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Successful Treatment of Estrogen Excess in Primary Bilateral Macronodular Adrenocortical Hyperplasia with Leuprolide Acetate

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Abstract

Primary bilateral macronodular adrenocortical hyperplasia (PBMAH) is an uncommon cause of adrenal Cushing syndrome (CS) in which cortisol and occasionally other steroid hormones can be secreted under the influence of aberrantly expressed G-protein coupled receptors (GPCRs) in the adrenal cortex. We describe the unique case of a 64-year-old postmenopausal female with PBMAH whose adrenal lesions expressed luteinizing hormone receptors (LHr). She presented initially with CS and underwent right adrenalectomy; a few years later she presented with macromastia and mastodynia, possibly due to estrogen excess from her remaining left adrenocortical masses. Testing before and after treatment with quarterly leupro-lide acetate therapy and immunohistochemistry on tissue and targeted sequencing of the genes of interest were performed. Tissue from the patient's right adrenal was tested for P450 aromatase (CYP19A1) and LHr expression; both were expressed throughout the hyperplastic cortex, although expression was more intense in the adenomatous areas. Targeted sequencing revealed a pathogenic *PDE11A* mutation, as well as variants in the *ARMC5* and *INHA* genes. PDE11A expression was decreased in the adenoma but there was no loss of hete-rozygosity for the *PDE11A* locus. Because of the clinical presentation and LHr expression, quarterly leuprolide acetate therapy was started. Shortly

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Author Contribution Statement

All authors have contributed to the design and conception of this study. FRF analyzed exome data and performed targeted sequencing. AB performed the immunohistochemical staining.

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Conflict of Interest

The authors declare that they have no conflict of interest.

after initiation of therapy, the patient reported decreased breast size and pain; she remains well controlled to date, after 10 years of treatment. This is the first description of a patient with PBMAH presenting with se-vere macromastia and mastodynia from what appears to be excess estrogen production from her adrenal tumor. The pa-tient had a long-lasting response to chronic leuprolide acetate treatment, showing that drug therapy exploiting the aberrant receptor expression in PBMAH is possible even in the absence of cortisol overproduction.

Keywords

adrenocortical tumors; Cushing syndrome; genetics; mastodynia; macromastia; phosphodiesterase; leuprolide

Introduction

Primary bilateral macronodular adrenocortical hyperplasia (PBMAH) is an uncommon cause of adrenal Cushing syndrome (CS). Inactivating mutations in Armadillo repeat containing 5 (*ARMC5*), a putative tumor suppressor gene, is the primary genetic cause of PBMAH, being mutated in approximately half of the known cases [1] Other genetic defects implicated in the pathogenesis of PBMAH include loss of protein kinase A (PKA) type I regulatory subunit alpha (*PRKAR1A*) expression or activating mutations of the guanine nucleotide binding protein alpha stimulating subunit (*GNAS*) gene both at the somatic (tumor) level only, and germline mutations in the phosphodiesterase 11 A (*PDE11A*) or 8B (*PDE8B*), menin (*MEN1*), adenomatous polyposis coli (*APC*), and fumarate hydrat (*FH*) genes [2].

In PBMAH, the synthesis of cortisol, and/or other steroid hormones, such as aldosterone, testosterone, and estrogen, can be stimulated by aberrant G-protein coupled receptors (GPCRs). Identification of aberrant receptors, such as luteinizing hormone receptor (LHr) [3–6], vasopressin [7–9], or gastric inhibitory polypeptide [3, 10, 11], offers the opportunity to control hormone production in PBMAH using medical therapy. The gonadotropin-releasing hormone (GnRH) agonist, leuprolide acetate, has been successfully used in patients with LH-responsive CS [3, 11, 12]. Herein, we report the first case of PBMAH presenting with macromastia and mas-todynia due to what appeared to be excess estrogen secretion from the nodular left adrenal gland; she has been successfully treated with leuprolide acetate therapy with long-lasting remission of her symptoms and signs.

Patient and Methods

Participant and data collection

In 2006, a 64-year-old Caucasian female presented to the National Institutes of Health Clinical Center for evaluation of progressive macro-mastia and mastodynia (▶Fig. 1a). Her past medical history was no-table for recurrent nephrolithiasis, partial hysterectomy due to uterine prolapse (premature menopause), and a right adrenalectomy for Cushing syndrome (CS) due to a right adrenocortical adenoma [maximum diameter 2 cm, density of 20 Hounsfield units (HU)] that was discovered during the workup of hypertension at age 55 years. Over a period of 5 years prior to her diagnosis of CS, she had noted central weight

gain of 40 lbs, hair loss, insomnia, palpitations, emotional liability and clitoromegaly. Her family history was unremarkable for any endocrinopathies. She underwent an uneventful laparoscopic right adrenalectomy and required hydrocortisone replacement therapy for adrenal insufficiency for approximately two years. Her CS appearance completely resolved post operatively. Her bone mineral density was normal and she did not have diabetes mellitus.

From the age of 58 to 64, she noted increasing breast size (32C in her 20's, 36C in her 40's to 42 DDD at time of presentation), breast tenderness, a 25 lbs weight gain, and skin changes, including decreased number and depth of wrinkles, decreased skin dryness, and increased firmness, particularly on the face. Her menstrual history was unremarkable until the partial hysterectomy. At presentation to our institution she was hypertensive (150/80 mm Hg) and felt uncomfortable due to her macromastia and mastodynia. She had no stigmata of CS. She was taking a thiazide diuretic and an angiotensin-converting-enzyme inhibitor with suboptimal con-trol; she was also on statin therapy.

Biochemical evaluation is summarized in ▶Table 1. The patient's serum estradiol was elevated, despite her being post-menopausal. Her elevated LH and follicular stimulating hormone (FSH) were con-gruent with her post-menopausal state. Baseline 24-h urinary 17-hy-droxysteroids were high at 24.2 mg/24 h (reference 2–6 mg/24 h, ▶Table 1, 2), but otherwise her evaluation for CS was unremarkable with late night serum cortisol, 24-h urine free cortisol, and adreno-corticotropic hormone (ACTH) levels within the normal range. Serum inhibin A (INHA) was < 1 pg/ml (postmenopausal reference value < 2.1 pg/ml). The Liddle's test was performed as described else-where [13] (▶Table 2 and Supplemental Table 1S). Given the suspicion for estrogen excess from the left adrenal gland as the cause of macromastia, serum and urine fractionated estrogens were evaluated during the Liddle's test (▶Table 2). Their pattern of secretion was consistent with non-ovarian origin and suggestive of an adrenal source, particularly given the elevations in estrone (E1). Adrenal CT showed a hyperplastic left adrenal gland with multiple nodules, with the largest nodule measuring 1.2 cm (10 HU) on its largest dimension (▶Fig. 2a). Of note, biochemical evaluation before the right adrenalectomy did not extend to other steroids and we have no androgen or estrogen values available for comparison. Because of the suspicion for possible estrogen-production from the left adrenal, additional molecular and genetic testing was performed on tissue from the previously resected right adrenal gland. Ethics approval was granted from the Institutional Review Board. Informed consent was obtained for all studies from the patient.

DNA investigations

DNA studies of peripheral blood cells and the adenomatous right adrenocortical tissue included whole-exome sequencing (WES) and targeted sequencing of *ARMC5*, *MEN1*, *GNAS*, *FH*, and *PRKAR1A*, as previously reported [1, 14]. PCR was performed for the 20 coding exons (exons 1–20) and the flanking intronic sequences of the *PDE11A* gene (OMIM: 610475) using specific primers and conditions as described previously [15, 16]. Analysis of *INHA* was performed as described elsewhere [17]. The *PDE8B* gene was sequenced as previously reported [18]. All amplified samples were validated by agarose gel

electrophoresis, and direct sequencing of the purified fragments was performed using the Genetic Sequencer ABI3100 apparatus (Applied Biosystems, Foster City, CA, USA).

Immunohistochemistry

Tissue from the patient's previously resected right adrenal gland was obtained: five-micron slides from formalin-fixed paraffin embedded tissue blocks were used for immunohistochemistry (IHC). Slides were deparaffinized in Histo-Clear (HS-202, National diagnostics, Atlanta, GA, USA) for 30 min followed by rehydration in graded ethanol. Antigen retrieval was achieved by heating the slides in Vector Antigen Retrieval Solution (H3300, Vector Laboratories, Burlingame, CA, USA) at 95 °C for 20 min. Endogenous peroxidase activity was blocked by incubation in 3 % hydrogen peroxide for 30 min. After 1 h blocking, sections were incubated over-night at 4 °C with the following primary antibodies: rabbit anti-luteinizing hormone receptor (1:2000, Sigma, Saint Louis, MO, USA), rabbit anti-Aromatase (1:1500, Sigma, Saint Louis, MO, USA), and rabbit anti-17-α-hydroxylase (1:1000, Abcam, Cambridge, MA, USA), and rabbit PDE11A (1:200, Abcam, Cambridge, MA, USA). Rabbit immunoglobulins were used at 1 μg/ml instead of primary antibodies as negative controls. Horseradish peroxidase-dextran polymer conjugate goat anti-rabbit (Envision™ PO System; Dako, Carpinteria, CA, USA) was used as secondary antibody and reporter as previously described, with 3,3-diaminobenzidine as chromo-gen. The sections were then briefly counterstained with Mayer's hematoxylin, dehydrated in graded alcohols, cleared in Histoclear and permanently mounted. Staining was evaluated in 3 high-power fields and samples scored as strongly positive (3 +), moderately positive (2 +), weakly positive (1 +) or negative (0). Staining pat-tern (membranous, cytoplasmic or nuclear) was also recorded.

Results

Medical therapy and follow up

The patient was initiated on 30 mg of intramuscular injections of leuprolide acetate every 4 months in 2007. She reported immedi-ate improvement in her symptoms with gradually decreasing breast size. She was followed yearly; currently, at the age of 75 years, her breast size is 38 DD without swelling or mastodynia (▶Fig. 1b) and she remains well, on the same therapy, without side effects. Serum gonadotropin and estrogen levels remained suppressed to this date. Repeat biochemical evaluations over the years of treatment are summarized in ▶Table 1 and ▶Table 2. Our patient's oldest daughter was found to have the same *PDE11A* mutation; she has PBMAH on imaging but retains normal bone mineral density, HbA1c, androgen, estrogen, and cortisol levels.

DNA sequencing results

WES from blood leukocytes revealed a non-novel p.Arg52Thr (c.155 G > C; rs77972073; NM_016953) pathogenic mutation in *PDE11A* which was also present in adrenocortical tissue from the right adrenalectomy (▶Fig. 3). There were two single nucleotide *INHA* polymorphisms: the first was located early in the 5′ non-cod-ing region of exon 1 (g.3081 G > T; NM_002191), and the second was a rare synonymous variant in exon 1 (c.207 C > T; rs371366906; NM_002191), corresponding to amino acid 69. No effect on the splice site

was identified using the splice site calculator by Desmet et al. [19]. Genetic analysis for germline *ARMC5* defects revealed only the common benign polymorphism (p.Ala705Val; c. 2114C > T; rs11150624; NM_024742). Genetic analysis for the other genes implicated in PBMAH in blood and hyperplastic adrenocortical tissues were negative for any other germline or somatic mutations.

Immunohistochemistry

Adrenocortical hyperplasia was evident, with a predominant cortical nodule extending centrally in the right adrenal gland (Supplemental Fig. 1a–f). Marked hyperplasia of the zona fasciculata with pale-staining, lipid-depleted cells was seen (Supplemental Fig. 1b). Table 3 shows a summary of the staining results. The hyperplas-tic area was negative for synaptophysin (SYN) staining; SYN instead was strongly positive (3 +) in the adrenal medulla as expected (Supplemental Fig. 1a–f, and 2a, b). Expression of estrogen receptor (ER) was consistently negative in all areas; P450 aromatase (CYP19A1) was expressed in both, the cortical non-hyperplastic area (1 + ; ▶Fig. 4a and c) and to a higher degree (2 +) in the adenomatous area (▶Fig. 4c and d). LHr showed a similar staining pattern (▶Fig. 5). Immunohistochemistry for 17-alpha hydroxylase showed moderate to strong (2–3 +) staining in the cells from the zona reticularis and fasciculata, and to a lesser degree in the zona glomerulosa. The expression of PDE11 A was lower in the adeno-matous regions compared to surrounding tissues (▶Fig. 6). However, loss of heterozygosity (LOH) for *PDE11 A* was not found in the hyperplastic, or normal adrenocortical tissues.

Discussion

This is the first report of a patient presenting with asynchronous PBMAH, who had initially presented with CS and subsequently with androgen and estrogen excess, the latter causing presumably macromastia and mastodynia. Biochemical and IHC testing were con-sistent with an adrenal source of estrogen excess. The patient had an immediate and sustained response to leuprolide acetate thera-py for over 10 years. Genetic testing revealed several variants in modifier genes, including what may be a pathogenic mutation in *PDE11A*, which could have potentiated adrenal steroid hormone production by increased PKA signaling through the LHr.

Feminizing adrenocortical tumors (ADTs) are rare, with an esti-mated prevalence of 1–2 % among patients with adrenocortical cancer [20]. However, excess estrogen production has never before been reported in the context of PBMAH. Pre-menopausal patients with estrogen-producing ADTs generally present with menstrual changes, whereas post-menopausal present often with vaginal bleeding. Patients can have elevation of serum estradiol and/or serum adrenal androgens, or the adrenal androgen-derived estrone; increased urinary estrogen along with 17-ketosteroid excretion is often detected in these cases [21–24]. The patient in this report, had mild elevations in serum and urinary estrogen levels and large elevations of urinary 17-hydroxysteroids; the latter is common among patients with PBMAH, as we have reported previously [14].

After the initial discovery of LH/hCG receptors in cells that contain cytochrome P450 side chain cleavage enzyme in zona reticularis [25], several reports have confirmed the presence

of aberrant LHr expression in adrenocortical tumors. Lacroix et al. [3] first reported on the successful treatment with leuprolide acetate, a synthetic nonapeptide gonadotropin releasing hormone (GnRH) analogue, of a 63-year-old female with PBMAH; this patient's cortisol production was stimulated by hCG and LH, but not FSH, suggesting that a functional receptor for adrenocortical LH/hCG was coupled to steroidogenesis. Goodarzi et al. [26] then reported a viri-lized 59-year-old woman with PBMAH and LHr-mediated elevations in serum testosterone levels, with normalization of values post le-uprolide acetate [26]. Since, other cases have been described with successful treatment of PBMAH using medications targeting the corresponding aberrant receptor(s), including leuprolide acetate [6, 27]. The fact that leuprolide decreases adrenal androgen production has also been confirmed in other settings, such as men being treated for prostate cancer [28]. Our case is unique given the asynchronous presentation, elevations in androgens and estrogens, and long-term clinical improvement on chronic leuprolide therapy which apparently successfully stopped any further aromatization of androstenedione [29].

The LHr, which is predominantly found in gonadal tissues, was overexpressed in the adrenal gland of this patient. LH plays an important role in the regulation of steroid hormone production and steroid metabolizing enzymes [30, 31]. LHr is one of several G-protein couple receptors that have been associated with hypercortisolism in PBMAH [6], and hyperaldosteronism in aldosterone-producing adenomas [32, 33]. In one case series of patients with ad-renal CS, aberrant cortisol production was found frequently [34]. It has been suggested that aberrant receptors may be a frequent cause of increased steroid hormone production by ADTs [35]. Ab-errant LHr expression alone is sufficient to cause both cortisol excess and tissue hyperplasia in mice [31].

Many of the genetic mechanisms of adrenal tumorigenesis have yet to be elucidated. Recently, *ARMC5*, a tumor suppressor gene, was implicated in PBMAH [1]. *PDE11A* is frequently mutated among patients with PBMAH as well [16]. The *PDE11A* gene is located on chromosome 2q31.2 and encodes a dual-specificity PDE that de-grades both cAMP and cGMP [36]. This gene is highly polymorphic in the general population and was the first of the PDEs to be asso-ciated with a predisposition to ADTs [37]. Despite the presence of what may be a pathogenic variant in the *PDE11A* gene, our patient did not have LOH of *PDE11A* in the previously excised affected adrenal gland (▶Fig. 3), although there was decreased PDE11A expression in the adenomatous area (▶Fig. 5).

In conclusion, we have described the first patient with PBMAH to present with estrogen excess, macromastia and mastodynia that was most likely due to an estrogen-producing hyperplastic left adrenal gland, several years after her right gland was resected for CS. The report speaks volumes for the clinical heterogeneity of PBMAH over time even in the same patient; in addition, this case represents one of the longest successfully medically treated patients with a hormonal syndrome due to an aberrantly expressed GPCR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ACTH	Adrenocorticotropic hormone
CS	Cushing syndrome
GPCRs	G protein-coupled receptors
PBMAH	Primary bilateral macronodular adrenocortical hyperplasia

References

[1]. Assie G, Libe R, Espiard S, Rizk-Rabin M, Guimier A, Luscap W, Barreau O, Lefevre L, Sibony M, Guignat L, Rodriguez S, Perlemoine K, Rene-Co-rail F, Letourneur F, Trabulsi B, Poussier A, Chabbert-Buffet N, Borson-Chazot F, Groussin L, Bertagna X, Stratakis CA, Ragazzon B, Bertherat J. ARMC5 mutations in macronodular adrenal hyperplasia with Cushing's syndrome. N Engl J Med 2013; 369: 2105–2114 [PubMed: 24283224]

[2]. Yu B, Ragazzon B, Rizk-Rabin M, Bertherat J. Protein kinase A alterations in endocrine tumors. Horm Metab Res 2012; 44: 741–748 [PubMed: 22752956]

[3]. Lacroix A, Hamet P, Boutin JM. Leuprolide acetate therapy in luteinizing hormone--dependent Cushing's syndrome. N Engl J Med 1999; 341: 1577–1581 [PubMed: 10564687]

[4]. Carlson HE. Human adrenal cortex hyperfunction due to LH/hCG. Mol Cell Endocrinol 2007; 269: 46–50 [PubMed: 17363138]

[5]. Bertherat J, Contesse V, Louiset E, Barrande G, Duparc C, Groussin L, Emy P, Bertagna X, Kuhn JM, Vaudry H, Lefebvre H. In vivo and in vitro screening for illegitimate receptors in adrenocorticotropin-independent macronodular adrenal hyperplasia causing Cushing's syndrome: identification of two cases of gonadotropin/gastric inhibitory polypeptide-dependent hypercortisolism. J Clin Endocrinol Metab 2005; 90: 1302–1310 [PubMed: 15585558]

[6]. Bourdeau I, D'Amour P, Hamet P, Boutin JM, Lacroix A. Aberrant membrane hormone receptors in incidentally discovered bilateral macronodular adrenal hyperplasia with subclinical Cushing's syndrome. J Clin Endocrinol Metab 2001; 86: 5534–5540 [PubMed: 11701732]

[7]. Horiba N, Suda T, Aiba M, Naruse M, Nomura K, Imamura M, Demura H. Lysine vasopressin stimulation of cortisol secretion in patients with adrenocorticotropin-independent macronodular adrenal hyperplasia. J Clin Endocrinol Metab 1995; 80: 2336–2341 [PubMed: 7629226]

[8]. Gagliardi L, Hotu C, Casey G, Braund WJ, Ling KH, Dodd T, Manavis J, Devitt PG, Cutfield R, Rudzki Z, Scott HS, Torpy DJ. Familial vasopres-sin-sensitive ACTH-independent macronodular adrenal hyperplasia (VPs-AIMAH): clinical studies of three kindreds. Clin Endocrinol (Oxf) 2009; 70: 883–891 [PubMed: 19018784]

[9]. Hofland J, Hofland LJ, van Koetsveld PM, Steenbergen J, de Herder WW, van Eijck CH, de Krijger RR, van Nederveen FH, van Aken MO, de Groot JW, Links TP, de Jong FH, Feelders RA. ACTH-independent macronodular adrenocortical hyperplasia reveals prevalent aberrant in vivo and in vitro responses to hormonal stimuli and coupling of arginine-vasopressin type 1a receptor to 11b-hydroxylase. Orphanet J Rare Dis 2013; 8: 142 [PubMed: 24034279]

[10]. Lacroix A, Bolte E, Tremblay J, Dupre J, Poitras P, Fournier H, Garon J, Garrel D, Bayard F, Taillefer R, Flanagan RJ, Hamet P. Gastric inhibitory polypeptide-dependent cortisol

hypersecretion–a new cause of Cushing's syndrome. N Engl J Med 1992; 327: 974–980 [PubMed: 1325608]

[11]. Preumont V, Mermejo LM, Damoiseaux P, Lacroix A, Maiter D. Transient efficacy of octreotide and pasireotide (SOM230) treatment in GIP-dependent Cushing's syndrome. Horm Metab Res 2011; 43: 287–291 [PubMed: 21264796]

[12]. Feelders RA, Lamberts SW, Hofland LJ, van Koetsveld PM, Verhoef-Post M, Themmen AP, de Jong FH, Bonjer HJ, Clark AJ, van der Lely AJ, de Herder WW. Luteinizing hormone (LH)-responsive Cushing's syndrome: the demonstration of LH receptor messenger ribonucleic acid in hyperplastic adrenal cells, which respond to chorionic gonadotropin and serotonin agonists in vitro. J Clin Endocrinol Metab 2003; 88: 230–237 [PubMed: 12519858]

[13]. Stratakis CA, Sarlis N, Kirschner LS, Carney JA, Doppman JL, Nieman LK, Chrousos GP, Papanicolaou DA. Paradoxical response to dexameth-asone in the diagnosis of primary pigmented nodular adrenocortical disease. Ann Intern Med 1999; 131: 585–591 [PubMed: 10523219]

[14]. Hsiao HP, Kirschner LS, Bourdeau I, Keil MF, Boikos SA, Verma S, Robinson-White AJ, Nesterova M, Lacroix A, Stratakis CA. Clinical and genetic heterogeneity, overlap with other tumor syndromes, and atypical glucocorticoid hormone secretion in adrenocorticotropin-in-dependent macronodular adrenal hyperplasia compared with other adrenocortical tumors. J Clin Endocrinol Metab 2009; 94: 2930–2937 [PubMed: 19509103]

[15]. Horvath A, Boikos S, Giatzakis C, Robinson-White A, Groussin L, Griffin KJ, Stein E, Levine E, Delimpasi G, Hsiao HP, Keil M, Heyerdahl S, Matyakhina L, Libe R, Fratticci A, Kirschner LS, Cramer K, Gaillard RC, Bertagna X, Carney JA, Bertherat J, Bossis I, Stratakis CA. A ge-nome-wide scan identifies mutations in the gene encoding phosphodi-esterase 11A4 (PDE11A) in individuals with adrenocortical hyperplasia. Nat Genet 2006; 38: 794–800 [PubMed: 16767104]

[16]. Libe R, Fratticci A, Coste J, Tissier F, Horvath A, Ragazzon B, Rene-Co-rail F, Groussin L, Bertagna X, Raffin-Sanson ML, Stratakis CA, Bertherat J. Phosphodiesterase 11A (PDE11A) and genetic predisposition to adrenocortical tumors. Clin Cancer Res 2008; 14: 4016–4024 [PubMed: 18559625]

[17]. Longui CA, Lemos-Marini SH, Figueiredo B, Mendonca BB, Castro M, Liberatore R, Jr., Watanabe C, Lancellotti CL, Rocha MN, Melo MB, Monte O, Calliari LE, Guerra-Junior G, Baptista MT, Sbragia-Neto L, Latronico AC, Moreira A, Tardelli AM, Nigri A, Taymans SE, Stratakis CA. Inhibin alpha-subunit (INHA) gene and locus changes in paediatric adrenocortical tumours from TP53 R337H mutation heterozygote carriers. J Med Genet 2004; 41: 354–359 [PubMed: 15121773]

[18]. Rothenbuhler A, Horvath A, Libé R, Faucz FR, Fratticci A, Raffin Sanson ML, Vezzosi D, Azevedo M, Levy I, Almeida MQ, Lodish M, Nesterova M, Bertherat J, Stratakis CA. Identification of novel genetic variants in phosphodiesterase 8B (PDE8B), a cAMP-specific phosphodiesterase highly expressed in the adrenal cortex, in a cohort of patients with adrenal tumours. Clin Endocrinol (Oxf) 2012; 77: 195–199 [PubMed: 22335482]

[19]. Desmet FO, Hamroun D, Lalande M, Collod-Beroud G, Claustres M, Beroud C. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res 2009; 37: e67 [PubMed: 19339519]

[20]. Moreno S, Guillermo M, Decoulx M, Dewailly D, Bresson R, Proye C. Feminizing adreno-cortical carcinomas in male adults. A dire prognosis. Three cases in a series of 801 adrenalectomies and review of the literature. Ann Endocrinol (Paris) 2006; 67: 32–38 [PubMed: 16596055]

[21]. Millington DS, Golder MP, Cowley T, London D, Roberts H, Butt WR, Griffiths K. In vitro synthesis of steroids by a feminising adrenocortical carcinoma: effect of prolactin and other protein hormones. Acta Endocrinol (Copenh) 1976; 82: 561–571 [PubMed: 180740]

[22]. Phornphutkul C, Okubo T, Wu K, Harel Z, Tracy TF, Jr., Pinar H, Chen S, Gruppuso PA, Goodwin G. Aromatase p450 expression in a feminizing adrenal adenoma presenting as isosexual precocious puberty. J Clin Endocrinol Metab 2001; 86: 649–652 [PubMed: 11158024]

[23]. Gabrilove JL, Sharma DC, Wotiz HH, Dorfman RI. Feminizing adrenocortical tumors in the male. A review of 52 cases including a case report. Medicine (Baltimore) 1965; 44: 37–79 [PubMed: 14264352]

[24]. Wotiz HH, Chattoraj SC, Gabrilove JL. Urinary estrogen titers in a patient with feminizing adrenocortical carcinoma. J Clin Endocrinol Metab 1968; 28: 192–197 [PubMed: 5636151]

[25]. Pabon JE, Li X, Lei ZM, Sanfilippo JS, Yussman MA, Rao CV. Novel presence of luteinizing hormone/chorionic gonadotropin receptors in human adrenal glands. J Clin Endocrinol Metab 1996; 81: 2397–2400 [PubMed: 8964884]

[26]. Goodarzi MO, Dawson DW, Li X, Lei Z, Shintaku P, Rao CV, Van Herle AJ. Virilization in bilateral macronodular adrenal hyperplasia controlled by luteinizing hormone. J Clin Endocrinol Metab 2003; 88: 73–77 [PubMed: 12519832]

[27]. Bovenberg SA, Pieters GF, Hofland LJ, Hermus AR. Leuprolide acetate therapy in LH-dependent Cushing's syndrome: In vivo and in vitro observations. Neth J Med 2004; 62: 456–458 [PubMed: 15685898]

[28]. Nishii M, Nomura M, Sekine Y, Koike H, Matsui H, Shibata Y, Ito K, Oyama T, Suzuki K. Luteinizing hormone (LH)-releasing hormone agonist reduces serum adrenal androgen levels in prostate cancer patients: Implications for the effect of LH on the adrenal glands. J Androl 2012; 33: 1233–1238 [PubMed: 22492843]

[29]. Young J, Bulun SE, Agarwal V, Couzinet B, Mendelson CR, Simpson ER, Schaison G. Aromatase expression in a feminizing adrenocortical tumor. J Clin Endocrinol Metab 1996; 81: 3173–3176 [PubMed: 8784064]

[30]. Mazzuco TL, Chabre O, Feige JJ, Thomas M. Aberrant expression of human luteinizing hormone receptor by adrenocortical cells is sufficient to provoke both hyperplasia and Cushing's syndrome features. J Clin Endocrinol Metab 2006; 91: 196–203 [PubMed: 16249277]

[31]. Kero J, Poutanen M, Zhang FP, Rahman N, McNicol AM, Nilson JH, Keri RA, Huhtaniemi IT. Elevated luteinizing hormone induces expression of its receptor and promotes steroidogenesis in the adrenal cortex. J Clin Invest 2000; 105: 633–641 [PubMed: 10712435]

[32]. Saner-Amigh K, Mayhew BA, Mantero F, Schiavi F, White PC, Rao CV, Rainey WE. Elevated expression of luteinizing hormone receptor in aldosterone-producing adenomas. J Clin Endocrinol Metab 2006; 91: 1136–1142 [PubMed: 16332935]

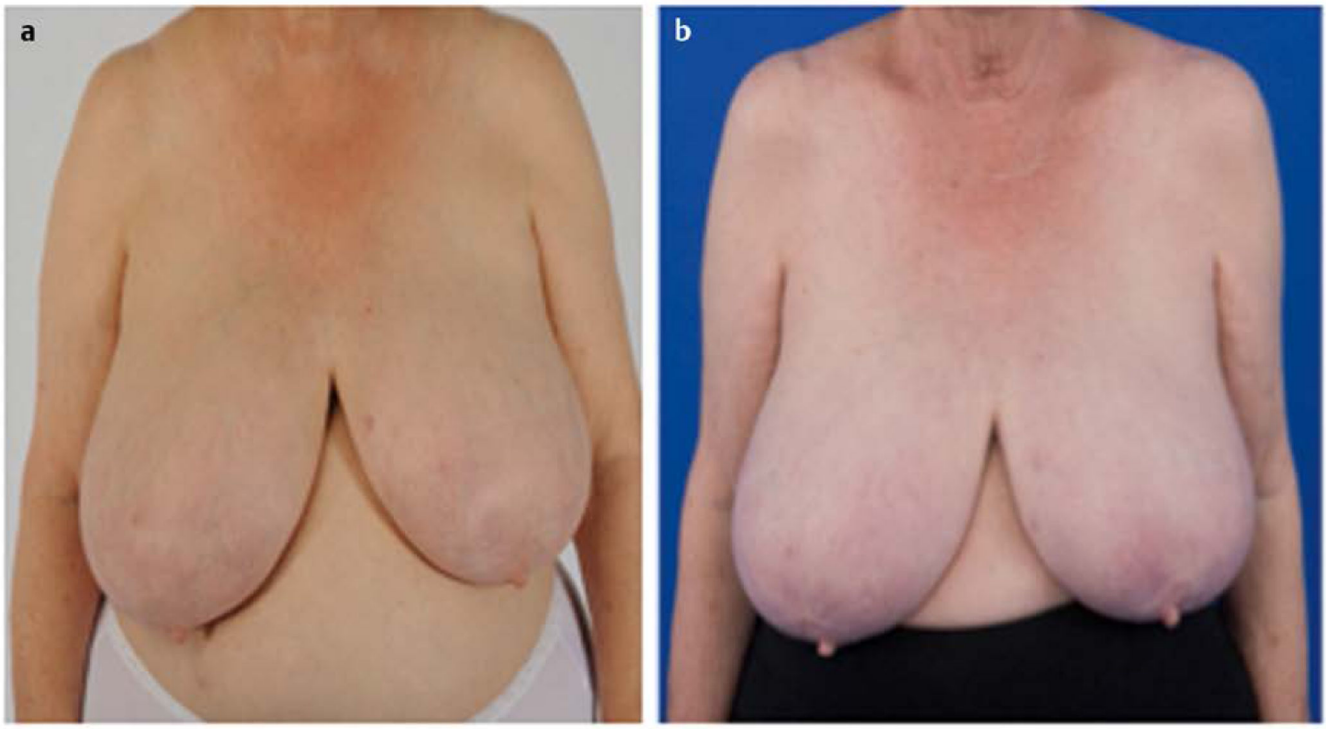
[33]. Ye P, Mariniello B, Mantero F, Shibata H, Rainey WE. G-protein-coupled receptors in aldosterone-producing adenomas: A potential cause of hyperaldosteronism. J Endocrinol 2007; 195: 39–48 [PubMed: 17911395]

[34]. Mircescu H, Jilwan J, N'Diaye N, Bourdeau I, Tremblay J, Hamet P, Lacroix A. Are ectopic or abnormal membrane hormone receptors frequently present in adrenal Cushing's syndrome? J Clin Endocrinol Metab 2000; 85: 3531–3536 [PubMed: 11061496]

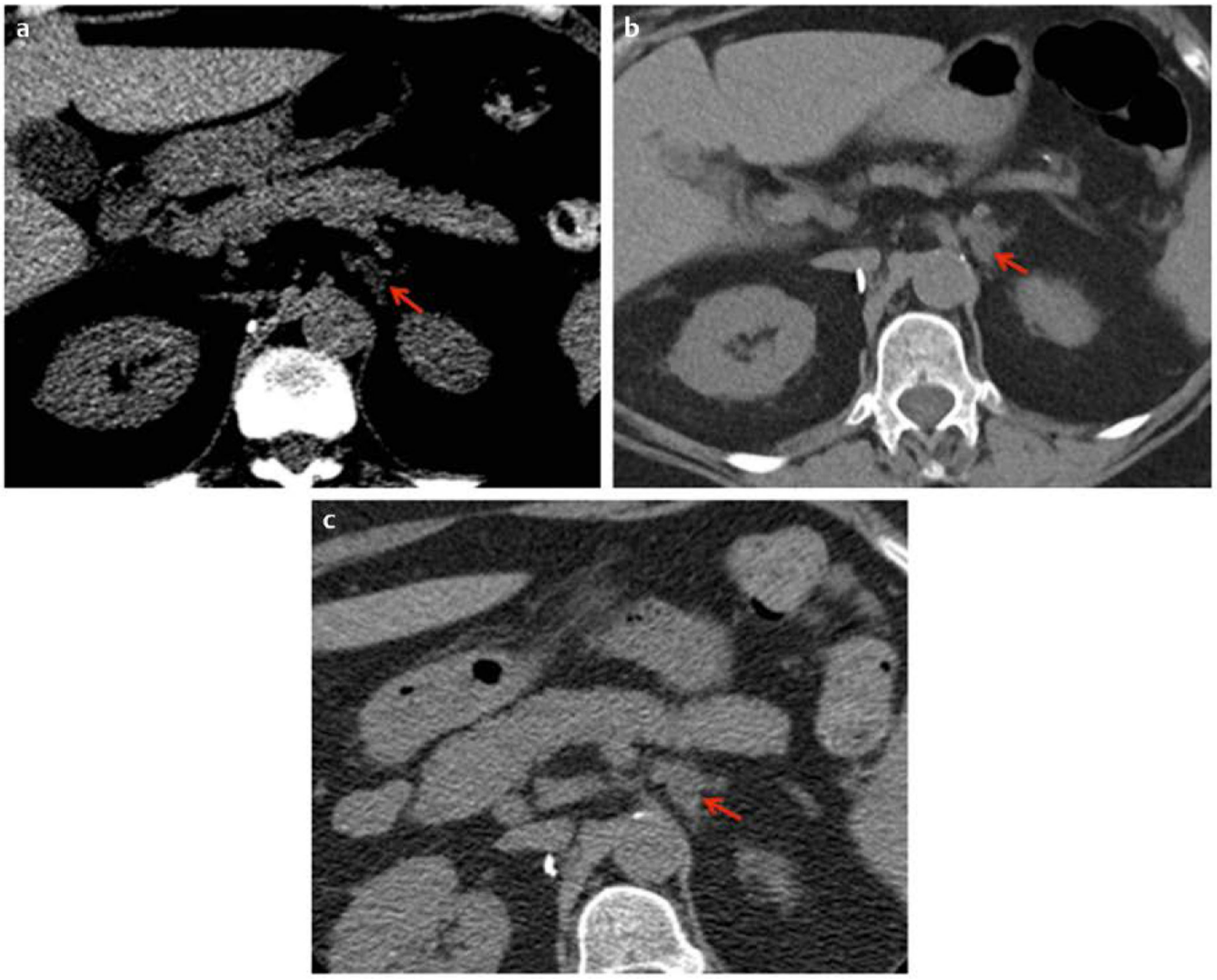
[35]. Christopoulos S, Bourdeau I, Lacroix A. Aberrant expression of hormone receptors in adrenal Cushing's syndrome. Pituitary 2004; 7: 225–235 [PubMed: 16010457]

[36]. Szarek E, Stratakis CA. Phosphodiesterases and adrenal Cushing in mice and humans. Horm Metab Res 2014; 46: 863–868 [PubMed: 25232906]

[37]. Horvath A, Giatzakis C, Robinson-White A, Boikos S, Levine E, Griffin K, Stein E, Kamvissi V, Soni P, Bossis I, de Herder W, Carney JA, Bertherat J, Gregersen PK, Remmers EF, Stratakis CA. Adrenal hyperplasia and adenomas are associated with inhibition of phosphodiesterase 11A in carriers of PDE11A sequence variants that are frequent in the population. Cancer Res 2006; 66: 11571–11575 [PubMed: 17178847]

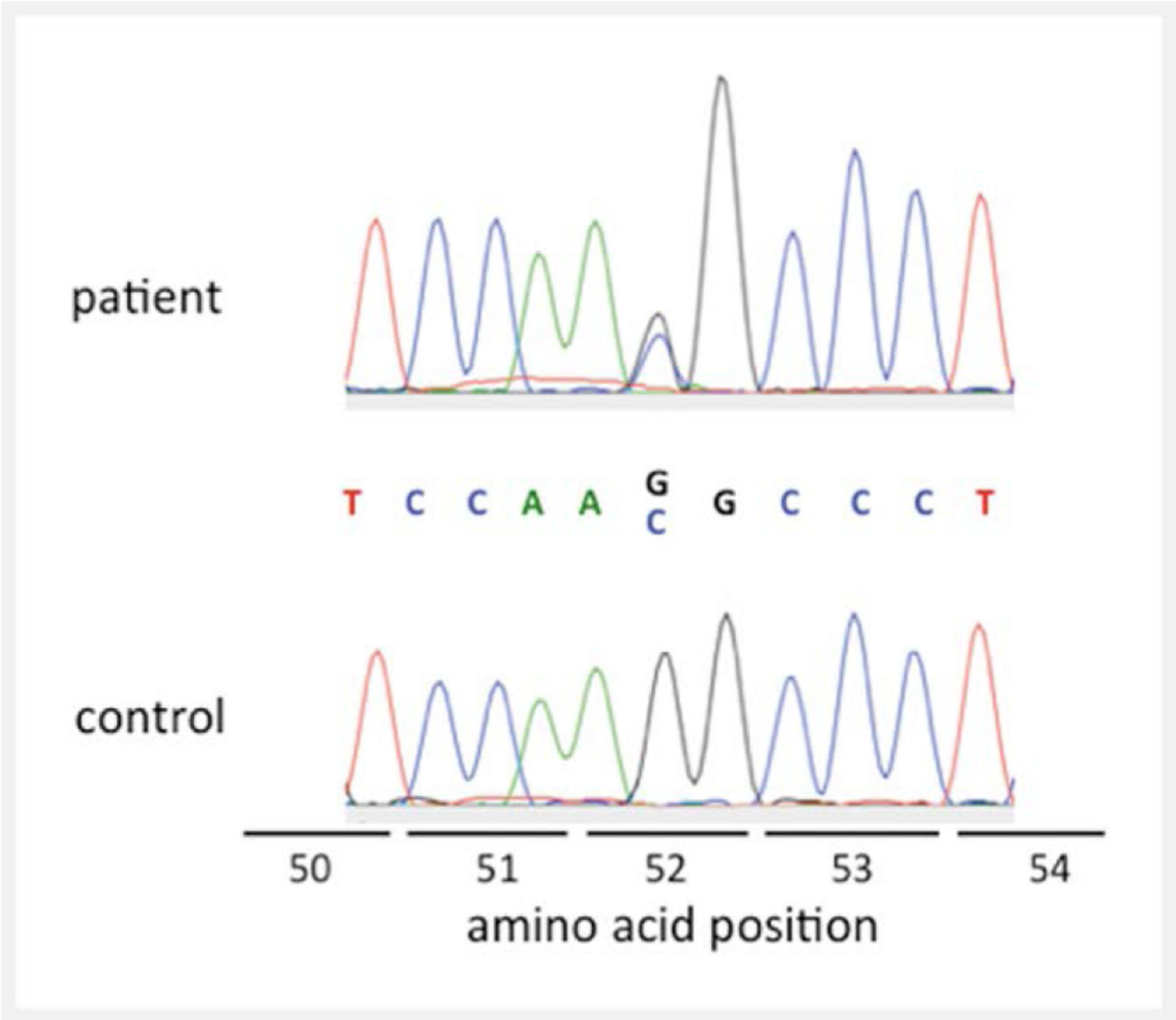


▶**Fig. 1.**
Improvement in bilateral macromastia post-leuprolide acetate therapy (**a** pre therapy, at 64-year-old, **b** post therapy, at 74-year-old).

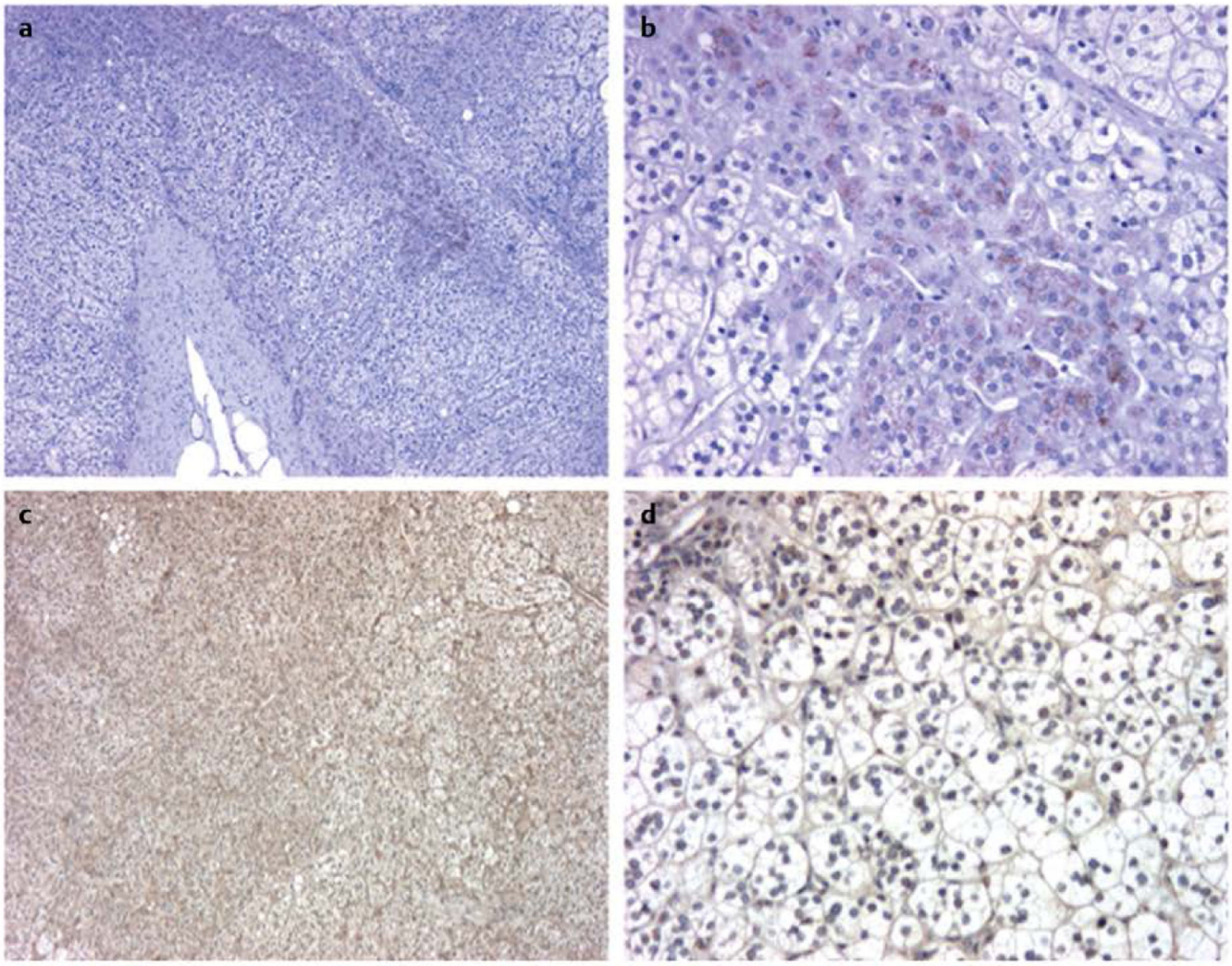


▶**Fig. 2.**

Adrenal computed tomography pre-adrenalectomy (**a** Initial presentation, 55-year-old) and several years later (**b** 64-year-old, **c**; 73-year-old) showing progressive enlargement and nodularity of the remaining left adrenal gland (arrows).

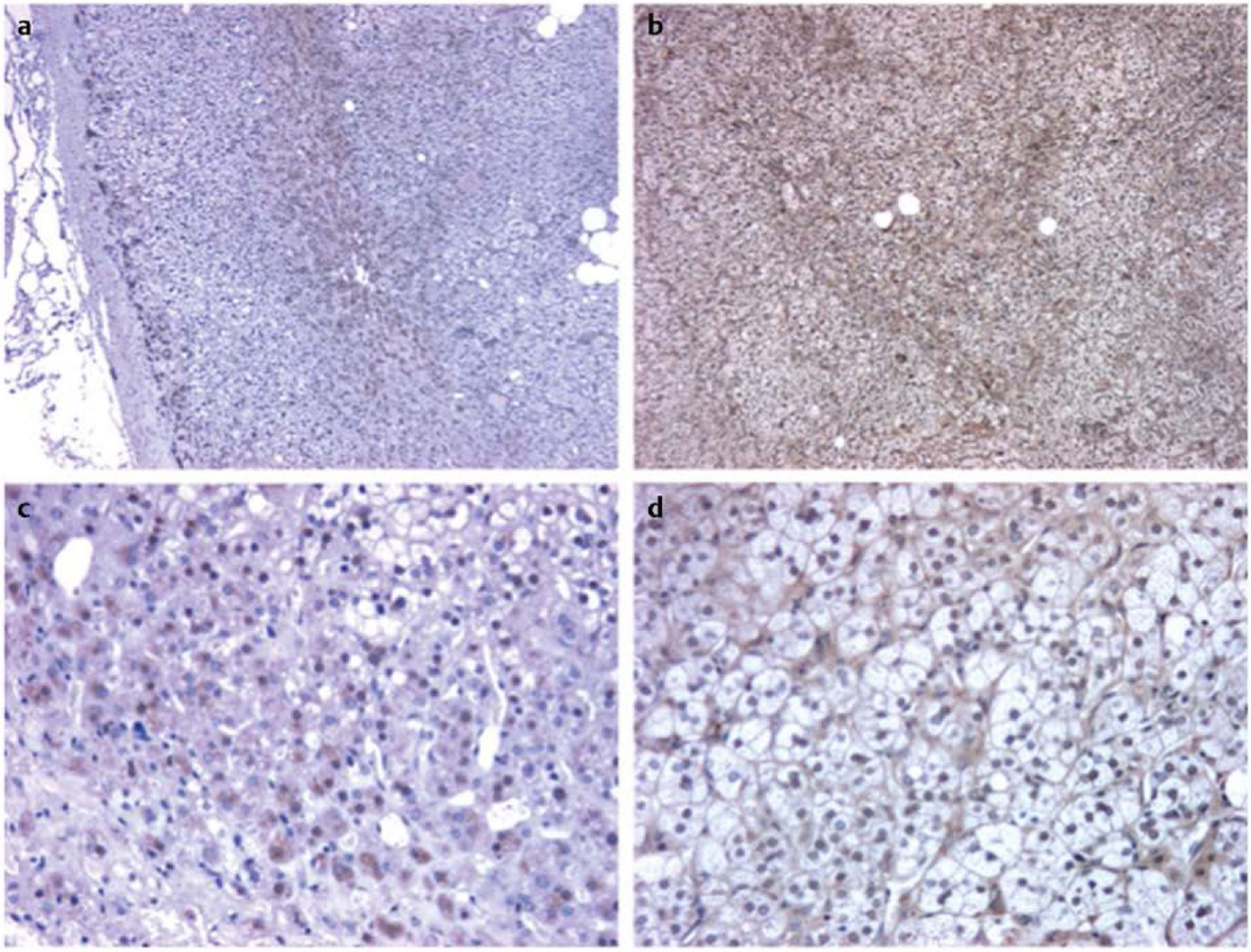


▶Fig. 3.
DNA sequencing from tumor tissue revealed a non-novel p.Arg52Thr mutation in *PDE11A*.



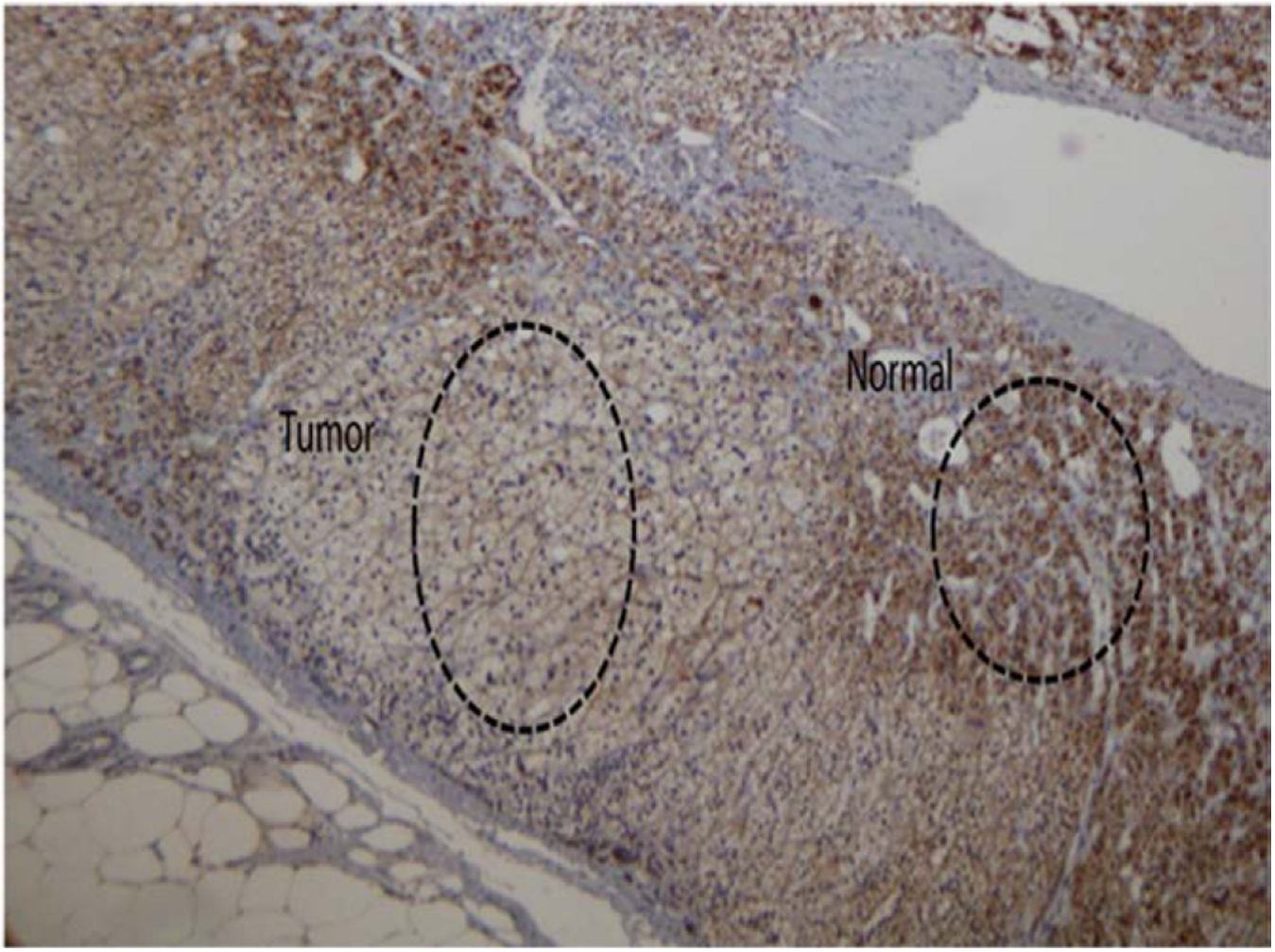
▶Fig. 4.

Immunohistochemistry for P450 aromatase expression in the previously resected right adrenal gland. Cortical cells (panel **a**, **c**; magnification 10 ×) showed less aromatase expression compared to cells from the hyperplastic nodule (**b**, **d**; magnification 10 ×).



▶Fig. 5.

Staining patterns of luteinizing hormone receptor (LHr) in the previously resected right adrenal gland. Granular staining inside the cytoplasm of cortical cells is seen (**a**, **b**; magnification 10 ×). Membranous staining was predominant in the cells from the nodular area (**c**, **d**; magnifica-tion 10 ×).



▶Fig. 6.

Lower expression of PDE11A in the nodular region (“tumor”) compared to other areas of the cortex (“normal”) of the previously resected right adrenal gland (magnification 10 ×).

▶Table 1

Biochemical evaluation of the patient.

Laboratory investigation	**2006**	**2008**	**2012**	**2015**	**2017**
11:30 PM Serum cortisol (< 1.8 μg/dl)	1.4 μg/dl	1.6 μg/dl	3.7 μg/dl	3.9 μg/dl	4.4 μg/dl
ACTH (5–46 pg/ml)	22.3 pg/ml	30.4 pg/ml	16.9 pg/ml	20.3 pg/ml	28.6 pg/ml
Enhanced serum estradiol (< 10 pg/ml)[a]	**14** pg/ml	< 10 pg/ml	5.5 pg/ml	8 pg/ml	< 5 pg/ml
Serum estrone (7–40 pg/ml)[a]	17 pg/ml	23 pg/ml	12.6 pg/ml	36 pg/ml	36 pg/ml
Androstenedione (30–200 ng/dl)	87 ng/dl	50 ng/dl	< 15 ng/dl	74 ng/dl	55 ng/dl
Dehydroepiandrosterone sulfate (2006–2008: 15–157 μg/dl; 2012–2017; 0.35–4.30 μg/dl)[b]	89 μg/dl	59 μg/dl	0.15 μg/dl	0.79 μg/dl	0.91 μg/dl
LH (11–40 U/l)[a]	**42** U/l	< 0.1 U/l	< 0.1 U/l	< 0.1 U/l	< 0.1 U/l
FSH (22–153 U/l)[a]	**58** U/l	10 U/l	6.9 U/l	6.4 U/l	5.1 U/l
Total testosterone (8–60 ng/dl)	**70** ng/dl	< 8 ng/dl	< 20 ng/dl	< 20 ng/dl	< 20 ng/dl
Urinary free cortisol (3.5–45 μg/24 h)	31 μg/24 h	23 μg/24 h	5.3 μg/24 h	7.1 μg/24 h	17.1 μg/24 h
17-Hydroxysteroids (2–6 mg/24 h)	**24.2** mg/24 h	8.2 mg/24 h	3.3 mg/24 h	3.4 mg/24 h	5.8 mg/24 h
Metanephrines, 24-h Ur (< 400 μg/24 h)[c]	36 μg/24 h	NP	28 μg/24 h	33 μg/24 h	28 μg/24 h
Normetanephrine, 24-h Ur (< 900 μg/24 h)[c]	322 μg/24 h	NP	204 μg/24 h	341 μg/24 h	312 μg/24 h
Total metanephrine, 24-h Ur (< 1300 μg/24 h)[c]	358 μg/24 h	NP	232 μg/24 h	374 μg/24 h	340 μg/24 h
Epinephrine, 24-h Ur (< 21 μg/24 h)	0.2 μg/24 h	NP	< 0.9 μg/24 h	1.3 μg/24 h	1.2 μg/24 h
Norepinephrine, 24-h Ur (15–80 μg/24 h)	45 μg/24 h	NP	39 μg/24 h	47 μg/24 h	43 μg/24 h
Dopamine, 24-h Ur (65–400 μg/24 h)	156 μg/24 h	NP	147 μg/24 h	188 μg/24 h	204 μg/24 h

ACTH: Adrenocorticotropic hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; NP: Not performed; Ur: Urine.

[a]References represent postmenopausal ranges. Test performed by Department of Laboratory Medicine, National Institutes of Health, Bethesda, MD, USA.

[b]Reference ranges for dehydroepiandrosterone sulfate (DHEA-S) have changed over the years. Test performed by Department of Laboratory Medicine, National Institutes of Health, Bethesda, MD, USA.

[c]Hypertensive reference ranges. Test performed by Mayo Clinic Department of Laboratory Medicine and Pathology, Rochester, MN, USA.

▶Table 2

Biochemical evaluation of the patient - continued.

Biochemical Studies	Baseline	Post low-dose Liddle's test	Post high-dose Liddle's test	% Reduction
2006 (64-year-old)				
24-h Urinary Fractionated Estrogens: [a,b]				
Total estrogens (10–120 μg/24 h)	47 μg/24 h	36 μg/24 h	40 μg/24 h	14.9 %
Estrone, E1 (4–30 μg/24 h)	**23** μg/24 h	19 μg/24 h	10 μg/24 h	56.5 %
Estradiol, E2 (< 15 μg/24 h)	9 μg/24 h	8 μg/24 h	6 μg/24 h	33.3 %
Estriol, E3 (< 75 μg/24 h)	15 μg/24 h	9 μg/24 h	10 μg/24 h	33.3 %
24-h Urinary Free Cortisol (3.5–45 μg/24 h) [b]	31 μg/24 h	3.8 μg/24 h	< 3.5 μg/24 h	> 89 %
17-Hydroxysteroids (2–6 mg/24 h) [b]	**24.2** μg/24 h	< 2 mg/24 h	< 2 mg/24 h	> 92 %
24-hr Urinary 17-Ketosteroid Fraction: [b,c]				
Androsterone (55–3198 μg/24 h)	473 μg/24 h	233 μg/24 h	155 μg/24 h	67.2 %
Etiocholanolone (151–3198 μg/24 h)	1133 μg/24 h	666 μg/24 h	380 μg/24 h	66.4 %
11-Hydroxyandrosterone (66–1032 μg/24 h)	227 μg/24 h	58 μg/24 h	61 μg/24 h	73.1 %
11-Hydroxyetiocholanolone (17–1006 μg/24 h)	409 μg/24 h	104 μg/24 h	59 μg/24 h	85.6 %
11-Ketoandrosterone (4–55 μg/24 h)	17 μg/24 h	7.5 μg/24 h	< 4.2 μg/24 h	> 75 %
11-Ketoetiocholanolone (51–1016 μg/24 h)	465 μg/24 h	174 μg/24 h	92 μg/24 h	80.2 %
Pregnanetriol (59–1391 μg/24 h)	594 μg/24 h	371 μg/24 h	246 μg/24 h	58.6 %
2008 (66-year-old)				
24-hr Urinary Fractionated Estrogens: [a,b]				
Total estrogens (10–120 μg/24 h)	16 μg/24 h	12 μg/24 h	14 μg/24 h	12.5 %
Estrone, E1 (4–30 μg/24 h)	**1** μg/24 h	2 μg/24 h	1 μg/24 h	0 %
Estradiol, E2 (< 15 μg/24 h)	7 μg/24 h	6 μg/24 h	9 μg/24 h	–28.6 %
Estriol, E3 (< 75 μg/24 h)	4 μg/24 h	8 μg/24 h	3 μg/24 h	25 %
24-h Urinary Free Cortisol (3.5–45 μg/24 h) [b]	23 μg/24 h	6.6 μg/24 h	< 3.5 μg/24 h	> 85 %
17-Hydroxysteroids (2–6 mg/24 h) [b]	8.2 mg/24 h	3.4 mg/24 h	4.3 mg/24 h	46.3 %

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Biochemical Studies	Baseline	Post low-dose Liddle's test	Post high-dose Liddle's test	% Reduction
24-h Urinary 17-Ketosteroid Fraction:[b,c]				
Androsterone (55–1589 μg/24 h)	388 μg/24 h	200 μg/24 h	< 55 μg/24 h	> 86 %
Etiocholanolone (151–3198 μg/24 h)	710 μg/24 h	573 μg/24 h	259 μg/24 h	63.5 %
11-Hydroxyandrosterone (66–1032 μg/24 h)	368 μg/24 h	254 μg/24 h	< 68 μg/24 h	> 81 %
11-Hydroxyetiocholanolone (17–1006 μg/24 h)	65 μg/24 h	68 μg/24 h	< 37 μg/24 h	> 43 %
11-Ketoandrosterone (4–55 μg/24 h)	13 μg/24 h	9.4 μg/24 h	5.5 μg/24 h	57.7 %
11-Ketoetiocholanolone (51–1016 μg/24 h)	151 μg/24 h	157 μg/24 h	< 59 μg/24 h	> 61 %
Pregnanetriol (59–1391 μg/24 h)	304 μg/24 h	286 μg/24 h	97 μg/24 h	68.1 %

N/A: Not applicable.

[a] References represent premenopausal ranges. Postmenopausal ranges are not available. Test performed by Inter Science Institute, Inglewood, CA, USA.

[b] 24-h urinary creatinine ~ 1 g/24 h.

[c] Test performed by Mayo Clinic Department of Laboratory Medicine and Pathology.

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▶Table 3

Summary of the staining results.

	LHr	Aromatase	17-alpha hydroxylase	Synaptophysin	Inhibin A
Adrenal					
Cortex	1 + , cytoplasmic	1–2 + , cytoplasmic	3 + , cytoplasmic	N/A	NP
Hyperplastic nodule	2–3 + , membranous	2–3 + , membranous	3 + , cytoplasmic	N/A	NP
Medulla	N/A	N/A	N/A	3 + , cytoplasmic	NP
Testes (control)					
Leydig	2–3 + , cytoplasmic	N/A	NP	NP	N/A
Sertoli	1–2 + , cytoplasmic	2–3 + , cytoplasmic	NP	NP	3 + , cytoplasmic
Germ cells	1 + , cytoplasmic	N/A	NP	NP	N/A

LHr: Luteinizing hormone receptor; N/A: Not applicable; NP: Not performed.

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