

Table S3: Clinical details of 120 patients with Cushing's disease.

Patient ID	USP8 mutation	Sex	Age (year)	BMI	Associated signs and symptoms	Accompanying diseases	Size (cm)	Tumor invasion	Plasma corticotropin (ACTH) (pg/ml)	Midnight serum cortisol (µg/dl)	Urinary free cortisol (µg/24 h)	LDST	HDST	Immunostain for ACTH	Available data for diagnosis	IPOBR	POR	RP (month)
WES-1	p.S718del	F	36	25.2	mf,f	HTN,OSTEO	0.8	No	68.8	21.20	632.84	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
WES-2	none	F	46	24.2	wg		4.0	Yes	208.0	31.22	981.04	Yes	N/A	2+	Clin,ACTH,Scort,UFC,LDST,MRI	No	N/A	
WES-3	p.P720R	F	38	22.8	wg,amen,mf,bh	HTN,DM, OSTEO	0.8	No	57.4	19.50	808.38	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
WES-4	none	F	36	25.0	wg,acne	HTN	2.2	Yes	21.1	39.07	1134.90	Yes	Yes	2+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
WES-5	p.S718P	F	43	24.4	f,emot, amen	HYKM	1.1	No	89.3	30.57	1164.00	Yes	Yes	2+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	Yes	22
WES-6	p.P720R	F	32	28.3	wg,acne,amen,pigm		1.5	No	136.0	19.12	344.70	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
WES-7	p.S718P	F	36	20.8	ed,f,ina	HTN	0.8	No	69.8	34.65	1147.86	Yes	N/A	3+	Clin,ACTH,Scort,UFC,LDST,MRI	Yes	No	
WES-8	p.S718P	F	43	22.2	wg,acne,mf,bh,ed		0.8	No	64.0	24.34	238.97	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
WES-9	p.S718P	F	41	23.8	wg,acne,amen,emot	HTN	1.0	No	86.6	52.94	325.80	LDST	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
WES-10	none	F	34	28.3	wg,acne, amen		1.5	No	95.9	27.16	1179.36	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
WES-11	none	F	40	21.5	amen,f,hair	DM,HTN, OSTEO	5.0	Yes	100.0	31.17	640.48	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	No	N/A	
WES-12	p.S718del	F	15	21.8	wg,mf,bh,amen,acne,f		0.5	No	70.4	20.57	247.25	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI,BIPSS	Yes	Yes	39
Val-1	p.S719P	F	43	29.6	wg,amen,str,acne	DM	N/A	N/A	40.3	18.60	N/A	N/A	N/A	+	Clin,ACTH, Scort	Yes	No	
Val-2	p.716-730del	F	38	24.4	wg,mf,bh,amen,str		0.6	No	29.7	16.60	192.80	Yes	Yes	2+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI,BIPSS	Yes	Yes	48
Val-3	p.S718del	F	33	26.6	wg,mf,bh	DM	1.2	No	269.0	N/A	N/A	N/A	N/A	3+	Clin,ACTH, MRI	Yes	No	
Val-4	p.PD720-721del	F	23	28.6	wg,mf		0.5	No	84.3	N/A	N/A	N/A	N/A	3+	Clin,ACTH, MRI	Yes	Yes	12
Val-5	p.Y717S,p.P720A	F	22	23.9	wg,str,amen,acne,mf,ecch	HTN,DM	0.3	No	63.2	27.06	679.77	Yes	Yes	+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	Yes	17
Val-6	p.S718P	F	30	28.4	mf,bh,f	HTN	0.7	No	62.6	19.85	N/A	N/A	N/A	2+	Clin,ACTH,Scort, MRI	No	N/A	
Val-7	p.S719P,p.D721N	F	41	23.9	amen,infx	HTN	1.5	No	N/A	22.86	N/A	N/A	N/A	3+	Clin,Scort,MRI	Yes	No	
Val-8	p.P720R	F	43	26.7	wg		0.5	No	N/A	N/A	N/A	N/A	N/A	3+	Clin,MRI,BIPSS	Yes	No	
Val-9	p.P720R	F	32	20.8	wg,amen,acne	HYKM	0.8	No	N/A	N/A	N/A	N/A	N/A	3+	Clin,MRI	Yes	No	
Val-10	p.S718del	F	37	20.6	wg,mf,acne,f,amen		0.8	No	90.3	N/A	1261.00	N/A	N/A	3+	Clin,ACTH,UFC, MRI	Yes	No	
Val-11	p.S718P	F	52	26.4	wg,amen,ed	HTN,DM, OSTEO	0.5	No	68.4	19.79	658.26	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI,BIPSS	Yes	No	
Val-12	p.S718F	F	60	24.7	wg,acne,f	HTN,DM	0.3	No	116.0	N/A	N/A	N/A	Yes	2+	Clin,ACTH,HDST,MRI	No	N/A	
Val-13	p.P720R	F	37	26.7	wg,ed,mf,str,f	HYKM	0.9	No	66.5	29.40	445.74	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
Val-14	p.S718P	F	24	29.3	wg,emot,hair,str,f	HTN	1.0	Yes	103.0	27.60	2653.79	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	No	N/A	
Val-15	p.P720R	M	18	24.0	wg,mf,acne,bh,f		0.6	No	N/A	29.41	2352.90	Yes	Yes	2+	Clin,Scort,UFC,LDST,HDST,MRI	Yes	No	
Val-16	p.P720R	F	33	26.2	wg,amen, pigm	HTN	0.6	No	75.8	19.33	954.00	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
Val-17	p.S718F	F	27	21.1	wg,acne		N/A	No	37.4	13.38	165.87	Yes	Yes	+	Clin,ACTH,Scort,UFC,LDST,HDST,BIPSS	Yes	No	

Table S3: (continued)

Patient ID	USP8 mutation	Sex	Age (year)	BMI	Associated signs and symptoms	Accompanying diseases	Size (cm)	Tumor invasion	Plasma corticotropin (ACTH) (pg/ml)	Midnight serum cortisol (µg/dl)	Urinary free cortisol (µg/24 h)	LDST	HDST	Immunostain for ACTH	Available data for diagnosis	IPOBR	POR	RP (month)
Val-18	p.S718del	F	26	29.4	wg,acne,amen,ed	HTN	1.0	No	47.6	20.96	573.36	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI,BIPSS	Yes	Yes	96
Val-19	p.S718P	M	38	23.2	ed,f	HTN,DM	0.6	No	230.0	44.86	1573.25	Yes	Yes	2+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI,BIPSS	Yes	No	
Val-20	p.S719del	F	18	34.4	wg,acne,str,amen		0.8	No	83.3	13.10	216.36	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	Yes	7
Val-21	p.S718del	F	43	25.3	f,mf,bh		0.8	No	51.1	37.01	1153.04	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI,BIPSS	Yes	No	
Val-22	p.S718del	F	54	24.8	f,ina	HTN,OSTEO	0.7	No	65.8	N/A	N/A	Yes	Yes	3+	Clin,ACTH,LDST,HDST,MRI	Yes	No	
Val-23	p.P720Q	F	35	23.6	wg,mf,acne,str,ed		0.6	No	194.0	43.93	N/A	Yes	Yes	3+	Clin,ACTH,Scort,LDST,HDST, MRI	Yes	No	
Val-24	p.PDIT720-723L	F	31	22.4	amen,wg,mf		0.8	No	64.2	N/A	N/A	Yes	N/A	3+	Clin,ACTH,LDST,MRI,BIPSS	Yes	Yes	16
Val-25	p.P720R	M	15	37.6	wg,acne,mf		0.7	No	26.7	24.53	1000.94	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI,BIPSS	No	N/A	
Val-26	p.P720R	F	24	22.8	ed,str,acne,mf,bh,f		0.5	No	81.8	28.35	356.24	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
Val-27	p.P720Q	F	26	19.9	acne,ed,amen		0.5	No	103.0	N/A	N/A	N/A	N/A	3+	Clin,ACTH,MRI	Yes	No	
Val-28	p.P720R	F	47	26.1	wg,mf,str	HTN	0.8	No	N/A	N/A	N/A	N/A	N/A	+	Clin,MRI,BIPSS	Yes	No	
Val-29	p.S718del	F	30	26.0	amen		0.5	No	30.9	41.60	219.46	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
Val-30	p.S718P	F	23	19.5	amen,acne,mf		0.8	No	61.0	29.20	755.86	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI,BIPSS	Yes	No	
Val-31	p.P720R,p.A725T	F	30	25.4	wg		0.4	Yes	42.8	9.36	N/A	N/A	N/A	+	Clin,ACTH,Scort,MRI	Yes	N/A	
Val-32	p.P720R	F	40	21.0	amen	HTN,OSTEO	N/A	No	45.0	9.29	N/A	N/A	N/A	3+	Clin,ACTH,Scort	Yes	N/A	
Val-33	p.P720R	F	33	24.1	wg,mf,amen,ed,str		N/A	No	108.0	N/A	N/A	Yes	N/A	3+	Clin,ACTH, LDST	Yes	Yes	36
Val-34	p.S718P	F	26	31.3	wg,amen,acne,str		N/A	No	N/A	10.53	N/A	N/A	N/A	2+	Clin,Scort	No	N/A	
Val-35	p.P720Q	F	43	32.9	wg,mf	HTN,DM	0.3	No	129.0	23.10	147.32	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI,BIPSS	No	N/A	
Val-36	p.P720R	F	51	28.5	wg,acne,ecch	HTN	0.4	No	95.0	22.07	416.85	Yes	N/A	3+	Clin,ACTH,Scort,UFC,LDST,MRI,BIPSS	No	N/A	
Val-37	p.P720R	M	21	23.0	wg,acne,str,ed	HTN,HYKM	0.4	No	108.0	N/A	724.56	Yes	Yes	2+	Clin,ACTH,UFC,LDST,HDST,MRI,BIPSS	Yes	No	
Val-38	p.S718P	F	48	27.7	wg	HTN,DM	0.4	No	N/A	23.30	698.11	Yes	Yes	3+	Clin,Scort,UFC,LDST,HDST,MRI	Yes	No	
Val-39	p.P720R	F	42	24.0	wg,mf,str	HTN	0.6	Yes	54.1	16.63	529.10	Yes	Yes	2+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
Val-40	p.S718del	F	50	28.4	wg,amen	HTN,OSTEO	0.8	No	103.0	N/A	N/A	N/A	N/A	2+	Clin,ACTH,MRI	Yes	No	
Val-41	p.S718P	F	53	17.3	f,ina,infx	HTN	0.6	No	121.0	44.96	166.78	Yes	Yes	+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI	Yes	No	
Val-42	p.P720R	M	16	29.4	wg,acne		0.5	No	158.0	N/A	N/A	Yes	Yes	2+	Clin,ACTH,LDST,HDS T,MRI	Yes	No	
Val-43	p.S718P	F	43	21.8	wg,mf,f,ina,amen		0.8	No	26.7	N/A	199.20	N/A	N/A	2+	Clin,ACTH,UFC, MRI	No	N/A	
Val-44	p.D721N	F	31	28.0	wg,amen,acne		0.8	No	129.0	N/A	N/A	N/A	N/A	3+	Clin,ACTH,MRI	Yes	Yes	36
Val-45	p.S718P	F	41	23.4	emot	HTN	1.5	Yes	46.6	27.70	N/A	N/A	N/A	2+	Clin,ACTH,Scort,MRI	Yes	N/A	
Val-46	p.S718del	M	15	25.4	wg		0.9	Yes	85.7	N/A	N/A	N/A	N/A	+	Clin,ACTH,MRI	No	N/A	
Val-47	p.S718del	F	26	22.8	wg,mf,bh,ecch,str		0.4	No	32.7	18.10	N/A	N/A	N/A	3+	Clin,ACTH,Scort,MRI	Yes	N/A	
Val-48	p.R715C,p.S718F	F	45	19.5	mf,bh,f		3.0	Yes	51.7	N/A	N/A	N/A	N/A	2+	Clin,ACTH,MRI	Yes	No	

Table S3: (continued)

Patient ID	USP8 mutation	Sex	Age (year)	BMI	Associated signs and symptoms	Accompanying diseases	Size (cm)	Tumor invasion	Plasma corticotropin (ACTH) (pg/ml)	Midnight serum cortisol (µg/dl)	Urinary free cortisol (µg/24 h)	LDST	HDST	Immunostain for ACTH	Available data for diagnosis	IPOBR	POR	RP (month)
Val-49	p.T735I	M	36	24.6	wg,skin		5.0	Yes	56.8	N/A	278.25	Yes	Yes	+	Clin,ACTH,UFC,LDST,HDST, MRI	Yes	No	
Val-50	p.P720R	F	33	21.9	amen,mf	HTN	1.2	No	50.1	14.27	176.30	N/A	N/A	3+	Clin,ACTH,Scort,UFC,MRI	No	N/A	
Val-51	p.P720R	F	23	19.2	mf,acne,amen,f		0.3	No	64.5	N/A	N/A	N/A	N/A	3+	Clin,ACTH,MRI	Yes	Yes	18
Val-52	p.S718del	F	36	24.2	wg,amen,mf,bh	HTN	2.5	Yes	N/A	N/A	431.38	N/A	N/A	3+	Clin,UFC,MRI	Yes	No	
Val-53	p.S718del,p.S716F	F	36	21.1	amen		N/A	N/A	72.0	12.55	552.64	N/A	Yes	3+	Clin,ACTH,Scort,UFC,HDST	Yes	No	
Val-54	p.P720R	M	36	25.6	wg,mf,f	HTN,DM	0.5	No	368.0	N/A	N/A	Yes	N/A	3+	Clin,ACTH,LDST,MRI,BIPSS	Yes	No	
Val-55	p.P720R	F	38	23.8	f,ed,str,mf,acne		0.6	No	N/A	N/A	N/A	Yes	Yes	3+	Clin,LDST,HDST,MRI	Yes	No	
Val-56	p.S718del	F	37	22.9	ed		0.4	No	61.0	N/A	2055.00	N/A	N/A	2+	Clin,ACTH,UFC,MRI,BIPSS	Yes	No	
Val-57	p.S718del	F	37	24.0	amen,hair,mf,bh		0.5	No	67.2	5.14	313.25	Yes	Yes	3+	Clin,ACTH,UFC,LDST,HDST, MRI,BIPSS	Yes	No	
Val-58	p.P720R	F	42	18.0	wg,mf,amen		N/A	N/A	146.0	N/A	N/A	N/A	N/A	3+	Clin,ACTH	Yes	No	
Val-59	p.P720R	F	30	31.2	wg,mf,amen,emot	HTN	N/A	N/A	35.3	32.82	N/A	N/A	N/A	+	Clin,ACTH,Scort	No	N/A	
Val-60	p.S718P	F	47	29.3	wg	HTN	N/A	N/A	164.0	N/A	N/A	N/A	N/A	2+	Clin,ACTH	No	N/A	
Val-61	p.S718P	F	51	29.3	wg	HTN	N/A	N/A	39.2	25.17	494.04	Yes	Yes	2+	Clin,ACTH,Scort,UFC,LDST, HDST	Yes	No	
Val-62	p.P720R	F	39	26.1	mf,bh	HTN,HYKM	N/A	N/A	91.0	N/A	N/A	N/A	N/A	3+	Clin,ACTH	Yes	No	
Val-63	p.P720R	F	27	20.2	ed		N/A	N/A	152.0	N/A	N/A	N/A	N/A	2+	Clin,ACTH	Yes	Yes	36
Val-64	p.S718del	F	31	24.4	ed	HTN	N/A	N/A	66.7	18.71	N/A	N/A	N/A	3+	Clin,ACTH,Scort	Yes	No	
Val-65	p.S718del	F	14	22.6	wg,amen,bh		N/A	N/A	44.5	N/A	N/A	N/A	N/A	3+	Clin,ACTH	Yes	No	
Val-66	p.S718P	F	22	21.1	amen,mf,str		N/A	N/A	N/A	N/A	750.94	N/A	N/A	3+	Clin,UFC	Yes	N/A	
Val-67	p.P720R	F	34	24.0	wg,acne,str	HTN,DM	N/A	N/A	238.0	32.91	2228.93	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST, HDST	Yes	No	
Val-68	none	F	57	32.3	wg		4.5	Yes	42.8	11.56	412.50	Yes	Yes	2+	Clin,ACTH,Scort,UFC,LDST, HDST,MRI	No	N/A	
Val-69	none	M	14	34.3	wg,str,acne,mf,f		1.1	No	133.0	7.26	385.44	Yes	Yes	2+	Clin,ACTH,UFC,LDST,HDST, MRI	Yes	No	
Val-70	none	F	53	20.2	wg,f,str,eomt	DM,HTN	0.8	No	67.1	14.93	207.18	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST,HDST,MRI,BIPSS	Yes	No	
Val-71	none	F	37	24.1	wg,amen,ed,str,f,ecch		N/A	No	69.8	21.82	397.61	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST, HDST,BIPSS	Yes	Yes	24
Val-72	none	M	36	23.9	wg,mf,str		1.8	No	281.0	18.80	1559.30	Yes	Yes	2+	Clin,ACTH,Scort,UFC,LDST, HDST,MRI	Yes	No	
Val-73	none	F	45	25.5	wg,f,ed	OSTEO	0.9	No	39.1	16.59	N/A	Yes	Yes	3+	Clin,ACTH,Scort,LDST,HDST, MRI	Yes	No	
Val-74	none	M	31	28.4	wg,str,acne,mf,bh	HTN	1.0	No	163.0	28.05	1353.00	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST, HDST,MRI	Yes	No	
Val-75	none	F	24	33.2	wg,mf,bh,ed	DM,HTN	1.1	No	74.4	22.31	1131.40	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST, HDST,MRI	Yes	No	
Val-76	none	M	51	20.8	f	DM,HTN	4.5	Yes	138.0	15.77	106.90	Yes	Yes	+	ACTH,Scort,LDST, HDST,MRI	Yes	No	
Val-77	none	M	19	29.0	wg,mf,ed,str,ecch		1.5	No	207.0	49.81	4100.00	Yes	Yes	3+	Clin,ACTH,Scort,UFC,LDST, HDST,MRI	Yes	Yes	12
Val-78	none	F	22	18.4	amen,acne,mf,emot	HTN	1.3	No	53.6	28.53	828.80	Yes	Yes	+	Clin,ACTH,Scort,UFC,LDST, HDST,MRI	No	N/A	

Table S3: (continued)

Patient ID	USP8 mutation	Sex	Age (year)	BMI	Associated signs and symptoms	Accompanying diseases	Size (cm)	Tumor invasion	Plasma corticotropin (ACTH) (pg/ml)	Midnight serum cortisol (µg/dl)	Urinary free cortisol (µg/24 h)	LDST	HDST	Immunostain for ACTH	Available data for diagnosis	IPOBR	POR	RP (month)
Val-79	none	F	30	25.1	amen,ed	HTN	2.5	Yes	142.0	39.10	1302.20	Yes	Yes	3+	Clin,ACTH,Scort, UFC,LDST, HDST,MRI	Yes	Yes	48
Val-80	none	M	56	24.2	wg,f		0.6	No	120.0	N/A	N/A	Yes	Yes	2+	Clin,ACTH,LDST, HDST,MRI	Yes	No	
Val-81	none	F	34	24.4	wg,amen,mf, pigm	DM,HTN	1.0	No	33.8	20.05	162.80	Yes	Yes	2+	Clin,ACTH,Scort, UFC,LDST, HDST,MRI	Yes	No	
Val-82	none	F	46	28.1	wg,mf,bh,f	OSTEO	N/A	No	85.7	N/A	N/A	N/A	N/A	3+	Clin,ACTH	Yes	N/A	
Val-83	none	F	29	21.6	wg,acne,str		5.0	Yes	250.0	N/A	N/A	N/A	N/A	2+	Clin,ACTH,MRI	Yes	Yes	48
Val-84	none	F	41	25.1	wg,mf,bh	DM	3.9	Yes	170.0	N/A	N/A	Yes	Yes	2+	Clin,ACTH,LDST, HDST,MRI	No	N/A	
Val-85	none	M	52	26.4	wg	HTN	2.7	Yes	80.5	N/A	N/A	N/A	N/A	3+	Clin,ACTH,MRI	Yes	No	
Val-86	none	M	25	30.2	wg		N/A	No	94.0	N/A	N/A	N/A	N/A	2+	Clin,ACTH	Yes	Yes	12
Val-87	none	M	43	26.7	wg		N/A	N/A	265.0	N/A	N/A	N/A	N/A	3+	Clin,ACTH	Yes	No	
Val-88	none	F	41	27.0	amen		3.1	Yes	86.7	N/A	N/A	N/A	N/A	3+	Clin,ACTH,MRI	Yes	No	
Val-89	none	M	53	27.2	wg,mf,bh,f		3.7	Yes	140.0	N/A	N/A	N/A	N/A	2+	Clin,ACTH,MRI	No	N/A	
Val-90	none	F	51	21.6	emot,f	HTN	2.5	Yes	21.2	9.10	N/A	N/A	N/A	3+	Clin,ACTH,Scort, MRI	Yes	No	
Val-91	none	F	58	26.7	wg,acne,hair		2.3	Yes	267.0	N/A	N/A	N/A	N/A	+	Clin,ACTH,MRI	No	N/A	
Val-92	none	F	28	19.0	wg,acne,amen		0.8	No	47.7	17.70	449.31	Yes	Yes	3+	Clin,ACTH,Scort, UFC,LDST, HDST,MRI	Yes	No	
Val-93	none	F	43	23.4	wg,amen	OSTEO	0.8	No	70.6	20.03	115.66	Yes	Yes	3+	Clin,ACTH,Scort, LDST, HDST,MRI	No	N/A	
Val-94	none	F	25	24.7	wg,ed,acne, amen		1.2	No	N/A	N/A	N/A	N/A	N/A	2+	Clin,MRI	Yes	No	
Val-95	none	F	25	25.8	wg,str,ed		1.3	No	37.1	N/A	479.76	N/A	N/A	3+	Clin,ACTH,UFC, MRI	Yes	Yes	84
Val-96	none	F	31	21.5	wg,amen,mf	HTN	N/A	N/A	60.6	20.43	721.20	Yes	Yes	2+	Clin,ACTH,Scort, UFC,LDST, HDST	No	N/A	
Val-97	none	F	43	21.3	wg,mf	HTN	0.5	No	111.4	24.20	660.00	Yes	Yes	3+	Clin,ACTH,Scort, UFC,LDST,HDST,MRI, BIPSS	Yes	N/A	
Val-98	none	F	54	25.4	f,ed	DM,HTN	1.6	No	60.2	35.96	1395.68	Yes	Yes	2+	Clin,ACTH,Scort, UFC,LDST, HDST,MRI	Yes	Yes	48
Val-99	none	F	19	18.7	wg,acne,amen, mf		N/A	No	20.4	8.47	438.84	Yes	Yes	3+	Clin,ACTH,Scort, UFC,LDST, HDST	Yes	No	
Val-100	none	F	39	19.7	mf	DM	N/A	N/A	171.0	37.68	1434.45	N/A	Yes	3+	Clin,ACTH,Scort, UFC,HDST	Yes	No	
Val-101	none	F	43	28.2	wg,amen		0.8	Yes	N/A	29.00	N/A	N/A	N/A	+	Clin,Scort,MRI	No	N/A	
Val-102	none	M	31	27.4	wg,acne	OSTEO,HTN	0.9	No	99.1	11.08	412.00	Yes	Yes	3+	Clin,ACTH,Scort, UFC,LDST, HDST,MRI	Yes	No	
Val-103	none	F	29	26.3	wg,skin,bh,acne	HTN	1.5	Yes	80.7	N/A	447.00	N/A	N/A	3+	Clin,ACTH,UFC, MRI	Yes	No	
Val-104	none	F	43	27.5	wg,amen,mf		N/A	N/A	119.0	N/A	N/A	N/A	N/A	3+	Clin,ACTH	Yes	No	
Val-105	none	F	34	21.2	acne	HTN	N/A	No	68.1	35.49	570.65	Yes	Yes	3+	Clin,ACTH,Scort, UFC,LDST, HDST	Yes	Yes	48
Val-106	none	M	40	25.9	wg,mf,bh,ed,str	HTN	4.5	Yes	163.0	17.07	994.29	Yes	Yes	+	Clin,ACTH,Scort, UFC,LDST, HDST,MRI	No	N/A	
Val-107	none	F	58	32.5	wg,mf,bh	HYKM,HTN	N/A	N/A	91.8	N/A	N/A	N/A	N/A	3+	Clin,ACTH	Yes	No	
Val-108	none	M	41	29.1	wg,mf,bh		N/A	N/A	476.0	N/A	N/A	N/A	N/A	3+	Clin,ACTH	No	N/A	

* The diagnosis of PAs in all cases was histologically confirmed by two senior pathologists after surgical resection. Each sample displayed positive immunostaining for ACTH and negative for other hormones, such as growth hormone (GH), prolactin (PRL), thyroid stimulating hormone (TSH), etc.

WES : whole exome sequencing cohort. **Val** : validation cohort, Val1-Val67 denote patients with mutation, Val68-V108 denote patients with no mutation. **BMI**: body mass index. **Size**: denote maximal adenoma diameter. **LDST**: denotes nonsuppressible serum cortisol levels following administration of 1 mg of dexamethasone. **HDST**: denotes suppressible 24-hr UFC following administration of 8mg of dexamethasone. **IPOBR**: immediate postoperative biochemical remission, which was defined on the basis of the biochemical evidence of adrenal cortical insufficiency as witnessed by the drop in the immediate postoperative serum cortisol (range, < 3µg/dL). **POR**: postoperative recurrence, which was defined when a patient with initial biochemical and clinical evidence for remission after operation developed symptomatic recurrence of Cushing disease with biochemical reconfirmation of pituitary-dependent endogenous hypercortisolism. **RP**: recurrence period.

N/A denotes not available: some clinical records were incomplete mainly owing to evaluation having been performed at outside hospitals, with detailed records missing from our hospital medical record. For these patients, the pathological and/or imageological evidence was especially crucial for the diagnosis.

Associated signs and symptoms: wg, recent weight gain; mf, moon facies, facial plethora; bh, buffalo hump; str, striae; ed, peripheral edema; amen, amenorrhea or menstrual abnormalities; hair, thinning of hair or alopecia; acne, increased acne; f, fatigue or weakness; ina, inappetence; emot, emotional lability or depression; ecch, Ecchymosis or easy bruisability; skin, thin skin; infx, recurrent or difficult-to-treat infections; pigm, pigmentation.

Accompanying diseases: DM, diabetes mellitus; HTN, hypertension; OSTEO, osteoporosis or osteopenia; HYKM, hypokalemia

Diagnostic criteria: Clin, documented clinical symptoms suggestive of Cushing syndrome; UFC, denotes elevated urinary free cortisol; ACTH, denotes elevated morning plasma ACTH; Scort, denotes elevated midnight serum cortisol; MRI, denotes visualized adenomas by MRI; BIPSS, denotes inferred microadenomas(<6mm) by bilateral inferior petrosal sinus sampling

Immunostain for ACTH : Two expert pathologists provided a semi-quantitative estimate of the ACTH immunopositive staining at high magnification (×400). Ten optimal fields with highest tumoral ACTH staining and minimal background reactivity were used to estimate the percentage of stained cells. Average percentage positivity was scored according as mean observed in ten fields and was categorized as: -(no reactivity), + (<30% of the neoplastic cells positive), 2+ (31%-60% positive), and 3+ (>60% positive).