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► **To cite this version:**

Antoine-Guy Lopez, Céline Duparc, Sylvie Renouf, Elise Machevin, Vincent Le Guillou, et al.. Expression of LHCGR in Pheochromocytomas Unveils an Endocrine Mechanism Connecting Pregnancy and Epinephrine Overproduction. *Hypertension*, 2022, 79 (5), pp.1006-1016. 10.1161/HYPERTENSIONAHA.121.18864 . hal-03936060

**HAL Id: hal-03936060**

<https://hal.science/hal-03936060v1>

Submitted on 24 Jan 2025

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# Expression of LHCGR in Pheochromocytomas Unveils an Endocrine Mechanism Connecting Pregnancy and Epinephrine Overproduction

Antoine-Guy Lopez<sup>1</sup>, Céline Duparc<sup>1</sup>, Sylvie Renouf, Elise Machevin, Vincent Le Guillou, Jean-Christophe Sabourin, Guillaume Defortescu, Alexandre Buffet, Anne-Paule Gimenez-Roqueplo<sup>1</sup>, Christophe Dubessy<sup>1</sup>, Estelle Louiset<sup>1</sup>,† Hervé Lefebvre<sup>1</sup>†

**BACKGROUND:** The mechanisms by which pregnancy may unmask pheochromocytomas and paragangliomas are not totally understood. We hypothesized that gestational hormones may participate in the pathophysiology of catecholamine excess during pregnancy. We report a case of silent pheochromocytoma revealed in a pregnant woman by life-threatening adrenergic myocarditis.

**METHODS:** In vitro studies were conducted to investigate the effect of estradiol and the pregnancy hormone hCG (human chorionic gonadotropin) on epinephrine secretion by cultured cells derived from the patient's tumor. Expression of LHCG (luteinizing hormone/chorionic gonadotropin) receptor was searched for in the patient's tumor, and a series of 12 additional pheochromocytomas by real-time reverse transcription polymerase chain reaction and immunohistochemistry. *LHCGR* expression was also analyzed in silico in the pheochromocytomas and paragangliomas cohorts of the *Cortico et Médullosurrénale: les Tumeurs Endocrines* and The Cancer Genome Atlas databases.

**RESULTS:** hCG stimulated epinephrine secretion by cultured cells derived from the patient's pheochromocytoma. The patient's tumor expressed the LHCG receptor, which was colocalized with catecholamine-producing enzymes. A similar expression pattern of the LHCG receptor was also observed in 5 out of our series of pheochromocytomas. Moreover, in silico studies revealed that pheochromocytomas and paragangliomas display the highest expression levels of LHCG receptor mRNA among the 32 solid tumor types of The Cancer Genome Atlas cohort.

**CONCLUSIONS:** Pregnancy may thus favor surges in plasma catecholamine and hypertensive crises through hCG-induced stimulation of epinephrine production by pheochromocytomas. (**Hypertension. 2022;79:1006–1016. DOI: 10.1161/HYPERTENSIONAHA.121.18864.**) • **Supplemental Material**

**Key Words:** chorionic gonadotropin ■ hypertension ■ pheochromocytoma ■ pregnancy ■ luteinizing hormone

**P**heochromocytomas/paragangliomas (PPGL) are rare catecholamine-producing neuroendocrine tumors that account for 0.1% to 0.6% of all hypertension cases.<sup>1,2</sup> Around 40% of PPGL are related to germline mutations in susceptibility genes.<sup>2,3</sup> The majority of these

mutations occur in *SDHB*, *SDHD*, *VHL*, *NF1*, and *RET* genes, whereas mutations of *SDHA*, *SDHAF2*, *SDHC*, *FH*, *MAX*, and *TMEM127* genes are less frequent.<sup>2,4</sup> Integrated genomic analyses also allowed identification of 5 molecular subgroups of PPGL which are organized

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Supplemental Material is available at <https://www.ahajournals.org/doi/suppl/10.1161/HYPERTENSIONAHA.121.18864>.

For Sources of Funding and Disclosures, see page 1015.

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## NOVELTY AND RELEVANCE

### What Is New?

The present study demonstrates expression of the LH (luteinizing hormone) receptor in pheochromocytomas, especially in epinephrine-producing tumors.

Our in vitro studies show that activation of the LH receptor by the gestational hormone hCG (human chorionic gonadotropin) is able to stimulate epinephrine production by pheochromocytomas.

### What Is Relevant?

Pregnancy can trigger hypertensive crises in women with undiagnosed pheochromocytoma through hCG-induced activation of the LH receptor expressed by tumor cells.

### Clinical/Pathophysiological Implications?

Illicit expression of LH receptor by epinephrine-producing cells represents a new mechanism by which pregnancy can unmask silent pheochromocytoma as early as the first trimester of gestation.

## Nonstandard Abbreviations and Acronyms

<b>ACTH</b>	adrenocorticotropic hormone
<b>CGA</b>	chromogranin A
<b>COMETE</b>	<i>Cortico et Médullosurrénale: les Tumeurs Endocrines</i>
<b>CRH</b>	corticotropin-releasing hormone
<b>hCG</b>	human chorionic gonadotropin
<b>LH</b>	luteinizing hormone
<b>LHCGR</b>	luteinizing hormone/chorionic gonadotropin receptor
<b>PNMT</b>	phenylethanolamine N-methyltransferase
<b>PPGL</b>	pheochromocytomas and paragangliomas
<b>TCGA</b>	The Cancer Genome Atlas
<b>TH</b>	tyrosine hydroxylase

into 2 main clusters. The cluster 1 contains *SDHx*- and *VHL*-mutated tumors which are associated with a pseudohypoxic phenotype and do not produce epinephrine.<sup>5</sup> Conversely, cluster 2 includes the PPGL related to *RET*, *NF1*, *TMEM127*, *MAX* mutations which enhance multiple kinase signaling pathways, tumors harboring mutations that activate the Wnt signaling pathway and mixed corticomedullary tumors.<sup>3,4,6,7</sup>

Although rare, these tumors must be early diagnosed and treated to prevent adrenergic crises which are potentially life-threatening.<sup>8</sup> Unfortunately, the diagnosis of PPGL remains frequently missed during pregnancy as the symptoms of the disease can be ascribed to other pregnancy-associated conditions like sympathetic hyperactivity, preeclampsia and hyperemesis.<sup>9,10</sup> Unrecognition of the diagnosis is particularly concerning in this context since PPGL are associated with a high risk of either maternal or fetal complications.<sup>11,12</sup> Reciprocally, it is well established

that pregnancy can favor sudden surges in plasma catecholamines in patients with PPGL.<sup>12,13</sup> It is generally considered that these potentially dangerous events are triggered by tumor compression resulting from fetus growth and movements, or labor and delivery.<sup>13,14</sup> However, the observation that symptoms of catecholamine excess may occur during the first trimester of gestation<sup>12,15–17</sup> suggests that pregnancy may also activate the secretory activity of PPGL through the involvement of nonmechanical factors. We hypothesized that catecholamine release by PPGL may be stimulated by gestational hormones, such as estradiol or hCG (human chorionic gonadotropin).

Here, we report a case of silent pheochromocytoma revealed by pregnancy. Epinephrine secretion by cultured tumor cells was investigated in response to estradiol and hCG. We also examined expression of the LHCGR (luteinizing hormone/chorionic gonadotropin) receptor in a series of pheochromocytoma tissues and PPGL cohort of The Cancer Genome Atlas (TCGA).

## METHODS

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Patient

A pregnant 33-year-old woman (gravida 2, para 1) with no history of cardiovascular disease including arterial hypertension was hospitalized for a sensation of discomfort rapidly followed by an acute chest pain associated with headaches occurring at 31 weeks of gestation. The course of pregnancy had been uneventful before with normal blood pressure and heart rate but the patient had noticed appearance of palpitations during the first trimester, which persisted thereafter. Confusion and emesis rapidly developed, and blood pressure increased to 166/134 mmHg. Electrocardiography showed

diffuse ST depression followed by junctional tachycardia which necessitated verapamil administration. Severe dyspnea due to pulmonary edema appeared and was controlled by furosemide injection. Cardiac ultrasonography showed a left ventricular ejection fraction of 35% without ventricular dilation. Fifty-five minutes later, the patient presented an acute cardiogenic shock, and fetal bradycardia to 13 bpm led to an emergency cesarean section which resulted in birth of a 2000 g boy. After transient improvement of hemodynamic parameters, cardiogenic shock relapsed. Cardiogenic shock was tentatively but unsuccessfully treated with catecholamine (epinephrine: 1.2 µg/[kg·min] and norepinephrine: 1 µg/[kg·min]) administration. Then, venoarterial-extracorporeal membrane oxygenation was implanted and secondarily associated with ventricular circulatory support. Emergency heart transplantation was considered and enhanced thoracoabdominal and pelvic computerized tomography scan performed for pretransplantation assessment, revealed a 27 mm left adrenal tumor which could not be characterized (Figure S1A in the [Supplemental Material](#)). The patient progressively recovered and extracorporeal membrane oxygenation and ventricular circulatory support could be removed on days 3 and 4, respectively. Cardiac ultrasonography showed normal contractility of the left ventricle. Blood pressure and cardiac rhythm remained normal and the patient was then discharged. Three months later, ambulatory blood pressure monitoring and Holter recording of the cardiac rhythm showed no abnormality. The patient underwent magnetic resonance imaging which showed that the adrenal tumor exhibited high signal intensity on T2-weighted sequences suggestive of a pheochromocytoma (Figure S1B). Biological investigations revealed a mild elevation of plasma chromogranin A (CGA) concentration and urinary fractionated metanephrine level associated with a subnormal normetanephrine rate (Figure S1C). <sup>123</sup>I-metaiodobenzylguanidine single-photon emission computed tomography/computed tomography showed intense uptake of the tracer by the adrenal mass (Figure S1D). Left retroperitoneoscopic adrenalectomy was performed and pathological examination of the tumor confirmed the diagnosis of pheochromocytoma (Figure S2). The

adrenal tissue did not exhibit morphological characteristics of mixed corticomedullary tumor.<sup>18,19</sup> Recovery was uneventful and both plasma chromogranin A and urinary metanephrine levels returned to normal values. Immunohistochemical phenotyping (SDHA, SDHB [succinohydrogenase types A and B], 5-hydroxymethylcytosine, CA9 [carbonic anhydrase 9], and FH [fumarate hydratase]) of the tumor suggested the absence of mutation in *SDHx* and *VHL* genes and was thus in favor of a cluster 2-related pheochromocytoma.<sup>4</sup> Analysis of PPGL susceptibility genes performed in leukocyte and tumor DNA revealed the presence of a heterozygous germline [c.2531G>A, p(Arg844Gln)] *RET* variant of uncertain significance. No genetic variations in other PPGL susceptibility genes were detected.<sup>20</sup> The patient had no familial history of endocrine neoplasia and showed no clinical or biological clues for multiple endocrine neoplasia type 2.

### Adrenal Tissues

Adrenal tumors from the patient and 12 additional pheochromocytomas were collected at surgery in the Rouen University Hospital and immediately dissected by the pathologist (Table). Adrenal explants were fixed in formalin for histological study and frozen for molecular analysis by the Tumor BioBank-Biological Resource Centre of the Rouen University Hospital. In addition, an adrenal sample from the patient 1 was immersed in DMEM culture medium until cell dissociation for primary culture.

Adrenal samples were collected in accordance with the procedures of the Tumor-BioBank-Centre for Biological resources of the University Hospital of Rouen. Written informed consent was obtained from all patients for scientific study of adrenal specimens including genetic analyses. The authors vouch for the accuracy and completeness of the data.

### Biological Assays

Twenty-four-hour urinary excretions of normetanephrine and metanephrine were measured by automated high-performance liquid chromatographic coupled to electrochemical detection (Chromsystems Instruments & Chemicals; Germany) or liquid chromatography with tandem mass spectrometry

**Table. Clinical, Genetic and Biological Characteristics of Patients with Pheochromocytomas**

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13
Sex	F	M	F	M	F	F	M	F	F	M	F	M	F
Age, y	33	56	62	61	40	53	39	41	57	38	53	53	49
Hypertension	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Germline mutation	<i>RET</i> c.2531G>A, p.(Arg844Gln)	No	No	No	<i>RET</i> c.1858T>C, p.(Cys620Arg)	No	No	No	No	No	No	No	<i>TMEM127</i> c.556G>C, p.(Ala186Pro)
Urinary Normetanephrine concentration (nmol/24 h)	2283* N<2100	1117†	8650†	4448*	2621*	3236‡	15 000*	27 159*	11 248*	17 961*	3510*	18 963*	2909*
Urinary Metanephrine concentration (nmol/24 h)	3124* N<1000	1721†	2670†	1866*	3510*	1160‡	952*	16 258*	1143*	46 975*	7970*	2782*	21 445*
Tumor size, mm	15	26	30	20	15	40	40	50	25	100	40	60	80
Positive MIBG scintigraphy	Yes	ND	ND	Yes	Yes	ND	No	Yes	Yes	ND	Yes	Yes	Yes

F indicates female; M, male; MIBG, <sup>123</sup>I-metaiodobenzylguanidine; N, normal values; and ND, not determined.

\*Quantification by high-performance liquid chromatographic coupled to electrochemical detection.

†Quantification by liquid chromatography with tandem mass spectrometry.

‡Plasma norepinephrine or epinephrine concentrations (pmol/L).

(Chromsystems Instruments & Chemicals). Plasma chromogranin A was measured by immunoassay (Kryptor assay, BRAHMS; Thermo Fisher).

## Cell Culture

Tumor sample from the patient 1 was dissociated with collagenase type 1A and desoxyribonuclease 1 type 4 (Sigma-Aldrich). Tumor cells were cultured in culture medium (50% DMEM, 50% Ham-F12; Thermo Fisher Scientific) supplemented with 1% antibiotic-antimycotic solution (Thermo Fisher Scientific), 1% insulin-transferring-selenium solution (Corning, Thermo Fisher Scientific), 10% horse serum, and 10% fetal calf serum (Sigma-Aldrich) at 37°C in a 5% CO<sub>2</sub>-95% air atmosphere with 100% relative humidity. Pheochromocytoma cells were purified by overnight differential plating to remove adherent nonchromaffin cells and then plated at a density of 10<sup>6</sup> cells/mL in 24 well plates. After 1 day, pheochromocytoma cells were treated with hCG (from 10<sup>-10</sup> to 10<sup>-6</sup> M; Organon, Eragny-sur-Epte, France) or estradiol (from 0.003 to 30 ng/mL; E8875; Sigma-Aldrich). Incubation experiments were conducted in quadruplicate at 37°C for 24 h. Secretion of noradrenaline (HEA907Ge), epinephrine (CEA858Ge), and chromogranin A (SEB212 Hu) in culture supernatants were quantified by using ELISA kits (Cloud-Clone Corp, CliniSciences, Nanterre, France). No significant cross-reactivity or interference between epinephrine or norepinephrine and their respective analogs were observed.

## Immunohistology

Immunohistology was performed on formalin-fixed and deparaffinized tissue sections. Sections were heated at 95°C for 20 minutes in 10 mmol/L citrate buffer (pH 6) for antigen retrieval. Tissue sections were treated with peroxidase blocking reagent (Dako-Agilent, Les Ulis, France) and normal donkey serum. Sections were then successively incubated with primary antibodies and anti-immunoglobulin coupled to peroxidase or fluorescent probes (Table S1). For immunohistochemistry, immunoreactivities were revealed with diaminobenzidine (Dako-Agilent), and the tissue sections were counterstained with hematoxylin. Sections were examined on an Eclipse E600D microscope (Nikon) and a Confocal SP8 MP (Leica). Images were obtained on PRIMACEN, the Cell Imaging Platform of Normandie, University of Rouen Normandie.

## Real-Time Reverse Transcription Polymerase Chain Reaction

Total RNA from adrenal tissue specimens was extracted using Tri Reagent (Sigma-Aldrich, Saint-Quentin-Fallavier, France) and purified on Nucleospin RNAII (Macherey Nagel, Hoerd, France). Human adrenal and testis polyA mRNAs (Clontech, Ozyme, Montigny-le-Bretonneux, France) were used for validation of primers and as control specimen. Purified RNA and polyA mRNAs were converted into cDNA by using ImProm-II Reverse Transcription System (Promega, Charbonnières-les-Bains, France). Real-time polymerase chain reaction amplifications were performed using SYBR Green I Master Mix (Applied Biosystem, Courtaboeuf, France) in a QuantStudio 3 System (Thermo Fisher Scientific, Illkirch, France) with specific primers (Table S2). Samples were analyzed in duplicates in 3 different experiments. Quantification of cDNAs was

normalized to *GAPDH* by using standard curves established by using dilution series of cDNAs derived from polyA adrenal or testis mRNAs.

## In Silico Analyses

TCGA Pan-Cancer Atlas gene expression profiles, mutation data, and relevant clinical data were retrieved from the cBioPortal website (<https://www.cBioPortal.org>).<sup>21,22</sup> TCGA Pan-Cancer Atlas database contains genomic and transcriptomic analyses of 10967 samples from 32 types of solid tumors, including 178 samples of PPGL.<sup>23</sup> Data were retrieved for further analysis, among which genomic alterations are provided for 9279 samples. RNA-sequencing data (preprocessed with the RNA-sequencing by expectation-maximization algorithm) were expressed as Log<sub>2</sub>(value + 1). The French *Cortico et Médullosurrénale: les Tumeurs Endocrines* (COMETE) database contains transcriptomic data from 188 PPGL samples and related clinical information.<sup>24</sup> Data normalized using the Robust Multiarray Average are available online as ArrayExpress entry E-MTAB-733 (<http://www.ebi.ac.uk/arrayexpress/>). Expression data obtained from multiple probes targeting a single gene were compiled using geometric mean. Clinical characteristics of the patients with PPGL included in the two cohorts are provided in Table S4.

## Statistical Analysis

Data obtained in vitro were expressed as mean or mean±SEM of repetitive measurements. They were analyzed using the Prism program (GraphPad Software). Statistical significance was assessed by nonparametric 2-tailed Mann-Whitney test. Correlation between data were analyzed by using 2-tailed Spearman correlation test. *P*<0.05 were considered significant.

Comparison of LHCG receptor (*LHCGR*) expression levels between PPGL and other tumor types from the TCGA Pan-Cancer Atlas were analyzed using 1-way ANOVA with a Benjamini-Hochberg false discovery rate applied for multiple testing correction. A *Q*<0.05 was considered statistically significant. Comparison of *LHCGR* expression levels between PPGL belonging either to cluster 1 or cluster 2 or between pheochromocytoma and paraganglioma was performed using a Mann-Whitney *U* test in both TCGA and COMETE cohorts. Alterations (mutation, amplification, deep deletion, fusion and multiple alterations) of the *LHCGR* gene were expressed according to their frequency of occurrence in each TCGA cancer type. In 2-tailed tests, a *P*<0.05 was considered statistically significant. Plots and statistical analyses were performed with Prism (version 9, GraphPad Software).

## RESULTS

### Effect of Pregnancy-Associated Hormones on Catecholamine Production by Cultured Pheochromocytoma Cells

The action of estradiol and hCG on catecholamine production was evaluated on cultured tumor cells derived from the patient 1. Epinephrine release by pheochromocytomas was not influenced by estradiol at concentrations



reaching  $10^{-7}$  M which reflect the maximum plasma levels of the hormone observed during pregnancy (Figure 1A). Recombinant hCG stimulated dose-dependently epinephrine production by cultured cells (Figure 1B), at concentrations ( $10^{-7}$ – $10^{-6}$  M) similar to those commonly observed in the plasma during the first trimester of gestation (up to 200 000 mUI/mL, that is, around  $4 \times 10^{-7}$  M). Conversely, hCG was without effect on norepinephrine and chromogranin A production (data not shown).

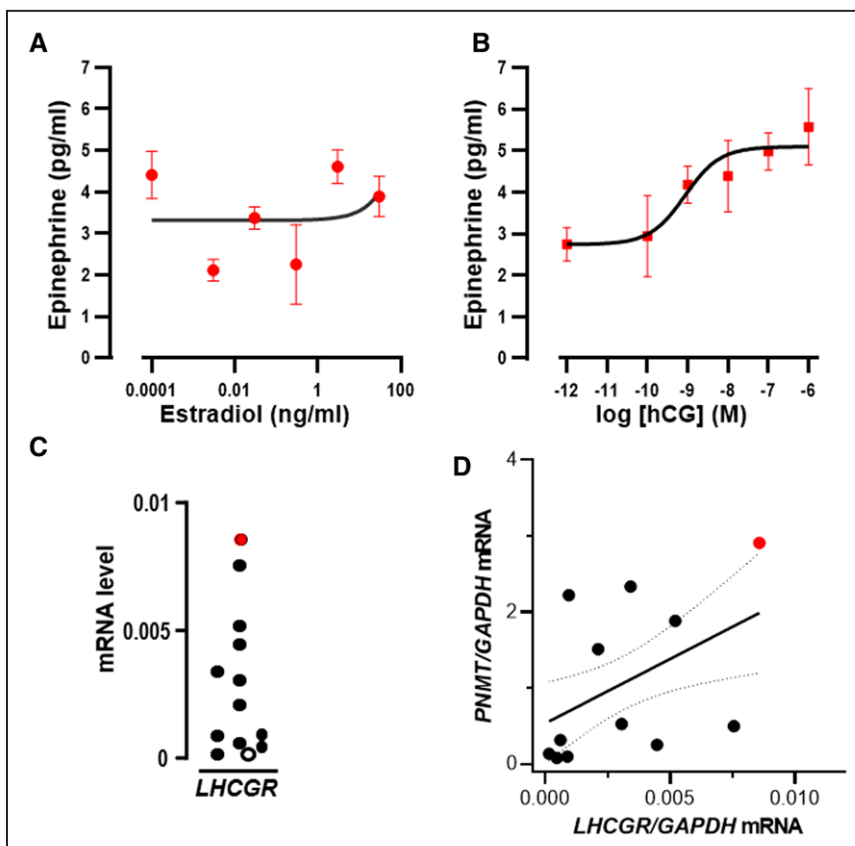
Quantitative RT-polymerase chain reaction study revealed that the patient's tumor highly expressed *LHCGR* mRNA which was detectable at various levels in an additional series of 12 pheochromocytomas (Figure 1C). The rate of *LHCGR* mRNAs was positively correlated to that of phenylethanolamine N-methyltransferase (*PNMT*) mRNAs ( $r=0.64$ ;  $P=0.02$ ; Figure 1D).

Immunohistochemistry showed that LHCGR immunoreactivity was heterogeneously distributed in tumor tissue from patient 1 (Figure 2A). Groups of LHCGR-labeled cells were also immunopositive for CGA and the catecholamine-producing enzymes TH (tyrosine hydroxylase), and PNMT (responsible for epinephrine synthesis; Figure 2B and 2C). These data indicated that the LHCGR is expressed by epinephrine-producing pheochromocytes consistently with the stimulatory action of hCG on epinephrine release by cultured pheochromocytoma cells. LHCGR immunoreactivity was also detected at various levels in PNMT-immunopositive areas in 5

pheochromocytoma tissues from the 12 studied samples (Figures 3 and Figures S3 and S4). In particular, LHCGR immunoreactivity was largely distributed throughout the tumor tissue from patient 5 who displayed a *RET* mutation (c.1858T>C, p.[Cys620Arg]). By contrast, LHCGR was not detected in pheochromocytoma tissues from 7 other patients, as well as in the medulla from the patient 1 peritumoral tissue and normal adrenal medulla (Figures S3 through S6).

Expression of *LHCGR* mRNA in PPGL was further analyzed using the transcriptomic data from TCGA and COMETE cohorts. Among the 32 solid cancer types included in TCGA Pan-Cancer Atlas, the PPGL have the highest level of *LHCGR* mRNA ( $P<0.001$ ) compared with the other cancer types (Figure 4A). In both TCGA and COMETE cohorts, expression levels of *LHCGR* mRNA were higher in PPGL belonging to cluster 2 which are also known to produce higher amounts of PNMT than those in pseudohypoxic cluster 1 ( $P<0.0001$ ; Figure 4B). Analysis of tumor subtypes revealed a higher level of *LHCGR* mRNA expression in pheochromocytomas compared to paragangliomas (TCGA,  $P<0.001$ ; COMETE,  $P<0.01$ ; Figure 4C).

In contrast with 23 other tumor types showing one or more alteration(s) of the *LHCGR* gene, no fusion, deletion, amplification, or mutation of this gene was reported in whole-exome sequencing data of PPGL in TCGA cohort (Figure 4D).

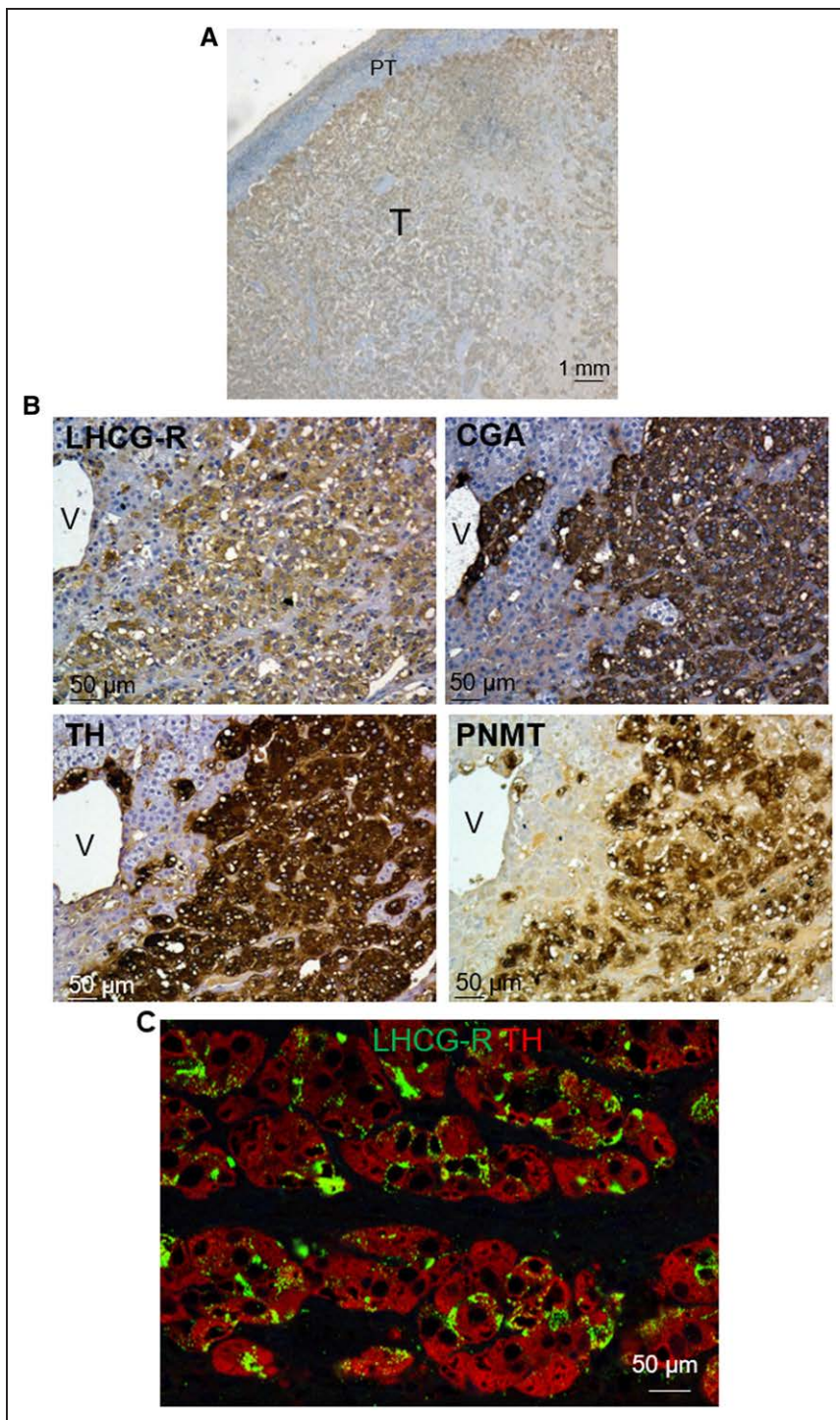


**Figure 1. Expression of LHCGR (luteinizing hormone/chorionic gonadotropin receptor) in patient 1 tumor and a series of pheochromocytomas.**

**A**, Absence of effect of increasing concentrations of estradiol on epinephrine production by cultured tumor cells from patient 1 pheochromocytoma.

**B**, Stimulatory effect of increasing concentrations of hCG (human chorionic gonadotropin) on epinephrine secretion by cultured tumor cells derived from patient 1. Data are expressed as mean $\pm$ SEM (N=4 replicates). \* $P<0.05$ .

**C**, Expression of *LHCGR* in normal adrenal (open circle) and a series of pheochromocytomas (black circles) including that of patient 1 (red circle). *LHCGR* mRNA levels were normalized to *GAPDH*. Data are expressed as mean of 3 replicates. **D**, Positive correlation between *LHCGR* and *PNMT* mRNA levels in a series of pheochromocytoma tissues (black circles) including that of patient 1 (red circle;  $r=0.643$ ,  $P=0.027$ , 2-tailed Spearman correlation test). Linear regression (solid line) and 95% confidence bands (dotted line).



**Figure 2. Distribution of LHCGR (luteinizing hormone/chorionic gonadotropin receptor) in patient 1 pheochromocytoma.**

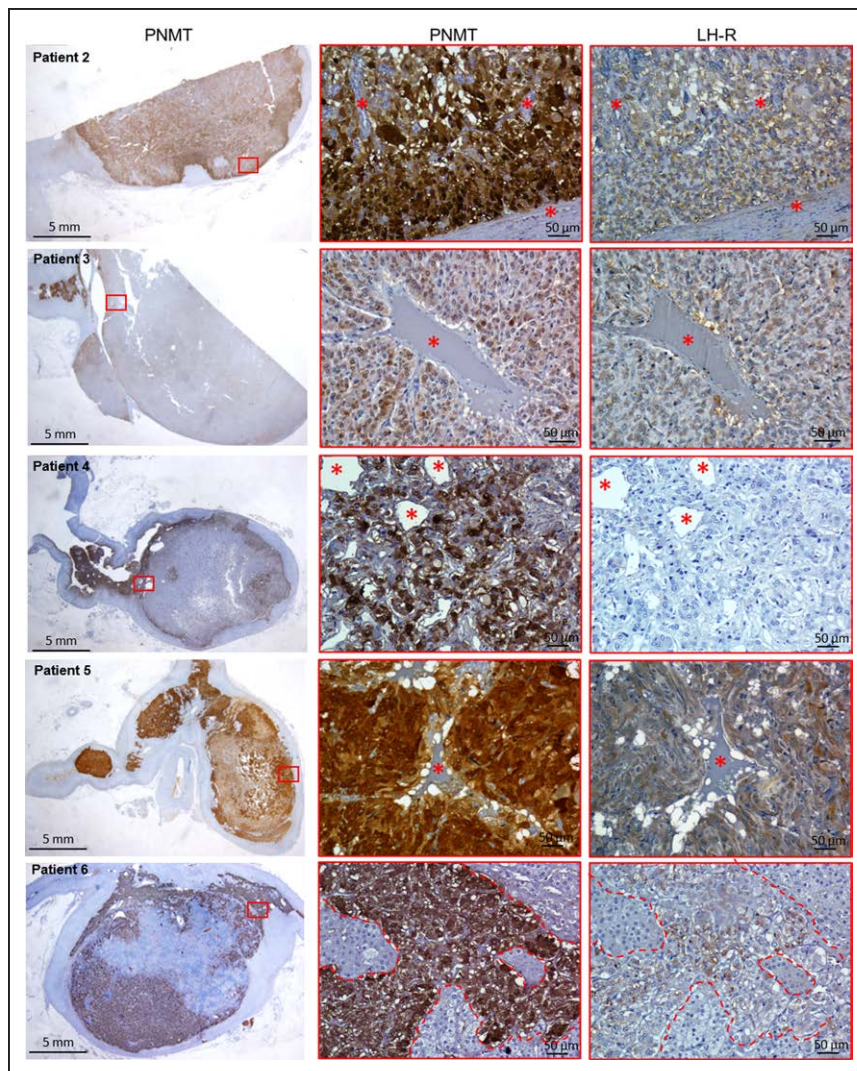
**A**, Detection of LHCGR immunoreactivity in the tumor region (T) of adrenal tissue from patient 1. **B**, Similar distribution of LHCGR (**upper left**), CGA (chromogranin A; **upper right**), TH (tyrosine hydroxylase; **lower left**) and PNMT (phenylethanolamine N-methyltransferase; **lower right**) immunoreactivities in the pheochromocytoma from patient 1. **C**, Colocalization of LHCGR (in green) and TH (in red) immunoreactivities in numerous cells of the adrenal tissue from patient 1. PT indicates peritumour tissue; and V, vein.

## DISCUSSION

It is well documented that gestation can trigger pheochromocytoma crises.<sup>12–14</sup> In the present study, retrospective analysis of the clinical course of the pregnant woman's disease allowed concluding that the patient experienced life-threatening acute pheochromocytoma-induced Takotsubo-like cardiomyopathy. Diffuse ST depression and junctional tachycardia at electrocardiography, decreased ventricular ejection fraction without

ventricle dilation or thickening at echocardiography as well as pulmonary edema have been described in this context together with possible evolution towards cardiogenic shock.<sup>25,26</sup> Peripartum cardiomyopathy associated with preeclampsia and hemolysis, elevated liver enzyme, low platelets syndrome appeared unlikely owing to the rapid regression of left ventricle failure after cesarean section which strongly argues against this hypothesis. In fact, in a recent study, 87% of patients affected by peripartum cardiomyopathy were still in clinical heart failure





**Figure 3. Detection of LHCGR (luteinizing hormone/chorionic gonadotropin receptor) in pheochromocytomas of patients 2-6.** Distribution of PNMT (phenylethanolamine N-methyltransferase; **left and middle**) and LHCGR receptor (**right**) immunoreactivities in consecutive sections of pheochromocytoma tissues from patients 2, 3, 4, 5, and 6. High-magnification views correspond to the region delimited by a red square in the left panels. LHCGR immunoreactivity was detected in tissue sections from patients 2, 3, 5, and 6. Dotted lines indicate PNMT-negative areas in patient 6 tissue sections. \*Provide histological landmarks.

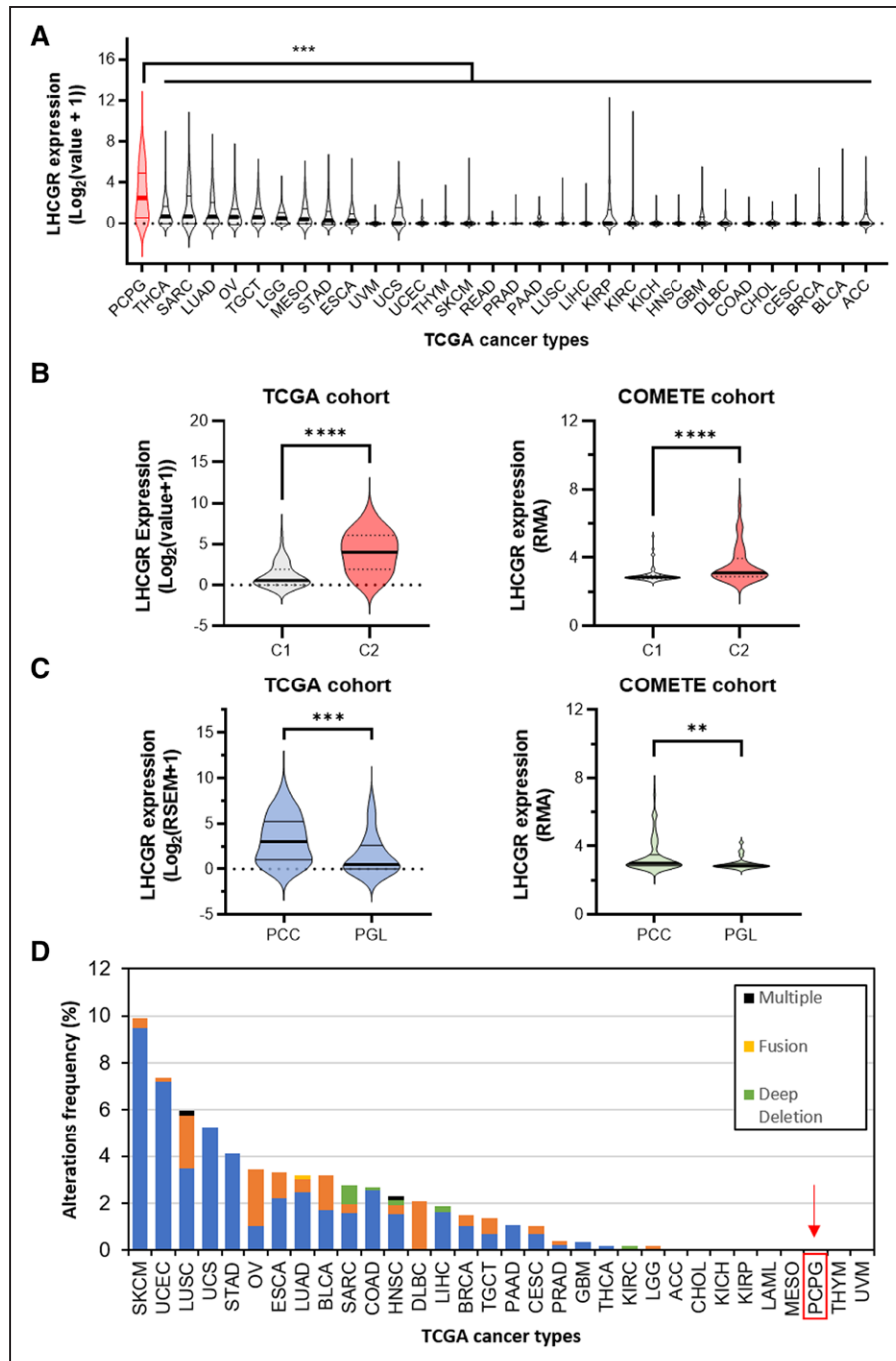
several weeks after the initial diagnosis.<sup>27</sup> In the same way, the echocardiographic presentation of the cardiomyopathy affecting our patient was not consistent with that of viral cardiomyopathy which classically includes ventricle dilation or thickening.<sup>28</sup>

The patient has been rescued by extracorporeal membrane oxygenation which is now considered as a valuable alternative to heart transplantation in the management of the most severe cases of pheochromocytoma-evoked myocarditis.<sup>29</sup> It is noteworthy that cardiac function and arterial blood pressure returned to normal after cesarean section although the adrenal tumor had not still been resected. In fact, a review of the literature revealed that the lack of adrenal surgery is associated with no improvement in ventricular ejection fraction in the great majority of patients with pheochromocytoma-induced cardiomyopathy.<sup>30</sup> This observation indicated that the patient's pheochromocytoma did not produce high amounts of catecholamines outside the period of gestation and that pregnancy was thus the major cause of adrenergic crisis. Consequently, it was possible to further perform adrenal surgery after a convalescence period allowing biological

and radiological evaluation of the incidentally discovered adrenal mass.

It is generally considered that mechanical factors, such as increased abdominal pressure due to fetus growth, represent the main mechanism by which pregnancy unmasks previously undiagnosed pheochromocytoma.<sup>9,12,13</sup> However, the fact that palpitations, which can retrospectively be considered as the first signs of catecholamine excess, appeared during early gestation in our patient led us to consider a potential role of pregnancy-associated hormones in stimulating the secretory activity of the pheochromocytoma, although the symptoms may have been related to pregnancy-induced sympathetic activation. Diverse hormones produced by placenta are released in the maternal blood, including CRH (corticotropin-releasing hormone), corticotropin (ACTH [adrenocorticotrophic hormone]), estrogens, progesterone, and hCG.<sup>31</sup> Absence or low expression of ACTH and CRH receptors previously reported in pheochromocytoma,<sup>32-34</sup> argues against a possible role of these hormones in the control of epinephrine secretion. Furthermore, the lack of effect of progesterone on tyrosine hydroxylase activity in





**Figure 4.** In silico analysis of expression of the luteinizing hormone/chorionic gonadotropin receptor (*LHCGR*) gene in pheochromocytomas/paragangliomas (PPGL).

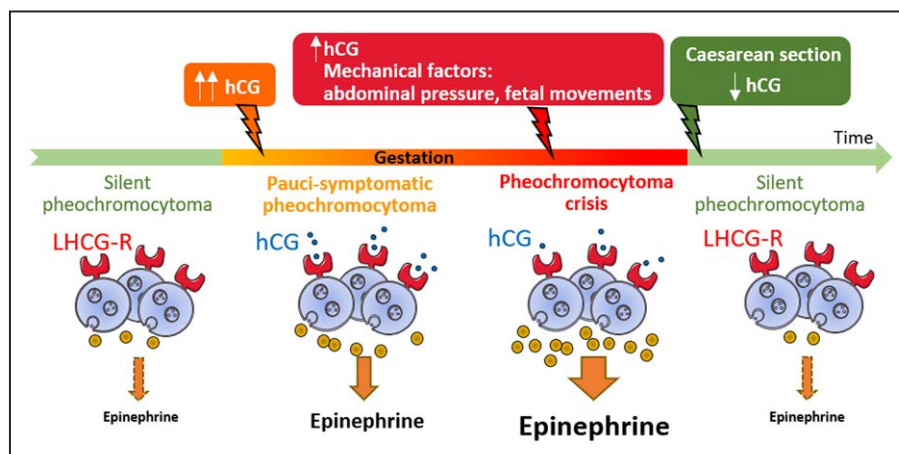
**A**, Violin plot shows that *LHCGR* mRNA is more expressed (median=2.487;  $***q < 0.001$ , 1-way ANOVA with Benjamini-Hochberg correction) in PPGL (PCPG; red violin) compared to the other 31 types of TCGA (The Cancer Genome Atlas) tumor types (gray violins; see Table S3). Cancer types are ranked according to the median expression of *LHCGR*. The data are expressed as  $\text{Log}_2$  of the value of RNA-sequencing by expectation-maximization (RSEM)+1. **B**, *LHCGR* mRNA expression levels in PPGL from cluster 1 (C1, pseudohypoxic phenotype; gray violins) and cluster 2 (C2, enhancement of MAP kinase or Wnt signaling pathway; red violins) in TCGA and *Cortico et Médullosurrénale: les Tumeurs Endocrines* (COMETE) cohorts.  $****P < 0.0001$ . **C**, *LHCGR* mRNA expression levels in pheochromocytomas and paragangliomas (PGL) in TCGA (left; blue violins;  $***P < 0.001$ ) and the COMETE (right; green violins;  $**P < 0.01$ ) cohorts. The data are expressed as  $\text{Log}_2$  of the value of RSEM+1 (left) and as the robust multiarray average value (right) in **B** and **C**. *P* was computed by 2-tailed Mann-Whitney *U* test in **B** and **C**. Median and quartiles are, respectively, indicated as thick and thin lines in violin plots in all parts. **D**, Frequency of alterations in the *LHCGR* gene among different types of cancers in TCGA cohort. Absence of alteration in the *LHCGR* gene in the samples of PPGL (denoted PCPG in TCGA cohort; red arrow) as well as in 8 other types of cancers. Conversely, one or more types of alterations were found among the 23 other cancer types. ACC indicates adrenocarcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; (Continued)

**Figure 4 Continued.** COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe carcinoma; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; and UVM, uveal melanoma.

the rat pheochromocytoma cell line PC12,<sup>35</sup> suggested that progesterone does not enhance the secretory activity of pheochromocytoma. By contrast, estrogens have been shown to reduce apoptosis in the pheochromocytoma cell line 12 cell line which is known to express the three types of estrogen receptors,<sup>36,37</sup> indicating that estrogens may favor both the growth and clinical expression of pheochromocytomas. Here, we observed that estradiol did not influence epinephrine release by cultured pheochromocytoma cells. Conversely, hCG stimulated in vitro epinephrine secretion by pheochromocytoma cells and the tumor was found to express high levels of LHCGR. This finding indicates that the initially silent tumor progressively evolved to an epinephrine-secreting pheochromocytoma under stimulation by hCG, leading to appearance of mild signs of catecholamine excess (Figure 5). In the late gestation, pheochromocytoma crisis was precipitated by mechanical factors, that is, elevation of abdominal pressure and fetus movements.<sup>14</sup> Cesarean section allowed regression of acute myocarditis, hypertension and heart rhythm disorder through suppression of mechanical stress and hCG production which induced a decrease in catecholamine secretion, as shown by the mild elevation of urinary metanephrine concentration found a few months after delivery (Figure 5). The latter finding is consistent with the previously reported biological profile of normotensive incidentally discovered pheochromocytoma.<sup>38</sup>

Interestingly, expression of the LHCGR was detected in some cases of our series of pheochromocytoma tissues. In these tumors, the occurrence of LHCGR was associated with high expression of PNMT. In addition, PPGL expressed the highest levels of *LHCGR* mRNA among the diverse solid tumor types appearing in the TCGA. Interestingly, expression of *LHCGR* was higher in cluster 2 PPGL which are also known to produce higher amounts of PNMT.<sup>7</sup> The higher expression of *LHCGR* mRNA in pheochromocytomas than paragangliomas is consistent with the observation that paragangliomas mainly belong to cluster 1.<sup>4,7,39</sup> It is also coherent with the recently published clinical data showing that functional head and neck or thoracic paragangliomas are not associated with maternal or fetal complications of catecholamine excess during gestation contrary to abdominal or pelvic catecholamine-producing paragangliomas which are submitted to mechanical stress in the late gestation period.<sup>12</sup> Finally, it is noteworthy that, like in patient 1 tumor, the LHCGR may be functional in most pheochromocytomas expressing the *LHCGR* mRNA, as suggested by the observation that these tumors do not exhibit mutations of the *LHCGR* gene.

To summarize, expression of LHCGR and its activation by hCG in cluster 2 pheochromocytomas provide an explanation for adrenergic crises occurring during the first trimester of gestation. To our knowledge, there is no evidence in the current literature that preovulatory plasma



**Figure 5.** Time course of the pathophysiological mechanisms that precipitated pregnancy-associated pheochromocytoma crisis in patient 1.

Stimulation by hCG (human chorionic gonadotropin) of the previously silent pheochromocytoma resulted in mild catecholamine hypersecretion. During the third trimester of gestation, mechanical factors, including fetal motor activity and increased abdominal pressure, triggered pheochromocytoma crisis which resulted in life-threatening acute adrenergic myocarditis. Cesarean section allowed recovery of clinical signs through the decrease of epinephrine production that followed hCG and mechanical stress suppression. LHCGR indicates luteinizing hormone/chorionic gonadotropin receptor.

LH (luteinizing hormone) surge may favor increases in catecholamine levels and blood pressure through a similar mechanism. It is possible that the plasma LH peak may be too transient to induce such effects. By contrast, we can speculate that sustained elevation of plasma LH levels after menopause may trigger adrenergic crises and unmask cluster 2 pheochromocytomas during the fifth and sixth decades.

## PERSPECTIVES

The present data indicate that pregnancy can unmask undiagnosed pheochromocytoma through stimulation of tumor catecholamine production by hCG. It is recommended that women at risk for developing pheochromocytoma must be carefully evaluated before planning pregnancy for early detection and treatment. Our findings show that preconceptional diagnosis is particularly important in patients with gene mutations that predispose to cluster 2 pheochromocytoma, that is, epinephrine-secreting pheochromocytoma, who may develop signs of catecholamine excess and related complications as soon as the first trimester of gestation.

## ARTICLE INFORMATION

Received December 10, 2021; accepted February 6, 2022.

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### Acknowledgments

Supported by in-kind payments from the Rouen University Hospital, Institut National de la Santé et de la Recherche Médicale, the Conseil Régional de Normandie and the European Regional Development Fund. Images were obtained on the Plate-Forme de Recherche en Imagerie Cellulaire de Normandie (PRIMACEN, (<http://www.primacen.fr>)). We are indebted to the Tumor BioBank-Biological Resource Centre of Rouen University Hospital. We thank the TCGA (The Cancer Genome Atlas) project team and the *Cortico et Médullosurrénale: les Tumeurs Endocrines* (COMETE) Network for generating genomic and transcriptomic profiles. We thank all members of the Genetics Department, Biological Resources Center and Tumor Bank Platform, Hôpital Européen Georges Pompidou (BB-0033-00063) for technical support. We are grateful to Dr Judith Favier for immunohistochemical phenotyping of the patient's tumor. We also thank the technical staff of the Department of Pathology of the Rouen University Hospital for skillful assistance.

### Sources of Funding

This work was supported by Institut National de la Santé et de la Recherche Médicale Unité 1239, the Institute for Research and Innovation in Biomedicine, Rouen University Hospital, and the Conseil Régional de Normandie and the European Regional Development Fund. The *Cortico et Médullosurrénale: les Tumeurs Endocrines* (COMETE) was funded by Institut National du Cancer and Direction Générale de l'Offre de Soins (DGOS), Programme de Recherche Translationnelle en cancérologie (PRT-K 2014, COMETE-TACTIC [Towards an Easy-to-use Adrenal Cancer/Tumor Identity Card]), INCA\_DGOS\_8663).

## Disclosures

None.

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