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# Clinical Case Study

# Pheochromocytoma Masked by Mutation in the *TH* Gene

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## CASE DESCRIPTION

A 51-year-old woman consulting within the framework of investigation for abdominal discomfort, nausea, and vomiting underwent a computed tomography examination that revealed a well-delimited right adrenal heterogeneous mass measuring 8.2 × 8.3 cm with a native density of 40 Hounsfield units (HU)[8]. An 18F-deoxyglucose positron emission tomography scan showed a hypercaptation on the adrenal tumor of 7.4 SUVmax. Apart from these symptoms, the patient had no other complaint. Familial history was unremarkable. On examination, the patient was in good physical condition, arterial blood pressure was 136/85 mmHg, heart rate at 74 bpm.

The measurement of plasma renin activity and aldosterone and the results of a 1-mg overnight dexamethasone suppression test ruled out primary hyperaldosteronism and Cushing syndrome. Catecholamine and metanephrine concentrations in multiple blood and urine samples were consistently within the reference interval and not compatible with the diagnosis of a pheochromocytoma (Table 1).

Because the adrenal mass was not hormonally active, its surgical resection was performed without preoperative care. The removal of a mass weighing 356 g in the right adrenal gland was performed by open laparotomy.

Histopathological analysis of tissue sections of the adrenal mass yielded an unexpected result: a pheochromocytoma was diagnosed on the basis of typical well-arranged nests called zellballen. This encapsulated adrenal tumor exhibits a low proliferation index (MIB-1, 1%–2%; 1 mitosis/high power field) without vascular invasion. Immunohistochemistry of the tumor sections revealed its neuroendocrine feature, with cells highly expressing CD56, chromogranin A, NSE, synaptophysin, and vimentin. Unfortunately, blood samples obtained before surgery were collected on heparin-coated tubes precluding serum chromogranin A assay. Because the unusual presentation of a "non–catecholamine-secreting pheochromocytoma" was in complete contradiction with the biochemical feature expected for these tumors, we studied the intratumoral protein expression of the main enzymes involved in catecholamine synthesis by means of immunohistochemistry on paraffin-embedded sections of the tumor biopsy. Tyrosine hydroxylase (TH) was not expressed in tumor tissue compared to normal adrenal gland medulla (Fig. 1 upper panel). Dopamine β-hydroxylase (DBH) and phenylethanolamine-*N*-methyl transferase (PNMT) protein expressions were lower in the tumor than in the adrenal medulla control, but the cells were clearly stained (Fig. 1 middle and lower panels). The *TH* (tyrosine hydroxylase)[9] gene expression was approximately 20-fold lower in this tumor than in the adrenal pheochromocytoma ($P = 0.016$), whereas expressions for *DBH*

### QUESTIONS TO CONSIDER

1. How can one define an adrenal pheochromocytoma that is unable to secrete catecholamines?
2. What alternative may be proposed to monitor a non–catecholamine-secreting pheochromocytoma?
3. How can one monitor for a possible relapse of a pheochromocytoma for this patient?
4. Are special measures needed preoperatively before removal of a non–catecholamine-producing adrenal pheochromocytoma?

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[8] Nonstandard abbreviations: HU, Hounsfield units; DBH, dopamine β-hydroxylase; PNMT, phenylethanolamine-*N*-methyl transferase.

[9] Human genes: *TH,* tyrosine hydroxylase; *DBH,* dopamine beta-hydroxylase; *PNMT,* phenylethanolamine N-methyltransferase; *SDHB,* succinate dehydrogenase complex iron sulfur subunit B; *SDHD,* succinate dehydrogenase complex subunit D; *VHL,* von Hippel-Lindau tumor suppressor; *RET,* ret proto-oncogene.

**Table 1. Concentrations of renin, aldosterone, cortisol, catecholamine, and their metabolites in urine and plasma found in the patient.[a]**

| | Concentration | | |
|---|---|---|---|
| | 43 Days before surgery | 32 Days postsurgery | Reference interval |
| Plasma catecholamines, nmol/L | | | |
| Norepinephrine | 3.11 | ND[b] | 0.64–6.55 |
| Epinephrine | 0.11 | ND | 0.02–1.23 |
| Dopamine | 0.02 | ND | 0.01–0.38 |
| Plasma free metanephrines, nmol/L | | | |
| Normetanephrine | 0.47 | 0.28 | 0.04–1.39 |
| Metanephrine | 0.19 | 0.05 | 0.03–0.85 |
| Methoxytyramine | 0.01 | 0.01 | <0.06 |
| Plasma total metanephrines, nmol/L | | | |
| Normetanephrine | 5.67 | 7.83 | 2.14–36.65 |
| Metanephrine | 2.86 | 0.32 | 0.66–13.45 |
| Methoxytyramine | 1.4 | 0.58 | 0.59–4.19 |
| Urine metanephrines, nmol/24 h | | | |
| Normetanephrine | 1657 | ND | <3800 |
| Metanephrine | 750 | ND | <1880 |
| Methoxytyramine | 4546 | ND | <1900 |
| Plasma renin activity, $ng \cdot mL^{-1} \cdot h^{-1}$ | 0.3 | ND | 0.2–2.8 |
| Plasma aldosterone, pg/mL | 82 | ND | 42–202 |
| Plasma cortisol, nmol/L | 14 | ND | <138 |

[a] The measurements were performed in plasma and urine 43 days before (left column) and 32 days after (right column) the removal of the pheochromocytoma. All concentrations were within the normal range. Plasma aldosterone was determined by RIA (ALDO-RIACT, Cisbio Bioassays) and plasma renin activity using an RIA kit (DiaSorin). Plasma cortisol concentrations were determined on a Cobas Elecsys 2010 E170 analyzer (Roche Diagnostics). Plasma catecholamines and metanephrines were quantified with the patient in a supine position after a 20-min rest by liquid chromatography tandem mass spectrometry and urine metanephrine by liquid chromatography coupled to electrochemical detection. Reference intervals for bioamines have been published previously [Grouzmann et al. *(10)*].

[b] ND, not done.

(dopamine beta-hydroxylase) and *PNMT* (phenylethanolamine N-methyltransferase) were unchanged ($P = 0.29$ and $P = 0.67$, respectively). Routine blood DNA analyses of the most common genes associated with a familial risk for a pheochromocytoma, *SDHB* (succinate dehydrogenase complex iron sulfur subunit B), *SDHD* (succinate dehydrogenase complex subunit D), *VHL* (von Hippel-Lindau tumor suppressor), and *RET* (ret proto-oncogene), were performed and revealed no mutations.

To investigate the molecular mechanism associated with the absence of expression of *TH*, the sequence of the coding exons and the exon–intron boundaries of *TH* were analyzed using the DNA from both blood lymphocytes and tumoral tissue of the patient (Fig. 1A and B). Sequencing of *TH* in the tumor tissue uncovered a homo/hemizygous mutation, c.669 + 5 G>C, in the exon 5/intron 5 junction of *TH* (Fig. 1B). The genomic location of this mutation is at Chr11: 2189316 (http://exac.broadinstitute.org/). This mutation has also been detected in the DNA from the lymphocytes, but only in the heterozygous state. This mutation was located in the conserved region for RNA splicing (donor site), at the 3′-junction of exon and intron 5, and was likely to affect RNA splicing efficiency.

## CASE DISCUSSION

Pheochromocytomas are tumors of the adrenal medulla that produce and usually secrete large amounts of catecholamines often associated with high fatality rates when undiagnosed *(1)*. The usual recommendations for assessing a hormonally active pheochromocytoma rely on the measurement of metanephrines, the results of which have high diagnostic sensitivity *(1)*. Therefore, a negative result allows excluding a pheochromocytoma with a high level of confidence. When metanephrines are positive, imaging studies must be used to locate the tumor, and premedication with $\alpha$ and $\beta$ adrenergic blockers is mandatory

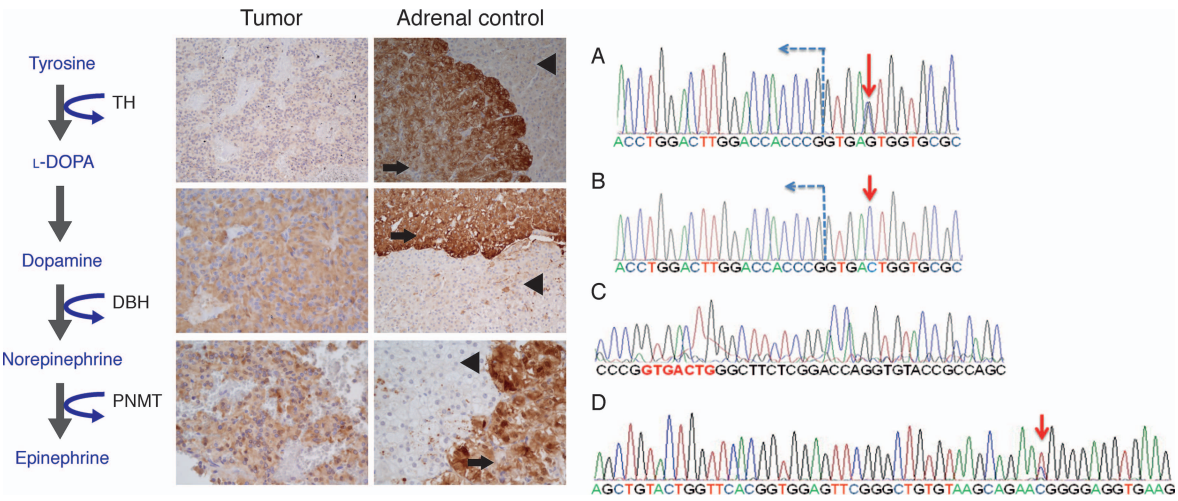


**Fig. 1. Immunohistochemical studies of TH in the patient tumor contrasting with the expression of DBH and PNMT and genetic analysis of TH in blood and pheochromocytoma.**

Upper panel shows the patient tumor, right panel staining with a healthy adrenal gland as a positive control. TH protein expression is not present in the tumor, whereas the adrenal control is producing TH. TH is the rate-limiting step in catecholamine synthesis involved in the hydroxylation of L-tyrosine to 3,4-dihydroxy phenylalanine (DOPA). DOPA is decarboxylated to dopamine by L-aromatic amino acid decarboxylase. Dopamine is hydroxylated into norepinephrine by dopamine $\beta$-hydroxylase (DBH). Phenylethanolamine N-methyl transferase (PNMT), an enzyme localized in adrenal chromaffin cells and pheochromocytes, leads to the methylation onto the amino group of norepinephrine to produce epinephrine. Magnification for TH and DBH: 200× and 400× for PNMT. Arrows indicate adrenal medulla tissue and arrowheads adrenal cortex section. Antibodies are described in Grouzmann et al. *(2)* and anti-PNMT antibody was purchased from Protos (Protos Biotech Corp). (A) and (B) Nucleotide sequences of the exon 5 and intron 5 junction of the *TH* gene (NM_199292; ENST00000381178). The heterozygous mutation c.669 + 5G>C (downward arrow) is observed in the exon 5–intron 5 junction (leftward dashed arrow) in lymphocyte-derived DNA (A), while the mutation is homo/hemizygous (arrow marked) in tumor DNA (B). (C), cDNA from the tumor shows incorporation of seven intronic nucleotides "GTGACTG" (in bold) in the cDNA. (D) Presence of a heterozygous SNP in the c.1239 position (downward arrow) in exon 12 of *TH* from the tumor, which is 2364 bp downstream of the mutation detected in the patient.

to prevent hypertensive crises during surgical removal of the tumor and fluid correction to prevent postsurgical hypotension *(1)*. Finally, the tumor is definitively confirmed to be a pheochromocytoma based on histologic analysis of the tumor by an experienced pathologist and the expression of neuroendocrine markers *(1)*. There are circumstances in which an adrenal mass is fortuitously found during radiological examination of a patient in the absence of symptoms or clinical signs suggestive of a pheochromocytoma, possibly due to a high tumor rate of catecholamine metabolism or tachyphylaxia *(2, 3)*. Mannelli et al. found in a retrospective study conducted on 298 Italian patients affected by pheochromocytomas/paragangliomas that 11.2% of the tumors were incidentally diagnosed, and among these, 62.5% of patients were normotensive *(3)*. The most dreaded presentation of pheochromocytoma occurs in an asymptomatic patient undergoing a procedure such as a surgical intervention when a fortuitous adrenal mass has been discovered *(4)*. In these situations, mortality rates as high as 80% have been reported owing to massive secretion of catecholamines, leading to paroxysmal hypertensive crises and multiorgan failure. Paragangliomas are extra-adrenal chromaffin tumors that can be either sympathetic or parasympathetic. Most of these are catecholamine-secreting tumors and primarily located in the abdomen (more rarely in the chest) in the extraadrenal paraganglia, whereas the remainder are generally non–catecholamine secreting and mostly located in the head and neck paraganglia *(5)*. However, metanephrine measurements are warranted to assess/exclude their hormonal activity. The prevalence of these adrenal incidentalomas increases with age, from <1% to 7% between 30 and 70 years of age *(6)*.

## RESOLUTION OF THE CASE

To further characterize the consequence of the c.669 + 5G>C mutation, sequence analysis of the cDNA generated from the tumor tissue was performed. The sequence of the cDNA revealed that in *TH* from the tumor tissue a cryptic donor splice site, c.669 + 8_9 GT was used for mRNA splicing rather than the usual c.669 + 1_2 GT. As a consequence, 7 nucleotides (GTGACTG) from in-

tron 5 were incorporated into the new cDNA (Fig. 1C), resulting in a frameshift in the amino acids with a premature stop codon (p.Gly223Valfs*18). Normal TH protein contains 528 amino acids, and in the tumor tissue the putative TH protein had only 223 N-terminal amino acids followed by a frameshift and premature truncation (Fig. 1C). When the corresponding DNA sequence from blood cells was investigated, the same mutation was observed in a heterozygous state (Fig. 1A), indicating that a second mutation, leading to homo/hemizygosity for the c.669 + 5G>C mutation, took place in the early stages of tumor development. In the tumor DNA, we also observed a heterozygous polymorphism in exon 12 of *TH*, 2364-bp downstream of the mutation (Fig. 1D).

From the genomic perspective, this patient had a heterozygous germline mutation in *TH*, which turned homozygous in the tumor tissue. Homo/hemizygosity of the splice site mutation in *TH* in the tumor cells may arise from a double-strand break that happens close to the mutation site on the wild-type chromosome and is repaired using the second chromosome (mutated). This appears to be a common repair mechanism at G2 *(7)*. The event would be similar to the homologous recombination mechanism during meiosis and the same as gene conversion, involving just a few hundred bp of the heteroduplex during the repair. This would explain the presence of one heterozygous SNP at approximately 2364 bp downstream of the mutation (Fig. 1D). In other words, the double-strand break and repair happened locally in an early stage of tumor development. qPCR data demonstrated that *TH* mRNA expression for this tumor is only 5% of the level of all pheochromocytomas (n = 50) recorded in our database, suggesting that transcribed mRNA from *TH* in the tumor is subsequently destroyed by the nonsense-mediated mRNA decay (NMD) pathway before being translated to TH protein, a phenomenon usually observed in RNAs with a premature stop codon *(7, 8)*.

*TH* deficiency led to depressed concentrations of L-Dopa and prevented the synthesis of the pharmacologically active catecholamines and their surrogate markers metanephrines within the tumor. Interestingly, no clinical signs suggestive of a sympathetic system default were noticed in this patient despite the presence of only one functional allele of *TH* for proper catecholamine synthesis. Similarly, catecholamine synthesis is also normal in heterozygous parents of children suffering from *TH* deficiency, referred to as autosomal recessive Segawa disease; since it is a recessive disease, we expect an absence of symptoms in heterozygote carriers as observed with this patient, who exhibited resting catecholamine concentrations in the normal range *(9)*.

**POINTS TO REMEMBER**

- Some histologically proven adrenal pheochromocytomas are tumors that do not produce or secrete catecholamines and metanephrines.
- If chromogranin A concentrations are found to be increased initially, chromogranin A measurement might be the test of choice to diagnose a recurrence in the follow-up (although not performed in the present study).
- Adrenal incidentaloma must be investigated biochemically to assess/exclude a pheochromocytoma. Plasma or urine metanephrines are the biochemical tests of choice. In the present case genetic studies elucidated "this exception to the rule" and reconciled the findings of clinical chemists and pathologists.
- Metanephrine assays are unreliable to monitor a noncatecholamine-secreting pheochromocytoma and imaging studies must be considered for follow-up. If the tumor described here were to relapse, it would be interesting to see whether it still had the same somatic mutation.
- When a pheochromocytoma is clearly suspected, the patient must be meticulously prepared with $\alpha$-blocking agents to prevent hypertensive crises during surgery and fluid correction to prevent postsurgical hypotension.

## CASE FOLLOW-UP

This finding has proven that the concerns initially raised regarding the relevance of measuring metanephrines for the biochemical diagnosis of pheochromocytoma were groundless. Fortunately, this missed pheochromocytoma diagnosis had no other clinical consequences than the late diagnosis of a large mass, since the tumor was not producing catecholamines. Finally and most importantly, the patient has fully recovered and, after a 24-month follow-up CT scan, there is no evidence of recurrence. In conclusion, *TH* mutation is a rare cause of nonsecreting pheochromocytoma that should, however, not be ignored.

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## References

1. Lenders JW, Duh QY, Eisenhofer G, Gimenez-Roqueplo AP, Grebe SK, Murad MH et al. Pheochromocytoma and paraganglioma: an Endocrine Society clinical practice guideline 2014. J Clin Endocrinol Metab 2014;99:1915–42.
2. Grouzmann E, Matter M, Bilz S, Herren A, Triponez F, Henzen C et al. Monoamine oxidase A down-regulation contributes to high metanephrine concentration in pheochromocytoma. J Clin Endocrinol Metab 2012;97:2773–81.
3. Mannelli M, Lenders JW, Pacak K, Parenti G, Eisenhofer G. Subclinical pheochromocytoma. Best Pract Res Clin Endocrinol Metab 2012;26:507–15.
4. Myklejord DJ. Undiagnosed pheochromocytoma: the anesthesiologist nightmare. Clin Med and Research 2004;1:59–62.
5. Manger WM, Gilford RW. Clinical and experimental pheochromocytoma. 2nd ed. Cambridge: Blackwell Sciences; 1996.
6. Kloos RT, Gross MD, Francis IR, Korobkin M, Shapiro B. Incidentally discovered adrenal masses. Endocrine Rev 1995;16:460–84.
7. Zhang J, Lindroos A, Ollila S, Russell A, Marra G, Mueller H et al. Nonhomologous end-joining Gene conversion is a frequent mechanism of inactivation of the wild-type allele in cancers from MLH1/MSH2 deletion carriers. Cancer Res 2006;66:659–64.
8. Nagy E, Maquat LE. A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. Trends Biochem Sci 1998;23:198–9.
9. Bräutigam C, Wevers RA, Jansen RJ, Smeitink JA, de Rijk-van Andel JF, Gabreëls FJ, Hoffmann GF. Biochemical hallmarks of tyrosine hydroxylase deficiency. Clin Chem 1998;44:1897–904.
10. Grouzmann E, Drouard-Troalen L, Baudin E, Plouin PF, Muller B, Grand D, Buclin T. Diagnostic accuracy of free and total metanephrines in plasma and fractionated metanephrines in urine of patients with pheochromocytoma. Eur J Endocrinol 2010;162:951–60.


## Commentary

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The morbidity and mortality of pheochromocytomas are mainly due to hypersecretion of catecholamines, which results in various hypertensive crises. These tumors may be identified by symptomatology or incidentally on radiographs ordered for unrelated indications. Pheochromocytoma is further confirmed by biochemical abnormalities and is then cured by surgical resection. The current case illustrates that there are a few patients with nonfunctional adrenal tumors (clinically considered pheochromocytomas and resected by surgery) who do not secrete excess amounts of catecholamines and have no hypertensive symptoms. Such patients will not be detected by the routine testing of catecholamines/metanephrines. The synthesis of catecholamine is a complex process and involves conversion of tyrosine to L-Dopa by the enzyme TH. Tumors with mutations of tyrosine hydroxylase will not be able to complete the synthesis of catecholamines. In such cases, anatomical pathologists confirm the tumor using immune-histochemical, ultrastructural, and local rare chemical properties of the tumors in these patients. This may need further confirmation by molecular genetics, using either the single mutation or combination of the most common mutations related to pheochromocytoma. Whole genome next generation sequencing is becoming very popular; however, the technique may have the challenges of confirming whether a found mutation is benign or has any clinical significance.

The Endocrine Society recommends succinate dehydrogenase gene testing for individuals with pheochromocytomas. However, there are no specific guidelines for *TH* genetic testing for pheochromocytomas, especially for rare nonfunctional benign pheochromocytomas. This case is a reminder that chemical or genetic laboratory tests may never be 100% sensitive or specific, especially for rare diseases. The ultimate care of patients with pheochromocytomas will be the combined assessment of the physicians, radiologists, pathologists, and surgeons involved.

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