biochemical response in normetanephrine.

84 *Conclusions:* This study has demonstrated the translation into clinical practice of *in vivo*
85 metabolomic analysis using ¹H-MRS in patients with SDH-deficient tumours. Potential
86 applications include non-invasive diagnosis and disease stratification, as well as monitoring
87 of tumour response to targeted treatments.

88 Introduction

89 The succinate dehydrogenase (SDH) enzyme is composed of four subunits (A-D) and has a
90 key role in the Krebs cycle and oxidative phosphorylation⁶. In the past two decades germline
91 mutations in the genes encoding the four SDH subunits (*SDHA/SDHB/SDHC/SDHD*),
92 collectively known as *SDHx* have emerged as an important cause of human neoplasia and a
93 paradigm for the role of disordered cellular metabolism in oncogenesis^{1-5,7}. *SDHx* mutations
94 were described initially in association with head and neck paragangliomas (derived from
95 parasympathetic ganglia) and in pheochromocytomas and paragangliomas (PPGL, derived
96 from sympathetic ganglia and often secreting catecholamines)^{1,2}. It is now recognised that
97 approximately 40% of PPGL patients harbour a germline mutation in an inherited PPGL gene
98 and *SDHx* mutations are the most common cause of PPGL predisposition⁹. In addition,
99 germline *SDHB* mutations are associated with a high risk of malignancy in PPGL⁹. Other
100 tumour types associated with *SDHx* mutations include gastrointestinal stromal tumours
101 (GISTs) and renal cell carcinomas (RCCs)¹⁰⁻¹³. GISTs are mesenchymal tumours of the
102 gastrointestinal tract and in adults usually associated with somatic activating mutations in the
103 *KIT* or *PDGFRA* genes^{3,11}. However GISTs without *KIT* and *PDGFRA* gene mutations³,
104 known as wild-type (wtGIST), account for 15% of adult and 85% of paediatric GIST tumours
105 and recent studies suggest that up to 88% of wtGIST are SDH-deficient¹¹. wtGIST with
106 SDH-deficiency may harbour a germline *SDHx* mutation (75% of cases) or an *SDHC* gene
107 epimutation with hypermethylation of the promoter region¹¹. Only about a third of patients
108 with SDH-deficient wtGIST achieve disease stabilisation with imatinib therapy¹² and the risk
109 of metastatic disease is higher for SDH-deficient GIST compared to conventional GIST^{11,12}.
110 *SDHx*-associated RCC may present in patients with a personal or family history of PPGL or
111 may present with an RCC-only phenotype¹³. Finally germline *SDHx* mutations have been
112 described in rare patients with pituitary adenomas¹⁰. Despite recent advances in the

113 understanding of the *SDHx* genes, there are many areas of unmet clinical need including a
114 lack of robust biomarkers to predict aggressive biological behaviour and to inform on clinical
115 surveillance and management¹⁴.
116 Succinate has been shown to be elevated by 100-fold in *SDHx*-mutated PPGL tumours *ex-*
117 *vivo* compared with non-*SDHx* mutated PPGL tumours¹⁵. Recently, *in vivo* detection of
118 succinate by MR spectroscopy was reported in two patient cohorts with SDH deficient
119 PPGL^{16, 17}. Similarly, the non-invasive detection of 2-hydroxyglutarate with ¹H-MRS has
120 been demonstrated in glioma in patients with a gain of function mutation in another citric
121 acid cycle enzyme, isocitrate dehydrogenase 1 (IDH1)¹⁸. The ability to measure succinate *in*
122 *vivo* has a number of important potential clinical applications including early identification of
123 SDH deficiency, which can enable tailored patient surveillance and management. *In vivo*
124 detection of succinate accumulation could also serve to verify genetic variant pathogenicity in
125 the era of next generation sequencing. The aim of this study was to investigate the role of ¹H-
126 MRS in detecting abnormally elevated succinate *in vivo* in patients with suspected SDH
127 deficient tumours, expanding the applications of ¹H-MRS in SDH deficient tumorigenesis to
128 include GIST and pituitary adenoma for the first time and to explore the technique as a
129 potential non-invasive biomarker of treatment response.

130

131 **Methods**

132 Patient selection

133 This study was performed as a prospective case series and subjects were recruited from a
134 dedicated neuroendocrine tumour clinic and a national paediatric and adult wild-type
135 (PAWS) GIST clinic in Cambridge University NHS Foundation Trust. Suitable patients were
136 identified based on *SDHx* germline status, suspicious clinical phenotype (metastatic PPGL,

137 paraganglioma or wtGIST) and/or immunohistochemistry of tumour tissue showing absent
138 SDHB immunostaining. A minimum tumour size threshold of 1.5cm was applied for
139 inclusion into the study. All participants gave written informed consent and the study was
140 approved by Cambridge South Research Ethics Committee.

141

142 MRS Analysis

143 Both SAGE (GE Healthcare, Waukesha, WI) and LCModel¹⁹ spectroscopy analysis
144 programmes were used to reconstruct, analyze and display spectra. For each metabolite,
145 LCModel reports both peak area and the estimated uncertainty in fitting of the peak (%SD).
146 This uncertainty measure was used to stratify the results using the following algorithm: 1) if
147 %SD of choline was >15%, the spectrum was discarded as a technical failure, because it was
148 assumed that choline should be detectable in a metabolically active tumour, such that
149 SD>15% would indicate probable data quality issues; 2) succinate detection was taken as
150 positive if its %SD was <50%, and negative if it was >50%. The succinate to choline ratio
151 was quantified (SCR), the full width at half maximum height (FWHM) of the water peak in
152 Hz was measured in SAGE and recorded as an additional data quality metric, and an expert
153 spectroscopist was asked to rate whether detected succinate peaks were convincing or
154 unconvincing based on data displayed both in LCModel and in SAGE.

155

156 Statistical methods, ¹H-MRS data acquisition, Germline genetic analysis, SDHB

157 Immunohistochemistry, SDHC hypermethylation analysis and measurement of succinate in ex 158 vivo tissue samples

159 See supplementary data.

160

161 **Results**

162 Patients and clinical phenotype

163 Fifteen subjects (6 females, 9 males; mean age 40 years (range 21-80 years) were studied.
164 Seven wtGIST, three unilateral adrenal pheochromocytomas, three abdominal PGL's, a
165 large left glomus PGL and a non-functioning pituitary macroadenoma were examined. Nine
166 patients (60%) had metastatic disease: six with wtGIST, two with an abdominal
167 paraganglioma and one with a unilateral pheochromocytoma. The liver was the most
168 common site for metastases (7/9, 77.7%). Three patients had multicentric primary tumours,
169 including subject #5 who presented with a metastatic wtGIST and was subsequently
170 diagnosed with a 1.9 cm carotid body PGL (figure 2d, case 5), subject #9 with an abdominal
171 paraganglioma and a small left sided 1.5 cm carotid paraganglioma (figure 3b, case 9), and
172 subject #8 with a large left sided glomus paraganglioma and a 2 cm prolactin secreting
173 pituitary adenoma (figure S1, case 8). Only two patients had a positive family history, (Table
174 1: case 2 and case 6).

175 Genotype

176 A germline mutation in a *SDHx* gene was identified in 9/15 (60%) of subjects: 5 in *SDHB* (4
177 missense variants and 1 truncating variant) and 4 in *SDHA* (1 missense and 3 truncating).
178 Two further patients were diagnosed with a somatic *SDHC* epimutation (Table 1).

179

180 ¹H-MRS succinate analysis

181 The ¹H-MRS characteristics of the 15 patients are shown in Supplementary Table S1. The
182 mean size of the tumour selected for spectra acquisition was 5.5 cm (median: 3.3 cm, range:
183 1.8-12 cm). The liver was the most common site to be assessed (n = 6), but good quality

184 spectra were also obtained from the pituitary (n = 1), and PPGL tumours (n = 5). The subjects
185 were divided into four groups according to whether a succinate tumour peak was: present,
186 absent, a borderline peak was detected, or technical failure prevented interpretation of the
187 spectra.

188

189 Succinate peak detected

190 Succinate was detected at 2.4 ppm in 6 patients (50 %). The mean SCR in these patients was
191 1.3 (SD \pm 0.71) and the mean tumour size in those six patients with reliable succinate peak
192 detection was 4.8 cm (SD \pm 2.94, range 2.3-9 cm). The *in vivo* detection of succinate on ¹H-
193 MRS correlated with tumour SDH deficiency: 4 of the 6 cases had a germline *SDHx* mutation
194 and loss of SDHB expression on immunohistochemistry and a somatic *SDHC* epimutation
195 was detected in 2 of the 6 (Figure 1).

196

197

198 Borderline succinate peak detected

199 A borderline succinate peak was detected in two subjects. Patient #8 with a germline *SDHB*
200 mutation (c.600G>T p.Trp200Cys) and a glomus paraganglioma, demonstrated an SCR of
201 1.19; however the linewidth (29 Hz) was so broad due to the proximity of metallic dental
202 work that the peak assignments were not reliable (Figure S1). Patient #7 with a metastatic
203 pheochromocytoma and no detectable germline *SDHx* mutation demonstrated an SCR of
204 0.18 but the LCModel detected a very small succinate peak at 2.4 ppm; this patient did not
205 undergo surgery or a diagnostic biopsy and therefore no tissue was available for further
206 analysis and therefore we have classified this case as borderline.

207 No succinate peak

208 No succinate peak was detected in three subjects. Patient #4 had a metastatic wtGIST with no
209 detectable germline *SDHx* mutation and preserved SDHB protein expression in the tumour
210 tissue; choline was confidently fitted on LCModel but no succinate was seen. Patient #6
211 demonstrated a good quality spectrum from the remnant pituitary adenoma; choline was
212 detected on LCModel and SAGE processing but no succinate was detected and this finding
213 was consistent with the preservation of SDHB protein expression in the pituitary tumour by
214 immunohistochemistry (Figure 4). Patient #10 had no detectable germline *SDHx* mutation
215 and preserved SDHB protein expression in the tumour tissue; choline was detected in the
216 tumour on ¹H-MRS but succinate was not detected.

217

218

219 Technical failure

220 Technical failure occurred in four patients (26%). Patient #12 demonstrated no reliable
221 detection of succinate or choline due to motion artefact and a low signal-to-noise ratio (SNR),
222 which was probably due to inconsistent breathing as the voxel was at the edge of the liver. A
223 small rib metastasis was imaged in patient #13 but only a pure lipid spectrum was obtained
224 from this challenging location. A metastasis on the edge of the liver was imaged in patient
225 #14, where again inconsistent respiration probably led to displacement of the voxel into
226 adjacent adipose tissue. Finally, patient #15 had a unilateral pheochromocytoma with a large
227 volume of blood, whose paramagnetic properties may have affected acquisition leading to
228 low SNR (Supplementary Table S1).

229

230 Sequential ¹H-MRS succinate analysis

231 Subject #2 with a metastatic paraganglioma to the lung, bone and lymph node and a germline
232 *SDHB* mutation (c.268C>T p.Arg90*) underwent ¹H-MRS on a large pelvic nodal metastasis
233 prior to treatment with four cycles of lutetium 177-labelled peptide receptor radionuclide
234 therapy. Succinate and choline peaks were detected with an SCR of 1.32 (Figures 5a, 5b).
235 Following four cycles of treatment, a repeat ¹H-MRS examination on the same pelvic nodal
236 metastases revealed a choline peak but no succinate peak (Figure 5c). Though the MRI
237 imaging features of the metastatic lesions were unchanged pre- and post-treatment, the loss of
238 a succinate peak was correlated with a reduction in plasma normetanephrine levels (from
239 1861 to 1193 pmol/L) and tumour avidity on ¹⁸F-fluorodeoxyglucose Positron Emission
240 Tomography/Computed Tomography (FDG-PET/CT; standard uptake value of 16.1 pre-
241 treatment and 9.3 post-treatment; Figure 5d-f). The detection of choline on the acquired
242 spectra both before and after treatment indicates that tumour necrosis is unlikely to account
243 for the absent succinate peak post treatment.

244 A sequential ¹H-MRS study was performed on patient #5 due to evidence of progressive
245 disease on surveillance CT, despite treatment with a multi-kinase inhibitor, regorafenib.
246 Serial ¹H-MRS demonstrated a larger succinate peak compared to the first study (Figure 2d
247 and 2e) and this correlated with the FDG avidity on PET/CT pre-treatment and ten months
248 post-treatment, which demonstrated an increase in disease burden and avidity (SUV: 15.1 and
249 27.1 respectively, Figures 2f-g).

250 Repeatability of ¹H-MRS was evaluated in two patients by investigating different tumour
251 deposits during the same study examination (case#5) and the same tumour deposit twice
252 during the same study examination (case#1). The results for succinate: choline were almost
253 identical in these two cases, suggesting good test reproducibility (Supplementary Table 2)

254

255 **Discussion**

256 This proof-of-principle study has demonstrated that detection of a succinate peak and an
257 increased succinate to choline ratio were specific for a variety of SDH-deficient tumour
258 types. All six tumours with a positive succinate peak and elevated SCR were associated with
259 a germline *SDHx* mutation (n = 4) or an *SDHC* epimutation (n = 2). In addition, the three
260 subjects with absent succinate peaks but adequate ¹H-MRS, demonstrated preservation of
261 SDHB expression in the tumour analyzed. Our findings are complementary to a previous
262 study in which ¹H-MRS was applied to 9 patients with paraganglioma and a succinate peak
263 was detected in all 5 with an *SDHx* mutation but not in the 4 patients without a mutation¹⁶.
264 We have demonstrated for the first time that ¹H-MRS can also be used to determine the SDH
265 status of GISTs and pituitary adenomas and that a succinate peak can be detected in SDH-
266 deficient tumours with epigenetic inactivation of *SDHC*. There are a wide variety of
267 situations in which ¹H-MRS might have clinical utility. Potential diagnostic applications of
268 this new approach include: (a) assessing the pathogenicity of patients with a germline SDHx
269 variants of uncertain significance and a potentially SDH-related tumour; (b) investigating
270 possible metastatic lesions e.g. in the liver, in patients with a germline *SDHx* mutation and a
271 primary SDH-deficient tumour; (c) assessing patients with multiple primary tumours to
272 determine if all are SDH-deficient; (d) identifying patients without a detectable germline
273 *SDHx* mutation who might benefit from specialist genetic investigations such as *SDHC*
274 promoter methylation status; and (e) assessing SDH tumour status pre-operatively
275 particularly for patients with possible wtGIST as standard adjuvant treatment with imatinib
276 has proven to be less effective in patients with SDH-deficient disease¹².
277 Notably, here we have used the presence of a choline signal as an internal control for viable
278 tissue to discriminate technical failures from a negative finding. To avoid issues of partial
279 voluming effects within smaller tumours, the voxel for MRS analysis was chosen to fully

280 include tumour where possible. We did not detect a statistically significant correlation
281 between tumour size and succinate/choline ratio although there was a trend towards
282 significance. This trend is the opposite of what would be expected if necrosis was artificially
283 lowering the overall succinate levels in large tumours, and therefore suggests that the method
284 is measuring real differences in succinate, which are independent of tumour size. However,
285 we recommend using a size threshold of greater than 2 cm where possible to improve the
286 sensitivity of the test.

287 There is increasing interest in understanding the metabolic adaptations that occur during
288 tumorigenesis and how these might be exploited for novel therapeutic interventions.
289 Increased production of lactate during aerobic glycolysis in most cancers, or the Warburg
290 effect, is the best known example of this. SDH-related cancers provides a paradigm for
291 investigating tumour metabolism as succinate is thought to act as an oncometabolite and to
292 drive tumorigenesis⁶. Succinate inhibits 2-oxoglutarate-dependent dioxygenases including
293 DNA and histone demethylases and hypoxic gene response regulators. As a consequence,
294 SDH-deficient tumours demonstrate epigenetic abnormalities, an activated hypoxic gene
295 response and more recently there is evidence that succinate may have a paracrine effect on
296 stromal tissue^{20, 21, 22}. Understanding the molecular mechanisms of SDH-related
297 tumorigenesis provides a rationale for novel therapeutic interventions such as reversing the
298 epigenetic abnormalities or exploiting metabolic vulnerabilities, similar to the recent
299 discovery that tumoral 2-hydroxyglutarate accumulation may increase responsiveness to
300 olaparib, a poly-ADP-ribose polymerase (PARP) inhibitor²³. The availability of sensitive
301 non-invasive biomarkers would greatly facilitate precision medicine-based clinical trials.
302 Imaging with ¹⁸F-FDG PET to measure the uptake and phosphorylation of a glucose-
303 analogue to probe the increased glucose utilisation that occurs in many metabolically-active
304 cancers, is a useful form of *in vivo* metabolic imaging and has been employed for the

305 detection of primary and metastatic disease in many tumour types including PPGL and
306 GIST^{24, 25} and is in widespread clinical use. However, despite being a very sensitive imaging
307 tool, ¹⁸F-FDG PET lacks specificity and cannot differentiate individual metabolites. ¹H-MRS
308 is highly specific and allows *in vivo* detection of individual metabolites without the use of
309 ionising radiation, however, ¹H-MRS is significantly less sensitive than PET, which could
310 limit the detection of low levels of succinate and it can be challenging to differentiate
311 intracellular from extracellular metabolites. In the future, ¹H-MRS may be complemented by
312 other techniques such as hyperpolarised ¹³C-MR spectroscopic imaging, which can increase
313 MR signal-to-noise by several orders of magnitude allowing assessment of enzyme flux *in*
314 *vivo*²⁶.

315

316 We have shown that ¹H-MRS could be a valuable tool for the assessment of tumour response
317 in the context of radionuclide and other therapies as alterations in succinate levels were
318 detected despite stable appearances of the tumour diameter. This important application of ¹H-
319 MRS could be expanded to include other tumours with specific metabolic defects including
320 fumarate hydratase deficient tumours²⁷, *IDH1* mutant tumours²⁸ and the recently identified
321 malate dehydrogenase 2 (MDH2) deficient tumours²⁹. However, important limitations of *in*
322 *vivo* metabolomic analysis using ¹H-MRS were also revealed by our study: for example,
323 spectral quality was poor in close proximity to metal dental work, adjacent to air spaces
324 including the lung, in bone metastases, and was susceptible to motion artefact. In this study,
325 the technical failure rate was 26%, which is similar to the failure rate reported in previous
326 studies using ¹H-MRS¹⁶. Importantly, no cases was excluded from this prospective study,
327 with the intention that this would inform on the translation of this imaging modality into
328 clinical practice. Based on the evidence from this exploratory study, we would recommend
329 that tumours were selected for ¹H-MRS analysis based on: (i) ideally the largest tumour

330 deposit but at least a size greater than 2 cm, (ii) tumours located close to bone or lung should
331 be avoided, (iii) tumours with significant necrosis or hemorrhage should be avoided, (iv)
332 superficial tumour deposits should be selected preferentially, and (v) respiratory triggered
333 acquisition should be used for tumours in the upper abdomen, such as hepatic metastases.
334 Although the use of ¹H-MRS as a diagnostic tool is likely to be limited to specialist centres,
335 the number of scan averages in our study during spectral acquisition was less than half those
336 reported in a previous study¹⁶ (200 versus 512), without demonstrating a reduction in
337 sensitivity. Using fewer scan averages reduces the acquisition time, making it more cost
338 effective and convenient for the patient. This is a particularly important consideration if this
339 imaging technique is to be considered for routine clinical practice or for sequential follow-up
340 as part of a clinical trial. Furthermore this imaging modality could be used to investigate
341 other metabolically-driven tumours.

342

343 In conclusion, this study is the largest to date to evaluate ¹H-MRS in patients with SDH
344 deficiency. It has revealed that ¹H-MRS has the potential to be used as a non-invasive
345 biomarker in the precision management of SDH-deficient disease and could have a role as a
346 biomarker of successful treatment response. Lessons learned from this study could be applied
347 to other similar metabolically-driven tumours.

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441

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453

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464 **Tables:**

465 **Table 1:** Clinical characteristics of the cohort. PA = pituitary adenoma, PC =

466 pheochromocytoma.

Case number	Genetic mutation	Sex	Age	Primary tumour	Metastatic disease	Site of metastatic disease	Family history	Other primary tumour
1	<i>SDHC</i> epimutation	F	21	GIST	Yes	Liver, lung	No	No
2	<i>SDHB</i> c.268C>T p.(Arg90*)	F	53	Abdominal PGL	Yes	Lymph nodes, bone	Yes- mother (GIST)	No
3	<i>SDHC</i> epimutation	F	25	GIST	Yes	Liver	No	No
4	No mutation detected	F	27	GIST	No	NA	No	No
5	<i>SDHB</i> c.137G>A p.(Arg46Gln)	M	38	GIST	Yes	Liver, peritoneum	No	No
6	<i>SDHB</i> c.380G>T p.(Ile127Ser)	M	80	PA	No	NA	Yes nephew (PPGL)	No
7	No mutation detected	M	70	PC	Yes	Liver, bone	No	No

8	<i>SDHB</i> c.600G>T p.(Trp200Cys)	M	41	Glomus PGL	No	NA	No	Yes, PA
9.	<i>SDHB</i> c.302G>A p.(Cys101Tyr)	M	26	Abdomin al PGL	No	NA	No	Carotid PGL
10.	No mutation detected	M	23	PC	No	NA	No	No
11.	<i>SDHA</i> c.91C>T p.(Arg31Ter)	F	21	GIST	Yes	Liver	No	No
12.	<i>SDHA</i> c.1765C>T p.(Arg589Trp)	F	37	GIST	Yes	Liver	No	No
13	<i>SDHA</i> c.91C>T p.(Arg31Ter)	M	46	PGL	Yes	Bone	No	No
14	<i>SDHA</i> c.91C>T p.(Arg31Ter)	M	24	GIST	Yes	Liver	No	No
15	No mutation	M	67	PC	No	NA	No	No

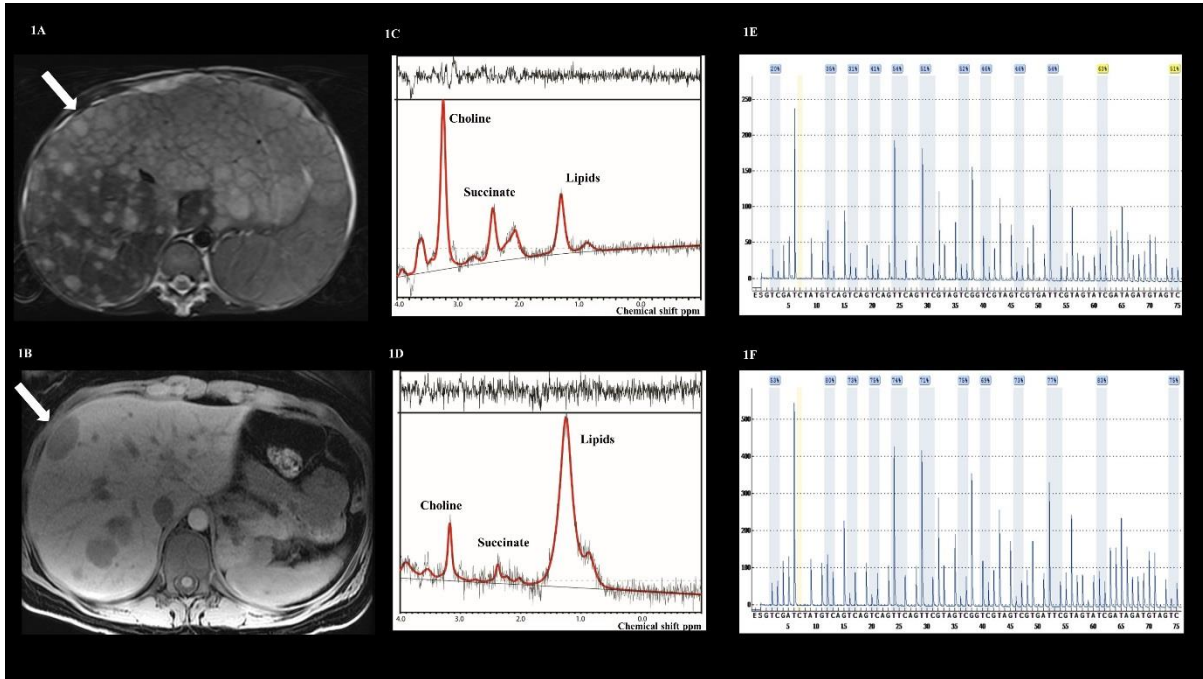
467

468 **Figure legends**

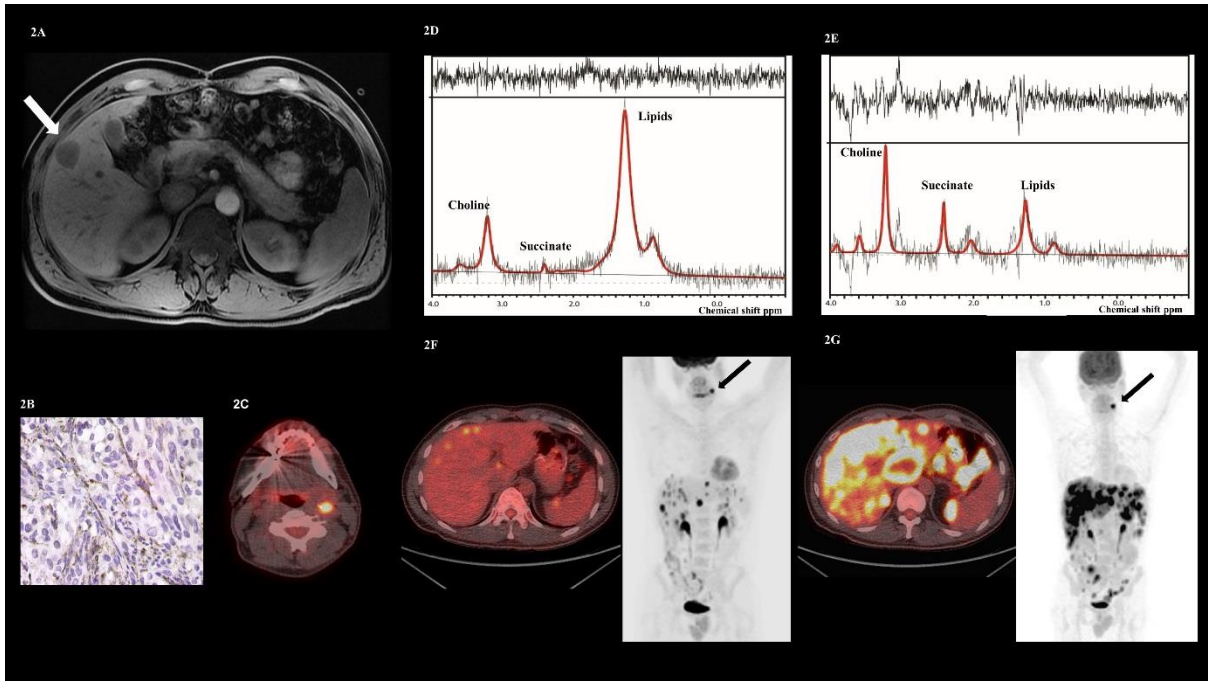
469 Figure 1. (A): T₂-weighted MR image from case 1 and (B) T₁-weighted image from case 3

470 demonstrating liver metastases from which spectra were acquired in the locations indicated

471 by the white arrows. (C-D) show the spectra from case 1 and case 3 demonstrating a
 472 succinate peak at 2.4 ppm. (E-F) demonstrate hypermethylation of the promoter region of the
 473 SDHC gene in tumour DNA from cases 1 and 3, confirming a somatic SDHC epimutation:
 474 55% mean methylation in case 1 and 75% mean methylation in case 3.



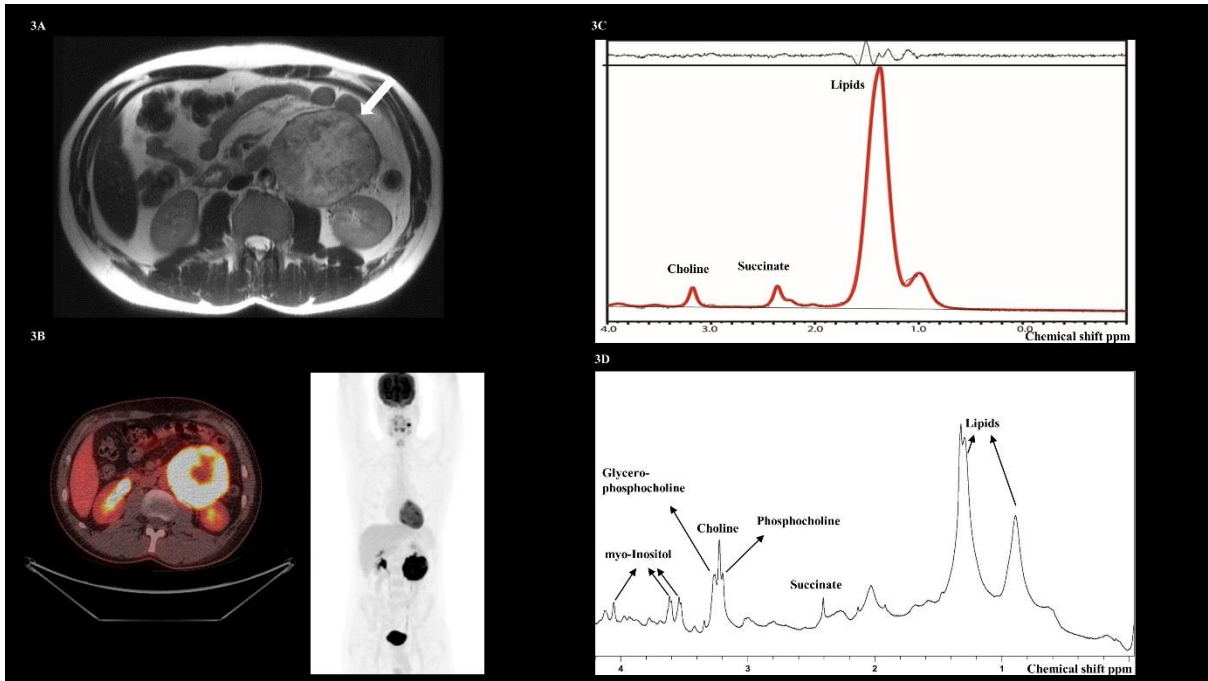
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 476
 477 Figure 2. (A) T₁-weighted MR image of a metastatic GIST to the liver (arrow) from case 5.
 478 (B) SDHB immunonegativity on SDHB immunohistochemistry performed on the wt GIST
 479 tumour from the same patient. (C) Axial fused ¹⁸F-FDG PET/CT image demonstrating an
 480 FDG-avid carotid body PGL after SDH deficiency was demonstrated on ¹H-MRS. (D-E)
 481 Spectra acquired at ¹H-MRS from the same case before and during treatment with a multi-
 482 kinase inhibitor. (F-G) Axial fused ¹⁸F-FDG PET/CT images and corresponding coronal PET
 483 projections illustrating the increase in disease burden and FDG avidity over time (SUV: 15.1
 484 and 27.1) which correlates with the increase in the succinate peak demonstrated on ¹H-MRS.



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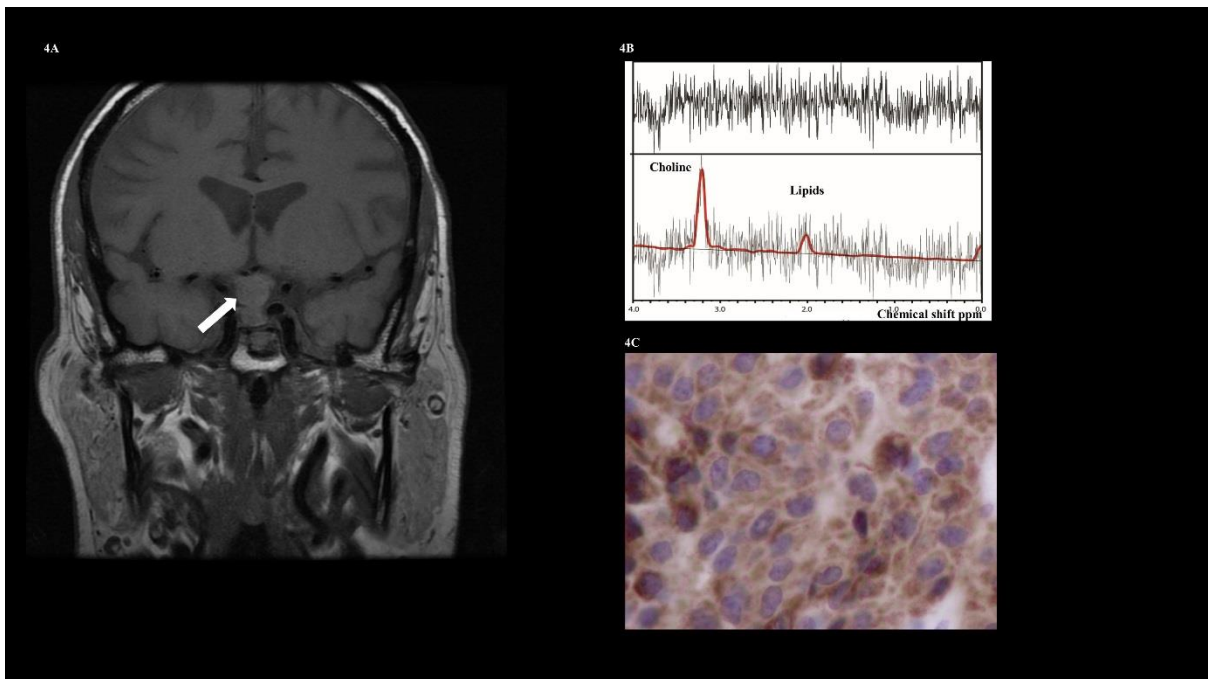
487 Figure 3. (A) T₂-weighted MRI showing a large non-secretory abdominal paraganglioma
 488 from case 9 (arrow). (B) ¹H-MR spectra demonstrating a succinate peak at 2.4 ppm. (C) Axial
 489 fused ¹⁸F-FDG PET/CT image. The corresponding coronal maximum intensity projection
 490 (MIP) PET image demonstrates a synchronous left sided carotid paraganglioma. (D) Spectra
 491 acquired by High Resolution Magic Angle Spinning (HR-MAS) *in vitro* on the
 492 paraganglioma tumour sample, again confirming a succinate peak at 2.4 ppm.



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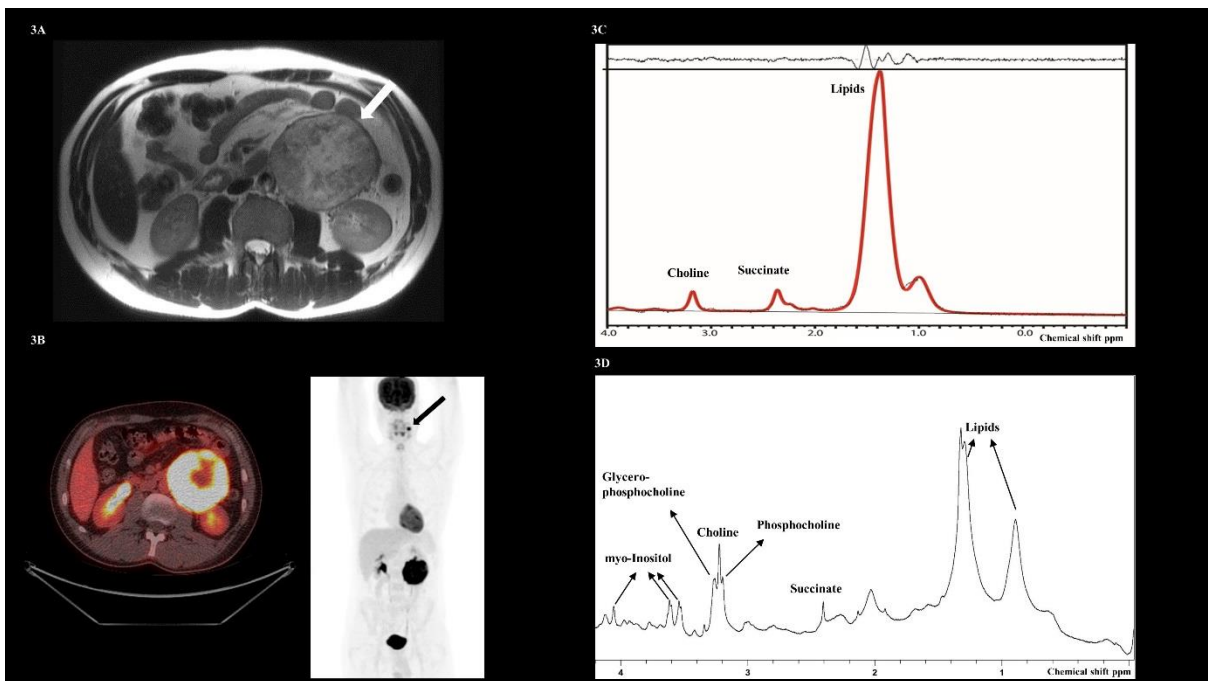
494

495 **Figure 4:** (A) Coronal T₁-weighted MRI demonstrating a remnant pituitary adenoma in case
 496 6 (white arrow). (B) Spectra acquired from the pituitary tumour at ¹H-MRS, with evidence of
 497 choline detection but no succinate. (C) SDHB IHC demonstrating preservation of the SDHB
 498 protein performed on a section of tumour tissue debulked from the pituitary tumour.



499

500 Figure 5. (A) Axial T₂-weighted MRI image of a retroperitoneal nodal metastases from case 2
501 (arrow). (B) Spectra acquired before treatment illustrating succinate accumulation at 2.4 ppm.
502 (C) Spectra acquired following 4 cycles of [¹⁷⁷Lu]-DOTATATE with no detectable succinate
503 peak at 2.4 ppm. (D) Plasma metanephrine and methoxytyramine levels before and after
504 treatment with [¹⁷⁷Lu]-DOTATATE. (E) Axial fused ¹⁸F-FDG PET/CT image and
505 corresponding coronal PET projection showing the FDG-avid nodal metastases (SUV = 16.1,
506 arrowed). (F) The same nodal metastases following treatment with [¹⁷⁷Lu]-DOTATATE
507 demonstrating reduced tracer uptake in keeping with the biochemical findings (SUV = 9.3).



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515 **Supplementary data:**

516 Figure S1: (A) Coronal MRI image of a large left sided glomus paraganglioma from case 8
517 demonstrated by the white arrow. (B) Spectra processed with LCModel from the same patient
518 showing a broad unreliable peak at 2.4 ppm, which was not convincing for succinate.

519 Table S1: Characteristics of the 15 tumours analysed by ¹H-MRS. TF: technical failure,
520 defined as an estimated uncertainty (%SD) > 15% in automated peak fitting of choline using
521 LCModel. ND: not detected. NA: not applicable.

522 Table S2: Characteristics of the two patients in whom ¹H-MRS was repeated during the same
523 examination to evaluate test reproducibility.

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