Supplementary methods

Immunohistochemistry

Immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded sections of 5 μm thickness. After deparaffinization, slides were boiled for 15 minutes in pH 9, Tris-based buffer (Vector Laboratories) for epitope retrieval. Peroxidase blocking (GBI Labs) was performed for 10 minutes followed by 10% normal goat serum (Abcam) blocking for 1 hour. Primary antibodies were incubated for 1 hour at room temperature. The following primary antibodies were used: 17α-hydroxylase/17,20 lyase (CYP17A1; diluted 1:2000; LifeSpan Biosciences, LS-B14227; RRID, AB_2857939), cytochrome b5 type A (CYB5A; diluted 1:5000; Acris Antibodies, AM31963PU-N; RRID, AB_11148731), 3β-hydroxysteroid dehydrogenase/isomerase type 2 (HSD3B2; diluted 1:5000; from Dr. Celso Gomez-Sanchez at University of Mississippi Medical Center; RRID, AB_2868546) (1), 11β-hydroxylase (CYP11B1 clone 80-7-3; diluted 1:10; from Dr. Celso Gomez-Sanchez; RRID, AB_2650563) (2), aldosterone synthase (CYP11B2; diluted 1:1250; Millipore, MABS1251; RRID, AB_2783793) (2), 11β-hydroxysteroid dehydrogenase type 2 (HSD11B2; diluted 1:2000; Santa Cruz, sc-365529; RRID, AB_10846196), and Aldo-keto reductase family 1 member C3 (AKR1C3; diluted 1:2000; R&D Systems, MAB7678; RRID, AB_2923127). The Polink-2 HRP Plus DAB Kits (GBI Labs) were used for detection and sections were counterstained with Harris hematoxylin, dehydrated, and coverslipped.

DNA isolation and targeted next-generation sequencing

Serial FFPE tumor sections were scraped using a sterile scalpel under an AmScope 3.5X-45X Stereo Zoom Microscope. Genomic DNA was extracted from scraped material using the

AllPrep DNA/RNA FFPE kit (Qiagen) as described previously (3). To determine somatic mutations in tumor DNA, targeted amplicon-based next-generation sequencing was performed using Ion Torrent System (Thermo Fisher Scientific) (4).

References

1. Gomez-Sanchez CE, Lewis M, Nanba K, Rainey WE, Kuppusamy M, Gomez-Sanchez EP. Development of monoclonal antibodies against the human 3beta-hydroxysteroid dehydrogenase/isomerase isozymes. Steroids. 2017;127:56-61.

2. Gomez-Sanchez CE, Qi X, Velarde-Miranda C, Plonczynski MW, Parker CR, Rainey W, Satoh F, Maekawa T, Nakamura Y, Sasano H, et al. Development of monoclonal antibodies against human CYP11B1 and CYP11B2. Mol Cell Endocrinol. 2014;383(1-2):111-7.

3. Rege J, Hoxie J, Liu CJ, Cash MN, Luther JM, Gellert L, Turcu AF, Else T, Giordano TJ, Udager AM, et al. Targeted Mutational Analysis of Cortisol-Producing Adenomas. J Clin Endocrinol Metab. 2021.

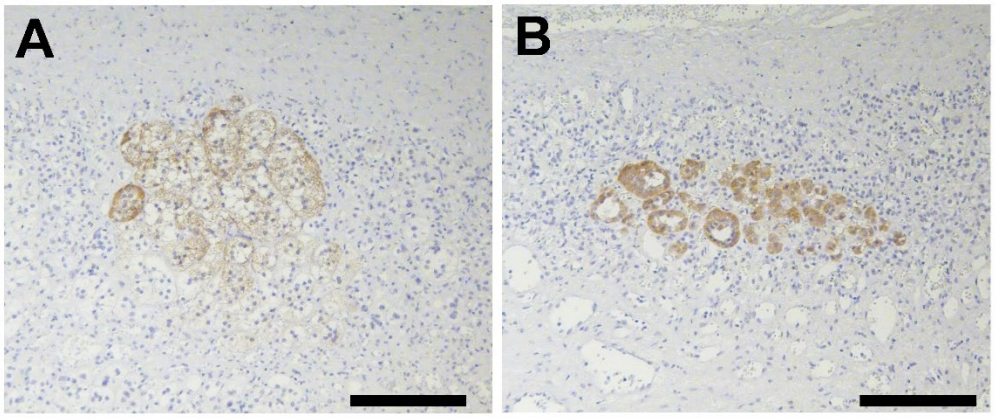
4. Nanba K, Omata K, Else T, Beck PCC, Nanba AT, Turcu AF, Miller BS, Giordano TJ, Tomlins SA, Rainey WE. Targeted Molecular Characterization of Aldosterone-Producing Adenomas in White Americans. J Clin Endocrinol Metab. 2018;103(10):3869-76.

Supplementary Table 1. Summary of pre- and post-operative clinical data

	Baseline	Post-operative follow-up data				
		day 6[a]	7 weeks[b]	3 months[b]	4 months[b]	8 months[b]
ACTH (pg/mL)	<1.5	2.9	NA	NA	33.4	142
Serum cortisol (μg/dL)	11.8	0.1	NA	NA	1.5	8
DHEA-S (μg/dL)	2250	16	7	5	7	5
Free testosterone (pg/mL)	7.1	<0.2	<0.2	<0.2	<0.2	<0.2
Plasma aldosterone concentration (pg/mL)	248.1	52.2	NA	NA	NA	NA
Plasma renin activity (ng/mL/hr)	2.9	2.7	NA	NA	NA	NA
Serum creatinine (mg/dL)	0.81	0.76	0.84	0.98	0.87	0.83
Serum sodium (mmol/L)	143	139	140	142	142	139
Serum potassium (mmol/L)	3.7	3.4	4.5	4.4	4.0	3.9
Serum chloride (mmol/L)	106	106	106	106	107	102

Dose of hydrocortisone as glucocorticoid replacement therapy; a, 30 mg/day; b, 15 mg/day;

ACTH, adrenocorticotropic hormone; DHEA-S, dehydroepiandrosterone sulfate; NA, not available.



Supplementary Figure 1. Aldosterone-producing micronodules in the adjacent adrenal tissue.

A and **B**, High-magnification photomicrographs of adjacent adrenal tissue with CYP11B2 immunohistochemistry are shown. CYP11B2, brown. Scale bars, 200 μm.