Al-Azzawie H.F et al /J. Pharm. Sci. & Res. Vol. 10(7), 2018, 1663-1667

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Genetic study of AHR exonic part and GNAS intronic part mutations in some of Iraqi acromegalic patients.

Al-Azzawie H.F[1], Nadhim M. H[2]., Al-kharasani A.M. R.[3], Albermani M. S. K.[2]

[1]College of Science, Baghdad University, Iraq
[2]College of biotechnology, Al-Qasim Green University, Babylon Province, Iraq.
[3]Iraqi National Diabetic Center, Al-Mustansiryia University, Iraq

Summary

Acromegaly is a rare syndrome categorized by extreme excretion of growth hormone via a pituitary adenoma, happening everywhere in the world also in Iraqi population. Practically all conditions of acromegaly are produced by a benign tumor in the pituitary that creates much growth hormone. In additions, some tumors in the body may yield an element known as growth hormone releasing hormone which lead the pituitary to oversecrion of growth hormone. So this research proposed to observe the frequency of guanine nucleotide binding protein, alpha stimulating (GNAS), and aryl hydrocarbon receptor (AHR) genes mutations.

Consequently, this cross sectional biochemical and molecular genetic study was achieved from December 2016 to September 2017, enrolled seventy patients (36 males, and 34 females) with somatotrophic pituitary adenoma continuously attended to the National diabetic center in Baghdad. Their ages were between 20 to 70 years old.

Polymerase chain reaction (PCR) was used for the detection of gsp and AHR mutations as a cause of acromegaly, by using specific primers for amplification of partially intronic and exonic 7 of gsp gene on chromosome no.20 and exon 10 of AHR gene on chromosome no.7. Sequencing was applied for PCR products of AHR and gsp genes, six different alterations in AHR gene and two different mutations in GNAS gene have been recognized. Two were recognized in 6/70 (9%) patients which are codon 705 with substitution thymine by adenine (T/A), and codon 410 with substitution of adenine by thymine (A/T) for 817bp fragment. In addition another four recognized alteration were established in (22/70) (31%) which are codon 193 with substitution of cytocine by adenine(C/A), codon 344 with substitution of thymine by adenine(T/A), codon 459 with substitution of guanine by cytocine(G/C), and codon 518 with substitution of adenine by cytocine(A/C) and this for 706bp fragment of AHR exon 10.Two mutations in GNAS gene were recognized in 5/70 (7%) which their codon 91 with substitution cytocine by guanine(C/G), and codon 128 with substitution guanine by adenine(G/A).

We concluded the novelties SNP were identified in Iraqi acromegalic patient. Genetic variations possibly encouraging functional irregularities of the aryl hydrocarbon receptor (AHR) pathway and guanine nucleotide binding protein, alpha stimulating (GNAS) are connected with a more severe acromegaly increased pituitary tumor mass, and somatostatin analog resistance in patients living in HR areas.

INTRODUCTION

Acromegaly is a rare neuroendocrine disorder caused by high-secretion levels of growth hormone (GH) that lead to the overproduction of insulin-like growth factor 1 (IGF-1). More than 90% of cases, the origin of GH over production are a non-cancerous pituitary somatotroph adenoma (1). ACM is initiated through pituitary somatotroph tumors or rarely by additional pituitary syndromes. The expansion of ACM is assessed to range from 38 to 80 patient for each million, and the yearly frequency of new cases is 3 to 4 per million (2). At the beginning ACM was discovered and diagnosed by Pierre Marie in 1886, the first scientific explanation of somatic growth tumor and its percentage, and suggested the term acromegaly (3). The exact time of disease creation is un- detectable and signs cannot shows and improve for of long period, cause a late identification of at least five years (4).

Tumor formation may originate by effect of chemical, physical, and environmental condition or arise because of a genetic problem. In any case the tumor start from a single cell which can grow and divide to form a tumor. As mentioned above the somatotroph tumor is a benign tumor and it is not genetically inherited. Genetic mutation, polymorphisms or alteration in a single gene or many genes which control the cell division and proliferation of pituitary cells leading to formation of tumor. As a result this defect in genes can effect on formation, secretion, and transmission of some chemical proteins as GH and IGF-1, the main reasons of acromegaly (5). The mutations or polymorphisms of the genes like AIP, AHR, Gsp, MENI, MENII, GPR101 have been recognized in somatotroph pituitary tumor (6-11) and clinically non-functioning pituitary adenomas (12, 13). More than 40% of diagnosed cases who is suffering of acromegaly they are having mutations in their genetics (9, 14). The frequency of somatotroph tumor mutations differs through the communities and locations (14). Usually the tumor can exist in any period of human life including childhood but mostly happens in adulthood or elders (15).

As a result to the increase level of acromegalic patient in IRAQ encourage us to study the genetic alteration of some genes like (Gsp, AHR). This review was proposed to analyze the genetic mutation and polymorphisms in the Gsp, and AHR genes in some of Iraqi acromegalic patient.

MATERIALS AND METHODS

Genetic material was obtained from 70 patients (36 male and 34 female) and from 30 normal people (15 male and 15 female). All the patients within more than one year of GH-somatotrope adenoma and all of them are getting long act octreotide injection treatment monthly. Acromegaly in all cases was identified measuring the level of GH and IGF-1 and establishing the size of tumor by using the MRI.

DNA was extracted from white blood cells using Genaid Kit as stated by manufacturer's information. The purity of DNA and concentration were estimated by a NanoDrop, whereas the DNA integrity was tested via 0.8% agarose gel electrophoresis. The extracted DNA was used as a template for PCR.

PCR amplification

Three total PCR fragments were selected for amplification. The first two of them were covered single exonic DNA positions of the AHR gene while the third PCR fragment covered intronic position of GNAS gene. Primers of AHR gene 5′-GTATGAGGCAACCAACCCT-3′ (forward primer), 5′-GAACTCTTGACTGATCCCAT-3′ (revers primer) for 817bp fragment and 5′-CAGTCCTTGGCTCTGAACT-3′ (forward primer), 5′-GAATGCTGTAGATAACCGAT-3′ for 706bp fragment of exon 10 of AHR (10). Also for GNAS gene 5′-GTGATCAAGCAGGCTGACTATGTG-3′ (forward primer) and 5′-CAGGCGGTTGGTCTGGTT-3′ (revers primer) of 583bp intronic part of gsp gene (7). The PCR reaction was performed using AccuPower PCR premix. The reaction mixture was completed with 10 pmol of each primer and 50 ng of genomic DNA. The amplification was began by initial denaturation at 94°C

for 5 min, followed by 30 cycles of denaturation at 94˚C, annealing at 62˚C, and elongation at 72˚C, and was finalized with a final extension at 72°C for 10 min. Amplification was verified by electrophoresis o, using a 100-bp ladder (Cat # D-1010, Bioneer) as a molecular weight marker. It was made sure that all PCR resolved bands are specific and consisted of only one clean and sharp band in order to be submitted into sequencing successfully.

DNA sequence for one hundred samples corresponding to seventy patients' (No. 1 – 70) and thirty control (No. 71 – 100) amplicons were commercially sequenced from both ends according to instruction manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea).

The sequencing results of the PCR products of the all sequenced 100 samples were edited, aligned, and analyzed as long as with their respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). Each observed variation was translated into amino acids in a reading frame corresponds to the reference amino acid sequences using the Expasy online program (http://web.expasy.org/translate/). Multiple amino acid sequence alignment was made between the reference amino acid sequences and its observed variations using the Clustal Omega program from the UniProt website (http://www.uniprot.org/align/).

RESULT

The polymorphisms of two improved loci of AHR gene and single amplified locus in GNAS gene in the patients with GH-hypersecretion adenoma was studied in this research. These three positions involved both exonic and intronic fragments in AHR gene and GNAS gene respectively. Over PCR-sequencing amplifications many SNPs were identified in all observed fragments. This work was actually done to recognize the relationship between the detected SNPs in the cases with normal members to find – if any – the possible occurrence of the disorder causal SNP(s). This direct detection procedure was relied on to try to correlate the potential relationship between the disease and the observed mutations. This study has discovered several point mutations in the three investigated genetic loci. These variations were varied in their nature from intronic SNPs, as found in GNAS gene and exonic SNPs, as found in AHR gene. Within the exonic portions of AHR gene, two types of SNPs were observed as well. The first one is synonymous SNP or silent mutation, in which no change in the amino acid was taken place. While the other SNP was non-synonymous SNP (nsSNP) or missense mutation, in which the native amino acid is substituted into another one. Totally 14 SNPs are detected in AHR exon 10 and GNAS (6 in 817bp, 6 in 706 bp and 2 in a 583bp)

AHR amplicons results

As mentioned above, two amplicons were studied in this gene that present in chromosome number 7. This gene has 11 exons, and the protein encoded by this gene is called aryl hydrocarbon receptor, which is a ligand-activated helix-loop-helix transcription factor involved in the regulation of biological responses to planar aromatic hydrocarbons. This receptor has been shown to regulate xenobiotic-metabolizing enzymes such as cytochrome P450. Before ligand binding, the encoded protein is sequestered in the cytoplasm; upon ligand binding, this protein moves to the nucleus and stimulates transcription of target genes. The exact positions of the studied PCR amplicons were graphically highlighted in the NCBI website. From this figure, all exonic positions were identified. Both of the two selected amplicons were covered variable regions of the tenth exon of AHR gene.

In our study the first fragment A705T is the most frequent mutation (7.1%), and T410A (1.4%) in the AHR 817bp piece. Also for the second fragment C193A is the most frequent mutation (30%), A518C (11.4%), T344A (1.4%), and G459C (1.4%) in the AHR 706bp piece.

The 817 bp AHR amplicons

The first studied PCR amplicon is 817 bp, which partially portion of exon 10. This fragment codes only 262 amino acids only from the exon 10. The alignment results of all the patient samples of exon 10 revealed the presence several SNPs among both patient and control samples. The sequencing chromatogram of each observed SNP is documented in this amplicon (Fig. 1).

The novelty of each observed SNP was detected using the NCBI SNP detector (Table 1).

The 706 bp AHR amplicons

The second studied PCR amplicon is 706 bp, which partially covers exon 10. This fragment codes only 190 amino acids only from the exon 10. The alignment results of all the patient samples of exon 10 revealed the presence several SNPs also among both patient and control samples. The sequencing chromatogram of each observed SNP is documented in this amplicon (Figure 2).

The novelty of each observed SNP was detected using the NCBI SNP detector (Table 2).

Table 1. The observed SNPs in the reference of the 817 bp exon10/AHR gene DNA sequences.

No.	Native	Allele	Position in the PCR fragment	Position in the reference genome	Amino acid change	Novelty of SNP
1	A	G	230	17339293	M490V	Known missense SNP (rs61755968)*
2	A	T	404	17339467	I548F	Novel
3	T	A	410	17339473	F550I	Novel
4	G	A	423	17339486	R554K	Known missense SNP (rs2066853)*
5	G	A	470	17339533	V570I	Known missense SNP (rs4986826)*
6	A	T	705	17339768	N648I	Novel

Table 2. The observed SNPs in the reference of the 706 bp exon10/AHR gene DNA sequences.

No.	Native	Allele	Position in the PCR fragment	Position in the reference genome	Amino acid change	Novelty of SNP
1	C	A	193	17339824	Q667K	Novel
2	T	A	344	17339975	L717Q	Known missense SNP (rs868478703)*
3	G	C	459	17340090	Q755H	Novel
4	A	C	483	17340114	-	Known synonymous SNP (rs776489857)*
5	A	C	518	17340149	Q775P	Novel
6	C	A	536	17340167	T781N	Known missense SNP (rs781710007)*

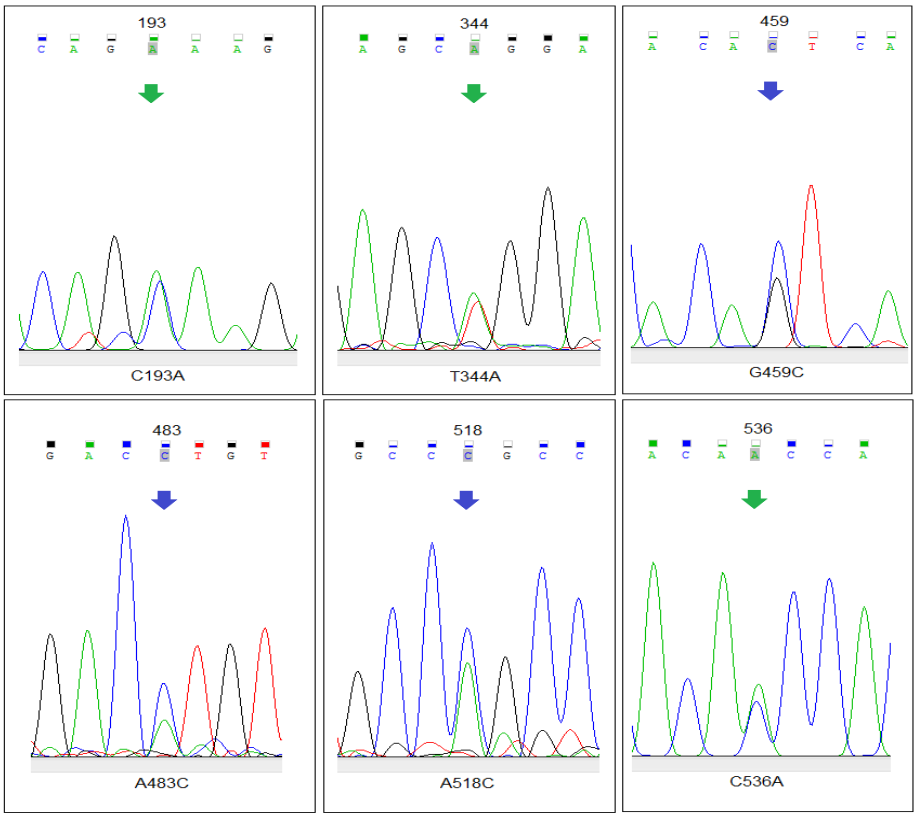

Fig. 2. The pattern of DNA chromatogram of all one hundred DNA specimens of the 706 bp amplicon of the exon-10/AHR gene. Each substitution SNP was highlighted according to their positions in the PCR products.

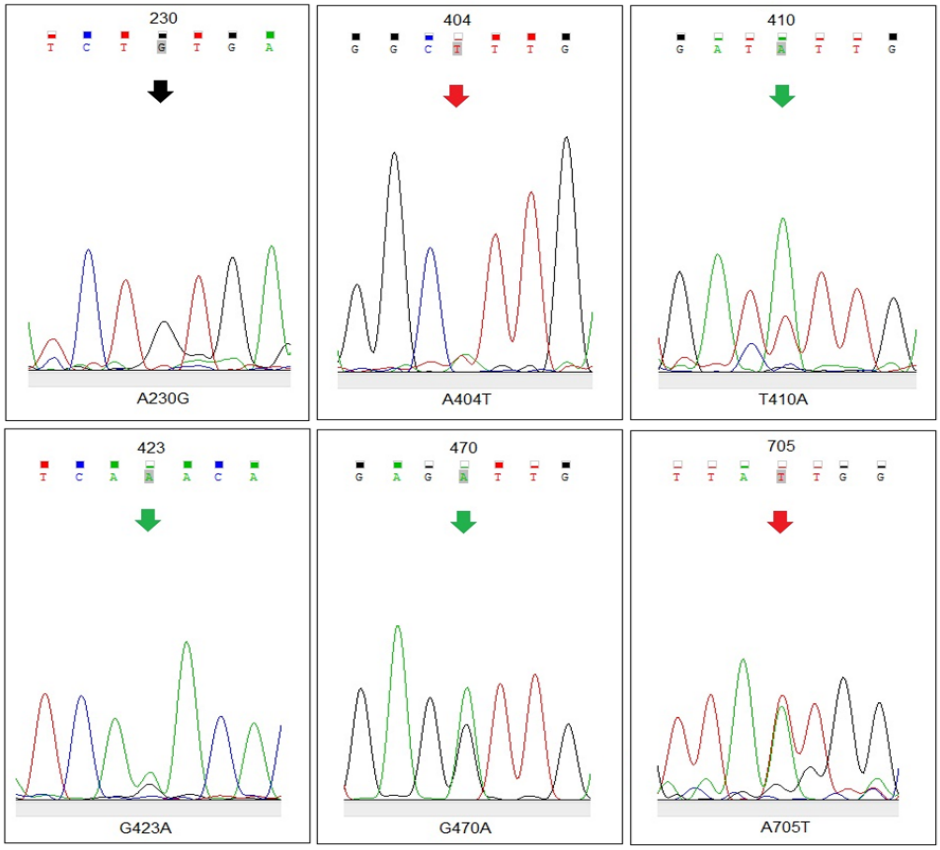

Fig. 1. The pattern of DNA chromatogram of all one hundred DNA specimens of the 817 bp amplicon of the exon-10/AHR gene. Each substitution SNP was highlighted according to their positions in the PCR products.

Table 3. The observed SNPs in the reference of the 583 bp of the partial exon7/GNAS gene DNA sequences.

No.	Native	Allele	Position in the PCR fragment	Position in the reference genome	Amino acid change	Novelty of SNP
1	C	G	91	58909271	-	Known intronic SNP (rs3730172)*
2	G	A	128	58909308	-	Known intronic SNP (rs1004902)*

GNAS amplicons results

As mentioned above, only one amplicon was studied in this gene that present in chromosome number 20. This gene has 22 exons, and the peptide chain encoded via this gene is known as G-protein alpha subunit, a main part of the traditional signal transduction pathway connecting receptor-ligand connections with the stimulation of adenylyl cyclase and an assortment of cellular reactions. Several transcription variations encoding many different forms have been detected for this gene. The only studied PCR amplicon is 583 bp, which partially covers intron 6 / exon 7 / intron 8. This fragment codes only 12 amino acids only from the exon 7. In our study G128A is the most frequent mutation (5.7%), and C91G (1.4%) in the GNAS gene. The sequencing chromatogram of each observed SNP is documented in this amplicon (Figure 3).

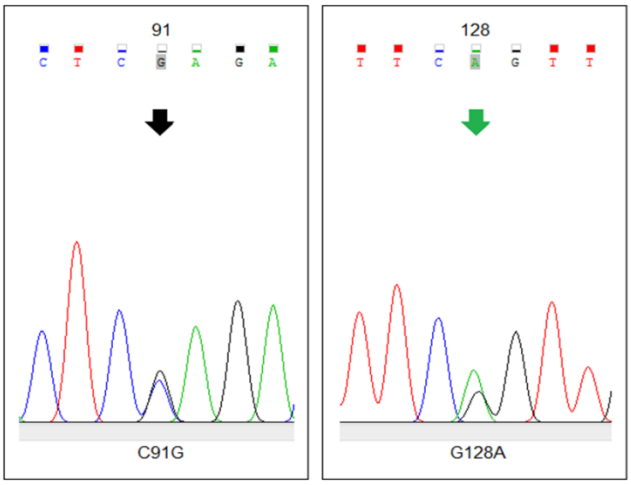

Fig. 3. The pattern of DNA chromatogram of all one hundred DNA specimens of the 583 bp amplicon of the partial exon-7/GNAS gene. Each substitution SNP was highlighted according to their positions in the PCR products.

The novelty of each observed SNP was detected using the NCBI SNP detector (Table 3).

DISCUSSION

Genetic single nucleotide polymorphism (SNP) could change a person's response to DNA damage derivative from food, life style, and surroundings. The greatest public genetic differences are characterized by SNPs, happening with a rate of about 1 in 500-800 nucleotides. A number of SNPs have been established to disturb health products produced by environmental contact, being considered as important susceptibility factors. The AHR gene, including about 50 kilobases at the genomic level, is thus estimated to contain about 60-100 SNPs. So far, 120 SNPs have been documented within the coding parts of the AHR gene and associated with the common database by the National Center for Biotechnology Information (NCBI) at www.ncbi.nlm.nih.gov/SNP (16).

The AHR gene, SNP variants occur commonly in exon 10, a region that codes a main portion of the trans-activation domain of the receptor that is responsible for regulating expression of target genes (17).

In our study we got a high level of frequency of polymorphisms in AHR gene especially in 706bp fragment, A705T is the most frequent mutation (5/70) (P< 0.005) this mutation will change the amino acid from asparagine to isoleucine, and T410A (1/70) (Non- significant) it will change the amino acid from phenylalanine to the isoleucine in the AHR 817bp fragment. Also for the second fragment C193A is the most frequent mutation (21/70) (P< 0.001) it will change the amino acid glutamine to lysine, A518C (8/70) (P< 0.005) it will change the amino acid glutamine to proline, T344A (1.4%), and G459C (1.4%) (Non – significant) will change the amino acid leucine to glutamine and glutamine to histidine respectively in the AHR 706bp piece.

Several studies have recently proposed that the AHR pathway is engaged in the change from a nonmalignant to a cancerous tumor, as also established in vitro by the greater nuclear AHR expression in aggressive than in unaggressive tumor cells or by amplified tumor infestation related with AHR down-regulation (18). In a previous studies, (19) established only double SNPs of exon 10, rs2066853 and rs4986826, in acromegalic patients the occurrence of rs2066853 and rs4986826 amongst acromegalic cases was much greater (22.4 and 2.9%, respectively) than what was described in the ExAC database for the European population (9.8 and 0.2%, respectively). The rs2066853 variation, involving in a G>A substitution and producing an arginine to lysine replacement in the transactivating domain, was found in a (25%) of patients and was associated with higher IGF-I levels and tumor invasiveness (10). Gu, established that the rs4986826 polymorphism was particularly uncommon and was always linked with the rs2066853 alteration (20). In this study G128A is the most frequent mutation (4/70) and C91G (1/70) in the GNAS gene. As both the mutations in intronic part of GNAS gene thus there is no change in the amino acid of gsp protein coded. The reported frequencies of *gsp* mutations in patients with GH-secreting pituitary adenomas was 1% for G128A and 12% for C91G of Portuguese examined cases (21).

REFERENCES

1. Sanno, N., Teramoto, A., Osamura, R. Y., Horvath, E., Kovacs, K., Lloyd, R. V., and Scheithauer, B. W. (2003). Pathology of pituitary tumors. Neurosurgery Clinics of North America, 14(1), 25-39.
2. Fernandez, A., Karavitaki, N., and Wass, J. A. (2010). Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). Clinical endocrinology, 72(3), 377-382.
3. Marie, P. (1886). On two cases of acromegaly: Marked hypertrophy of the upper and lower limbs and the head. Rev Med, 6, 297-333.
4. Ribeiro-Oliveira Jr, A., and Barkan, A. (2012). The changing face of acromegaly—advances in diagnosis and treatment. Nature Reviews Endocrinology, 8(10), 605-611.
5. Tamburrano, G., Durante, C., and Baldelli, R. (2002). Therapy of diabetes and dyslipidemia in acromegaly. Pituitary, 5(1), 27-31.
6. Vallar, L. ; Spada, A. and Giannattasio, G. (1987). Altered Gs and adenylate cyclase activity in human GH-secreting pituitary adenomas. Nature 330 566–568.
7. Landis, C. A., Masters, S. B., Spada, A., Pace, A. M., Bourne, H. R., and Vallar, L. (1989). GTPase inhibiting mutations activate the α chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. Nature, 340(6236), 692-696.
8. Clementi, E., Malgaretti, N., Meldolesi, J., and Taramelli, R. (1990). A new constitutively activating mutation of the Gs protein alpha

subunit-gsp oncogene is found in human pituitary tumours. Oncogene, 5(7), 1059-1061.
9. Lyons, J., Landis, C.A., Harsh, G., Vallar, L., Grunewald, K., Feichtinger, H., Duh, Q.Y., Clark, O.H., Kawasaki, E., Bourne, H.R. and McCormick, F. (1990). Two G protein oncogenes in human endocrine tumors. Science, 249(4969), 655-659.
10. Cannavo, S., Ferrau, F., Ragonese, M., Romeo, P.D., Torre, M.L., Puglisi, S., De Menis, E., Arnaldi, G., Salpietro, C., Cotta, O.R. and Albani, A. (2014). Increased frequency of the rs2066853 variant of aryl hydrocarbon receptor gene in patients with acromegaly. Clinical endocrinology, 81(2), 249-253.
11. Ferraù, F., Romeo, P.D., Puglisi, S., Ragonese, M., Torre, M.L., Scaroni, C., Occhi, G., De Menis, E., Arnaldi, G., Trimarchi, F. and Cannavo, S. (2016). Analysis of GPR101 and AIP genes mutations in acromegaly: a multicentric study. Endocrine, 54(3), 762-767.
12. Tordjman, K.A.R.E.N., Stern, N.A.F.T.A.L.I., Ouaknine, G.E.O.R.G.E.S., Yossiphov, Y.O.S.S.E.P.H., Razon, N.I.S.S.I.M., Nordenskjöld, M. and Friedman, E.I.T.A.N. (1993). Activating mutations of the Gs alpha-gene in nonfunctioning pituitary tumors. The Journal of Clinical Endocrinology & Metabolism, 77(3), 765-769.
13. Williamson, E. A., Daniels, M., Foster, S., Kelly, W. F., Kendall-Taylor, P., and Harris, P. E. (1994). Gsα and Gi2α mutations in clinically non-functioning pituitary tumours. Clinical endocrinology, 41(6), 815-820.
14. Hosoi, E., Yokogoshi, Y., Hosoi, E., Horie, H., Sano, T., Yamada, S. and Saito, S. (1993) Analysis of the Gsα gene in growth hormone-secreting pituitary adenomas by the polymerase chain reaction–direct sequencing method using paraffin-embedded tissues. Acta Endocrinologica, 129, 301–306.
15. Andreassen, T. T., & Oxlund, H. (2001). The effects of growth hormone on cortical and cancellous bone. Journal of Musculoskeletal and Neuronal Interactions, 2(1), 49-58.
16. Ridolfi, R., Cozzolino, R., Boscaino, F., Malorni, L., Canzanella, S., Malorni, A., Palmieri, G. and Malorni, L., (2014). AhR (aryl hydrocarbon receptor) polymorphisms: A possible role in TCDD (dioxins)-AhR binding and carcinogenesis. *International Journal of Biology*, *6*(4), 82.
17. Harper, P.A., Wong, J.M., Lam, M.S. and Okey, A.B., (2002). Polymorphisms in the human AH receptor. *Chemico-biological interactions*, *141*(1-2), 161-187.
18. Yang, X., Liu, D., Murray, T.J., Mitchell, G.C., Hesterman, E.V., Karchner, S.I., Merson, R.R., Hahn, M.E. and Sherr, D.H. (2005). The aryl hydrocarbon receptor constitutively represses c-myc transcription in human mammary tumor cells. *Oncogene*, *24*(53), 7869.
19. Cannavo, S., Ragonese, M., Puglisi, S., Romeo, P.D., Torre, M.L., Alibrandi, A., Scaroni, C., Occhi, G., Ceccato, F., Regazzo, D. and De Menis, E. (2016). Acromegaly is more severe in patients with AHR or AIP gene variants living in highly polluted areas. *The Journal of Clinical Endocrinology & Metabolism*, *101*(4), 1872-1879.
20. Gu, A., Ji, G., Jiang, T., Lu, A., You, Y., Liu, N., Luo, C., Yan, W. and Zhao, P. (2012). Contributions of aryl hydrocarbon receptor genetic variants to the risk of glioma and PAH-DNA adducts. *Toxicological Sciences*, *128*(2), 357-364.
21. Foltran, R.K., (2015). Molecular study of the genes GNAS, PTTG, AIP, CDKN1B and MEG3 in sporadic pituitary adenomas (Doctoral dissertation, University of São Paulo).