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ORIGINAL ARTICLE

Analysis of *GPR101* and *AIP* genes mutations in acromegaly: a multicentric study

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Abstract This multicentric study aimed to investigate the prevalence of the G protein-coupled receptor 101 (*GPR101)* p.E308D variant and aryl hydrocarbon receptor interacting protein (*AIP)* gene mutations in a representative cohort of Italian patients with acromegaly. 215 patients with GH-secreting pituitary adenomas, referred to 4 Italian referral centres for pituitary diseases, have been included. Three cases of gigantism were present. Five cases were classified as FIPA. All the patients have been screened for germline *AIP* gene mutations and *GPR101* gene p.E308D variant. Heterozygous *AIP* gene variants have been found in 7 patients (3.2 %). Five patients carried an *AIP* mutation (2.3 %; 4 females): 3 patients harboured the p.R3O4Q mutation, one had the p.R304* mutation and the last one the IVS3+1G>A mutation. The prevalence of *AIP* mutations was 3.3 % and 2.8 % when considering only the patients diagnosed when they were $\leq$30 or $\leq$40-year old, respectively. Furthermore, 2.0 % of the patients with a pituitary macroadenoma and 4.2 % of patients resistant to somatostatin analogues treatment were found to harbour an *AIP* gene mutation. None of the patients was found to carry the *GPR101* p.E308D variant. The prevalence of *AIP* gene mutations among our sporadic and familial acromegaly cases was similar to that one reported in previous studies, but lower when considering only the cases diagnosed before 40 years of age. The GPR101 p.E308D change is unlikely to have a role in somatotroph adenomas tumorigenesis, since none of our sporadic or familial patients tested positive for this variant.

Keywords Acromegaly · AIP · GPR101 · FIPA · Somatotropinoma

Introduction

Somatotropinomas are the most frequent cause of growth hormone excess leading to acromegaly/gigantism [1]. They can occur sporadically or, more rarely, in a familial setting [2, 3]. The genetic background involved in their pathogenesis is still largely unknown [2]. Somatic activating mutations in the *GNAS* gene, which encodes for the Gsα subunit of G-proteins, are found in up to 40 % of sporadic somatotroph adenomas [4]. Familial acromegaly/gigantism can occur in the context of rare inherited syndromes such as familial isolated pituitary adenoma (FIPA), which is caused in 15–20 % of cases by aryl hydrocarbon receptor interacting protein (AIP) gene germline mutations [3]. FIPA syndrome includes isolated familial somatotropinomas in which *AIP* germline mutations are found in approximately 40 % of families [2, 3]. *AIP* gene germline mutations account also for a variable proportion of patients with apparently sporadic somatotroph adenoma, more frequently if young-onset and/or resistant to conventional

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treatments [5–7]. Other rare familial syndromes are associated with acromegaly/gigantism such as, the multiple endocrine neoplasia 1 (MEN1), in most of the cases due to loss of function mutations of the *MEN1* gene which lead to the development of pituitary and/or pancreatic and/or parathyroid tumours; the McCune-Albright syndrome which is caused by mosaicism for a mutation in the *GNAS* gene; the Carney complex, in the majority of cases due to inactivating mutations of *PRKAR1A* gene; the multiple endocrine neoplasia type 4 due to mutations of *CDKN1B* gene which codes for p27; and the so called '3Pas' related to succinate dehydrogenase mutations, a condition in which pituitary adenoma associates with pheochromocytoma/paraganglioma [8, 9]. Moreover, very recently, Trivellin et al. described a disorder that is caused by an Xq26.3 genomic duplication and is characterized by early-onset gigantism resulting from an excess of growth hormone [10]. The same authors found a recurrent variant (p.E308D) in *GPR101* gene, which is located on Xq26.3, in 4 % of patients with non-familial acromegaly. A following study found 1 % of sporadic acromegalic patients harbouring the *GPR101* p.E308D variant [11].

This multicentric study aimed to investigate the prevalence of the *GPR101* p.E308D variant and *AIP* gene mutations in a representative cohort of Italian patients with acromegaly and to describe the clinical phenotype of mutation carriers.

Patients and methods

Patients

In this study, 215 consecutive Caucasian patients (136 females) with acromegaly due to somatotropinomas, referred to 4 Italian referral centres for pituitary diseases (Endocrinology Units of the University Hospital of Messina, Ancona and Padova and the Department of Internal Medicine of the General Hospital of Montebelluna, Treviso) from 2005 to 2014, have been included. Acromegaly was diagnosed according to the current diagnostic guidelines [12]. Serum GH and IGF1 levels were assayed by commercial methods in each referral centre. The mean age at diagnosis was 46.9 ± 14.1(SD). Three case of gigantism, defined as history of abnormal growth velocity for age or final height >2 SD above country normal means, were present: a female patient diagnosed at 15 years of age with a macroadenoma, a male with a macroadenoma diagnosed at 28 years of age and a female diagnosed with a macroadenoma when she was 18-year old. A macroadenoma was found in 67.5 % of the patients. Forty-seven patients out of 159 for whom this information was available were resistant to SSA treatment, as they did not achieve biochemical control after at least 6 months of treatment at the highest allowed/tolerated dose [12, 13]. Five unrelated cases were classified as FIPA, while no one was diagnosed with MEN1 syndrome. In the sporadic cases, familial and personal history was not suggestive of other inheritable pituitary tumour-related syndromes. DNA from peripheral blood leukocytes has been obtained from each patient for genetic analysis. Local ethic committee approved the study and each patient has signed informed consent.

Methods

All the patients have been screened for germline *AIP* gene variants (in exons 1-6 and paraxonic intron sequences) and *GPR101* gene p.E308D variant. DNA was obtained from leukocytes of peripheral blood anti-coagulated with EDTA as previously described [14]. PCR was performed in a total volume of 25 µl, containing 5 µl Taq buffer (5X Colorless GoTaq® Reaction Buffer, Promega, Madison, WI, USA), 1.25 U Taq polymerase (GoTaq® DNA Polymerase, Promega), 0.5 µl dNTP Mix 10 mM (Promega) and 0.5 µl of forward and reverse primer (Table 1) at concentration of 10 µM. The reaction was carried out in a Thermocycler Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA) with an initial denaturation step at 95 °C for 5 min and then 36 cycles at 95 °C for 45 s, at the temperatures detailed in Table 1 for 45 s, and at 72 °C for 45 s, followed by a final extension of 5 min at 72 °C. PCR reactions were purified using Exo-Sap reaction (United States Biochemical (USB) Corporation, Cleveland, OH, USA). Bidirectional sequencing of PCR products was performed using the BigDyev.3.1 kit chemistry, visualized with ABI 3730 capillary sequencer (Applied Biosystems) and analysed with Mutation Surveyor Software® v. 4.0.5 (SoftGenetics®, Pennsylvania, USA) in comparison to the wild-type sequence (RefSeq NG_008969.1 and NG_016367.1).

Results

AIP gene variants have been found in 7 patients (3.2 %), five of whom were found with an *AIP* gene mutation known to be pathogenic (2.3 %; 4F/1 =M) (Tables 2, 3). Three patients harboured the p.R3O4Q mutation (c.911G>A): one case was a female diagnosed, when she was 62-year old, with a macroadenoma resistant to SSA treatment; the second one was a female found to have a microadenoma at 53 years of age; and the third was a female included in a previous study [15], diagnosed with a macroadenoma when she was 67-year old. One case with the p.R304* mutation (c.910C>T) was a FIPA patient included in a previous study [16], diagnosed when he was

Table 1 Primers and PCR conditions used to amplify the coding region of the *AIP* gene and *GPR101* gene

Gene	Primer name	Sequence (5′–3′)	Amplicon size (bp)	Temperature annealing (C°)
AIP	AIP-1F	CCGAGACATTCCTAGGCTCC	397	56
	AIP-1R	CTCTCGCCTAAGGCCTCC		
	AIP-2F	GGACTGGACTTCTCCTTGGG	346	60
	AIP-2R	GTCTAGCAGAGGGTGGAGGG		
	AIP-3F	GATGGTGGTGGGGAAGG	359	62
	AIP-3R	ACCCCTGGGTGGACAGG		
	AIP-4/5F	ATGTGGGTCAGGTCTGCTG	587	58
	AIP-4/5R	AAAGGCTAGGTCTTGACCCC		
	AIP-6F	TGTAAAACGACGGCCAGT	477	62
	AIP-6R	CAGGAAACAGCTATGACC		
GPR101	GPR101-1F	GATGAAGAGGGAGCAGAG	778	60
	GPR101-1R	CAGAATCGTAGGAAGGGA		

bp base pairs

Table 2 Prevalence of *AIP* gene and *GPR101* p.E308D mutations in our cohort of patients with acromegaly

Patients	*n*°	*AIP* mut	*GPR101* p.E308D mut
Patients	215	5 (2.3 %)	0
Apparently sporadic patients	210	4 (1.9 %)	0
Patients ≤30 years old at diagnosis	30	1 (3.3 %)	0
Patients ≤40 years old at diagnosis	71	2 (2.8 %)	0
Patients with macroadenoma	145	3 (2.0 %)	0
Patients resistant to SSA treatment	47	2 (4.2 %)	0
FIPA patients	5	1 (20 %)	0

Mut mutations known to be pathogenic, *SSA* somatostatin analogues, *FIPA* familial isolated pituitary adenoma

Table 3 Clinical characteristics of patients with *AIP* variants

Case *n*°	*AIP* variant	Gender (M/F)	Age[§] (years)	Adenoma size	SSA response	FIPA
1	p.R304*	M	32	Micro	R	Yes
2	p.R304Q	F	53	Micro	S	No
3	p.R16H	F	26	Micro	na	Yes
4	p.R304Q	F	62	Macro	R	No
5	p.R16H	M	50	Macro	S	No
6	p.R304Q	F	67	Macro	S	No
7	IVS3+1G>A	F	28	Macro	S	No

FIPA familial isolated pituitary adenoma, *SSA* somatostatin analogues treatment (*S* sensible/*R* resistant), *na* not applicable

[§] At diagnosis

32-year old with a microadenoma resistant to SSA treatment. The last one harboured the IVS3+1G>A mutation and was a female with a macroadenoma diagnosed at 28 years of age (Table 3). Overall, among the patients with *AIP* gene mutations, 40 % were resistant to SSA treatment and 60 % were diagnosed with a macroadenoma (Table 3). Two patients were found with the p.R16H variant (c.47G > A) which is still doubted to be pathogenic: one was a female FIPA patient, who had been included in a previous study [17], with a microadenoma diagnosed at 26 years of age; the other one was a 50-year-old male with a macroadenoma (Table 3). We have not found *AIP* gene mutations in any of the 3 giants. When considering only the patients with ≤30 years of age at diagnosis (30 cases), one

(3.3 %) was found with an *AIP* gene mutation (IVS3+1G>A), while a second one had the p.R16H variant (Table 2). If only the cases diagnosed at ≤40 years of age (71 cases) were considered, the prevalence of *AIP* gene mutations was 2.8 % (one case with the p.R304* and the other one with the IVS3+1G>A) (Table 2). When considering only the patients with *apparently* sporadic acromegaly, the prevalence of *AIP* mutations was 1.9 %. Among the FIPA cases, 1 patient (20 %) was found with an *AIP* gene mutation (p.R304*). Among the patients with macroadenoma, an *AIP* gene mutation was found in 3 cases (2.0 %), whereas the prevalence of *AIP* mutations was 4.2 % when considering exclusively the patients resistant to SSA treatment (Table 2). None of the patients was found to harbour the *GPR101* gene p.E308D variant.

Discussion

The first aim of this study was to screen a representative number of Italian acromegalic patients for the *GPR101* p.E308D variant, which has not been found in any of them. Recently, Trivellin et al. found microduplications on chromosome Xq26.3 to cause a new pituitary gigantism syndrome (X-linked acrogigantism, X-LAG) characterized by infant onset. In this study, they also demonstrated that, among the duplicated genes on Xq26.3, only the GPR101 was highly up regulated in pituitary tumours obtained from patients with X-LAG [10]. On the basis of this finding they screened a large international cohort of patients with acromegaly for genetic variants of *GPR101* and found a missense change, c.924G>C (p.E308D; rs73637412) in 4.4 % of patients with acromegaly. Of the 11 mutation carriers, 3 appeared to carry a constitutive mutation, which was detected in DNA from peripheral blood leukocytes (1.9 %), while in the remaining 8 patients they detected the mutation in the tumour DNA. In one patient, they determined that the mutation was a de novo somatic mutation. They also screened 13 families with familial isolated pituitary adenomas but none of them carried the GPR101 p.E308D variant [10]. In the same paper, they showed the results of functional studies proving the pathogenic role of this genetic variant. Indeed, transfection of a construct expressing GPR101 containing the p.E308D mutation increased proliferation and growth hormone secretion in a rat pituitary cell line. Moreover, they showed that GPR101 can strongly activate the cAMP pathway, for which the mitogenic effects in pituitary somatotrophs are well established [10]. Furthermore, Kameinicky et al. screened 263 patients with gigantism or acromegaly for germline mutations in *GPR101* and *AIP* genes. Only 3 patients (1.1 %), including 2 patients who were previously reported [10], had the *GPR101* p.E308D variant. These 3 patients had adult-onset sporadic acromegaly. In addition, they found in a patient with sporadic acromegaly (0.4 %) a novel *GPR101* variant (p.D366E). In their study, germline *AIP* mutations were identified in 8 of 263 patients with somatotropinomas (3.0 %), 6 of whom (75 %) had gigantism. None of the 263 patients carried both *GPR101* and *AIP* germline mutations [11]. On the other hand, Roohi suggested caution in interpreting the c.924G>C change as disease-associated considering that in the ExAC database the allele frequency of this variant is 0.55 % in the European population and 0.36 % regardless of ethnicity and that acromegaly prevalence is around 6 per 100,000 population [18]. However, Daly et al. pointed out that the *GPR101* p.E308D could be a low penetrance variant [19].

In our study, *AIP* gene mutations (known to be pathogenic) were found in 2.3 % of our unselected acromegalic patients, in 1.9 % of subjects with *apparently* sporadic acromegaly, in 1 out of 5 familial cases, and in none of the 3 giants. The FIPA patient carried the *AIP* p.R304*, as the mother who harboured a PRL-secreting microadenoma [16]. Among the *AIP* mutation positive patients, 3 were found with the p.R304Q, but, interestingly, none of them were <40-year old at diagnosis, all of them were females, 2 had a macroadenoma and only one was resistant to SSA treatment. In this regard, it's worth to be mentioned that in the ExAC database the frequency of the p.R304Q variant is 0.14 % including 2 homozygous subjects. Moreover, there are conflicting evidences from in silico analyses on the pathogenicity of the p.R304Q mutation, since according to PolyPhen-2 prediction tool this variant would not have a deleterious effect on AIP, whereas according to SIFT prediction tool the mutation would be damaging. The prevalence of *AIP* mutations was 3.3 and 2.8 % if considering only the patients diagnosed when they were ≤30 or ≤40-year old, respectively. Furthermore, 2.0 % of the patients with macroadenoma and 4.2 % of patients resistant to SSA treatment were found to harbour an *AIP* gene mutation. All the *AIP* gene variants found in our cohort of patients were previously described [20, 21].

Previous studies showed that in non-familial acromegaly, germline *AIP* mutations can be found especially but not exclusively in young patients with large aggressive somatotropinomas resistant to SSA treatment. Indeed, the reported prevalence of *AIP* gene mutations in sporadic acromegaly ranges from 0 to 4.1 % in unselected population of patients [22–27]. Schofl et al. found 5.5 % of acromegaly patients diagnosed at ≤30 years of age with *AIP* gene variants, but more FIPA patients were included in their cohort as compared to our study, and the prevalence of *AIP* mutation positive patients among their sporadic cases was actually 2.3 % [28]. Tichomirowa et al. found *AIP* mutations in 13.3 % of 83 young patients (<30 years of age at diagnosis) with a GH-secreting macroadenoma

[7]. In our unselected population of patients, only 30 cases were diagnosed when they were ≤30 years old and 56.6 % of them harboured a macroadenoma, thus partially explaining the lower prevalence of *AIP* mutations (5.9 %), we found in this subgroup of our patients. Furthermore, *AIP* gene variants have been found in up to 8 % of sporadic acromegaly patients diagnosed before 40 years of age, but only 4.2 % carried a mutation known to be pathogenic [29]. When considering only sporadic acromegalics resistant to conventional treatments, a previous study found an *AIP* gene variants prevalence of 8 %, but only 4 % carried a mutation, similarly to what we found [6]. Among sporadic somatotropinomas, the highest prevalence of *AIP* mutations has been found in paediatric cases (in up to 42.8 %) [24], but in our study only 3 giants were enrolled (2 out of them were females) and tested negative for *AIP* variants.

The limitations of the present study are that (i) we did not search for the very rare big deletions of the *AIP* gene [30]; (ii) we did not screen the whole *GPR101* gene, although the p.E308D variant is that one predominantly found in the two previous studies as well as the one thought to be pathogenic on the basis of functional studies; and (iii) we did not search for *GPR101* p.E308D variant in somatic DNA from patients pituitary tumours, so the results of the present study should be compared to the findings of Kamenicky et al. and the germline prevalence data reported by Trivellin et al. [10, 11].

In conclusion, in our cohort of patients with acromegaly, the prevalence of *AIP* gene mutations among the sporadic and familial cases was similar to that one reported in previous studies but was slightly lower when considering only the cases diagnosed before 40 years of age. Differently from previous reports, we did not find any germline *GPR101* p.E308D mutation in sporadic as well as in the few cases of familial acromegaly. Therefore, limited to our findings, the germline GPR101 p.E308D variant is unlikely to have a role in somatotroph tumorigenesis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. S. Melmed, Acromegaly pathogenesis and treatment. J. Clin. Invest. **119**(11), 3189–3202 (2009). doi:10.1172/JCI39375
2. C. Capatina, J. Wass, 60 Years of neuroendocrinology: acromegaly. J. Endocrinol. **226**(2), T141–T160 (2015). doi:10.1530/JOE-15-0109
3. A. Beckers, L.A. Aaltonen, A.F. Daly, A. Karhu, Familial isolated pituitary adenomas (FIPA) and the pituitary adenoma predisposition due to mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene. Endocr. Rev. **34**(2), 239–277 (2013). doi:10.1210/er.2012-1013
4. J. Lyons, C.A. Landis, G. Harsh, L. Vallar, K. Grünewald, H. Feichtinger, Q.Y. Duh, O.H. Clark, E. Kawasaki, H.R. Bourne, F. Mc Cormick, Two G protein oncogenes in human endocrine tumors. Science **249**(4969), 655–659 (1990)
5. M. Georgitsi, E. De Menis, S. Cannavò, M.J. Mäkinen, K. Tuppurainen, P. Pauletto, L. Curtò, R.J. Weil, R. Paschke, G. Zielinski, A. Wasik, J. Lubinski, P. Vahteristo, A. Karhu, L.A. Aaltonen, Aryl hydrocarbon receptor interacting protein (AIP) gene mutation analysis in children and adolescents with sporadic pituitary adenomas. Clin. Endocrinol. (Oxf) **69**(4), 621–627 (2008)
6. J. Oriola, T. Lucas, I. Halperin, M. Mora, M.J. Perales, C. Alvarez-Escola, M.N. de Paz, G. DiazSoto, I. Salinas, M.T. Julian, I. Olaizola, I. Bernabeu, M. Marazuela, M. Puig-Domingo, Germline mutations of AIP gene in somatotropinomas resistant to somatostatin analogues. Eur. J. Endocrinol. **168**, 9–13 (2012)
7. M.A. Tichomirowa, A. Barlier, A.F. Daly, M.L. Jaffrain-Rea, C. Ronchi, M. Yaneva, J.D. Urban, P. Petrossians, A. Elenkova, A. Tabarin, R. Desailloud, D. Maiter, T. Schurmeyer, R. Cozzi, M. Theodoropoulou, C. Sievers, I. Bernabeu, L.A. Naves, O. Chabre, C.F. Montanana, V. Hana, G. Halaby, B. Delemer, J.I. Aizpun, E. Sonnet, A.F. Longas, M.T. Hagelstein, P. Caron, G.K. Stalla, V. Bours, S. Zacharieva, A. Spada, T. Brue, A. Beckers, High prevalence of AIP gene mutations following focused screening in young patients with sporadic pituitary macroadenomas. Eur. J. Endocrinol. **165**, 509–515 (2011)
8. M.H. Schernthaner-Reiter, G. Trivellin, C.A. Stratakis, Men1 Men4 and carney complex: pathology and molecular genetics. Neuroendocrinology (2015). doi:10.1159/000371819
9. J. Dénes, F. Swords, E. Rattenberry, K. Stals, M. Owens, T. Cranston, P. Xekouki, L. Moran, A. Kumar, C. Wassif, N. Fersht, S.E. Baldeweg, D. Morris, S. Lightman, A. Agha, A. Rees, J. Grieve, M. Powell, C.L. Boguszewski, P. Dutta, R.V. Thakker, U. Srirangalingam, C.J. Thompson, M. Druce, C. Higham, J. Davis, R. Eeles, M. Stevenson, B. O'Sullivan, P. Taniere, K. Skordilis, P. Gabrovska, A. Barlier, S.M. Webb, A. Aulinas, W.M. Drake, J.S. Bevan, C. Preda, N. Dalantaeva, A. Ribeiro-Oliveira Jr, I.T. Garcia, G. Yordanova, V. Iotova, J. Evanson, A.B. Grossman, J. Trouillas, S. Ellard, C.A. Stratakis, E.R. Maher, F. Roncaroli, M. Korbonits, Heterogeneous genetic background of the association of pheochromocytoma/paraganglioma and pituitary adenoma: results from a large patient cohort. J. Clin. Endocrinol. Metab. **100**(3), E531–E541 (2015)
10. G. Trivellin, A.F. Daly, F.R. Faucz, B. Yuan, L. Rostomyan, D.O. Larco, M.H. Schernthaner-Reiter, E. Szarek, L.F. Leal, J.H. Caberg, E. Castermans, C. Villa, A. Dimopoulos, P. Chittiboina, P. Xekouki, N. Shah, D. Metzger, P.A. Lysy, E. Ferrante, N. Strebkova, N. Mazerkina, M.C. Zatelli, M. Lodish, A. Horvath, R.B. de Alexandre, A.D. Manning, I. Levy, M.F. Keil, M.L. Sierra, L. Palmeira, W. Coppieters, M. Georges, L.A. Naves, M. Jamar, V. Bours, T.J. Wu, C.S. Choong, J. Bertherat, P. Chanson, P. Kamenický, W.E. Farrell, A. Barlier, M. Quezado, I. Bjelobaba, S.S. Stojilkovic, J. Wess, S. Costanzi, P. Liu, J.R. Lupski, A. Beckers, C.A. Stratakis, Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. N. Engl. J. Med. **371**(25), 2363–2374 (2014). doi:10.1056/NEJMoa1408028
11. P. Kamenický, J. Bouligand, P. Chanson, Gigantism, acromegaly, and GPR101 mutations. N. Engl. J. Med. **372**(13), 1264 (2015). doi:10.1056/NEJMc1500340#SA1

12. S. Melmed, F.F. Casanueva, A. Klibanski, M.D. Bronstein, P. Chanson, S.W. Lamberts, C.J. Strasburger, J.A. Wass, A. Giustina, A consensus on the diagnosis and treatment of acromegaly complications. Pituitary **16**(3), 294–302 (2013). doi:10.1007/s11102-012-0420-x
13. D. Cuevas-Ramos, M.J. Fleseriu, Somatostatin receptor ligands and resistance to treatment in pituitary adenomas. Mol. Endocrinol. **52**(3), R223–R240 (2014). doi:10.1530/JME-14-0011
14. S. Cannavo, F. Ferrau, M. Ragonese, P.D. Romeo, M.L. Torre, S. Puglisi, E. De Menis, G. Arnaldi, C. Salpietro, O.R. Cotta, A. Albani, R.M. Ruggeri, F. Trimarchi, Increased frequency of the rs2066853 variant of aryl hydrocarbon receptor gene in patients with acromegaly. Clin. Endocrinol. (Oxf). **81**(2), 249–253 (2014). doi:10.1111/cen.12424
15. G. Occhi, G. Trivellin, F. Ceccato, P. De Lazzari, G. Giorgi, S. Dematte, F. Grimaldi, R. Castello, M.V. Davì, G. Arnaldi, L. Salviati, G. Opocher, F. Mantero, C. Scaroni, 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. **163**(3), 369–376 (2010)
16. G. Occhi, M.L. Jaffrain-Rea, G. Trivellin, N. Albiger, F. Ceccato, E. De Menis, M. Angelini, S. Ferasin, A. Beckers, F. Mantero, C. Scaroni, The R304X mutation of the aryl hydrocarbon receptor interacting protein gene in familial isolated pituitary adenomas: mutational hot-spot or founder effect? J. Endocrinol. Invest. **33**(11), 800–805 (2010)
17. M.C. Zatelli, M.L. Torre, R. Rossi, M. Ragonese, F. Trimarchi, E. Degli Uberti, S. Cannavò, Should aip gene screening be recommended in family members of FIPA patients with R16H variant? Pituitary **16**(2), 238–244 (2013). doi:10.1007/s11102-012-0409-5
18. J. Roohi, Gigantism, acromegaly, and GPR101 mutations. N. Engl. J. Med. **372**(13), 1264–1265 (2015)
19. A.F. Daly, G. Trivellin, C.A. Stratakis, Gigantism, acromegaly, and GPR101 mutations. N. Engl. J. Med. **372**(13), 1265 (2015)
20. F. Martucci, G. Trivellin, M. Korbonits, Familial isolated pituitary adenomas: an emerging clinical entity. J. Endocrinol. Invest. **35**(11), 1003–1014 (2012)
21. L.C. Hernández-Ramírez, P. Gabrovska, J. Dénes, K. Stals, G. Trivellin, D. Tilley, F. Ferraù, J. Evanson, S. Ellard, A.B. Grossman, F. Roncaroli, M.R. Gadelha, M. Korbonits, International FIPA consortium: landscape of familial isolated and young-onset pituitary adenomas: prospective diagnosis in AIP mutation carriers. J. Clin. Endocrinol. Metab. **100**(9), 1242–1254 (2015)
22. A. Barlier, J.F. Vanbellinghen, A.F. Daly, M. Silvy, M.L. Jaffrain-Rea, J. Trouillas, G. Tamagno, L. Cazabat, V. Bours, T. Brue, A. Enjalbert, A. Beckers, Mutations in the aryl hydrocarbon receptor interacting protein gene are not highly prevalent among subjects with sporadic pituitary adenomas. J. Clin. Endocrinol. Metab. **92**, 1952–1955 (2007)
23. M. Georgitsi, A. Raitila, A. Karhu, K. Tuppurainen, M.J. Makinen, O. Vierimaa, R. Paschke, W. Saeger, R.B. van der Luijt, T. Sane, M. Robledo, E. De Menis, R.J. Weil, A. Wasik, G. Zielinski, O. Lucewicz, J. Lubinski, V. Launonen, P. Vahteristo, L.A. Aaltonen, Molecular diagnosis of pituitary adenoma predisposition caused by aryl hydrocarbon receptor-interacting protein gene mutations. Proc. Natl. Acad. Sci. USA **104**, 4101–4105 (2007)
24. L. Cazabat, J. Bouligand, S. Salenave, M. Bernier, S. Gaillard, F. Parker, J. Young, A. Guiochon-Mantel, P. Chanson, Germline AIP mutations in apparently sporadic pituitary adenomas: prevalence in a prospective single-center cohort of 443 patients. J. Clin. Endocrinol. Metab. **97**, E663–E670 (2012)
25. L. Cazabat, R. Libe, K. Perlemoine, F. Rene-Corail, N. Burnichon, A.P. Gimenez-Roqueplo, L. Dupasquier-Fediaevsky, X. Bertagna, E. Clauser, P. Chanson, J. Bertherat, M.L. Raffin-Sanson, 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. **157**, 1–8 (2007)
26. T. Iwata, S. Yamada, N. Mizusawa, H.M. Golam, T. Sano, K. Yoshimoto, The aryl hydrocarbon receptor-interacting protein gene is rarely mutated in sporadic GH-secreting adenomas. Clin. Endocrinol. **66**, 499–502 (2007)
27. G. Occhi, G. Trivellin, F. Ceccato, P. De Lazzari, G. Giorgi, S. Dematte, F. Grimaldi, R. Castello, M.V. Davi, G. Arnaldi, L. Salviati, G. Opocher, F. Mantero, C. Scaroni, 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. **163**, 369–376 (2010)
28. C. Schofl, J. Honegger, M. Droste, M. Grussendorf, R. Finke, U. Plockinger, C. Berg, H.S. Willenberg, A. Lammert, D. Klingmuller, C. Jaursch-Hancke, A. Tonjes, S. Schneidewind, J. Flitsch, C. Bullmann, C. Dimopoulou, G. Stalla, B. Mayr, W. Hoeppner, J. Schopohl, Frequency of AIP gene mutations in young patients with acromegaly: a registry-based study. J. Clin. Endocrinol. Metab. **99**, E2789–E2793 (2014)
29. V. Preda, M. Korbonits, S. Cudlip, N. Karavitaki, A.B. Grossman, Low rate of germline AIP mutations in patients with apparently sporadic pituitary adenomas before the age of 40: a single-centre adult cohort. Eur. J. Endocrinol. **171**, 659–666 (2014)
30. M. Georgitsi, E. Heliövaara, R. Paschke, A.V. Kumar, M. Tischkowitz, O. Vierimaa, P. Salmela, T. Sane, E. De Menis, S. Cannavò, S. Gündogdu, A. Lucassen, L. Izatt, S. Aylwin, G. Bano, S. Hodgson, C.A. Koch, A. Karhu, L.A. Aaltonen, Large genomic deletions in AIP in pituitary adenoma predisposition. J. Clin. Endocrinol. Metab. **93**(10), 4146–4151 (2008)