

SNPs in the aryl hydrocarbon receptor-interacting protein gene associated with sporadic non-functioning pituitary adenoma

YESHUI HU, JUN YANG, YONGKAI CHANG, SHUNCHANG MA and JIANFA QI

Department of Neurosurgery, Fuxing Hospital Affiliated to Capital Medical University, Beijing 100038, P.R. China

Received November 15, 2014; Accepted December 8, 2015

DOI: 10.3892/etm.2016.3002

Abstract. Mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene have previously been associated with a predisposition to pituitary adenomas. However, to the best of our knowledge, mutations in *AIP* that relate specifically to sporadic non-functioning pituitary adenomas (NFPAs) have yet to be reported. Therefore, the present study aimed to identify single nucleotide polymorphisms (SNPs) in the *AIP* gene that may be associated with NFPAs. Peripheral blood samples and the entire coding sequence of the *AIP* gene from 56 patients with NFPAs and 56 controls were analyzed in triplicate. Of the 56 patients with NFPAs, 9 patients (16.1%) were identified as harboring five different SNPs, although no germline mutations in the *AIP* gene were detected in any of the patients. Three different SNPs (7051C>T, 8012G>C and 8020G>C) were identified in exons 4 and 6 in 3 different patients (each in 1 patient). Two different SNPs (7318C>A and 7886A>G) were identified in exons 5 and 6, respectively, in 6 different patients (each in 3 patients). No SNPs or germline mutations in the *AIP* gene were identified in the controls. The results of the present study suggested that mutations in the *AIP* gene might not have an important role in the tumorigenesis of NFPAs. However, further studies are required in order to investigate potential molecular and genetic mechanisms that may underlie the involvement of *AIP* in NFPA.

Introduction

Pituitary adenomas occur with an estimated prevalence of ~16.7% in the general population (1), and they account for ~15% of all intracranial tumors (2,3). Sporadic non-functioning pituitary adenomas (NFPAs) are a common subtype of pituitary adenoma (4,5), which account for 25% of all

pituitary adenomas (6). They are not associated with clinical hypersecretory syndromes, rather with symptoms induced by an intracranial mass, including headaches, hypopituitarism or visual-field disturbances (6). Furthermore, they never show a genetic predisposition, as compared with familial NFPAs. Pituitary adenomas are rarely malignant; however, they may exhibit various invasive behaviors that have been associated with the particular pathological subtype (7) and with varying degrees of morbidity (1). Previous studies have investigated the molecular and genetic mechanisms underlying these tumors (2-6); however, the exact pathogenesis underlying the tumorigenesis of pituitary adenomas, in particular NFPAs, is currently unclear.

In a previous study, based on the detection of three clusters of familial pituitary adenomas in Northern Finland, germline mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene were associated with the oncogenesis of pituitary adenomas (8). This finding suggested that the *AIP* gene may act as a tumor suppressor in pituitary adenomas (8). Conversely, Yu *et al* (9) reported that mutations in the *AIP* gene were not prevalent among US patients with sporadic pituitary adenomas. Investigations of *AIP* mutations in patients with sporadic or familial pituitary tumors have been conducted in other populations worldwide, including: The Netherlands (10), Belgium (10,11), Italy (10,11,12), France (10,11), USA, Spain, Argentina, The Netherlands, Czech Republic (11), Germany, Turkey, Canada (12), Finland (13), UK (12,14,15), Brazil (11,16) and Ireland (17). Although the results of these studies suggested that the degree of germline mutations rate in the *AIP* gene varied, they also demonstrated that *AIP* has a significant role in the tumorigenesis of pituitary adenomas. A previous study has demonstrated that *AIP* forms a complex with the aryl hydrocarbon receptor (AHR) and two 90-kDa heat-shock proteins. Furthermore, mutations in *AIP* induced the downregulation of *AIP* and AHR, which lead to tumor predisposition (8). It has also been demonstrated that *AIP* is capable of interact with at least two phosphodiesterase (PDE) isoforms, PDE2A and PDE4A5, peroxisome proliferation-activated receptor- α , survivin, translocase of the outer membrane of mitochondria 20, and thyroid hormone receptor β 1. Therefore, mutations in *AIP* may have various important roles in the tumorigenesis of pituitary adenomas (13). However, to the best of our knowledge, mutations in *AIP* relating specifically to NFPAs have yet to be investigated. Therefore, the present study screened the genomes of 56 patients with sporadic NFPAs and 56 controls,

Correspondence to: Professor Jun Yang, Department of Neurosurgery, Fuxing Hospital Affiliated to Capital Medical University, 7th Floor of South Tower, 20 Fu Xing Men Wai Street, Beijing 100038, P.R. China
E-mail: junhou2000@163.com

Key words: aryl hydrocarbon receptor-interacting protein, non-functioning pituitary adenoma, single nucleotide polymorphism

in order to identify single nucleotide polymorphisms (SNPs) in the *AIP* gene that may be associated with the tumorigenesis of NFPAs.

Materials and methods

Patients. A total of 56 consecutive patients with sporadic NFPAs from the Department of Neurosurgery at the Fuxing Hospital (Beijing, China) were prospectively enrolled in the present study between September 2012 and May 2014. A total of 56 controls were also recruited. In the control group, age, gender, symptoms, physical examinations and medical history were recorded. All controls were healthy without any disease and lacked a familial history of related diseases. Enhanced head MRI was conducted and peripheral blood samples were collected from all controls in order to ensure they met the criterion of the study. The inclusion criteria for the patients were as follows: i) No familial history of pituitary adenomas; ii) the patient had not accepted treatment; and iii) laboratory tests for the growth hormones prolactin and adrenocorticotrophic hormone were normal for both patients and controls. Written informed consent was obtained from all subjects. The study was approved by the Ethics Committee of the Capital Medical University (Beijing, China; no ECCMU201105250263).

Materials. Peripheral blood samples were collected from the patients and controls prior to surgery or treatments and were immediately frozen at -80°C . Patients underwent either craniotomy or transsphenoidal surgical procedures, during which pituitary adenoma tissue samples were collected. Subsequently, tissue slices were fixed in formalin for light microscopy and immunocytochemistry, following staining with hematoxylin and eosin. The avidin-biotin-peroxidase complex technique was used to identify pituitary tumor cells. Patients with symptoms of an intracranial mass, including headaches, hypopituitarism or visual-field disturbances, were required to undergo an enhanced head MRI. Following the identification of occupying lesions in the saddle area, peripheral blood samples were harvested from corresponding patients in order to assess the pituitary hormone levels and in preparation for the detection of *AIP* gene polymorphisms. Upon classification of the tumors as pituitary adenomas via postoperative immunohistochemical pathology, peripheral blood samples were used in all subsequent experiments.

DNA extraction and polymerase chain reaction (PCR) amplification. Genomic DNA was extracted from the peripheral blood samples using the Animal Blood Genomic DNA Extraction Magnetic Bead kit (Bioeasy Biotechnologies, Co., Ltd., Shenzhen, China), in order to conduct germline mutation analyses. Briefly, the exons and flanking intronic sequences of the *AIP* gene (GenBank accession number Hs. 412433) from patients and controls were amplified by PCR using the Applied Biosystems GeneAmp[®] PCR System 9700 (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The following primers were used: Exon 1 forward, 5'-CGCAGA GAACCAATCACCAT-3' and reverse, 5'-AAACCCAGA TACCCGAGGAC-3'; exon 2 forward, 5'-AGGTGTAAG GTCAGGTGGTG-3' and reverse, 5'-CAGAGCAAGACTCC ATCTCA-3'; exon 3 forward, 5'-CTGTGCTTAAACGGA

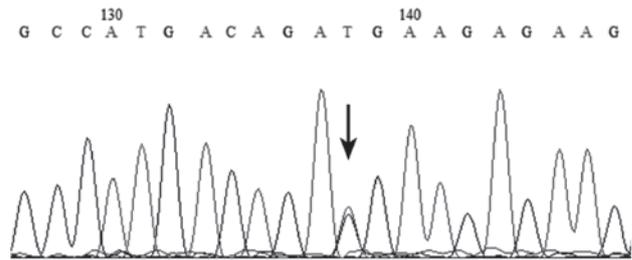


Figure 1. Single nucleotide polymorphism (7051C>T) was identified in exon 4 of the aryl hydrocarbon receptor-interacting protein gene in 1 patient and the base sequence was altered from C to T in some copies of the gene, suggesting that the base sequence was of the C/T heterozygous genotype.

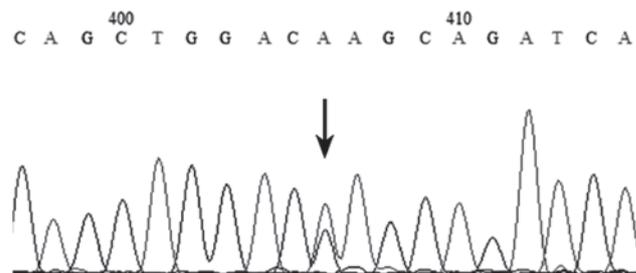


Figure 2. Single nucleotide polymorphism (7318C>A) was identified in exon 5 of the aryl hydrocarbon receptor-interacting protein gene in 3 patients and the base sequence was altered from C to A in some copies of the gene, suggesting that the base sequence was of the C/A heterozygous genotype.

GTAGGGT-3' and reverse, 5'-AACAGTGAACAAGACGGT GAAAA-3'; exon 4-5 forward, 5'-CTCTGCTGCTGGTGT GTGAT-3' and reverse, 5'-CATTTCATGCTTCATTGGC ACA-3'; and exon 6 forward, 5'-ATGGTGCCAGGAGAC ATGAG-3' and reverse, 5'-AACAGCCACCCAAGTACCA-3' (Takara Biotechnology, Co., Ltd., Dalian, China). All primers included the entire coding region of the *AIP* gene, and extended from the exon/intron junction in opposite directions.

The PCR cycling conditions were as follows: Denaturation at 95°C for 5 min, followed by 32 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 45 sec, followed by a final extension step at 72°C for 7 min. Subsequently, DNA sequencing was conducted using the MegaBASE 4500 DNA analyzer (GE Healthcare Life Sciences, Shanghai, China). The sequences were analyzed using Sequence Pilot software, version 3.1 (JSI medical systems GmbH, Ettenheim, Germany). All samples were analyzed in triplicate.

Results

Clinical data. In order to investigate whether specific mutations in the *AIP* gene were associated with NFPAs, the genomes of 56 patients with NFPAs and 56 controls were analyzed. The patient cohort consisted of 30 males (53.6%) and 26 females (46.4%); whereas the control group consisted of 29 males (51.8%) and 27 females (48.2%). The age average of patient group was 38.3 ± 6.5 years old (range, 22-56 years), whereas the controls were 40.6 ± 4.6 years old (range, 19-62 years). Of the 56 patients, 13 (23.2%) were diagnosed with microadenomas and 43 (76.8%) were diagnosed with macroadenomas (in the

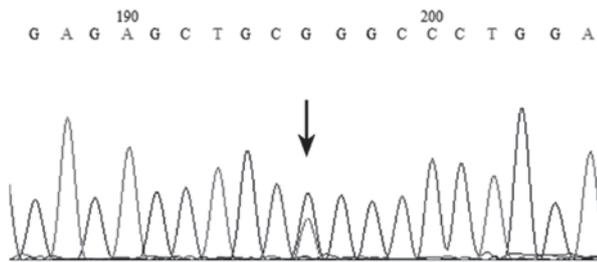


Figure 3. Single nucleotide polymorphism (7886A>G) was identified in exon 6 of the aryl hydrocarbon receptor-interacting protein gene in 3 patients and the base sequence was changed from A to G in some copies of the gene, indicating that the base sequence was of the A/G heterozygous genotype.

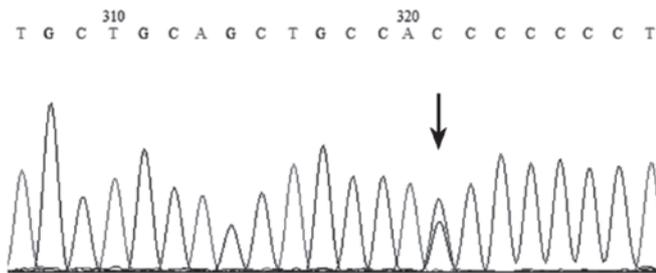


Figure 4. Single nucleotide polymorphism (8012G>C) was identified in exon 6 of the aryl hydrocarbon receptor-interacting protein gene in 2 patients and the base sequence was altered from G to C in some copies of the gene, suggesting that the base sequence was of the G/C heterozygous genotype.

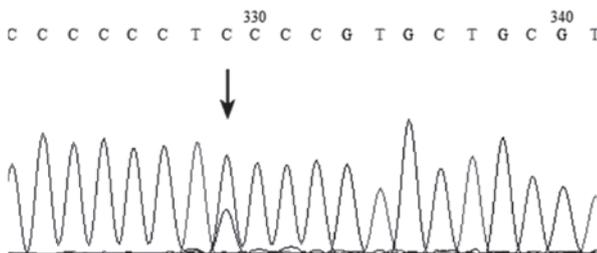


Figure 5. Single nucleotide polymorphism (8020G>C) was identified in exon 6 of the aryl hydrocarbon receptor-interacting protein gene in 2 patients and the base sequence was altered from G to C in some copies of the gene, indicating that the base sequence was of the G/C heterozygous genotype.

present study, a microadenoma was defined as having a tumor diameter <1 cm, and a macroadenoma as having a diameter of ≥1 cm).

SNPs in the AIP gene. Of the 56 patients with NFPAs, 4 were identified as harboring three SNPs, but no germline mutations, in the AIP gene. The 7051C>T SNP was identified in exon 4 of the AIP gene in 1 patient; the base sequence was changed from cytosine (C) to thymine (T) in some copies of the gene, indicating that the base sequence was of the C/T heterozygous genotype (Fig. 1). The 7318C>A SNP was identified in exon 5 of the AIP gene in 3 patients; the base sequence was changed from C to adenine (A) in some copies of the gene, indicating that the base sequence was of the C/A heterozygous genotype (Fig. 2). The 7886A>G SNP was identified in exon 6 of the AIP gene in 3 patients; base sequence was changed from A to guanine (G) in some copies of the gene, indicating that the

base sequence was of the A/G heterozygous genotype (Fig. 3). Furthermore, the SNPs (8012G>C; 8020G>C) were each identified in exon 6 of the AIP gene in 2 different patients; the base sequences were changed from G to C in some copies of the gene, which suggested that the base sequences were of the G/C heterozygous genotype (Figs. 4 and 5). No SNPs or germline mutations were identified in the AIP gene of the 56 controls.

Discussion

Pituitary adenomas, which are relatively common intracranial tumors that are second only to gliomas and meningiomas, represent ~15% of primary intracranial neoplasms by histology (3). Although they are typically benign and seldom transform into malignant tumors, pituitary adenomas exhibit a collection of invasive behaviors that are associated with varying degrees of morbidity (7). These tumors grow aggressively, characterized by gross invasion of the surrounding tissues. Patients may experience vision and visual-field disturbances when the optic nerves are invaded. Patients may also experience diplopia, ocular movement disorder and increased intracranial pressure; whereas nausea and vomiting are common in patients with pituitary tumors (6).

Vierimaa *et al* (8) identified germline mutations in the AIP gene in three clusters of familial pituitary adenomas from Northern Finland. In this isolated population, three AIP mutations were identified, which accounted for 16% of all patients diagnosed with pituitary tumors. The findings suggested that a predisposition to pituitary adenomas may be associated with mutations in the AIP gene, and that the AIP gene may act as a tumor suppressor in pituitary tumors (8).

The AIP gene is located on chromosome 11q13 and encodes a 330-amino-acid protein, which interacts with the aryl hydrocarbon receptor (AHR) and the heat shock protein 90 dimer (15,18). In a previous study, the α -helical carboxyl (C)-terminus of the AIP gene, which is adjacent to the tetratricopeptide repeat domain, was shown to be essential for binding to AHR, since deleting the five amino acids at the C-terminal resulted in a truncated protein that was unable to bind to the AHR; alanine-scanning mutagenesis confirmed the absolute requirement of the α -helical C-terminus domain for binding to AhR (18). AHR is a ligand-activated transcription factor involved in responses to hypoxemia, cellular differentiation and cell cycle regulation (19). The AHR may bind to exogenous ligands, including carcinogenic and teratogenic hydrocarbons and their derivatives, such as dioxin, and can be activated by cyclic adenosine monophosphate (cAMP) via protein kinase A, which may cause the receptor to translocate to the nucleus and stimulate AHR-dependent gene expression (20). Heterozygous inactivating mutations are likely to downregulate expression of the AIP protein. In addition, AIP has been shown to bind to phosphodiesterase 4A5 and attenuate its effect (21). The consequences of AIP inactivation on cAMP signaling require further study.

At present, the mechanisms underlying the induction of oncogenesis by mutations in the AIP gene remain hypothetical. Subsequent to the initial study (8), a series of mutations in the AIP gene were discovered in patients with sporadic or familial pituitary adenomas from various populations (9-17). Yu *et al* (9) reported that AIP mutations were not

prevalent among US patients with sporadic pituitary tumors. Daly *et al* (11) investigated the genetic and clinical features of 73 families with familial isolated pituitary adenomas, and demonstrated that 10 *AIP* mutations were present among these families, of which nine were novel mutations. Furthermore, Jennings *et al* (22) reported that familial isolated pituitary tumors in a large Samoan kindred population from Australia/New Zealand contained an R271W mutation that was associated with aggressive pituitary adenomas. In addition, Naves *et al* (23) reported the A195V mutation, Pinho *et al* (24) the E24X mutation, and Villa *et al* (25) the E216X mutation in the *AIP* gene. Tichomirowa *et al* (26) reported the existence of a germline mutation in the *AIP* gene in 11.8% of patients with pituitary adenomas, and Cazabat *et al* (27) detected *AIP* mutations in 16/443 patients, including 6/148 patients with acromegaly. However, to the best of our knowledge, mutations in the *AIP* gene specifically relating to sporadic NFPAs have not been reported previously.

The present study demonstrated that there were five different SNPs among exons 4, 5 and 6 of the *AIP* gene in 56 patients, although no germline mutations in the *AIP* gene were identified. Three SNPs (7051C>T; 8012G>C; 8020G>C) were identified in exons 4 and 6 in 3 different patients, each within 1 patient. Two SNPs (7318C-A; 7886A-G) were identified in exons 5 and 6 of 6 different patients, each within 3 patients.

In conclusion, the present study analyzed the occurrence of SNPs in the *AIP* gene from the genomes of patients with sporadic NFPAs. The results suggest that mutations or SNPs in the *AIP* gene are unlikely to have an important role in NFPAs. However, the present study had certain limitations, which were the lack of familial and other types of pituitary adenomas among the subjects, and the absence of any analysis of the mechanism underlying any effects of the *AIP* gene in the tumorigenesis of NFPAs. Furthermore, given the 7.1% prevalence of SNPs in the *AIP* gene, the molecular and genetic mechanisms underlying the involvement of the *AIP* gene in the tumorigenesis of NFPAs require further investigation.

Acknowledgements

The authors of the present study would like to thank Dr Qian Ma and Mr. Yang Li (Laboratory of Biochemistry, Capital Medical University) for skillful technical assistance. They also thank the medical and nursing staff in the Department of Neurosurgery of Fuxing Hospital Affiliated to Capital Medical University.

References

- Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML and McCutcheon IE: The prevalence of pituitary adenomas: A systematic review. *Cancer* 101: 613-619, 2004.
- Heaney AP and Melmed S: Molecular targets in pituitary tumours. *Nat Rev Cancer* 4: 285-295, 2004.
- Melmed S: Medical progress: Acromegaly. *N Engl J Med* 355: 2558-2573, 2006.
- Toledo RA, Lourenço DM Jr, Liberman B, Cunha-Neto MB, Cavalcanti MG, Moyses CB, Toledo SP and Dahia PL: Germline mutation in the aryl hydrocarbon receptor interacting protein gene in familial somatotropinoma. *J Clin Endocrinol Metab* 92: 1934-1937, 2007.
- Daly AF, Tichomirowa MA and Beckers A: Genetic, molecular and clinical features of familial isolated pituitary adenomas. *Horm Res* 71: 116-122, 2009.
- Pierantoni GM, Finelli P, Valtorta E, Giardino D, Rodeschini O, Esposito F, Losa M, Fusco A and Larizza L: High-mobility group A2 gene expression is frequently induced in non-functioning pituitary adenomas (NFPAs), even in the absence of chromosome 12 polysomy. *Endocr Relat Cancer* 12: 867-874, 2005.
- Dworakowska D and Grossman AB: The pathophysiology of pituitary adenomas. *Best Pract Res Clin Endocrinol Metab* 23: 525-541, 2009.
- Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, Tuppurainen K, Ebeling TM, Salmela PI, Paschke R, *et al*: Pituitary adenoma predisposition caused by germline mutations in the *AIP* gene. *Science* 312: 1228-1230, 2006.
- Yu R, Bonert V, Saporta I, Raffel LJ and Melmed S: Aryl hydrocarbon receptor interacting protein variants in sporadic pituitary adenomas. *J Clin Endocrinol Metab* 91: 5126-5129, 2006.
- Daly AF, Jaffrain-Rea ML, Ciccarelli A, Valdes-Socin H, Rohmer V, Tamburrano G, Borson-Chazot C, Estour B, Ciccarelli E, Brue T, *et al*: Clinical characterization of familial isolated pituitary adenomas. *J Clin Endocrinol Metab* 91: 3316-3323, 2006.
- Daly AF, Vanbellinghen JF, Khoo SK, Jaffrain-Rea ML, Naves LA, Guitelman MA, Murat A, Emy P, Gimenez-Roqueplo AP, Tamburrano G, *et al*: Aryl hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: Analysis in 73 families. *J Clin Endocrinol Metab* 92: 1891-1896, 2007.
- DiGiovanni R, Serra S, Ezzat S and Asa SL: *AIP* mutations are not identified in patients with sporadic pituitary adenomas. *Endocr Pathol* 18: 76-78, 2007.
- Georgitsi M, Heliövaara E, Paschke R, Kumar AV, Tischkowitz M, Vierimaa O, Salmela P, Sane T, De Menis E, Cannavò S, *et al*: Large genomic deletions in *AIP* in pituitary adenoma predisposition. *J Clin Endocrinol Metab* 93: 4146-4151, 2008.
- Igreja S, Chahal HS, Akker SA, Gueorguiev M, Popovic V, Damjanovic S, Burman P, Wass JA, Quinton R, Grossman AB and Korbonits M: Assessment of p27 (cyclin-dependent kinase inhibitor 1B) and aryl hydrocarbon receptor-interacting protein (*AIP*) genes in multiple endocrine neoplasia (MEN1) syndrome patients without any detectable MEN1 gene mutations. *Clin Endocrinol (Oxf)* 70: 259-264, 2009.
- Ozfirat Z and Korbonits M: *AIP* gene and familial isolated pituitary adenomas. *Mol Cell Endocrinol* 326: 71-79, 2010.
- Toledo RA, Mendonça BB, Fragoso MC, Soares IC, Almeida MQ, Moraes MB, Lourenço DM Jr, Alves VA, Bronstein MD and Toledo SP: Isolated familial somatotropinoma: 11q13-LOH and gene/protein expression analysis suggests a possible involvement of *AIP* also in non-pituitary tumorigenesis. *Clinics (Sao Paulo)* 65: 407-415, 2010.
- Chahal HS, Stals K, Unterländer M, Balding DJ, Thomas MG, Kumar AV, Besser GM, Atkinson AB, Morrison PJ, Howlett TA, *et al*: *AIP* mutation in pituitary adenomas in the 18th century and today. *N Engl J Med* 364: 43-50, 2011.
- Bell DR and Poland A: Binding of aryl hydrocarbon receptor (AhR) to AhR-interacting protein. The role of hsp90. *J Biol Chem* 275: 36407-36414, 2000.
- Gu YZ, Hogenesch JB and Bradfield CA: The PAS superfamily: Sensors of environmental and developmental signals. *Annu Rev Pharmacol Toxicol* 40: 519-561, 2000.
- Oesch-Bartlomowicz B, Huelster A, Wiss O, Antoniou-Lipfert P, Dietrich C, Arand M, Weiss C, Bockamp E and Oesch F: Aryl hydrocarbon receptor activation by cAMP vs. dioxin: Divergent signaling pathways. *Proc Natl Acad Sci USA* 102: 9218-9223, 2005.
- Bolger GB, Peden AH, Steele MR, MacKenzie C, McEwan DG, Wallace DA, Huston E, Baillie GS and Houslay MD: Attenuation of the activity of the cAMP-specific phosphodiesterase PDE4A5 by interaction with the immunophilin XAP2. *J Biol Chem* 278: 33351-33363, 2003.
- Jennings JE, Georgitsi M, Holdaway I, Daly AF, Tichomirowa M, Beckers A, Aaltonen LA, Karhu A and Cameron FJ: Aggressive pituitary adenomas occurring in young patients in a large Polynesian kindred with a germline R271W mutation in the *AIP* gene. *Eur J Endocrinol* 161: 799-804, 2009.
- Naves LA, Jaffrain-Rea ML, Vêncio SA, Jacomini CZ, Casulari LA, Daly AF and Beckers A: Aggressive prolactinoma in a child related to germline mutation in the ARYL hydrocarbon receptor interacting protein (*AIP*) gene. *Arq Bras Endocrinol Metabol* 54: 761-767, 2010.

24. Pinho LK, Vieira Neto L, Wildemberg LE, Moraes AB, Takiya CM, Frohman LA, Korbonits M and Gadelha MR: Familial isolated pituitary adenomas experience at a single center: Clinical importance of AIP mutation screening. *Arq Bras Endocrinol Metabol* 54: 698-704, 2010.
25. Villa C, Lagonigro MS, Magri F, Koziak M, Jaffrain-Rea ML, Brauner R, Bouligand J, Junier MP, Di Rocco F, Sainte-Rose C, *et al*: Hyperplasia-adenoma sequence in pituitary tumorigenesis related to aryl hydrocarbon receptor interacting protein gene mutation. *Endocr Relat Cancer* 18: 347-356, 2011.
26. Tichomirowa MA, Barlier A, Daly AF, Jaffrain-Rea ML, Ronchi C, Yaneva M, Urban JD, Petrossians P, Elenkova A, Tabarin A, *et al*: High prevalence of AIP gene mutations following focused screening in young patients with sporadic pituitary macroadenomas. *Eur J Endocrinol* 165: 509-515, 2011.
27. Cazabat L, Bouligand J, Salenave S, Bernier M, Gaillard S, Parker F, Young J, Guiochon-Mantel A and Chanson P: 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.