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

[8] Nonstandard abbreviations: HU, Hounsfield units; DBH, dopamine _-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.

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(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* (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 protooncogene), 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.

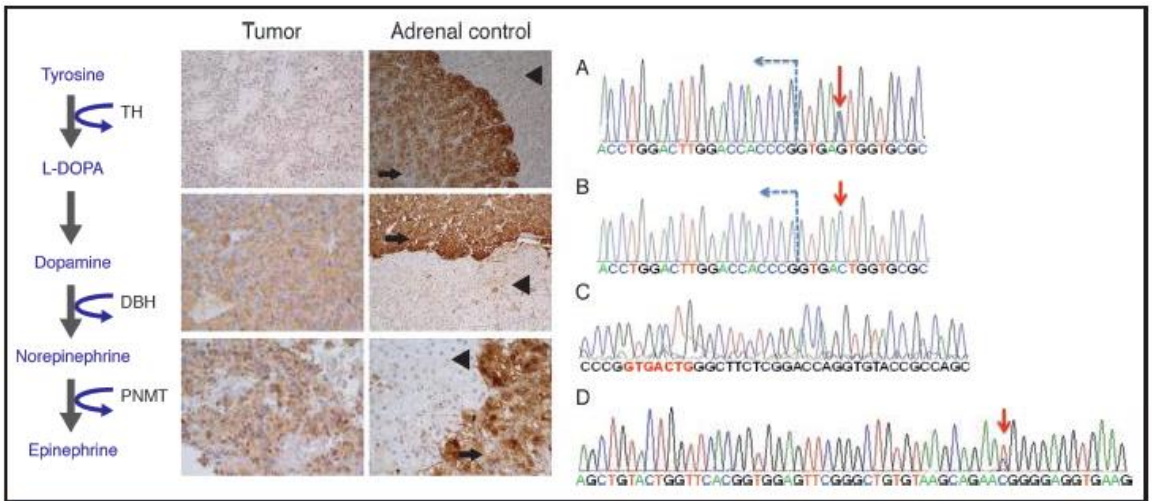

Fig. 1. Immunohistochemical studies of TH in the patient tumor contrasting with the expression of DBH and PNMT and genetic analysis of TH in blood and pheochromocytoma.

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 β-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 *(3)* 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.

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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/mL/h	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 a 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 *(10)*.

[b] ND, not done.

QUESTIONS TO CONSIDER
• How can one define an adrenal pheochromocytoma that is unable to secrete catecholamines?
• What alternative may be proposed to monitor a non-catecholamine-secreting pheochromocytoma?
• How can one monitor for a possible relapse of a pheochromocytoma for this patient?
• Are special measures needed preoperatively before removal of a non-catecholamine-producing adrenal pheochromocytoma?

Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the July 2016 issue of *Clinical Chemistry*. To view the case and comments online, go to http://www.clinchem.org/content/vol62/issue7 and follow the link to the Clinical Case Study and Commentaries.

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