

Supplementary Table 1. Description of study cohort.

Variable	mean/median	SD/IQR	Total n
Age at diagnosis (years), mean $\pm$ SD, [total n]	42	$\pm$ 15.2	86
Sex (female), n (%), [total n]	62	(72%)	86
BMI (kg/m ²), mean $\pm$ SD, [total n]	28.9	$\pm$ 6.3	74
Disease presentation, n (%), [total n]			86
Cushing	66	(77%)	
Nelson	20	(23%)	
Number of prior pituitary surgeries, n (%), [total n]			80
0	50	(63%)	
1	23	(29%)	
$\geq$ 2	7	(9%)	
Total number of pituitary surgeries, n (%), [total n]			82
1	46	(56%)	
2	23	(28%)	
$\geq$ 3	13	(16%)	
Complete tumor resection, n (%), [total n]	32	(60%)	53
Postoperative remission, n (%), [total n]	46	(59%)	78
Postoperative tumor control, n (%), [total n]	34	(60%)	57
Radiation therapy, n (%), [total n]	24	(34%)	70
Radiation therapy before sample collection, n (%), [total n]	7	(13%)	53
Bilateral adrenalectomy, n (%), [total n]	23	(27%)	86
Pharmacological treatments ^a , n (%), [total n]	18	(42%)	43
Preoperative hormone levels			
Plasma ACTH (pg/mL), median (IQR)	98	(570.4)	75
Serum cortisol (μ g/dl), median (range)	29.1	(168.6)	50
24h-urinary free cortisol (μ g/24h), median (range)	432.5	(598.3)	30
Serum cortisol after low-dose DST (μ g/dl), median (IQR)	20	(20.7)	46
Postoperative hormone levels			
Plasma ACTH (pg/mL), median (IQR)	20	(107.6)	57
Serum cortisol nadir (μ g/dl), median (range)	8.8	(19.4)	58
Tumor size (mm), median (IQR), [total n]	15	(13.0)	85
Microadenoma	19	(22%)	
Macroadenoma	66	(78%)	
Granulation, n (%), [total n]			30
Sparsely	9	(30%)	
Densely	21	(70%)	
Ki67 index, median (IQR), [total n]	2.0	(3.8)	36
Ki67 index $\geq$ 3%, n (%)	14	(39%)	36
p53 positivity, median (IQR), [total n]	1	(26.5)	9
Invasion, n (%), [total n]	34	(53%)	64
Hardy grade, n (%), [total n]			61
1	13	(21%)	
2	22	(36%)	
3	18	(30%)	
4	8	(13%)	
Knosp grade, n (%), [total n]			35
0	5	(14%)	
1	12	(34%)	
2	3	(9%)	
3	7	(20%)	
4	8	(7%)	
Disease-specific death, n (%), [total n]	5	(9%)	58

^a Pharmacological treatments: pasireotide (n=6), ketoconazole (n=5), mitotane (n=5), temozolamide (n=4) metyrapone (n=5), cabergoline (n=3), bevacizumab (n=1). Five patients received >1 pharmacological agent.

Supplementary Table 2. Primers used for *TP53* amplification and Sanger sequencing.

Primer	Sequence	DNA source
TP53-1	5'-TCTCATGCTGGATCCCCACT-3'	FF, FFPE
TP53-1rv	5'-GACCAGGTCCTCAGCC-3'	FFPE
TP53-2fw	5'-GGGGGCTGAGGACCTGGT-3'	FFPE
TP53-2rv	5'-ATACGGCCAGGCATTGAAGT-3'	FFPE
TP53-2	5'-AGAGGAATCCCAAAGTTCCA-3'	FF
TP53-3	5'-GTGCCCTGACTTTCAACTC-3'	FF, FFPE
TP53-3rv	5'-GGCAACCAGCCCTGTC-3'	FFPE
TP53-4fw	5'-GCCTCTGATTCCTCACTGAT-3'	FFPE
TP53-4	5'-CAGGAGAAAGCCCCCCTACT-3'	FF, FFPE
TP53-5	5'-CTTGCCACAGGTCTCCCCAA-3'	FF, FFPE
TP53-6	5'-AGGGGTCAGAGGCAAGCAGA-3'	FF, FFPE
TP53-7	5'-TAGGACCTGATTCCTTA-3'	FF, FFPE
TP53-7rv	5'-AGTGAATCTGAGGCATAAC-3'	FFPE
TP53-7Bfw	5'-TGGAGGAGACCAAGGGTG-3'	FFPE
TP53-7Brv	5'-CGGCATTTTGAGTGTTAGAC-3'	FFPE
TP53-8	5'-TAAGCTATGATGTTCTTAG-3'	FF, FFPE
TP53-8rv	5'-GACTGTTTTACCTGCAATTG-3'	FFPE
TP53-9	5'-CAATTGTAACCTGAACCATC-3'	FF, FFPE
TP53-10	5'-GGATGAGAATGGAATCCTAT-3'	FF, FFPE
TP53-11	5'-TCTCACTCATGTGATGTCATC-3'	FF, FFPE
TP53-12	5'-CACACCTATTGCAAGCAAGG-3'	FF, FFPE

FF, fresh frozen; FFPE, formalin-fixed paraffin embedded.

Supplementary Table 3. Common *TP53* variants in the study cohort.

Variant in chromosome 17 (GRCh38/hg38)		Present study			ALFA ^a (version 20201027095038)			gnomAD ^b (v3.1.2, genomes)			1000 Genomes Project ^a (phase 3)		
SNP ID		Allele frequency	Count	Allele frequency	Count	P-value ^c	Allele frequency	Count	P-value ^c	Allele frequency	Count	P-value ^c	
rs1800370	7676261					0.603			1.000			1.000	
	C	0.986	71	0.987	54859		0.986	63667		0.983	989		
	T	0.014	1	0.013	707		0.014	905		0.017	17		
rs1042522	7676154					0.708			0.613			1.000	
	G	0.280	23	0.263	23805		0.256	16523		0.285	287		
	C	0.720	59	0.737	66583		0.744	48001		0.715	719		
rs1642785	7676483					0.692			0.496			0.892	
	G	0.300	21	0.281	5733		0.261	16851		0.288	290		
	A	0.700	49	0.719	14665		0.739	47621		0.712	716		
rs540683791	7676324					0.072			-			0.187	
	C	0.986	71	0.999	9629		NA	NA		0.998	1004		
	G	0.014	1	0.001	9		NA	NA		0.002	2		
rs59758982	7676335_7676366					0.634			1.000			<0.001 ^d	
	(CCCCAGCCCTCC AGGT)(C) ₄ AGCCC	0.141	10	0.167	886		0.146	9404		0.378	380		
	(CCCCAGCCCTCC AGGT) ₂ (C) ₄ AGCCC	0.859	61	0.833	4414		0.854	54872		0.622	626		
rs17883323	7676301					0.800			0.799			0.793	
	G	0.965	70	0.942	34434		0.946	61022		0.942	948		
	T	0.035	3	0.058	2122		0.054	3510		0.058	58		
rs1625895	7674797					0.341			0.419			0.254	
	T	0.089	8	0.135	29617		0.128	8250		0.143	144		
	C	0.911	77	0.865	189339		0.872	56295		0.857	862		

^a European cohort only

^b Non-Finnish European cohort only

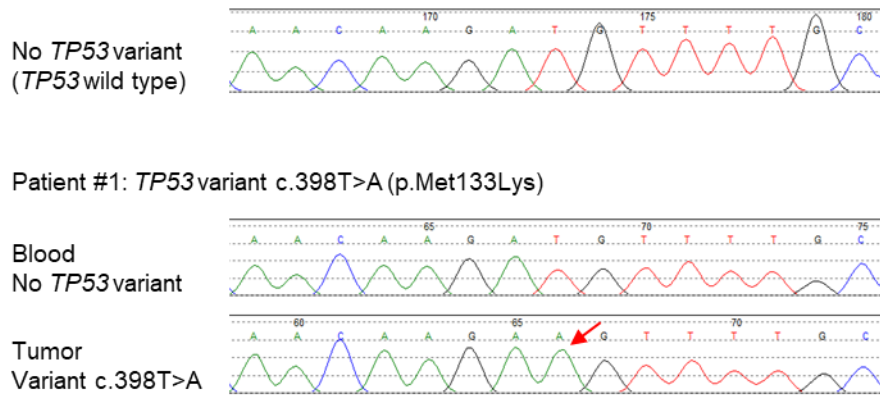
^c Exact two sided P-value for comparison with the present study (Chi-square test).

^d rs59758982 allele frequencies differ in 1KG from all other databases.

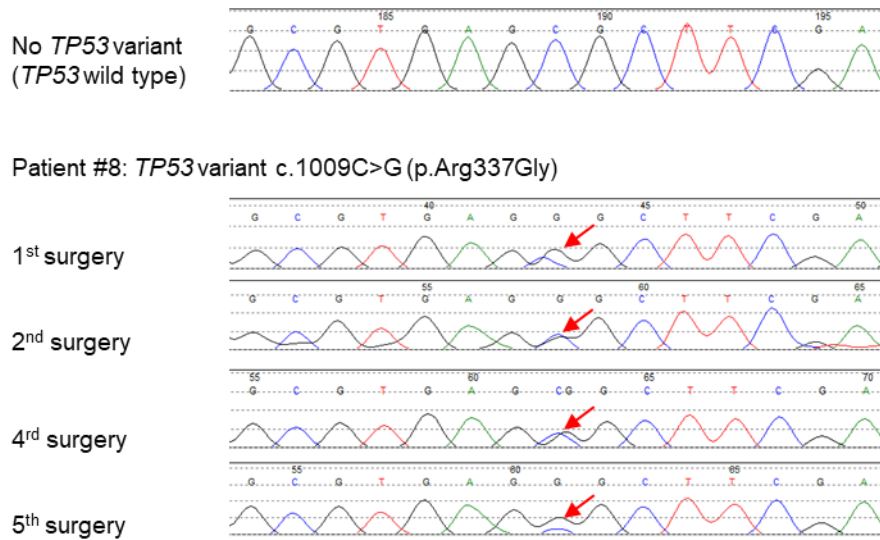
Supplementary Table 4. Comparison of *TP53* mutant versus *TP53* wild type group.

Variable	<i>TP53</i> mutant (n=9)	<i>TP53</i> Wild-type (n=77)	P-value
Age at diagnosis (years), mean $\pm$ SD	53 $\pm$ 18	41 $\pm$ 21	0.023
Sex (female), n (%)	6/9 (67%)	56/77 (73%)	0.705
BMI (kg/m ²), mean $\pm$ SD	30.6 $\pm$ 6.0	28.7 $\pm$ 6.4	0.412
Disease presentation, n (%)			0.203
CD	5/9 (56%)	61/77 (79%)	
CTP-BAD _X /NS	4/9 (44%)	16/77 (21%)	
Number of prior surgeries, n (%)			0.007
0	2/9 (22%)	48/71 (67%)	
1	4/9 (44%)	19/71 (27%)	
≥ 2	3/9 (33%)	4/71 (6%)	
Total number of surgeries, n (%)			0.021
1	2/9 (22%)	44/73 (60%)	
2	3/9 (33%)	20/73 (28%)	
≥ 3	4/9 (44%)	9/73 (12%)	
Complete tumor resection, n (%)	1/8 (13%)	31/45 (69%)	0.001
Postoperative remission, n (%)	4/9 (44%)	42/69 (61%)	0.475
Postoperative tumor control, n (%)	4/8 (50%)	30/49 (61%)	0.429
Radiation therapy, n (%)	7/9 (78%)	17/61 (28%)	0.014
Radiation therapy before sample collection, n (%)	3/8 (38%)	4/5 (9%)	0.043
Bilateral adrenalectomy, n (%)	4/9 (44%)	19/76 (25%)	0.240
Pharmacological treatments, n (%)	4/6 (67%)	14/37 (38%)	0.379
Preoperative hormone levels			
Plasma ACTH (pg/mL), median (IQR)	105 (741)	98 (448)	0.855
Serum cortisol (μ g/dl), median (range)	29 (19)	29 (175)	0.753
24h-urinary free cortisol (μ g/24h), median (range)	1680 (2860)	433 (591)	0.607
Serum cortisol after low-dose DST (μ g/dl), median (IQR)	8.9 (24.2)	20.0 (20.3)	0.439
Postoperative hormone levels			
Plasma ACTH (pg/mL), median (IQR)	106.0 (216.0)	19.0 (75.3)	0.225
Serum cortisol nadir (μ g/dl), median (range)	5.7 (20.8)	9.0 (20.2)	0.857
Tumor size (mm), median (IQR)	20.0 (14.0)	15.0 (12.1)	0.026
Tumor size, n (%)			0.194
Microadenoma	0/9 (0%)	19/76 (25%)	
Macroadenoma	9/9 (100%)	57/76 (75%)	
Granulation, n (%)			0.188
Sparsely	3/5 (60%)	6/25 (24%)	
Densely	2/5 (40%)	19/25 (76%)	
Ki67 index, median (IQR)	15.0 (14.8)	2.0 (2.0)	0.014
Ki67 index $\geq 3\%$, n (%)	5/6 (83%)	9/30 (28%)	0.024
p53 positivity, median (IQR)	27.5 (41.8)	1.0 (0.5)	0.111
Invasion, n (%)	8/8 (100%)	26/56 (46%)	0.011
Hardy grade, n (%)			0.001
1	0/8 (0%)	13/53 (24%)	
2	1/8 (13%)	21/53 (40%)	
3	3/8 (38%)	15/53 (28%)	
4	4/8 (50%)	4/53 (8%)	
Knosp grade, n (%)			≤ 0.001
0	0/7 (0%)	5/28 (18%)	
1	1/7 (14%)	11/28 (39%)	
2	0/7 (0%)	3/28 (11%)	
3	0/7 (0%)	7/28 (25%)	
4	6/7 (86%)	2/28 (7%)	
Disease-specific death, n (%)	3/7 (43%)	2/51 (4%)	0.006

A



B



Supplementary Figure 1. Chromatograms showing the *TP53* variants found in the corticotroph tumor of patient #1 and #8 (Table 1). A. The variant c.398T>A was present in homozygosity in the tumor and absent in the blood. B. The variant c.1009C>G is detected in all available surgical specimens in this patient. First and 2nd surgeries were Cushing's disease tumors and 4th and 5th CTP-BADX/NS.