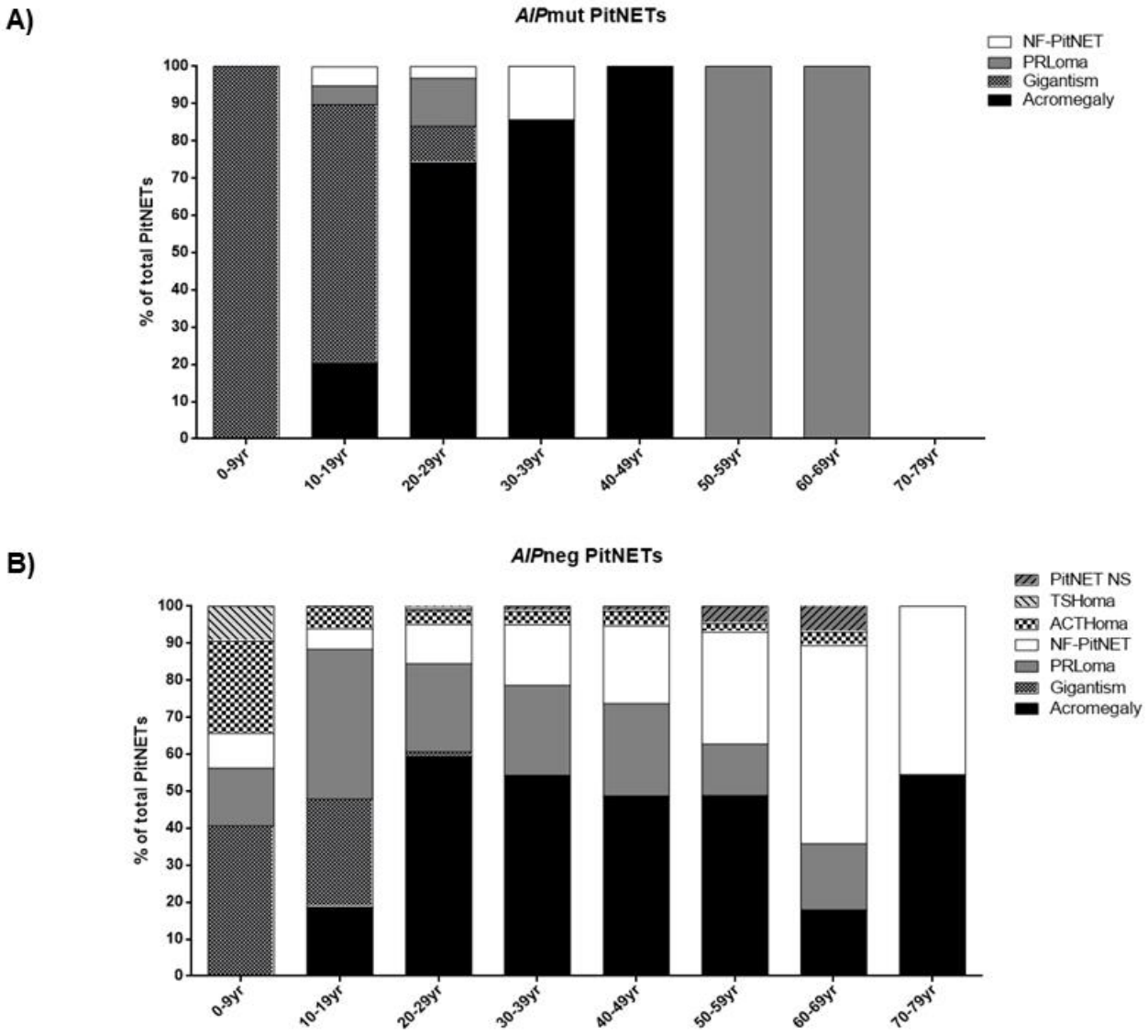
Supplemental materials for manuscript: Marques P, et al. (2020) "Significant benefits of AIP testing and clinical screening in familial isolated and young-onset pituitary tumors". Submission to *Journal of Clinical Endocrinology and Metabolism.*



Supplemental Figure 1: Distribution of clinical diagnosis according to age of onset among patients with *AIP*mut (A) and *AIP*neg (B) PitNETs. ACTHoma, ACTH-secreting adenoma or Cushing´s disease; *AIP*mut, *AIP* mutation-positive; *AIP*neg, *AIP* mutation-negative; NF-PitNET, non-functioning PitNET; NS, not specified; PitNET, pituitary neuroendocrine tumor; PRLoma, prolactinoma; TSHoma, thyrotropinoma; yr, years. In this comparison, GH/PRL positive tumors were added to the gigantism or acromegaly group, as appropriate.

	Prolactinomas			NF-PitNETs		
	***AIP*mut** n=17	***AIP*neg** n=377	***p* value**	***AIP*mut** n=14	***AIP*neg** n=172	***p* value**
Cohort type based on family history of PitNETs						
Familial cohort	82.4%	48.5%	**0.006**	100%	68.6%	**0.013**
Sporadic cohort	17.6%	51.5%		0%	31.4%	
Gender						
Male	52.9%	33.2%	0.092	64.3%	57.0%	0.595
Female	47.1%	66.8%		35.7%	43.0%	
Age at disease onset ≤ 18 yr	45.5%	40.6%	0.747	50.0%	12.4%	**0.003**
Age at first symptoms (yr)	27.5 ± 17.9	24.1 ± 10.8	0.959	22.6 ± 7.7	36.6 ± 17.3	**0.016**
Age at diagnosis (yr)	30.7 ± 16.5	26.2 ± 11.2	0.619	29.2 ± 14.8	39.4 ± 17.3	**0.038**
Delay in diagnosis (yr)	4.5 ± 8.8	2.4 ± 4.6	0.665	1.1 ± 2.3	1.5 ± 3.3	0.761
Pituitary apoplexy	16.7%	2.8%	**0.009**	0	8.6%	0.270
Hypopituitarism at diagnosis	62.5%	59.0%	0.846	9.1%	41.5%	**0.041**
Number of pituitary deficiencies at diagnosis	1.38 ± 1.51	0.93 ± 1.04	0.470	0.18 ± 0.60	1.00 ± 1.40	**0.045**
Macroadenoma	75.0%	63.7%	0.358	30.8%	85.0%	**<0.001**
Maximum tumor diameter (mm)	14.4 ± 16.1	20.6 ± 19.7	0.270	9.0 ± 9.8	22.8 ± 15.9	**0.001**
Suprasellar extension	42.9%	34.9%	0.665	27.3%	56.2%	0.071
Cavernous sinus invasion	16.7%	22.7%	0.728	18.2%	22.4%	0.753
Ki-67 > 3%	0	52.9%	0.303	33.3%	63.2%	0.907
Number of treatments	1.12 ± 0.78	1.39 ± 0.90	0.212	0.46 ± 0.78	1.19 ± 1.01	**0.005**
Number of surgeries	0.35 ± 0.49	0.35 ± 0.68	0.609	0.31 ± 0.48	0.89 ± 0.66	**0.001**
Reoperation	0	18.6%	0.246	0	13.9%	0.423
Radiotherapy	5.9%	7.2%	0.838	7.7%	20.8%	0.257
Dopamine agonists	70.6%	86.6%	0.068	7.7%	7.2%	0.948
Multimodal treatment	28.6%	22.8%	0.618	50.0%	28.9%	0.365
≥ 3 treatments	7.1%	8.0%	0.907	0	13.3%	0.435
Active disease at last follow-up	15.4%	29.1%	0.289	10.0%	18.8%	0.494
Hypopituitarism at last follow-up	25.0%	23.0%	0.897	10.0%	45.7%	**0.040**
Number of pituitary deficiencies at last follow-up	0.75 ± 1.49	0.58 ± 1.16	0.847	0.10 ± 0.31	1.16 ± 1.50	0.067
Follow-up duration (yr)	13.6 ± 12.5	9.3 ± 10.1	0.150	7.5 ± 7.1	8.1 ± 11.3	0.551

Supplemental Table 1: Comparative analysis between *AIP*mut vs *AIP*neg prolactinomas and *AIP*mut vs *AIP*neg NF-PitNETs. Categorical data are shown as %; continuous variables are shown as mean ± standard deviation. *AIP*mut, *AIP* mutation-positive; *AIP*neg, *AIP* mutation-negative; NF-PitNET, non-functioning pituitary neuroendocrine tumor; PitNET, pituitary neuroendocrine tumor; yr, years.

***AIP* mutation**	**Prevalence within *AIP*mut PitNETs (n=167)**	**Mutation type**	**Location in the AIP protein**	**References to previously published mutations / brief description of patients with novel mutations**
g.4856_4857CG>AA (p.?)	2 (1.2%)	*Promoter*	5-UTR (not in protein)	(1-3)
c.1-?_993+?del- (p.0?) (whole gene deletion)	8 (4.8%)	*Large genomic deletion*	Absence of whole protein	(1)
c.(?-50)_(99+1_100-1)del (p.0?) (exon 1 deletion)	1 (0.6%)	*Large genomic deletion*	Absence of whole protein	**Female, age at onset 17yr, age at diagnosis 19yr, acromegaly, macroadenoma**
c.3G>A (p.?)	2 (1.2%)	*Start codon*	N-terminus	(4)
c.40C>T (p.Q14*)	2 (1.2%)	*Nonsense*	N-terminus	(5-8)
c.70G>T (p.E24*)	7 (4.2%)	*Nonsense*	N-terminus	(2,9)
c.74_81delins7 (p.L25Pfs*130)	4 (2.4%)	*Frameshift*	PPIase domain	(1,10)
c.100-1025_279+357del (p.A34_K93del) (exon 2 deletion)	6 (3.6%)	*Large genomic deletion*	PPIase domain	(11)
c.140_163del (p.G47_R54del)	1 (0.6%)	In-frame deletion	PPIase domain	(12)
c.240_241delinsTG (p.M80_R81delinsIG)	1 (0.6%)	In-frame deletion insertion	PPIase domain	(13)
c.241C>T (p.R81*)	7 (4.2%)	*Nonsense*	PPIase domain	(2,3,14-16)
c.249G>T (p.G83Afs*15)	3 (1.8%)	*Splice site*	PPIase domain	(1)
c.333delC (p.K112Rfs*44)	1 (0.6%)	*Frameshift*	PPIase domain	(13)
c.338_341dup (p.L115Pfs*16)	2 (1.2%)	*Frameshift*	PPIase domain	(6,17)
c.344delT (p.L115Rfs*41)	**1 (0.6%)**	***Frameshift***	**PPIase domain**	**Male, age at onset 15yr, age at diagnosis 16yr, prolactinoma, microadenoma**
c.376_377delCA (p.Q126Dfs*3)	1 (0.6%)	*Frameshift*	PPIase domain	(13)
c.427C>T (p.Q143*)	2 (1.2%)	*Nonsense*	Between PPlase and TPR1 domains	(6)
c.469-2A>G (p.E158_Q184del)	1 (0.6%)	*Splice site (resulting in in-frame deletion)*	TPR1 domain	(18-20)
c.490C>T (p.Q164*)	2 (1.2%)	*Nonsense*	Between PPlase and TPR1 domains	(1)
c.504G>A (p.W168*)	1 (0.6%)	*Nonsense*	TPR1 domain	(21)
c.562C>T(p.R188W)	1 (0.6%)	Missense	TPR1 domain	(22)
c.570C>G (p.Y190*)	4 (2.4%)	*Nonsense*	TPR1 domain	(6)
c.605A>G (p.Y202C)	1 (0.6%)	Missense	TPR1 domain	(13)
c.645+1G>C (p.?)	1 (0.6%)	*Splice site*	TPR1 domain	(13)

c.662dupC (p.E222*)	2 (1.2%)	*Frameshift*	Between TPR1 and TPR2 domains	(1)
c.713G>A (p.C238Y)	3 (1.8%)	Missense	TPR2 domain	(2,9)
c.760T>C (p.C254R)	1 (0.6%)	Missense	TPR2 domain	(22)
c.762C>G (p.C254W)	2 (1.2%)	Missense	TPR2 domain	(22)
c.773T>G (p.L258R)	**1 (0.6%)**	**Missense[#]**	**TPR2 domain**	**Male, age at onset 21yr, age at diagnosis 29yr, prolactinoma, macroadenoma**
c.779delA (p.K260Sfs*44)	**1 (0.6%)**	***Frameshift***	**PPIase domain**	**Male, age at onset 8yr, age at diagnosis 12yr, gigantism, macroadenoma**
c.783C>G (p.Y261*)	2 (1.2%)	*Nonsense*	TPR2 domain	(6,18,23)
c.804C>A (p.Y268*)	3 (1.8%)	*Nonsense*	TPR3 domain	(6,16,24)
c.805_825dup (p.F269_H275dup)	16 (9.6%)	In-frame insertion	TPR3 domain	(2,3,18)
c.811C>T (p.R271W)	8 (4.8%)	Missense	TPR3 domain	(1,25-27)
c.815G>A (p.G272D)	1 (0.6%)	Missense	TPR3 domain	(4,28)
c.816delC (p.K273Rfs*30)	1 (0.6%)	*Frameshift*	TPR3 domain	(6)
c.863_864del (p.F288Cfs*?)	**1 (0.6%)**	***Frameshift***	**TPR3 domain**	**Female, age at onset 16yr, age at diagnosis 31yr, acromegaly, macroadenoma**
c.868A>T (p.K290*)	1 (0.6%)	*Nonsense*	TPR3 domain	(6)
c.872_877delTGCTGG (p.V291_L292del)	1 (0.6%)	In-frame deletion	TPR3 domain	(29)
c.910C>T (p.R304*)	57 (34.1%)	*Nonsense*	C-terminal α-helix	(2,8,18,19,23,25,26,30)
c.967delC (p.R323Gfs*39)	1 (0.6%)	*Frameshift*	C-terminal α-helix	(6)
c.976_977insC (p.G326Afs*?)	1 (0.6%)	*Frameshift*	C-terminal α-helix	(6)
c.978dupG (p.I327Dfs*?)	1 (0.6%)	*Frameshift*	C-terminal α-helix	(6)
c.991T>C (p.*331R)	1 (0.6%)	Stop-loss	C-terminal α-helix	(13)

Supplemental Table 2: List of pathogenic/likely pathogenic *AIP* mutations identified in our cohort. Mutations in italic are truncating or predicted truncating mutations. Mutations in bold are novel mutations not previously described. None of these were found in the GnomAD database (https://gnomad.broadinstitute.org/gene/ENSG00000110711). All 5 patients with novel mutations were simplex cases. [#]Revel score (31) of this variant is 0.989 out of the maximum 1, strongly suggesting pathogenic status and Gavin score (32) is 'pathogenic'. *AIP*mut, *AIP* mutation-positive; GH, growth hormone; PitNET, pituitary neuroendocrine tumor; PPIase, peptidylprolyl isomerase; TPR, tetratricopeptide repeat; UTR, untranslated region.

Supplemental References (to Supplemental Table 2):

1. Igreja S, Chahal HS, King P, Bolger GB, Srirangalingam U, Guasti L, Chapple JP, Trivellin G, Gueorguiev M, Guegan K, Stals K, Khoo B, Kumar AV, Ellard S, Grossman AB, Korbonits M, International FC. Characterization of aryl hydrocarbon receptor interacting protein (AIP) mutations in familial isolated pituitary adenoma families. Hum Mutat 2010; 31:950-960
2. Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, Chahal HS, Igreja SC, Jordan S, Rowe J, Stolbrink M, Christian HC, Wray J, Bishop-Bailey D, Berney DM, Wass JA, Popovic V, Ribeiro-Oliveira A, Jr., Gadelha MR, Monson JP, Akker SA, Davis JR, Clayton RN, Yoshimoto K, Iwata T, Matsuno A, Eguchi K, Musat M, Flanagan D, Peters G, Bolger GB, Chapple JP, Frohman LA, Grossman AB, Korbonits M. The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic pituitary adenomas. J Clin Endocrinol Metab 2008; 93:2390-2401
3. Soares BS, Eguchi K, Frohman LA. Tumor deletion mapping on chromosome 11q13 in eight families with isolated familial somatotropinoma and in 15 sporadic somatotropinomas. J Clin Endocrinol Metab 2005; 90:6580-6587
4. Radian S, Diekmann Y, Gabrovska P, Holland B, Bradley L, Wallace H, Stals K, Bussell AM, McGurren K, Cuesta M, Ryan AW, Herincs M, Hernandez-Ramirez LC, Holland A, Samuels J, Aflorei ED, Barry S, Denes J, Pernicova I, Stiles CE, Trivellin G, McCloskey R, Ajzensztejn M, Abid N, Akker SA, Mercado M, Cohen M, Thakker RV, Baldeweg S, Barkan A, Musat M, Levy M, Orme SM, Unterlander M, Burger J, Kumar AV, Ellard S, McPartlin J, McManus R, Linden GJ, Atkinson B, Balding DJ, Agha A, Thompson CJ, Hunter SJ, Thomas MG, Morrison PJ, Korbonits M. Increased Population Risk of AIP-Related Acromegaly and Gigantism in Ireland. Hum Mutat 2017; 38:78-85
5. Georgitsi M, Raitila A, Karhu A, Tuppurainen K, Makinen MJ, Vierimaa O, Paschke R, Saeger W, van der Luijt RB, Sane T, Robledo M, De Menis E, Weil RJ, Wasik A, Zielinski G, Lucewicz O, Lubinski J, Launonen V, Vahteristo P, Aaltonen LA. Molecular diagnosis of pituitary adenoma predisposition caused by aryl hydrocarbon receptor-interacting protein gene mutations. Proc Natl Acad Sci U S A 2007; 104:4101-4105
6. Hernandez-Ramirez LC, Gabrovska P, Denes J, Stals K, Trivellin G, Tilley D, Ferrau F, Evanson J, Ellard S, Grossman AB, Roncaroli F, Gadelha MR, Korbonits M, International FC. Landscape of Familial Isolated and Young-Onset Pituitary Adenomas: Prospective Diagnosis in AIP Mutation Carriers. J Clin Endocrinol Metab 2015; 100:E1242-1254
7. Raitila A, Georgitsi M, Karhu A, Tuppurainen K, Makinen MJ, Birkenkamp-Demtroder K, Salmenkivi K, Orntoft TF, Arola J, Launonen V, Vahteristo P, Aaltonen LA. No evidence of somatic aryl hydrocarbon receptor interacting protein mutations in sporadic endocrine neoplasia. Endocr Relat Cancer 2007; 14:901-906
8. Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, Tuppurainen K, Ebeling TM, Salmela PI, Paschke R, Gundogdu S, De Menis E, Makinen MJ, Launonen V, Karhu A, Aaltonen LA. Pituitary adenoma predisposition caused by germline mutations in the AIP gene. Science 2006; 312:1228-1230
9. Gadelha MR, Prezant TR, Une KN, Glick RP, Moskal SF, 2nd, Vaisman M, Melmed S, Kineman RD, Frohman LA. Loss of heterozygosity on chromosome 11q13 in two families with acromegaly/gigantism is independent of mutations of the multiple endocrine neoplasia type I gene. J Clin Endocrinol Metab 1999; 84:249-256
10. Jones MK, Evans PJ, Jones IR, Thomas JP. Familial acromegaly. Clin Endocrinol (Oxf) 1984; 20:355-358
11. Georgitsi M, De Menis E, Cannavo S, Makinen MJ, Tuppurainen K, Pauletto P, Curto L, Weil RJ, Paschke R, Zielinski G, Wasik A, Lubinski J, Vahteristo P, Karhu A, Aaltonen LA. Aryl hydrocarbon receptor interacting protein (AIP) gene mutation analysis in children and adolescents with sporadic pituitary adenomas. Clin Endocrinol (Oxf) 2008; 69:621-627
12. Daly AF, Tichomirowa MA, Petrossians P, Heliovaara E, Jaffrain-Rea ML, Barlier A, Naves LA, Ebeling T, Karhu A, Raappana A, Cazabat L, De Menis E, Montanana CF, Raverot G, Weil RJ, Sane T, Maiter D, Neggers S, Yaneva M, Tabarin A, Verrua E, Eloranta E, Murat A, Vierimaa O, Salmela PI, Emy P, Toledo RA, Sabate MI, Villa C, Popelier M, Salvatori R, Jennings J, Longas AF, Labarta Aizpun JI, Georgitsi M, Paschke R, Ronchi C, Valimaki M, Saloranta C, De Herder W, Cozzi R, Guitelman M, Magri F, Lagonigro MS, Halaby G, Corman V, Hagelstein MT, Vanbellinghen JF, Barra GB, Gimenez-Roqueplo AP, Cameron FJ, Borson-Chazot F, Holdaway I, Toledo SP, Stalla GK, Spada A, Zacharieva S, Bertherat J, Brue T, Bours V, Chanson P, Aaltonen LA, Beckers A. Clinical characteristics and

therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. J Clin Endocrinol Metab 2010; 95:E373-383

13. Caimari F, Hernandez-Ramirez LC, Dang MN, Gabrovska P, Iacovazzo D, Stals K, Ellard S, Korbonits M, International Fc. Risk category system to identify pituitary adenoma patients with AIP mutations. J Med Genet 2018; 55:254-260

14. Guaraldi F, Corazzini V, Gallia GL, Grottoli S, Stals K, Dalantaeva N, Frohman LA, Korbonits M, Salvatori R. Genetic analysis in a patient presenting with meningioma and familial isolated pituitary adenoma (FIPA) reveals selective involvement of the R81X mutation of the AIP gene in the pathogenesis of the pituitary tumor. Pituitary 2012; 15, Suppl.:61-67

15. Luccio-Camelo DC, Une KN, Ferreira RE, Khoo SK, Nickolov R, Bronstein MD, Vaisman M, Teh BT, Frohman LA, Mendonca BB, Gadelha MR. A meiotic recombination in a new isolated familial somatotropinoma kindred. Eur J Endocrinol 2004; 150:643-648

16. Toledo RA, Lourenco DM, Jr., Liberman B, Cunha-Neto MB, Cavalcanti MG, Moyses CB, Toledo SP, Dahia PL. Germline mutation in the aryl hydrocarbon receptor interacting protein gene in familial somatotropinoma. J Clin Endocrinol Metab 2007; 92:1934-1937

17. Stratakis CA, Tichomirowa MA, Boikos S, Azevedo MF, Lodish M, Martari M, Verma S, Daly AF, Raygada M, Keil MF, Papademetriou J, Drori-Herishanu L, Horvath A, Tsang KM, Nesterova M, Franklin S, Vanbellinghen JF, Bours V, Salvatori R, Beckers A. The role of germline AIP, MEN1, PRKAR1A, CDKN1B and CDKN2C mutations in causing pituitary adenomas in a large cohort of children, adolescents, and patients with genetic syndromes. Clin Genet 2010; 78:457-463

18. Cazabat L, Bouligand J, Salenave S, Bernier M, Gaillard S, Parker F, Young J, Guiochon-Mantel A, Chanson P. Germline AIP mutations in apparently sporadic pituitary adenomas: prevalence in a prospective single-center cohort of 443 patients. J Clin Endocrinol Metab 2012; 97:E663-670

19. Cazabat L, Libe R, Perlemoine K, Rene-Corail F, Burnichon N, Gimenez-Roqueplo AP, Dupasquier-Fediaevsky L, Bertagna X, Clauser E, Chanson P, Bertherat J, Raffin-Sanson ML. Germline inactivating mutations of the aryl hydrocarbon receptor-interacting protein gene in a large cohort of sporadic acromegaly: mutations are found in a subset of young patients with macroadenomas. Eur J Endocrinol 2007; 157:1-8

20. Martucci F, Trivellin G, Korbonits M. Familial isolated pituitary adenomas: an emerging clinical entity. J Endocrinol Invest 2012; 35:1003-1014

21. Garcia WR, Cortes HT, Romero AF. Pituitary gigantism: a case series from Hospital de San Jose (Bogota, Colombia). Arch Endocrinol Metab 2019; 63:385-393

22. Hernandez-Ramirez LC, Martucci F, Morgan RM, Trivellin G, Tilley D, Ramos-Guajardo N, Iacovazzo D, D'Acquisto F, Prodromou C, Korbonits M. Rapid proteasomal degradation of mutant proteins is the primary mechanism leading to tumorigenesis in patients with missense AIP mutations. J Clin Endocrinol Metab 2016:jc20161307

23. Cuny T, Pertuit M, Sahnoun-Fathallah M, Daly A, Occhi G, Odou MF, Tabarin A, Nunes ML, Delemer B, Rohmer V, Desailloud R, Kerlan V, Chabre O, Sadoul JL, Cogne M, Caron P, Cortet-Rudelli C, Lienhardt A, Raingeard I, Guedj AM, Brue T, Beckers A, Weryha G, Enjalbert A, Barlier A. Genetic analysis in young patients with sporadic pituitary macroadenomas: besides AIP don't forget MEN1 genetic analysis. Eur J Endocrinol 2013; 168:533-541

24. Jorge BH, Agarwal SK, Lando VS, Salvatori R, Barbero RR, Abelin N, Levine MA, Marx SJ, Toledo SP. Study of the multiple endocrine neoplasia type 1, growth hormone-releasing hormone receptor, Gs alpha, and Gi2 alpha genes in isolated familial acromegaly. J Clin Endocrinol Metab 2001; 86:542-544

25. Daly AF, Vanbellinghen JF, Khoo SK, Jaffrain-Rea ML, Naves LA, Guitelman MA, Murat A, Emy P, Gimenez-Roqueplo AP, Tamburrano G, Raverot G, Barlier A, de Herder WW, Penfornis A, Ciccarelli E, Estour B, Lecomte P, Gatta B, Chabre O, Sabate MI, Bertagna X, Garcia BN, Stalldecker G, Colao A, Ferolla P, Wemeau JL, Caron P, Sadoul JL, Oneto A, Archambeaud F, Calender A, Sinilnikova O, Montanana CF, Cavagnini F, Hana V, Solano A, Delettieres D, Luccio-Camelo DC, Basso A, Rohmer V, Brue T, Bours V, Teh BT, Beckers A. Aryl hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. J Clin Endocrinol Metab 2007; 92:1891-1896

26. Tichomirowa MA, Barlier A, Daly AF, Jaffrain-Rea ML, Ronchi C, Yaneva M, Urban JD, Petrossians P, Elenkova A, Tabarin A, Desailloud R, Maiter D, Schurmeyer T, Cozzi R, Theodoropoulou M, Sievers C, Bernabeu I, Naves LA, Chabre O, Montanana CF, Hana V, Halaby G, Delemer B, Aizpun JI, Sonnet E, Longas AF, Hagelstein MT, Caron P, Stalla GK, Bours V, Zacharieva S, Spada A, Brue T, Beckers A. High prevalence of AIP gene mutations following focused screening in young patients with sporadic pituitary macroadenomas. Eur J Endocrinol 2011; 165:509-515

27. Jennings JE, Georgitsi M, Holdaway I, Daly A, Tichomirowa M, Beckers A, Aaltonen L, Karhu A, Cameron F. Aggressive pituitary adenomas occurring in young patients in a large Polynesian kindred with a germline R271W mutation in the AIP gene. Eur J Endocrinol 2009; 161:799-804

28. Karaca Z, Taheri S, Tanriverdi F, Unluhizarci K, Kelestimur F. Prevalence of AIP mutations in a series of Turkish acromegalic patients: are synonymous AIP mutations relevant? Pituitary 2015; 18:831-837

29. Ramirez-Renteria C, Hernandez-Ramirez LC, Portocarrero-Ortiz L, Vargas G, Melgar V, Espinosa E, Espinosa-de-Los-Monteros AL, Sosa E, Gonzalez B, Zuniga S, Unterlander M, Burger J, Stals K, Bussell AM, Ellard S, Dang M, Iacovazzo D, Kapur S, Gabrovska P, Radian S, Roncaroli F, Korbonits M, Mercado M. AIP mutations in young patients with acromegaly and the Tampico Giant: the Mexican experience. Endocrine 2016; 53:402-411

30. Occhi G, Trivellin G, Ceccato F, De Lazzari P, Giorgi G, Dematte S, Grimaldi F, Castello R, Davi MV, Arnaldi G, Salviati L, Opocher G, Mantero F, Scaroni C. Prevalence of AIP mutations in a large series of sporadic Italian acromegalic patients and evaluation of CDKN1B status in acromegalic patients with multiple endocrine neoplasia. Eur J Endocrinol 2010; 163:369-376

31. Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, Musolf A, Li Q, Holzinger E, Karyadi D, Cannon-Albright LA, Teerlink CC, Stanford JL, Isaacs WB, Xu J, Cooney KA, Lange EM, Schleutker J, Carpten JD, Powell IJ, Cussenot O, Cancel-Tassin G, Giles GG, MacInnis RJ, Maier C, Hsieh CL, Wiklund F, Catalona WJ, Foulkes WD, Mandal D, Eeles RA, Kote-Jarai Z, Bustamante CD, Schaid DJ, Hastie T, Ostrander EA, Bailey-Wilson JE, Radivojac P, Thibodeau SN, Whittemore AS, Sieh W. REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. Am J Hum Genet 2016; 99:877-885

32. van der Velde KJ, de Boer EN, van Diemen CC, Sikkema-Raddatz B, Abbott KM, Knopperts A, Franke L, Sijmons RH, de Koning TJ, Wijmenga C, Sinke RJ, Swertz MA. GAVIN: Gene-Aware Variant INterpretation for medical sequencing. Genome Biol 2017; 18:6

Variant HGVS nomenclature: DNA (protein)	dbSNP ID	American College of Medical Genetics and Genomics and the Association for Molecular Pathology category	Revel and Gavin scores	Number of subjects in our study population	MAF in our cohort - affected individuals (n=1216) (%)	MAF in GnomAD exomes and genomes (%)
c.47G>A (p.R16H)	rs145047094	benign	0.777 / benign	4 (all affected)	0.3289	0.2082
c.100-18C>T (p?)	rs202156895	likely benign	*n/a / benign	7 (all affected)	0.5757	0.3147
c.132C>T (p.D44=)	rs11822907	benign	*n/a / benign	3 (all affected)	0.2467	0.7984
c.144C>T (p.T48=)	rs772658134	benign	*n/a / benign	1 (affected)	0.0822	0.0064
c.468+9C>T (p?)	rs373159347	likely benign	*n/a / benign	1 (affected)	0.0822	0.0066
c.469-13C>T (p?)	n/a	VUS	*n/a / benign	1 (affected)	0.0822	n/a
c.516C>T (p.D172=)	rs2276020	benign	*n/a / benign	22 (nineteen affected, three unaffected [one homozygous])	1.56	3.4314
c.579G>T (p.G193=)	rs1194122725	likely benign	*n/a / benign	1 (unaffected)	0	n/a
c.682A>C (p.K228Q) †	rs641081	likely benign	0.117 / benign	18 (all affected)	1.4803	5.0202
c.787+9C>T (p?)	rs749392143	VUS	*n/a / benign	1 (affected)	0.0822	0.0047
c.807C>T (p.F269=)	rs139407567	VUS	*n/a / benign	11 (five affected)	0.4112	0.0550
c.831C>T (p.A277=)	rs531331351	VUS	*n/a / pathogenic	1 (affected)	0.0822	0.0016
c.891C>A (p.A297=)	rs35665586	benign	*n/a / benign	2 (affected)	0.1645	0.1813
c.896C>T (p.A299V)	rs148986773	likely benign	0.292 / pathogenic	5 (one affected)[#]	0.0822	0.0544
c.906G>A (p.V302=)	rs142912418	benign	*n/a / benign	2 (one affected)	0.0822	0.0086
c.911G>A (p.R304Q)	rs104894190	VUS	0.31 / benign	32 (sixteen affected)	1.32	0.1568

Supplemental Table 3: List of non-pathogenic *AIP* variants identified in the study population. n/a, not available; VUS, variant of uncertain significance. *n/a, Revel score not available as this scoring system only consider missense variants. †There is a Q at this position in the AIP reference sequence, but we consider K as the wild-type amino acid, due to its higher prevalence in the population screened so far (GnomAD, 1000Genomes); we considered A at this position as the reference allele when analyzing GnomAD data. [#]Two of the unaffected subjects carry the R304* and the A299V variants on 2 different alleles, strongly suggesting that the A299V variant is benign (Williams *et al.* JCEM, 2014). Variant nomenclature was based on transcript NM_003977.4. Categorization of variants was based on the combination of multiple *in silico* prediction tools, clinical and experimental data, as specified in the Methods section.