Three Novel *MEN1* Variants in *AIP*-Negative Familial Isolated Pituitary Adenoma Patients

Sema Yarman^a Feyza Nur Tuncer^b Esin Serbest^b

^aDivision of Endocrinology and Metabolic Diseases, Department of Internal Medicine, Faculty of Medicine, Istanbul University, Istanbul, Turkey; ^bDepartment of Genetics, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey

Keywords

Familial isolated pituitary adenoma · *MEN1* gene · Stop codon read-through variation · 3'UTR variation

Abstract

Objectives: Pituitary adenomas (PAs) may rarely occur in well-defined hereditary conditions, like multiple endocrine neoplasia type 1 (MEN1) syndrome and familial isolated pituitary adenoma (FIPA) associated with germline mutations in *MEN1* and *AIP*, respectively. This study aimed to assess *MEN1* genetic abnormalities in *AIP* mutation-negative FIPA patients, not associated with MEN1 components. **Methods:** Among 20 patients evaluated in 13 FIPA families, 12 were previously reported as *AIP* mutation-negative. In this study, 6 new families with 8 patients were recruited. All patients were subjected to multiplex ligation-dependent probe amplification to detect copy number variations in *AIP* and *MEN1*, and *AIP* sequencing was performed in additional patients. *AIP* mutation-negative patients were subjected to *MEN1* sequencing. **Results:** Our cohort revealed only 3 novel heterozygous *MEN1* variants including c.1846T>A p. (*616Argext*21), rs778272737:T>C, and rs972128957:C>T in 2 families, with patients diagnosed with Cushing disease, nonfunctional adenoma, and acromegaly, respectively. Among them,

c.1846T>A p. (*616Argext*21) is a stop codon read-through, whereas the others are 3'UTR variations. *MEN1* variation frequency was detected as 15%. **Conclusions:** *MEN1* alterations can be of significance in FIPA patients and screening could be offered to *AIP* mutation-negative patients without MEN1 features. Further studies are needed to clarify the role of *MEN1* in FIPA patients.

© 2019 S. Karger AG, Basel

Introduction

Pituitary adenomas (PAs) that occur in familial settings account for no more than 4–5%. They can be part of established endocrine-related tumor syndromes such as multiple endocrine neoplasia type 1 (MEN1), Carney complex (CNC) or familial isolated pituitary adenoma (FIPA) [1–4]. Taken together, MEN1 and FIPA, representing 2.7 and 2.5% of familial PA cases, respectively, are attributed to be the most common hereditary syndromes predisposing to PA [5]. MEN1 is an autosomal dominant inherited disease characterized by primary hyperparathyroidism (PHPT), functional or nonfunctional pancreatic neuroendocrine tumors, and PA in a patient [2, 6]. PHPT is the most common and the first

Table 1. Summary of clinical and radiological features of all FIPA patients and outcome of *MEN1* gene analysis

Family	Case	Sex	Clinical features					Genetic characteristics			
			age at onset, years	type of PA	maximum tumor diameter, mm	follow-up period, years	treatment, response to treatment	variant position (NM_003977.3)	region	MAF	genomic position (NC_000011.9 [hg19])
I	1	M	26	somatotrophinoma ^a	12	3	TSS, remission after operation	n.f.	n.a.	n.a.	n.a.
II	2	F	38	somatotrophinoma ^b	15	15	TSS, postoperatively RT + SSA + GHRA	n.f.	n.a.	n.a.	n.a.
III	3	F	59	somatotrophinoma	15	10	TSS, remission after operation	n.f.	n.a.	n.a.	n.a.
	4	F	33	somatotrophinoma	18	7	TSS, SSA after operation	n.f.	n.a.	n.a.	n.a.
IV	5	F	47	corticotrophinoma	12	14	TSS, remission after operation	c.1846 T>A (p.*616Argext*21) rs778272737:T>C	Exon 10	NA	g.64,571,808 A>T
	6	F	35	NFA	6	6	Unchanged tumor size, still nonfunctional		3'UTR	MAF<0.01 (C)	g.64,571,336 T>C
	7	F	20	PRLoma	10	13	Continued with DA	n.f.	n.a.	n.a.	n.a.
	8	F	43	somatotrophinoma	13	40	TSS, remission after operation	n.f.	n.a.	n.a.	n.a.
V	9	F	34	somatotrophinoma^c	12	35	TSS + RT + DA + SSA	rs972128957:C>T	3'UTR	MAF<0.01 (T)	g.64,571,394 C>T
VI	10	M	59	PRLoma	40	7	DA	n.f.	n.a.	n.a.	n.a.
	11	M	45	somatotrophinoma	n.a.	5	TSS + SSA	n.f.	n.a.	n.a.	n.a.
VII	12	F	28	somatotrophinoma ^d	20	16	TSS + RT, remission	n.f.	n.a.	n.a.	n.a.
VIII	13	F	17	PRLoma	18	22	DA, PA disappeared	n.f.	n.a.	n.a.	n.a.
	14	M	33	Somatotrophinoma	11	3	TSS, remission	n.f.	n.a.	n.a.	n.a.
IX	15	F	41	corticotrophinoma ^e	12	7	TSS, remission	n.f.	n.a.	n.a.	n.a.
X	16	M	45	corticotrophinoma	7	8	TSS, remission	n.f.	n.a.	n.a.	n.a.
	17	M	54	NFA	32	4	TSS, no recurrence	n.f.	n.a.	n.a.	n.a.
XI	18	M	22	PRLoma ^f	20	7	DA continued	n.f.	n.a.	n.a.	n.a.
XII	19	F	50	somatotrophinoma ^g	15	5	SSA, tumor disappeared and for the past 18 years the drug remained discontinued	n.f.	n.a.	n.a.	n.a.
XIII	20	M	21	somatotrophinoma ^h	60	14	2 TSS + RT + DA + SSA + GHRA	n.f.	n.a.	n.a.	n.a.

Bold type denotes patients detected to have *MEN1* variations. NFA, nonfunctional adenoma; PRLoma, prolactinoma; TSS, transsphenoidal surgery; SSA, somatostatin analog; DA, dopamine agonist; GHRA, growth hormone receptor antagonist; RT, radiotherapy; PA, pituitary adenoma; n.f., not found; n.a., not available; UTR, untranslated region; MAF, minor allele frequency; M, male; F, female.

^a Uncle has somatotrophinoma; ^b sister operated for NFA; ^c she participated in an international FIPA study [26] and her brother died at a young age from the same disease; ^d cousin has somatotrophinoma; ^e uncle (NFA) and cousin (PRLoma); ^f maternal grandmother had NFA; ^g cousin has somatotrophinoma; ^h cousin has somatotrophinoma.

clinical manifestation of the disease in 80–100% of *MEN1* patients under the age of 40 years, but PAs are detected in approximately 35–40% of *MEN1* patients [7–9]. Moreover, it has been reported that PHPT does not routinely manifest before other components of *MEN1* in different geographic regions, but in 15% of *MEN1* mutation carriers, PA is the first lesion to be diagnosed [9–11]. According to the guidelines on *MEN1* diagnosis, *MEN1* affects an age range of 5–81 years with 80% of the patients developing clinical manifestations and >98% of patients showing biochemical findings by 50 years of age [12]. In contrast, FIPA was originally described as *MEN1*-unrelated PAs, in which current FIPA diagnosis requires the occurrence of ≥ 2 cases of PAs in a single family without hypercalcemia and *MEN1* components or CNC lesions [13].

Genetic studies involving hereditary PAs have associated germline heterozygous loss-of-function mutations in the tumor suppressor genes aryl hydrocarbon receptor-interacting protein (*AIP*) and menin 1 (*MEN1*) with FIPA and *MEN1* conditions, respectively [14–17]. In the literature, 459 different germline *MEN1* mutations out of 1,133 were reported in 90% of familial cases [3, 6, 12, 15, 18, 19]. On the other hand, *AIP* gene mutations have been identified in 15–20% of FIPA cases [4, 5, 20, 21].

Recently, a plausible role of *MEN1* gene alterations in sporadic PA pathogenesis was suggested via the detection of *MEN1* mutations in *AIP* mutation-negative young pituitary macroadenomas [22]. Likewise, we recently reported that we could not detect *AIP* point mutations in our cohort of 12 FIPA patients [23]. This prompted us to screen *AIP* mutation-negative FIPA patients for *MEN1*

Fig. 1. The chromatograms of 3 novel *MEN1* variations are depicted. **a** Heterozygous *MEN1* c.1846 T>A alteration in Case 5. **b** Heterozygous change in case 6 is shown as rs778272737:T>C. **c** Heterozygous alteration in case 9 is shown as rs972128957:C>T.

gene alterations. For this study, 6 additional FIPA families were recruited, comprising 8 patients not associated with other *MEN1* syndrome components (without hypercalcemia and/or *MEN1*-associated other lesions) in the long-term clinical follow-up period.

Patients and Methods

Patients

A total of 20 patients in 13 FIPA families were included in this study and followed-up at the Outpatient Pituitary Clinic at the Istanbul University Faculty of Medicine. Patients from 7 families were reported on previously and 6 additional families were recruited for this study (Table 1) [23]. Since Istanbul is a cosmopolitan city, patients participating in the study come from different geographical regions of Turkey. One was a patient with familial acromegaly, who participated in the International multicenter FIPA study; no *AIP* mutation was reported in this patient [24]. Our FIPA families consisted of 6 homogeneous patients with somatotrophinoma, and 7 heterogeneous patients with somatotrophinoma, prolactinoma, corticotrophinoma, and nonfunctional adenoma (NFA) (Table 1).

Functional PA diagnosis was based on clinical signs and symptoms and laboratory tests demonstrating elevated basal serum hormone levels and/or abnormal outcomes of dynamic tests. The results were interpreted in accordance with current recommendations and guidelines [25–29]. PA types in our cohort of 20 FIPA patients consisted of somatotrophinoma ($n = 11$), prolactinoma ($n = 4$), NFA ($n = 2$), and corticotrophinoma ($n = 3$). Table 1 summarizes the diagnoses of the patients' relatives, who were not available for genetic analyses. Patients' MRIs were assessed by a senior radiologist experienced in evaluating MRIs of the pituitary gland.

Genetic Analyses

Given the family occurrence of PAs in this cohort, genetic materials were obtained from all patients following their written informed consent in accordance with the Istanbul University Faculty of Medicine Clinical Ethics Committee (2015/498). Genomic DNA was extracted from peripheral blood utilizing either the Qia-gen Maxi kit (Qiagen, Inc., Hilden, Germany) or the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA). In order to assess copy number variations (CNVs) in the *AIP* and *MEN1* genes, MLPA was performed. Commercially obtained reagents and probe-mixes were used according to the manufacturer's instructions (P244-AIP-MEN1-CDKN1B, MRC-Holland,

The Netherlands). Patients depicted negative for CNVs in the *AIP* and *MEN1* genes were subjected to Sanger sequencing of these genes. *AIP* gene sequencing was initially performed, followed by sequencing of the *MEN1* gene only in *AIP* mutation-negative patients. The Primer3 program was utilized for primer design to PCR-amplify all exons, exon-intron boundaries, and untranslated regions (UTRs) of both the *AIP* and *MEN1* genes. PCR amplicons were subjected to Sanger sequencing, at the service of Macrogen Korea, Inc.. CLC Main Workbench v6.5 was used in sequence analysis against the reference sequences NM_003977.3 and NM_000244.3 for the *AIP* and *MEN1* genes, respectively.

Evaluation of the Genetic Findings

MLPA results were analyzed using the Coffalyser Net platform. To determine minor allele frequencies (MAF) of genetic variants obtained from the *AIP* and *MEN1* mutational analyses, all available databases including Leiden Open Variation Database (LOVD), Exome Aggregation Consortium (ExAC), gnomAD, Exome Variant Server (EVS), Mine Data Browser, UMD-MEN1, and HGMD database were utilized [30–35]. A literature search was performed with an attempt to inspect the variants obtained in our patients, with particular reference to the review on *MEN1* mutations detected in 2007–2015 [36]. Additionally, an exome variant database of varying disorders comprising 1,182 individuals from Turkey was also screened as a population control set for the *MEN1* genetic variants detected in the patients.

Results

In our cohort of 13 FIPA families with 20 patients, the mean ($\pm$ SD) age at the diagnosis of PA was 38 ± 13 (range 17–59) years. The patients in our cohort were followed up for 18 ± 12 (range 6–34) years. Clinical and radiologic data along with genetic results of all patients are summarized in Table 1. We did not detect any germline *AIP* point variations in the 8 newly recruited FIPA patients. All patients were detected negative for CNVs in the *AIP* and *MEN1* genes through MLPA analysis. Three novel heterozygous *MEN1* variants, NM_000244.3:c.1846T>A p.(^{*}616Argext^{*}21), rs778272737:T>C, and rs972128957:C>T, were determined in a total of 3 patients from families IV and V (Fig. 1) and were submitted to ClinVar database with accession numbers SCV000611858, SCV000611858, and SCV000611860, respectively.

Genetic and Clinical Findings in Family IV

This highly inbred family has heterogeneous FIPA (Fig. 2). The *MEN1* NM_000244.3:c.1846T>A p.(^{*}616Argext^{*}21) variation was detected in Case 5 with a diagnosis of Cushing disease (Fig. 1a), and the *MEN1* 3'UTR rs778272737:T>C variation was detected in her cousin, Case 6 (Fig. 1b). Cases 7 and 8 did not reveal any *MEN1* variations, and were diagnosed with prolactinoma and somatotrophinoma, respectively (Fig. 2).

Among the carriers of *MEN1* variants, maximum tumor sizes were determined as 12 and 6 mm for Cases 5 and 6, respectively. Currently, Case 5 is in remission, following a single transsphenoidal surgery (TSS). In Case 6, microadenoma size did not change or gain function. Case 7, with a tumor size of 10 mm at diagnosis, is still on dopamine agonist therapy. Case 8, having a macrosomatotrophinoma of 13 mm, is in remission following transsphenoidal surgery (Table 1).

Genetic and Clinical Findings in Family V

A different novel *MEN1* 3'UTR rs972128957:C>T variant was detected in Case 9 of family V, who was diagnosed with acromegaly (Fig. 1c; Fig. 3). Case 9 developed normocalcemic PHPT 24 years after diagnosis (at the age of 58 years) and had normal vitamin D and 24-h urinary calcium excretion levels. She also had negative parathyroid imaging. This patient was under control with somatostatin analog. She remained normocalcemic until her death at the age of 69 years due to heart failure and arrhythmia. When asked, this patient told us that her brother who had been living in Europe had died from the same disease at a young age (Fig. 3).

To date, no endocrinopathies for MEN1 syndrome besides PA have manifested in any of the FIPA patients, including the carriers of *MEN1* variations. *MEN1* variant was detected at a frequency of 15% in our *AIP* mutation-negative FIPA patients.

Discussion

The genetic basis of FIPA has long been studied with mutations in the *AIP* gene. However, it has been determined that screening for *AIP* in the majority of FIPA families (80–85%) does not explain the genetic cause of the disease [37, 38]. In fact, two recent negative results obtained from genetic screening of tumor suppressor gene *CDKN1B* in FIPA patients exemplify the effort to discover new genetic variants related to this disease [39, 40]. In this study, we aimed to determine the genetic causes in 20 patients exhibiting only PA in our FIPA cohort. For this purpose, we initially screened for *AIP* mutations in these patients. Surprisingly, our cohort lacked any *AIP* gene alterations, which prompted us to examine the tumor-suppressor gene *MEN1* for any variations, despite the fact that none of the patients exhibited hypercalcemia or MEN1 components. We identified three novel heterozygous *MEN1* gene variations in a total of three patients in two of the FIPA families. Overall, the 15% frequency of

Fig. 2. Pedigree of family IV. NFA, nonfunctional adenoma; PRLoma, prolactinoma.

Fig. 3. Pedigree of family V.

the *MEN1* variant determined in our cohort was similar to the 15–25% frequency of *AIP* gene mutations detected in FIPA patients [24, 41, 42].

Four patients in family IV were available for genetic screening, and only Cases 5 and 6 were found to carry two distinct novel *MEN1* variants. Case 5 exhibited a heterozygous alteration in the stop codon (NM_000244.3:c.1846

T>A p.[*616Argext*21]) that leads to erroneous extension of the transcript into the next stop codon situated in the C-terminal of the *MEN1* gene. Recently, a total of 20 stop codon read-through mutations were associated with different hereditary disorders other than PAs in humans [43]. However, the functional effects of these read-through mutations are not yet clearly delineated. Only a limited number of functional studies showed varied effects of these mutations on the protein, including degradation by ubiquitin-proteasome system, conversion of protein function, and alteration of the subcellular localization [43–45]. Thus, it is deduced that C-terminal extension mutations impact genes and their functions, depending on the length of newly formed mutated transcripts. Here, we introduce a new stop codon read-through alteration in *MEN1* [NM_000244.3:c.1846 T>A p.[*616Argext*21]] with a plausible role in the onset of Cushing disease in Case 5, which requires validation through functional studies. In addition, Case 6 carried a heterozygous rs778272737:T>C variation with a MAF of 0.0004202 for the “C” allele in the gnomAD database [31]. This variant was reported in individuals with PHPT and multiple endocrine neoplasia (MEN) with a denotation of uncertain clinical significance in the ClinVar database [46]. On the basis of these findings, this variant could play a role in the predisposition to

micro-NFA in this case. Taking into account the consanguinity in successive generations within this family, one would expect to detect homozygous variations in these patients. However, homozygosity is rarely observed in disorders with autosomal dominant inheritance due to severe phenotypic outcomes [47]. Since FIPA is an autosomal dominant disease and family IV has affected individuals descending from a single grandfather and different grandmothers (Fig. 2), it was not surprising to detect two distinct novel heterozygous *MEN1* variations in Cases 5 and 6. Moreover, no *AIP* or *MEN1* gene alterations were detected in Cases 7 and 8, which strongly suggests the presence of other candidate genes yet to be discovered to play a role in FIPA onset. The patients in this family exhibited heterogeneous PAs. Therefore, clinical phenotypes were completely different from each other, independent of the presence of *MEN1* variants. Among the two *MEN1* variant-negative family members, Case 7 had a prolactinoma and we were not able to withdraw DA during the follow-up period of 13 years. The other patient (Case 8) is still in remission after the operation (in follow-up for 40 years). On the other hand, the *MEN1* variant-positive cases (Cases 5 and 6) also exhibit good prognosis (Table 1).

In family V, a different novel *MEN1* variant was detected in Case 9 as rs972128957:C>T. This 3'UTR variant, having an MAF <0.01 for the "T" allele, has not yet been associated with a clinical finding in the conventional databases. It was unfortunate that her deceased brother had the same PA but was unavailable for the genetic analyses. Case 9 differed from the patients in family IV discussed above, in the sense that she developed normocalcemic PHPT 24 years after her FIPA diagnosis. This could be coincidental since no hypercalcemia or other *MEN1* components were exhibited in the 11 years until her death.

Conclusions

This is the first study highlighting the importance of germline *MEN1* variations in *AIP* mutation-negative FIPAs, detected with 15% frequency in our cohort. Our cohort did not exhibit any other components of *MEN1* during the long-term clinical follow-up, suggesting a plausible impact of *MEN1* gene alterations in FIPA pathogenesis. To elucidate the role of *MEN1* variations in pituitary tumorigenesis, our results need to be replicated in larger cohorts, and functional validations should be performed. We therefore suggest screening of *MEN1* variations in *AIP* mutation-negative FIPA patients, to contribute to genotype-phenotype correlations in FIPA and elucidate the role of this tumor-suppressor gene in disease pathogenesis.

Acknowledgements

The authors thank the patients for taking part in our study. We would like to acknowledge Advanced Genomics and Bioinformatics Research Center (IGBAM), TUBITAK MAM-GMBE, for kindly providing variant frequency data of 1,182 exome sequenced individuals from Turkey with varying disorders and used as a population control set for the novel *MEN1* variations. This work was supported by Scientific Research Projects Coordination Unit of Istanbul University, Istanbul, Turkey, project No. 54222.

Statement of Ethics

Written informed consent was obtained from all participants in accordance with the Istanbul University Faculty of Medicine Clinical Ethics Committee (2015/498).

Disclosure Statement

The authors declare that they have no conflict of interest.

References

- 1 Wermer P. Genetic aspects of adenomatosis of endocrine glands. *Am J Med*. 1954 Mar;16(3): 363–71.
- 2 Thakker RV. Multiple endocrine neoplasia type 1 (MEN1) and type 4 (MEN4). *Mol Cell Endocrinol*. 2014 Apr;386(1-2):2–15.
- 3 Lemos MC, Thakker RV. Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. *Hum Mutat*. 2008 Jan;29(1):22–32.
- 4 Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, et al. Pituitary adenoma predisposition caused by germline mutations in the *AIP* gene. *Science*. 2006 May; 312(5777):1228–30.
- 5 Beckers A, Daly AF. The clinical, pathological, and genetic features of familial isolated pituitary adenomas. *Eur J Endocrinol*. 2007 Oct; 157(4):371–82.
- 6 Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J Clin Endocrinol Metab*. 2001 Dec;86(12): 5658–71.
- 7 Doherty GM, Olson JA, Frisella MM, Lairmore TC, Wells SA Jr, Norton JA. Lethality of multiple endocrine neoplasia type I. *World J Surg*. 1998 Jun;22(6):581–6.
- 8 Kouvaraki MA, Lee JE, Shapiro SE, Gagel RF, Sherman SI, Sellin RV, et al. Genotype-phenotype analysis in multiple endocrine neoplasia type 1. *Arch Surg*. 2002 Jun;137(6):641–7.
- 9 Vergès B, Boureille F, Goudet P, Murat A, Beckers A, Sassolas G, et al. Pituitary disease in MEN type 1 (MEN1): data from the France-Belgium MEN1 multicenter study. *J Clin Endocrinol Metab*. 2002 Feb;87(2):457–65.

- 10 Pieterman CR, Schreinemakers JM, Koppe-schaar HP, Vriens MR, Rinkes IH, Zonnen-berg BA, et al. Multiple endocrine neoplasia type 1 (MEN1): its manifestations and effect of genetic screening on clinical outcome. *Clin Endocrinol (Oxf)*. 2009 Apr;70(4):575–81.
- 11 Syro LV, Scheithauer BW, Kovacs K, Toledo RA, Londoño FJ, Ortiz LD, et al. Pituitary tumors in patients with MEN1 syndrome. *Clinics (São Paulo)*. 2012;67(S1 Suppl 1):43–8.
- 12 Thakker RV, Newey PJ, Walls GV, Bilezikian J, Dralle H, Ebeling PR, et al.; Endocrine Society. Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *J Clin Endocrinol Metab*. 2012 Sep;97(9):2990–3011.
- 13 Daly AF, Jaffrain-Rea ML, Ciccarelli A, Val-des-Socin H, Rohmer V, Tamburrano G, et al. Clinical characterization of familial isolated pituitary adenomas. *J Clin Endocrinol Metab*. 2006 Sep;91(9):3316–23.
- 14 Thakker RV, Bouloux P, Wooding C, Chotai K, Broad PM, Spurr NK, et al. Association of para-thyroid tumors in multiple endocrine neoplasia type 1 with loss of alleles on chromosome 11. *N Engl J Med*. 1989 Jul;321(4):218–24.
- 15 Agarwal SK, Kester MB, Debelenko LV, Hep-pner C, Emmert-Buck MR, Skarulis MC, et al. Germline mutations of the MEN1 gene in fa-milial multiple endocrine neoplasia type 1 and related states. *Hum Mol Genet*. 1997 Jul;6(7):1169–75.
- 16 Caimari F, Hernández-Ramírez LC, Dang MN, Gabrovská P, Iacovazzo D, Stals K, et al.; International FIPA consortium. Risk category system to identify pituitary adenoma patients with AIP mutations. *J Med Genet*. 2018 Apr;55(4):254–60.
- 17 Hernández-Ramírez LC, Morgan RM, Barry S, D'Acquisto F, Prodromou C, Korbonsits M. Multi-chaperone function modulation and as-sociation with cytoskeletal proteins are key fea-tures of the function of AIP in the pituitary gland. *Oncotarget*. 2018 Jan;9(10):9177–98.
- 18 Bassett JH, Forbes SA, Pannett AA, Lloyd SE, Christie PT, Wooding C, et al. Characteriza-tion of mutations in patients with multiple en-docrine neoplasia type 1. *Am J Hum Genet*. 1998 Feb;62(2):232–44.
- 19 Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, et al. Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science*. 1997 Apr;276(5311):404–7.
- 20 Daly AF, Tichomirowa MA, Beckers A. Genetic, molecular and clinical features of familial isolated pituitary adenomas. *Horm Res*. 2009 Apr;71 Suppl 2:116–22.
- 21 Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, et al. The role of the aryl hydrocarbon receptor-interacting pro-tein gene in familial and sporadic pituitary ad-enomas. *J Clin Endocrinol Metab*. 2008 Jun;93(6):2390–401.
- 22 Cuny T, Pertuit M, Sahnoun-Fathallah M, Daly A, Occhi G, Odou MF, et al. Genetic anal-ysis in young patients with sporadic pituitary macroadenomas: besides AIP don't forget MEN1 genetic analysis. *Eur J Endocrinol*. 2013 Mar;168(4):533–41.
- 23 Yarman S, Ogret YD, Oguz FS. Do the aryl hy-drocarbon receptor interacting protein vari-ants (Q228K and Q307R) play a role in patients with familial and sporadic hormone-secreting pituitary adenomas? *Genet Test Mol Biomark-ers*. 2015 Jul;19(7):394–8.
- 24 Igreja S, Chahal HS, King P, Bolger GB, Srira-ngalingam U, Guasti L, et al.; International FIPA Consortium. Characterization of aryl hydro-carbon receptor interacting protein (AIP) mu-tations in familial isolated pituitary adenoma families. *Hum Mutat*. 2010 Aug;31(8):950–60.
- 25 Katznelson L, Atkinson JL, Cook DM, Ezzat SZ, Hamrahian AH, Miller KK; American As-sociation of Clinical Endocrinologists. Ameri-can Association of Clinical Endocrinologists medical guidelines for clinical practice for the diagnosis and treatment of acromegaly – 2011 update. *Endocr Pract*. 2011 Jul-Aug;17 Suppl 4:1–44.
- 26 Casanueva FF, Molitch ME, Schlechte JA, Abs R, Bonert V, Bronstein MD, et al. Guidelines of the Pituitary Society for the diagnosis and management of prolactinomas. *Clin Endocri-nol (Oxf)*. 2006 Aug;65(2):265–73.
- 27 Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, et al. The di-agnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin En-docrinol Metab*. 2008 May;93(5):1526–40.
- 28 Melmed S, Casanueva FF, Hoffman AR, Klein-berg DL, Montori VM, Schlechte JA, et al.; En-docrine Society. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011 Feb;96(2):273–88.
- 29 Katznelson L, Laws ER Jr, Melmed S, Molitch ME, Murad MH, Utz A, et al.; Endocrine Soci-ety. Acromegaly: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2014 Nov;99(11):3933–51.
- 30 Fokkema IF, Taschner PE, Schaafsma GC, Cel-li J, Laros JF, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. *Hum Mutat*. 2011 May;32(5):557–63.
- 31 Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al; Exome Aggre-gation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016 Aug;536(7616):285–91.
- 32 Johnston JJ, Biesecker LG. Databases of ge-nomic variation and phenotypes: existing re-sources and future needs. *Hum Mol Genet*. 2013 Oct;22 R1:R27–31.
- 33 Wautot V, Vercherat C, Lespinasse J, Chambe B, Lenoir GM, Zhang CX, et al. Germline mu-tation profile of MEN1 in multiple endocrine neoplasia type 1: search for correlation be-tween phenotype and the functional domains of the MEN1 protein. *Hum Mutat*. 2002 Jul;20(1):35–47.
- 34 Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, Thomas NS, et al. Human Gene Mu-tation Database (HGMD): 2003 update. *Hum Mutat*. 2003 Jun;21(6):577–81.
- 35 Project MinE Data Browser. <http://databrows-er.projectmine.com/> 2018 Accessed May 24, 2018.
- 36 Concolino P, Costella A, Capoluongo E. Mul-tiple endocrine neoplasia type 1 (MEN1): an update of 208 new germline variants reported in the last nine years. *Cancer Genet*. 2016 Jan-Feb;209(1-2):36–41.
- 37 Beckers A, Aaltonen LA, Daly AF, Karhu A. Familial isolated pituitary adenomas (FIPA) and the pituitary adenoma predisposition due to mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene. *Endocr Rev*. 2013 Apr;34(2):239–77.
- 38 Toledo RA, Lourenço DM Jr, Toledo SP. Fa-milial isolated pituitary adenoma: evidence for genetic heterogeneity. *Front Horm Res*. 2010;38:77–86.
- 39 Tichomirowa MA, Lee M, Barlier A, Daly AF, Marinoni I, Jaffrain-Rea ML, et al. Cyclin-de-pendent kinase inhibitor 1B (CDKN1B) gene variants in AIP mutation-negative familial iso-lated pituitary adenoma kindreds. *Endocr Relat Cancer*. 2012 May;19(3):233–41.
- 40 Occhi G, Trivellini G, Ceccato F, De Lazzari P, Giorgi G, Demattè S, et al. Prevalence of AIP mutations in a large series of sporadic Italian acromegalic patients and evaluation of CD-KN1B status in acromegalic patients with mul-tiple endocrine neoplasia. *Eur J Endocrinol*. 2010 Sep;163(3):369–76.
- 41 Georgitsi M, Raitila A, Karhu A, Tuppurainen K, Mäkinen MJ, Vierimaa O, et al. Molecular diagnosis of pituitary adenoma predisposition caused by aryl hydrocarbon receptor-interact-ing protein gene mutations. *Proc Natl Acad Sci USA*. 2007 Mar;104(10):4101–5.
- 42 Daly AF, Tichomirowa MA, Petrossians P, He-liövaara E, Jaffrain-Rea ML, Barlier A, et al. Clinical characteristics and therapeutic re-sponses in patients with germ-line AIP mu-tations and pituitary adenomas: an international collaborative study. *J Clin Endocrinol Metab-ol*. 2010;95:E373–83.
- 43 Shibata N, Ohoka N, Sugaki Y, Onodera C, In-oue M, Sakuraba Y, et al. Degradation of Stop Codon Read-through Mutant Proteins via the Ubiquitin-Proteasome System Causes Heredi-tary Disorders. *J Biol Chem*. 2015 Nov;290(47):28428–37.
- 44 Abe S, Katagiri T, Saito-Hisaminato A, Usami S, Inoue Y, Tsunoda T, et al. Identification of CRYM as a candidate responsible for nonsyn-dromic deafness, through cDNA microarray analysis of human cochlear and vestibular tis-sues. *Am J Hum Genet*. 2003 Jan;72(1):73–82.
- 45 Eswarappa SM, Potdar AA, Koch WJ, Fan Y, Vasu K, Lindner D, et al. Programmed transla-tional readthrough generates antiangiogenic VEGF-Ax. *Cell*. 2014 Jun;157(7):1605–18.
- 46 Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res*. 2016 Jan;44 D1:D862–8.
- 47 Charfeddine C, Ktaifi C, Laroussi N, Hamma-mi H, Jmel H, Landoulsi Z, et al. Clinical and molecular investigation of Buschke-Fischer-Brauer in consanguineous Tunisian families. *J Eur Acad Dermatol Venerol*. 2016 Dec;30(12):2122–30.