Pituitary (2021) 24:252–261
https://doi.org/10.1007/s11102-020-01105-4

Genetics, clinical features and outcomes of non-syndromic pituitary gigantism: experience of a single center from Sao Paulo, Brazil

Ericka B. Trarbach[1,2] · Giampaolo Trivellin[3,9] · Isabella P. P. Grande[1] · Felipe H. G. Duarte[2] · Alexander A. L. Jorge[1] · Felipe Barjud Pereira do Nascimento[4,8] · Heraldo M. Garmes[5] · Marcia Nery[6] · Berenice B. Mendonca[7] · Constantine A. Stratakis[3] · Marcello D. Bronstein[2] · Raquel S. Jallad[1,2]

Accepted: 26 October 2020 / Published online: 6 November 2020

Abstract

Purpose Non-syndromic pituitary gigantism (PG) is a very rare disease. Aryl hydrocarbon receptor-interacting protein (*AIP*) and G protein-coupled receptor 101 (*GPR101*) genetic abnormalities represent important etiologic causes of PG and may account for up to 40% of these cases. Here, we aimed to characterize the clinical and molecular findings and long-term outcomes in 18 patients (15 males, three females) with PG followed at a single tertiary center in Sao Paulo, Brazil.

Methods Genetic testing for *AIP* and *GPR101* were performed by DNA sequencing, droplet digital PCR and array comparative genomic hybridization (aCGH).

Results Pathogenic variants in the *AIP* gene were detected in 25% of patients, including a novel variant in splicing regulatory sequences which was present in a sporadic male case. X-LAG due to GPR101 microduplication was diagnosed in two female patients (12.5%). Of interest, these patients had symptoms onset by age 5 and 9 years old and diagnosis at 5 and 15 years, respectively. X-LAG, but not *AIP*, patients had a significantly lower age of symptoms onset and diagnosis and a higher height Z-score when compared to non-X-LAG. No other differences in clinical features and/or treatment outcomes were observed among PG based on their genetic background.

Conclusion We characterize the clinical and molecular findings and long-term outcome of the largest single-center PG cohort described so far.

Keywords Pituitary gigantism · *GPR101* · *AIP* · Outcome · Prognosis

Introduction

Pituitary gigantism (PG) is a very rare disease caused by chronic growth hormone (GH) and insulin-like growth factor 1 (IGF-1) hypersecretion occurring before complete fusion of the epiphyseal growth plates. Commonly, GH overproduction in PG derives from a pituitary somatotropinomas [1, 2]. The large majority of PG occurs as a sporadic disease while a small number occurs in the context of genetic syndromic disorders, such as McCune Albright syndrome (MAS), Carney complex (CNC), multiple endocrine neoplasia types 1 and 4 (MEN 1 and MEN 4) and the paraganglioma, pheochromocytoma and pituitary adenoma association (3PA) [1, 3, 4].

The genetic background of non-syndromic PG includes inactivating germline mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene. These mutations were found in about 29% of gigantism cases either sporadically or in the setting of Familial Isolated Pituitary Adenoma (FIPA) [5, 6]. *AIP* mutations are more frequent in young, predominantly males, patients and have been associated with large and invasive tumors and pituitary apoplexy [5, 7–9]. Generally, these patients more often had GH excess, were more often resistant to treatment with somatostatin receptor ligands (SRL) and underwent more surgical interventions, requiring multimodal therapy [5, 7–9].

Recently, an additional cause of gigantism has been linked to microduplications of G protein-coupled receptor 101 (*GPR101*) in Xq26.3 and termed X-linked acrogigantism

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11102-020-01105-4) contains supplementary material, which is available to authorized users.

✉ Raquel S. Jallad
raquel.jallad@hc.fm.usp.br

Extended author information available on the last page of the article

(X-LAG) [10].*GPR101* microduplications were found in both sporadic and familial cases of patients with somatotropinomas [10]. Patients with X-LAG have a distinct phenotype characterized by extraordinarily early gigantism with a median age of onset of 12 months [10–12].

Here, our main objectives were to evaluate (a) the relationship between clinical characteristics, genetic abnormalities, and long-term outcomes of patients with non-syndromic PG followed at a single pituitary center in Sao Paulo, Brazil, and (b) the different therapeutic strategies used to induce disease remission. These results add new case reports of this very rare disease increasing knowledge of pituitary gigantism.

Materials and methods

Subjects

This study included 18 patients with non-syndromic pituitary gigantism followed at a quaternary referral Neuroendocrine Unit, Division of Endocrinology and Metabolism, Clinical Hospital—FMUSP, between 1990 to 2016. All patients were from Sao Paulo state. PG encompassed patients with pituitary lesions leading to GH/IGF-1 hypersecretion that presented: (a) a final height standard deviation score (Z-score) above + 2 or (b) elevated rates of height growth or (c) height above + 2 SD from the mid-parental height [2, 13]. This study was approved by the local Ethics Committee and an informed consent form was obtained from all patients or their legal guardian.

Study design

This study included a retrospective review and a cross-sectional evaluation of PG, presenting our experience in the diagnosis, management, and follow-up of these patients. The data were obtained from medical records at the time of first symptoms, at diagnosis, and at last follow-up. Anthropometric data were established at medical appointment, when height was measured using a stadiometer. Whenever possible, patient's parents underwent clinical evaluation for confirmation of their height.

Clinical and hormonal data

Height was measured using the stadiometer and expressed in centimeters and as sex and age specific Z-scores [14].Target height was based on sex adjusted mid-parental heights, − 6.5 cm for girls and + 6.5 cm for boys [15]. Body mass index (BMI) was calculated as kilograms per square meter (kg/m^2). Pubertal development was determined according to the classification proposed by Marshall and Tanner [16, 17]. Bone age was estimated based on non-dominant-hand/wrist radiographs using the atlas of Greulich and Pyle [18]. Familial tall stature and signs of obesity, precocious or delayed puberty signs and dysmorphisms, and stigmata of specific disorders known to be associated with tall stature were also evaluated.

Serum GH concentration was measured with immunoradiometric assay (IRMA) or by immunofluorometric assay (IFMA) (AutoDELFIA, Wallac, Turku, Finland) with monoclonal antibodies. IGF-1 was measured by RIA after ethanol extraction (Diagnostic Systems Laboratories, Webster, TX) or by chemiluminescence assays (CLIA) (IMMULITE; Diagnostic Products Corp., Los Angeles,CA). IGF-1 level was standardized for age and sex, according to reference values provided by the manufacturer's.

GH hypersecretion was diagnosed by lack of suppression of GH levels during oral glucose tolerance test (OGTT) and IGF-1 level above the age-adjusted normal range. In patients undergoing surgical approach, the status of the disease was also evaluated by OGTT and IGF-I levels performed four months after surgery. In patients on medical therapy, the status of the disease was defined by random GH and IGF-I levels in the age-adjusted normal range. The nadir GH cutoff used to distinguish active disease from control/remission was method-specific, depending on the assay available at the time of diagnostic. IGF-1 levels were expressed as a multiple above the upper upper limit of normal reference range for age (x ULNR IGF-I; normal = x ULNR IGF-I < 1). Based on ULNR-IGF-1 the disease was defined as controlled (IGF-1 ≤ ULNR) or active (IGF-1 > ULNR) [19].

MRI and histopathological studies

The maximum tumor diameter was measured preoperatively by computed tomography (CT) or magnetic resonance imaging (MRI) T1 weighted coronal view evaluated by a single neuroradiologist. Concerning histopathological analysis, tumor specimens were evaluated by routine eosin-hematoxylin stain and immunohistochemical staining for GH, PRL, ACTH, LH, and FSH hormones. Somatotropinomas were confirmed based on positive staining for GH. Tumor T2-weighted signal intensity was assessed by visual inspection on coronal plane and adenomas are classified as hypo-, iso- or hyper-intense in relation to the healthy pituitary gland or the temporal grey matter as reference tissue.

Genetic screening

Blood samples for DNA extraction (supplementary methods) was available for 16 patients. For these, the entire coding region of *AIP* (ENST00000279146), *GPR101* (ENST00000298110), *MEN1* (ENST00000312049), *CDKN1B* (ENST00000228872.9) and *GNAS* hotspots (exons

8 and 9; ENST00000371100) were amplified by polymerase chain reaction (PCR) and Sanger sequenced (supplementary methods). All variants identified were confirmed in two independent PCR products and by sequencing both DNA strands. The new genetics variants were categorized in different classes of pathogenicity according to the American College of Medical Genetics and Genomics (ACMG) guidelines [20].

Droplet digital PCR (ddPCR) was used to assess copy number variants (CNVs) at the *GPR101* and *AIP* genes using blood-derived DNA [21]. *GPR101* CNV analysis was also performed using DNA extracted from tumor or buccal cells, when available. Detection of a *GPR101* microduplication by ddPCR was also confirmed by array comparative genomic hybridization (aCGH), as previously described [10]. The term "non mutated patients" was used throughout this manuscript to define the patients without pathogenic variants in known genes.

Statistical analysis

All statistical analyses were performed using the Stata/SE 14.2 software (StataCorp LLC, Texas, USA). All data were expressed as median and lower–upper quartile and compared with two-samples Wilcoxon rank-sum (Mann–Whitney) non-parametric test. Categorical variables are presented as absolute values or percentages and were tested using the Fisher exact test. P values < 0.05 were considered statistically significant.

Results

Clinical and biochemical characteristics of patients

Table 1 presents the main clinical and biochemical characteristics of each patient. Fifteen patients were males (83%) and three were females (17%). Three cases (17%) had a familial history of pituitary adenomas (FIPA) but not of tall stature. The median age at time of diagnosis of PG was 17 (15–20) years with first signs and symptoms noticed at 14 (9–16) years. Therefore, the delay in the diagnosis of gigantism was 3.5 (2–8) years. The median of height Z-score was 3.6 (2.9–5), with adult height median of 198 (195–203) cm. The most common complaint was accelerated growth and tall stature in twelve patients (67%), followed by loss of libido in three (17%), diabetes mellitus in two (11%), and paresthesia in one case (5%).

Headache was present in fourteen (78%) and visual disturbance in thirteen (72%) patients, hyperhidrosis in fourteen (78%) patients, enlarged hands and feet in eleven patients (61%), arterial hypertension in nine patients (50%), arthralgias in eight (44%) patients, paresthesia in four (22%) patients, fatigue in nine (50%) patients. None of the patients had galactorrhea and in three cases (17%) diabetes mellitus was diagnosed. Eight patients (44%, age range 15–28) showed evidence of epiphyseal closure on hand radiography at the time of diagnosis. Two patients (11%) had a bone age delay higher than two years.

GH basal and x ULNR-IGF1 medians at diagnosis were 70 (35–108) and 1.9 (1.7–2.9), respectively. Biochemical evidences for gonadal, thyroid, and adrenal deficiencies were found in sixteen (89%), fifteen (83%) and ten (55.5%) cases, respectively.

Tumor features

All pituitary tumors were macroadenomas, three (17%) with a maximum diameter ≥ 4 cm (giant adenomas). Suprasellar extension was noted in fifteen adenomas (83%). Of these, 5/15 adenomas (33%) presented intrasellar, 3/15 (20%) parasellar, and 7/15 (47%) both intrasellar and parasellar extension. Interestingly, pituitary hyperplasia alongside adenoma was observed in one (5%) patient. All tumors were immunoreactive for GH, with six adenomas (33%) also expressing PRL (Table 1). At diagnosis, three, six and four tumors were T2-hypo-, iso- and hyper-intense, respectively (Table 1).

Treatments and outcomes

Seventeen patients underwent pituitary surgery (sixteen transsphenoidal and one transcranial) as primary therapy, which was completely effective in only two cases. A second surgical approach was unsuccessful in seven patients. Postoperative radiotherapy (RT) was performed in two cases, resulting in disease control. Postoperative medical therapy was administered in the remaining thirteen patients and included SRL alone or in combination with the dopamine agonist (DA) cabergoline. Ten out of these 13 patients remained uncontrolled. Of these, five patients have received RT as a third treatment modality, which was successful in four cases.

One patient received SRL as primary therapy, but required additional transsphenoidal surgery for disease control. This patient had a hyper-intense signal on T2 (#5, Table 1). Other eight patients from iso- and hyper-intense group, received SRL as adjuvant therapy. Of these, three (#1, #11 and #14, Table 1) were responsive and five (#4, #7, #13, #15 and #17, Table 1) unresponsive to medical treatment. Only one patient with hypo-intense tumors signal (#18, Table 1) used SRL as secondary therapy and showed no response. The other two patients with hypo-intense tumors (#6 and #8, Table 1) were successfully treated with primary TSS.

Overall, the rate of success of treatment was 11% (2/18 patients) with monomodal therapy, 37.5% (6/16) with bimodal therapy (surgery + SRL/DA or surgery + RT), and

Table 1 Clinical, hormonal and tumor features, outcomes, and germline genetic abnormalities identified in PG patients

ID	Clinical, genetic and hormonal							Tumor feature			Treatment and outcome				
	Sex	Mut. gene	Age (y) AD/FS	Main complaint	AD height (cm/Z-score)	Adult height (cm/Z-score)	GH (ng/mL)/ IGF-1 (xULNR)	MDS (cm)/ T2-signal intensity	Sellar extension	IHC	1st mod	2nd mod	3nd mod	DC	Time (y) follow-up/ DC
#1	M	*AIP*	15/15	AG and TS	198/3.8	198/3.5	108/1.8	3.0/hyper	S+I+P	GH	TCS	SRL[&]+DA	–	Yes	15/4
#2	M	*AIP**	18/16	AG and TS	190/2.3	197/3.3	191/1.8	3.8/N.A	S+I	GH	TSS	SRL+DA	RT-C	Yes	24/20
#3	M	*AIP**	11/9	AG and TS	180/5.7	198/3.5	50/1.8	4.1/N.A	S+I+P	GH	TSS	SRL+DA	RT-C	Yes	20/18
#4	M	*AIP**	19/7	AG and TS	205/4.6	205/4.5	26/1.1	2.4/iso	S+I	Mixed	TSS	SRL+DA	–	No	5/–
#5	M	No	26/16	Loss of libido	197/3.4	197/3.3	85.8/2.9	2.7/hyper	S+P	GH	SRL	TSS	–	Yes	18/13
#6	M	No	18/16	Paresthesia	195/3.1	195/3.0	3.5/1.8	2.1/hypo	S+I	Mixed	TSS	–	–	Yes	8/8
#7	M	No	16/13	AG and TS	193/2.9	196/3.2	81/1.6	1.9/iso	S+P	GH	TSS	SRL+DA	–	No	5/–
#8	M	No	16/15	AG and TS	185/1.8	188/2.0	61/1.2	1.2/hypo	No	GH	TSS	–	–	Yes	6/6
#9	M	No	14/12	AG and TS	202/5.0	223/7.3	11/1.1	3.2/hyper	S+P	GH	TSS	RT-S	–	Yes	14/6
#10	M	No	16/14	AG and TS	200/3.9	210/5.3	65/1.7	4.0/N.A	S+I+P	GH	TSS	SRL	RT-C	Yes	21/5
#11	M	No	27/17	Loss of libido	195/3.1	199/3.6	3.8/3.0	1.4/iso	No	Mixed	TSS	SRL+DA	–	Yes	8/7
#12	M	No	20/15	AG and TS	203/4.3	203/4.2	75**/2.9	3.1/N.A	S+I	GH	TSS	SRL+DA	RT-C	Yes	30/18
#13	M	No	19/14	AG and TS	195/3.1	200/3.8	114**/2.1	4.4/iso	S+I+P	Mixed	TSS	SRL+DA	–	No	30/–
#14	M	No	24/16	Loss of libido	194/2.9	194/2.9	35/3.4	2.0/iso	No	Mixed	TSS	SRL+DA	–	Yes	21/20
#15	M	N.A	28/14	DM	192/2.6	192/2.6	122/2.9	2.7/iso	S+I+P	GH	TSS	SRL[&]+DA	–	No	11/–
#16	F	Xq26.3	5/5	DM	134/5.7	190/4.6	44.6/3.3	3.5/N.A	S+I	GH***	TSS	RT-C	–	Yes	25/21
#17	F	Xq26.3	15/9	AG and TS	203/6.8	203/6.8	75.3/3.9	3.1/hyper	S+I+P	Mixed	TSS	SRL[&]+DA	RT-S	No	7/–
#18	F	N.A	11/7	AG and TS	181/5.5	N.A	110/2.3	3.0/hypo	S+I+P	GH	TSS	SRL+DA	–	No	8/–

AIP mutations: Patients #1 (c.788-2A>C), #2 (p.Arg304Ter), #3 (p.Gln217Ter) and #4 (p.Ala277Pro); *patients with familial history of pituitary adenoma: #2-father and paternal uncle with somatotropinoma/acromegaly, #3-father, sister and paternal aunt with somatotropinomas/acromegaly, #4-mother with prolactinoma; **GH concentration measured by immunoradiometric assay (IRMA); ***presence of hyperplastic areas; [&]pasireotide in combination with SRL first generation

AD Age at diagnosis, *FS* age at first symptoms, *AG and TS* accelerated growth and tall stature, *DM* diabetes mellitus, x*ULNR* multiple above upper limit of normal range, *MDS* maximum diameter size, *S* suprasellar *I* intrasellar *P* parasellar, *IHC* immunohistochemical, *Mixed* GH+PRL, *TCS* transcranial surgery, *TSS* transsphenoidal surgery, *SRL* somatostatin receptor ligands, *DA* dopamine agonist, *RT-C* conventional radiotherapy, *RT-S* stereotactic radiotherapy, *N.A.* not available, *DC* disease control

80% (4/5) with trimodal therapy (surgery + SRL/DA + RT). Patients were followed for a median of 12.5 years (7–21) and the median time to hormonal remission was 15.5 years (7–20).

Genetic abnormalities

Genetic analysis of *AIP* in DNA extracted from blood samples identified four heterozygous variants in PG patients (Supplementary Fig. 1). A novel c.788-2A > C variant (absent in gnomAD and 1000 Genome databases) was detected in one male patient. This variant is located in the canonical acceptor splice site of intron 5 and was classified as likely pathogenic according to ACMG criteria, weighted as very strong (PVS1). The three other *AIP* variants identified were previously described in patients with pituitary somatotropinomas as deleterious: p.Gln217Ter (c.649C > T, rs267606566, exon 5), p.Ala277Pro (c.829G > C, rs267606581, exon 6) and p.Arg304Ter (c.910C > T, rs104894195, exon 6). No *AIP* deletions were detected using ddPCR in our cohort of PG.

GPR101 single nucleotide variants (SNVs) were not observed in any case. Two sporadic female patients presented a germline microduplication in Xq26.3 as detected by ddPCR and confirmed by aCGH. In one of these patients, this microduplication was also observed in the tumor DNA. The CNVs identified in patients #16 and #17 also encompassed *CD40LG*, *ARHGEF6,* and *RBMX*, frequently duplicated with *GPR101* in X-LAG (Fig. 1). Mutations in *MEN1* and *GNAS* were excluded in all patients. The clinical and tumor characteristic of *AIP* mutated and X-LAG patients are described in Table 1. Figure 2 shows MRI pituitary images of one X-LAG patient.

Comparison of genetics, patients' and tumor features and outcomes

Among all patients there were significant differences in age at diagnosis when comparing X-LAG to non-mutated (10.0: 5–15 vs. 18.5:16–24 years, respectively, P = 0.04, Table2) but not with *AIP*-mutated patients 16.5:13–18.5 years, P = 0.25, Table 2). Significantly younger age at onset of symptoms was observed for X-LAG when compared to non-mutated (7.0: 5–9 vs. 15:14–16 years, respectively; P = 0.03, Table 2) but not with *AIP*-mutated patients (12.0: 8–15.5 years; P = 0.24, Table 2). No statistical significance was observed when differences of ages at diagnosis and at symptoms onset were compared between *AIP*-mutated and non-mutated patients (P = 0.24 and P = 0.23, respectively). Although no statistically significant difference was identified in height at diagnosis between X-LAG, *AIP* mutated, and non-mutated patients (Table 2), a significant height Z-score difference was identified between X-LAG and non-mutated patients (6.2:5.7–6.8 vs. 3.1:2.9–3.9, respectively; P = 0.03 Table 2).

No other significant differences, such as GH and IGF-1 levels, tumor features, multi-modal therapy or disease control, were observed among these three categories of PG patients (Table 2). However, patients receiving trimodal treatment had larger tumors when compared to those receiving monomodal (3.8:3.1–4 vs. 1.65:1.2–2.1; respectively; P = 0.03). Also, tumor size had a weak positive correlation with height Z-Score (rho = 0.6; P = 0.03).

Discussion

Pituitary gigantism is a very rare disease, with few cases being described worldwide [2]. Nevertheless, significant advances in understanding the genetic causes of PG were achieved in the last years. The *AIP* gene was considered the most common cause of non-syndromic PG, accounting for approximately 29%, and *GPR101* has been identified as an essential novel *locus* for this disease [2, 5, 10].

In the present study, a genetic investigation was undertaken in 16 out of 18 PG patients evaluated. *AIP* pathogenic variants were found in 4 cases (25%) and included a novel putative splicing variant (c.788-2A > C) in intron 5. This variant was observed in one male patient without familial history of accelerated growth or pituitary tumors, unlike the others *AIP*-mutated cases of our cohort who had family members with pituitary tumors. For familial cases, DNA samples from paternal uncle (patient #2, p.Arg304Ter) and father (patient #3, p.Gln217Ter), both with acromegaly, were available. The same *AIP* proband mutations were present in their respective relatives (data not shown). In fact, *AIP* mutations are frequently observed in familial cases of isolated somatotropinomas (FIPA) and were commonly found in PG patients [6, 8].

Surprisingly, in the most extensive multi-center international PG study involving 208 syndromic and non-syndromic cases, the majority of *AIP*-mutated patients were apparently sporadic [2]. However, the authors highlighted that these cases could be mistakenly interpreted as simplex, since *AIP* genetic family screening cannot be extensively performed and incomplete penetrance could lead to a family generation with no affected relatives [2, 5]. *AIP* mutated patients of our cohort showed invasive macroadenomas and did not achieve disease control with first-generation SRL monotherapy concurring with previous publications. *AIP* mutations were indeed described to confer resistance to first-generation SRL in patients with somatotropinomas [7, 22], and multi-modal therapy was required for hormonal control and/or residual size reduction of these tumors [23].

GH receptor antagonist pegvisomant (alone or combined with long-acting SRL) has been shown to be an effective

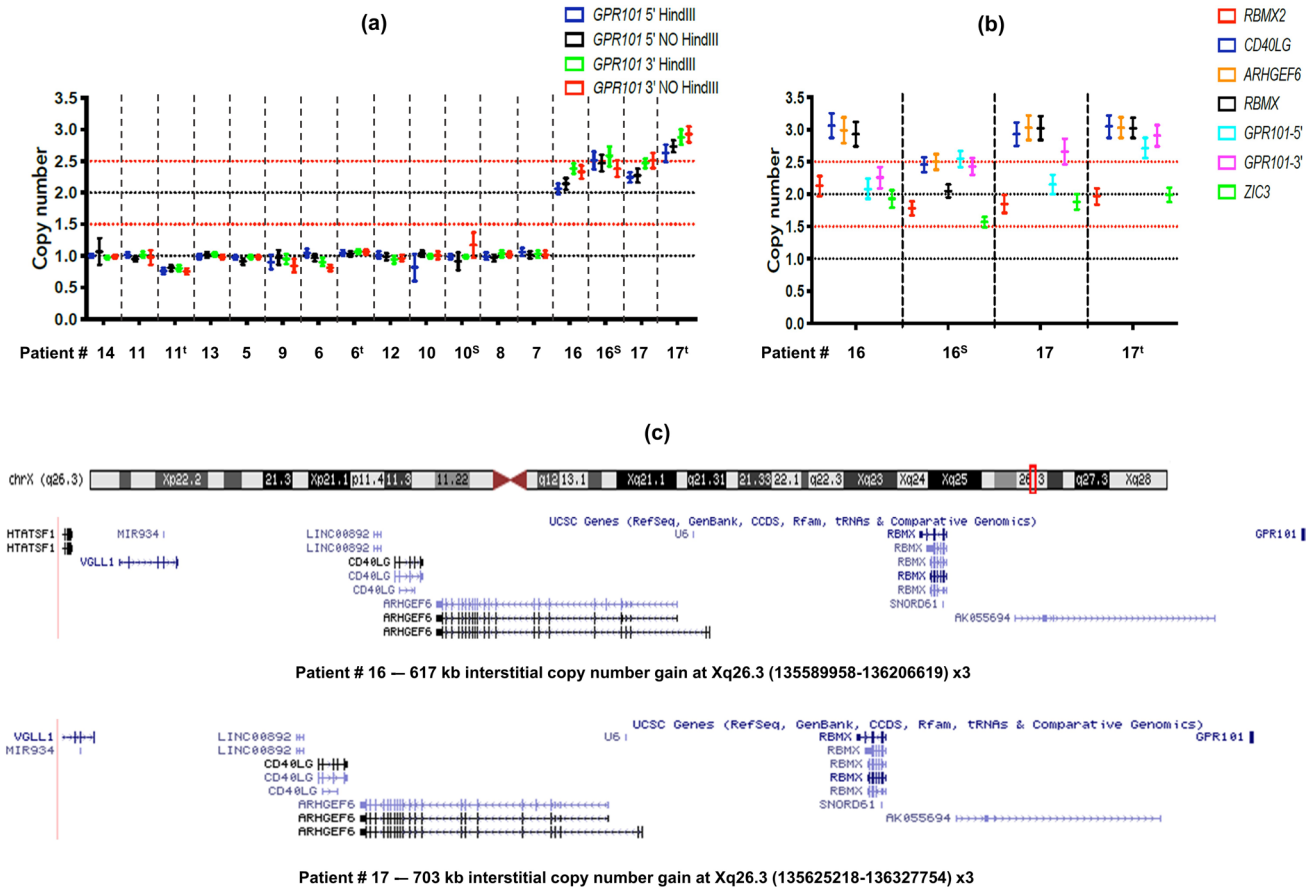

Fig. 1 Results of droplet digital polymerase chain reaction (ddPCR) and array comparative genomic hybridization (aCGH) analyses for the Xq26.3 region in the subset of *AIP*-mutation negative patients. **a** Copy number ddPCR assays for *GPR101*. Two copy number variation assays, Hs01818174_cn and Hs01730605_cn (Thermo Fisher Scientific), located respectively at the 5′ and 3′ end of the gene were employed. Reactions were performed both with and without the restriction enzyme HindIII, which was confirmed to not cut within the target and reference (*RNASEP*) amplicons. HindIII was used to reduce sample viscosity and off-target amplification, and by separating tandem gene copies, ensured proper random partitioning into droplets. Patients #16 and #17 showed a potential duplication of *GPR101*. **b** For patients #16 and #17, the ddPCR analysis was extended to include the centromerically adjacent *CD40LG*, *ARHGEF6* and *RBMX* genes, commonly duplicated alongside *GPR101* in X-LAG patients. The centromeric *RBMX2* and the telomeric *ZIC3*, two genes never included in the X-LAG duplications reported so far, served as controls. All reactions were performed using HindIII. As expected, *RBMX2* and *ZIC3* showed no copy number gains. While some variability, likely due to the quality of the DNA, was observed for the other genes, the presence of a Xq26.3 duplication was confirmed. In both panels **a** and **b**, bars correspond to 95% confidence intervals; the dotted red lines crossing the y axis at 1.5 and 2.5 copy numbers represent the threshold for deletion and duplication in females, respectively. *S* Oral swab-derived DNA, *T* tumor-derived DNA. **c** Genome view of the Xq26.3 duplication detected by aCGH in blood-derived DNA from Patients #16 and #17, according to the UCSC Genome Browser (hg19; GRCh37/hg19). aCGH confirmed the ddPCR results

modality for the treatment of pituitary gigantism [24–26]. However, this drug is not available in our public health system. Second-generation somatostatin multireceptor ligand pasireotide-LAR was administered with octreotide-LAR in three patients of cohort: one harboring the *AIP* c.788-2A > C intronic mutation (patient #1, Table 1), one with X-LAG (patient #17, Table 1) and one with unknow mutation status (patient #15, Table 1). Only the first one patient achieved disease remission. Recent reports have demonstrated a correlation between T2-tumor signal intensity and response to medical treatment with SRL, before or after surgery, in patients with somatotropinomas [27–30]. In particular, a better response to SRL therapy was observed in T2-hypointense compared to hyper/isointense adenomas [27–30]. However, only one patient with T2-hypointense signal of our cohort received SRL therapy and this relationship could not be evaluated in the current study.

Xq26.3 genomic abnormalities, encompassing *GPR101*, occur in approximately 10% of PG and define the newly described X-linked acrogigantism (X-LAG). X-LAG is a subtype of PG characterized by early-onset gigantism, mostly affecting females [10]. Usually, these patients

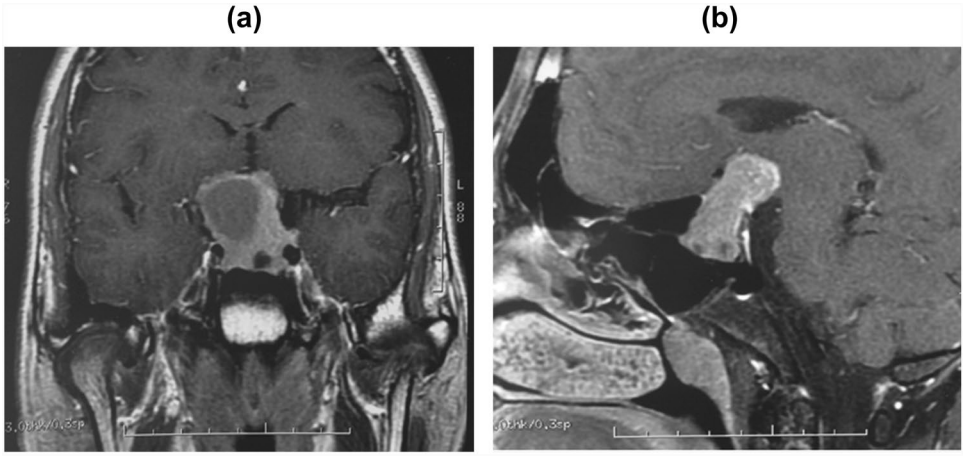

Fig. 2 T1-weighted **a** coronal and **b** sagittal MRI of patient #17 with X-LAG revealed a heterogeneous macro adenoma with suprasellar lesion extension

Table 2 The comparison of clinical, tumoral, and outcome data among *AIP*-mutated, X-LAG, and non-mutated patients with PG

	AIP mutated (n = 4)	X-LAG (n = 2)	Non-mutated (n = 10)	P[a]	P[b]	P[c]
Gender						
Male:female	4:0	0:2	10:0	–	–	0.06
Age (year)						
At diagnosis	16.5 (13–18.5)	10.0 (5–15)	18.5 (16–24)	0.24	**0.04**	0.25
Of first symptoms	12.0 (8–15.5)	7.0 (5–9)	15 (14–16)	0.23	**0.03**	0.24
Delay of diagnosis	2.0 (1–7)	3.0 (0–6)	4 (2–8)	0.47	0.51	0.81
Height						
At diagnosis (cm)	194 (185–201)	168 (134–203)	195 (190–202)	0.77	0.91	0.64
Z-score (at diagnosis)	4.2 (3–5.1)	6.2 (5.7–6.8)	3.1 (2.9–3.9)	0.31	**0.03**	0.10
Adult (cm)	198 (197–201)	196 (190–203)	198 (195–203)	0.72	0.74	0.63
Z-score (MPH)	3.5 (3.4–4)	5.7 (4.6–6.8)	3.4 (3–4.2)	0.72	0.13	0.06
Laboratorial						
GH (ng/mL)	79 (38–149.5)	59.9 (44.6–75)	63.1 (11–81)	0.39	0.82	0.64
IGF1 (xULNR)	1.8 (1.4–1.8)	3.6 (3.3–3.9)	1.9 (1.6–2.9)	0.92	0.06	0.05
PRL (ng/mL)	29 (17–73)	223 (26–420)	53.5 (14–78)	0.67	0.39	0.35
Tumor features						
Maximum diameter (cm)	3.4 (2.7–3.9)	3.3 (3.1–3.5)	2.4 (1.9–3.2)	0.26	0.33	0.99
> 4 cm (yes:no)	1:3	0:2	2:8	0.67	0.68	0.67
Sellar extension (yes:no)	4:0	2:0	7:3	0.33	0.54	-
GH:GH-PRL stain	3:1	1:1	6:4	0.64	0.68	0.60
Treatment						
Monomodal	0	0	2	0.55	0.98	0.82
Bimodal	2	1	6			
Trimodal	2	1	2			
Disease control (yes:no)	3:1	1:1	8:2	0.67	0.45	0.60
Time of disease control (year)	20 (4–20)	25 (25–25)	10.5 (7–19)	0.83	0.12	0.15
Follow-up (year)	16.5 (10–21)	14 (7–21)	11 (6–21)	0.83	0.82	0.98

MPH Mid-parental height; *xULNR* multiple above upper limit of normal range

[a]AIP vs. non-mutated

[b]X-LAG vs. non-mutated

[c]AIP vs. X-LAG

present with accelerated growth between 12 – 24 months and in all 36 cases described to date the disease onset occurred before four years of age [10–12, 21, 31]. Here, germline Xq26.3 microduplications were found in 2/16 (12,5%) of PG. Both were females with a sporadic disease and reported their symptoms at 5 and 9 years of age (patients #16 and 17, respectively; Table 1). We think this finding can be attributed to specific patients' factors leading to difficulty recognizing their health situation, such as low intellectual and socioeconomic status and psychological aspects. Pediatric growth charts would be useful to evaluate these patients' growth velocity and, consequently, indicate the onset of illness. Unfortunately, these data were not available for both X-LAG patients.

Recently, somatic mosaicism was shown to occur in sporadic X-LAG male patients. In some of these patients the *GPR101* duplication was identified in the pituitary DNA tissue, but not in leukocytes or saliva-derived DNA [32, 33]. Conceivably, postzygotic *GPR101* mutations could be present in some of the male patients described in our cohort. However, only two male patients had pituitary tumor tissue available for exclusion of somatic Xq26.3 microduplications. Of note, the clinical characteristics of mosaic males are similar to those of the females/males with X-LAG due to germline Xq26.3 microduplications [21, 31–33].

Comparing X-LAG to non-mutated PG patients, there were significant differences in the average age at diagnosis (P = 0.04), age of onset of symptoms (P = 0.03), and height Z-score (P = 0.03). However, such differences were not seen when compared to *AIP* mutated patients. Classically, both *AIP* mutated and X-LAG patients showed a more aggressive clinical presentation, challenging its treatment strategy [21–23, 34]. According to this data, in the present study's cohort, no patients harboring genetic abnormalities achieved disease control after transsphenoidal surgery and all of them required adjuvant therapy.

In conclusion, to our knowledge this report represents the largest cohort of PG patients followed at a single center. The prevalence of *AIP* mutations and *GPR101* genomic abnormalities were in line to previous studies. Although the age of onset of symptoms in our X-LAG patients is higher than previously described, this result must be viewed in the context of some limitations, including insufficient availability of birth and early childhood data and a relatively small number of patients with genetic abnormalities evaluated.

Funding This work was in part funded by the intramural research program, NICHD, NIH, Bethesda, MD, USA (to CAS).

Data availability Data available within the article or its supplementary materials.

Compliance with ethical standards

Conflicts of interest EBT, IPPG, FHGD, AALJ, FBPN, HMG, MN, BBM, MDB and RSJ have nothing to declare. CAS and GT hold a patent on the *GPR101* gene and its function and have received funding from Pfizer, Inc., on growth hormone and acromegaly research. CAS also holds patents on *PRKAR1A* and *PDE11A* genes and their function.

Ethics approval This study was approved by the local Ethics Committee and an Informed Consent form was obtained from All Patients or their Legal Guardian.

References

1. Rostomyan L, Daly AF, Beckers A (2015) Pituitary gigantism: causes and clinical characteristics. Ann Endocrinol (Paris) 76:643–649. https://doi.org/10.1016/j.ando.2015.10.002
2. Rostomyan L, Daly AF, Petrossians P et al (2015) Clinical and genetic characterization of pituitary gigantism: an international collaborative study in 208 patients. Endocr Relat Cancer 22:745–757. https://doi.org/10.1530/ERC-15-0320
3. Dénes J, Swords F, Rattenberry E et al (2015) Heterogeneous genetic background of the association of pheochromocytoma/paraganglioma and pituitary adenoma: results from a large patient cohort. J Clin Endocrinol Metab 100:E531–E541. https://doi.org/10.1210/jc.2014-3399
4. Xekouki P, Szarek E, Bullova P et al (2015) Pituitary adenoma with paraganglioma/pheochromocytoma (3PAs) and succinate dehydrogenase defects in humans and mice. J Clin Endocrinol Metab 100:E710–E719. https://doi.org/10.1210/jc.2014-4297
5. Rostomyan L, Potorac I, Beckers P et al (2017) AIP mutations and gigantism. Ann Endocrinol (Paris) 78:123–130. https://doi.org/10.1016/j.ando.2017.04.012
6. Vasilev V, Daly AF, Trivellin G et al (2020) The roles of AIP and GPR101 in familial isolated pituitary adenomas (FIPA). Endocr Relat Cancer 27:T77–T86. https://doi.org/10.1530/ERC-20-0015
7. Oriola J, Lucas T, Halperin I et al (2013) Germline mutations of AIP gene in somatotropinomas resistant to somatostatin analogues. Eur J Endocrinol 168:9–13. https://doi.org/10.1530/EJE-12-0457
8. Daly AF, Tichomirowa MA, Petrossians P et al (2010) Clinical characteristics and therapeutic responses in patients with germline AIP mutations and pituitary adenomas: an international collaborative study. J Clin Endocrinol Metab. https://doi.org/10.1210/jc.2009-2556
9. Marques P, Caimari F, Hernández-Ramírez LC, et al (2020) Significant benefits of AIP testing and clinical screening in familial isolated and young onset pituitary tumors. J Clin Endocrinol Metab 105:2247–2260. https://doi.org/10.1210/clinem/dgaa040
10. Trivellin G, Daly AF, Faucz FR et al (2014) Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. N Engl J Med 371:2363–2374. https://doi.org/10.1056/NEJMoa1408028
11. Beckers A, Lodish MB, Trivellin G, Rostomyan L, Lee M, Faucz FR et al (2015) X-linked acrogigantism (X-LAG) syndrome: clinical profile and therapeutic responses. Endocr Relat Cancer 22:353–367. https://doi.org/10.1530/ERC-15-0038
12. Beckers A, Rostomyan L, Potorac I et al (2017) X-LAG: How did they grow so tall? Ann Endocrinol (Paris) 78:131–136. https://doi.org/10.1016/j.ando.2017.04.013
13. Beckers A, Petrossians P, Hanson J, Daly AF (2018) The causes and consequences of pituitary gigantism. Nat Rev Endocrinol 14:705–720

14. Silva DAS, Pelegrini A, Petroski EL, Gaya ACA (2010) Comparison between the growth of Brazilian children and adolescents and the reference growth charts: Data from a Brazilian project. J Pediatr (Rio J) 86:115–120. https://doi.org/10.2223/JPED.1975
15. Tanner JM (1986) Normal growth and techniques of growth assessment. Clin Endocrinol Metab 15:411–451. https://doi.org/10.1016/S0300-595X(86)80005-6
16. Marshall WA, Tanner JM (1969) Variations in pattern of pubertal changes in girls. Arch Dis Child 44:291–303. https://doi.org/10.1136/adc.44.235.291
17. Marshall WA, Tanner JM (1970) Variations in the pattern of pubertal changes in boys. Arch Dis Child 45:13–23. https://doi.org/10.1136/adc.45.239.13
18. Pyle SI, Waterhouse AM, Greulich WW (1971) Attributes of the radiographic standard of reference for the National Health Examination Survey. Am J Phys Anthropol 35:331–337. https://doi.org/10.1002/ajpa.1330350306
19. Giustina A, Barkan A, Beckers A, Biermasz N, Biller BMK, Boguszewski C, Bolanowski M, Bonert V, Bronstein MD, Casanueva FF, Clemmons D, Colao A, Ferone D, Fleseriu M, Stefano Frara SM (2020) A consensus on the diagnosis and treatment of acromegaly comorbidities: an update—PubMed. J Clin Endocrinol Metab 105:e937–e946
20. Richards S, Aziz N, Bale S et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405–424. https://doi.org/10.1038/gim.2015.30
21. Iacovazzo D, Caswell R, Bunce B et al (2016) Germline or somatic GPR101 duplication leads to X-linked acrogigantism: a clinico-pathological and genetic study. Acta Neuropathol Commun 4:56. https://doi.org/10.1186/s40478-016-0328-1
22. Daly AF, Rostomyan L, Betea D et al (2019) Aip-mutated acromegaly resistant to first-generation somatostatin analogs: long-term control with pasireotide lar in two patients. Endocr Connect 8:367–377. https://doi.org/10.1530/EC-19-0004
23. Joshi K, Daly AF, Beckers A, Zacharin M (2018) Resistant paediatric somatotropinomas due to AIP mutations: role of pegvisomant. Horm Res Paediatr 90:196–202. https://doi.org/10.1159/000488856
24. Goldenberg N, Racine MS, Thomas P et al (2008) Treatment of pituitary gigantism with the growth hormone receptor antagonist pegvisomant. J Clin Endocrinol Metab 93:2953–2956. https://doi.org/10.1210/jc.2007-2283
25. García WR, Cortes HT, Romero AF (2019) Pituitary gigantism: A case series from hospital de san josé (Bogotá, Colombia). Arch Endocrinol Metab 63:385–393. https://doi.org/10.20945/2359-3997000000150
26. Mangupli R, Rostomyan L, Castermans E et al (2016) Combined treatment with octreotide LAR and pegvisomant in patients with pituitary gigantism: clinical evaluation and genetic screening. Pituitary 19:507–514. https://doi.org/10.1007/s11102-016-0732-3
27. Tortora F, Negro A, Grasso LFS et al (2019) Pituitary magnetic resonance imaging predictive role in the therapeutic response of growth hormone-secreting pituitary adenomas. Gland Surg 8:S150–S158. https://doi.org/10.21037/gs.2019.06.04
28. Potorac I, Petrossians P, Daly AF et al (2016) T2-weighted MRI signal predicts hormone and tumor responses to somatostatin analogs in acromegaly. Endocr Relat Cancer 23:871–881. https://doi.org/10.1530/ERC-16-0356
29. Potorac I, Petrossians P, Daly AF et al (2015) Pituitary MRI characteristics in 297 acromegaly patients based on T2-weighted sequences. Endocr Relat Cancer 22:169–177. https://doi.org/10.1530/ERC-14-0305
30. Heck A, Ringstad G, Fougner SL et al (2012) Intensity of pituitary adenoma on T2-weighted magnetic resonance imaging predicts the response to octreotide treatment in newly diagnosed acromegaly. Clin Endocrinol (Oxf) 77:72–78. https://doi.org/10.1111/j.1365-2265.2011.04286.x
31. Trivellin G, Faucz FR, Daly AF et al (2020) GPR101, an orphan GPCR with roles in growth and pituitary tumorigenesis. Endocr Relat Cancer 27:T87–T97. https://doi.org/10.1530/ERC-20-0025
32. Daly AF, Yuan B, Fina F, Caberg JH, Trivellin G, Rostomyan L, de Herder WW, Naves LA, Metzger D, Cuny T, Rabl W, Shah N, Jaffrain-Rea ML, Zatelli MC, Faucz FR, Castermans E, Nanni-Metellus I, Lodish M, Muhammad A, Palmeira L, Potorac I, Mantovani G, Neggers SJ, Klein M, Barlier A, Liu P, Ouafik L, Bours V, Lupski JR, Stratakis CA, Beckers A (2016) Somatic mosaicism underlies X-linked acrogigantism syndrome in sporadic male subjects. Endocr Relat Cancer 23(4):221–233
33. Rodd C, Millette M, Iacovazzo D et al (2016) Somatic GPR101 duplication causing X-linked acrogigantism (XLAG) - Diagnosis and management. J Clin Endocrinol Metab 101:1927–1930. https://doi.org/10.1210/jc.2015-4366
34. Trivellin G, Hernández-Ramírez LC, Swan J, Stratakis CA (2018) An orphan G-protein-coupled receptor causes human gigantism and/or acromegaly: molecular biology and clinical correlations. Best Pract Res Clin Endocrinol Metab 32:125–140. https://doi.org/10.1016/j.beem.2018.02.004

Affiliations

Ericka B. Trarbach[1,2] · Giampaolo Trivellin[3,9] · Isabella P. P. Grande[1] · Felipe H. G. Duarte[2] · Alexander A. L. Jorge[1] · Felipe Barjud Pereira do Nascimento[4,8] · Heraldo M. Garmes[5] · Marcia Nery[6] · Berenice B. Mendonca[7] · Constantine A. Stratakis[3] · Marcello D. Bronstein[2] · Raquel S. Jallad[1,2]

[1] Laboratorio de Endocrinologia Celular E Molecular/LIM25, Disciplina de Endocrinologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil

[2] Unidade de Neuroendocrinologia, Disciplina de Endocrinologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Av Dr Eneas de Carvalho Aguiar, 155, PAMB, 8 andar, São Paulo, SP CEP 05403-010, Brazil

[3] Section on Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD, USA

[4] Faculdade de Medicina, Instituto de Radiologia, Hospital das Clinicas HCFMUSP, Universidade de Sao Paulo, Sao Paulo, SP, Brazil

[5] Divisao de Endocrinologia, Departamento de Clinica Medica, Faculdade de Ciencias Medicas da Universidade Estadual de Campinas (FCM-Unicamp), Campinas, SP, Brazil

[6] Unidade de Diabetes, Disciplina de Endocrinologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil

[7] Unidade de Endocrinologia Do Desenvolvimento, Laboratorio de Hormonios E Genetica Molecular/LIM42, Disciplina de Endocrinologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil

[8] Departamento de Radiologia E Diagnóstico Por Imagem, Hospital Israelita Albert Einstein, São Paulo, SP, Brazil

[9] Endocrinology Unit and Laboratory of Cellular and Molecular Endocrinology, Humanitas Clinical and Research Center-IRCCS, Rozzano, MI, Italy