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ORIGINAL ARTICLE

Germline *AIP* variants in sporadic young acromegaly and pituitary gigantism: clinical and genetic insights from a Han Chinese cohort

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Abstract

Purpose Variants in the Aryl hydrocarbon receptor-interacting protein (*AIP*) gene have been identified in sporadic acromegaly and pituitary gigantism, especially in young patients, with a predisposition to aggressive clinical phenotype and poor treatment efficacy. The clinical characteristics of patients with sporadic acromegaly and pituitary gigantism as well as *AIP* variants in Han Chinese have been rarely reported. We aimed to identify *AIP* gene variants and analyze the clinical characteristics of patients with sporadic acromegaly and pituitary gigantism in Han Chinese.

Methods The study included 181 sporadic acromegaly (N = 163) and pituitary gigantism (N = 18) patients with an onset age of no more than 45 years old, who were diagnosed, treated, and followed up in Huashan Hospital. All 6 exons and their flanking regions of the *AIP* gene were analyzed with Sanger sequencing or NGS. The clinical characteristics were compared between groups with and without *AIP* variants.

Results Germline *AIP* variants were found in 15/181 (8.29%) cases. In patients with an onset age ≤30 years old, *AIP* variants were identified in 12/133 (9.02%). Overall, 13 variants were detected. The pathogenic (P) variants p.R304X and p.R81X were identified in four cases, with two instances of each variant. Six exon variants (p.C254R, p.K103fs, p.Q228fs, p.Y38X, p.Q213*, and p.1115 fs) have not been reported before, which were likely pathogenic (LP). Patients with P/LP variants had younger onset ages, a higher prevalence of pituitary gigantism, larger tumor volumes, and a higher percentage of Ki-67-positive cells in tumors. In addition, the group with P/LP variants showed a less significant reduction of GH levels in an acute octreotide suppression test (OST) [17.7% (0, 65.0%) *vs*. 80.5% (63.9%, 90.2%), $P = 0.001$], and a trend of less GH decrease after the 3-month treatment with long-acting somatostatin analogs (SSAs).

Conclusion Germline *AIP* variants existed in sporadic Chinese Han acromegaly and pituitary gigantism patients and were more likely to be detected in young patients. *AIP* variants were associated with more aggressive tumor phenotypes and less response to SSA treatment.

Keywords Aryl hydrocarbon receptor-interacting protein · Acromegaly · Pituitary gigantism · Somatostatin analogs

Introduction

Acromegaly and pituitary gigantism are caused by persistent hypersecretion of growth hormone (GH) and the following increased synthesis of insulin-like growth factor 1

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(IGF-1) by the liver. Over 95% of the patients harbor a GH-secreting pituitary adenoma. Patients present diverse clinical features and have a significantly increased prevalence of diabetes mellitus and glucose intolerance, hypertension, sleep apnoea, cardiac hypertrophy, osteopathy, cardiovascular events, and colorectal cancers, leading to an increased risk of mortality and a reduced life-span [1, 2].

Genetic variants have been investigated in patients with GH-secreting pituitary adenomas, especially in those who have a familial background. Mutations in *MEN1, PRKAR1A, GPR101*, and *GNAS* have been confirmed to cause familial pituitary tumors, including endocrine neoplasia type 1 (MEN1), Carney complex (CNC), X-linked acrogigantism (X-LAG), McCune-Albright syndrome [3]. Familial isolated pituitary adenomas (FIPA) is another condition where pituitary tumors manifest in two or more family members without affecting other organs [4].In the field of genetic research into FIPA, significant attention has been drawn to the role of the Aryl Hydrocarbon Receptor-Interacting Protein (*AIP*) gene. In 2006, Vierimaa et al. underscored the link between germline variants in the *AIP* gene and an increased predisposition to pituitary adenomas for the first time [5]. Subsequent studies further detailed this relationship within the context of FIPA. Daly et al. reported that approximately 15% of the FIPA families studied (73 in total) exhibited *AIP* mutations [6]. Leontiou et al. quantified this rate at about 35% within a cohort of 26 FIPA families [5]. In these studies, patients with *AIP* variants were also found to be more susceptible to GH-secreting pituitary adenomas [5, 6]. The connection between *AIP* and GH-secreting tumors then attracted considerable attention and has been explored in both familial and sporadic cases of somatotropinomas. In pituitary gigantism, Rostomyan et al. found the prevalence of *AIP* mutations could be as high as approximately 1/3, with 44% of the cases having FIPA [6]. Among sporadic somatotropinomas, the incidence of *AIP* variants varies between 2.5% to 20.5%, depending on the demographic characteristics of the study population [7]. Clinical characteristics have also been investigated in somatotropinomas with *AIP* variants. In an international collaborative study, somatotropinomas with *AIP* variants ($n = 75$) had a significantly younger age of onset, larger tumor size, higher incidence of extrasellar extension and extra-pituitary invasion, and a higher level of GH at diagnosis than the control group ($n = 232$) [8]. Furthermore, the responsiveness to somatostatin analogs (SSAs) in somatotropinomas with *AIP* variants is markedly diminished. This is evidenced by a reduced efficacy in lowering GH and IGF-1 levels, as well as in achieving tumor shrinkage [8]. The pivotal role of *AIP* gene variants in influencing the disease's severity and treatment outcomes was underscored.

The *AIP* gene is located on chromosome 11q13.3, having 6 exons. It encodes an intracellular 330 amino-acid co-chaperone protein [4]. AIP protein has an N-terminal immunophilin-like domain, a highly conserved C-terminal domain with three tetratricopeptide repeat (TPR) motifs, and an α-7 helix, which is important for its functional interactions with partners [9]. *AIP* variants encompass a wide range of types, including deletions, duplications, insertions, nonsense, missense, splice sites, promoter mutations, and large segmental deletions of exons or the entire gene. To date, nearly a hundred mutational sites have been reported, most of which are located in the coding region for the C-terminal of the protein, and substantially impact the TPR (Tetratricopeptide Repeat) motifs and the α-helix structure [3]. Among these, the variant of p.R304X has been the most frequently identified one [10]. Many other recurrent variants have been reported worldwide, such as p.R304Q, p.R271W, p.R81X, etc.

However, studies on *AIP* variants among Han Chinese patients with pituitary adenomas have been limited to date. In a Han Chinese cohort of six familial pituitary adenoma pedigrees and 216 sporadic pituitary adenomas, the occurrence of *AIP* pathogenic variants was 3.88%, while it was 9.30% in patients with somatotropinomas. Five novel variants (one synonymous variant and four missense variants) were found [11]. In another Chinese family with FIPA, a novel missense variant (c.512C>T, p.T171I) was discovered in 3 patients and was proven to be pathogenic [12]. To gain a more detailed understanding of *AIP* variants in Han Chinese patients with sporadic acromegaly and pituitary gigantism, we screened for *AIP* gene variants and analyzed the clinical characteristics in a Han Chinese sporadic acromegaly and gigantism cohort.

Method

Patients

Sporadic acromegaly or pituitary gigantism patients with age of onset under 45 years old admitted to Huashan Hospital from January 2011 to December 2020 were included in the study. They were diagnosed, treated, and followed up in Huashan Hospital. No history of known familial pituitary adenomas was reported in any of the study subjects. Demographic and clinical information was collected and analyzed. The diagnosis adhered to current diagnostic criteria [13], which included clinical manifestations, elevated levels of IGF-1, GH nadir ≥1 ng/ml in oral glucose tolerance test (OGTT), and imaging confirmation of a pituitary lesion. Pituitary gigantism diagnosis requires abnormal growth velocity or final height measurements beyond +2 standard deviations of the mean population, alongside evidence of elevated GH nadir ≥1 ng/ml in OGTT, IGF-1 levels, and imaging evidence of a pituitary lesion.

According to the inclusion criteria, one hundred and eighty-one patients were enrolled. The male/female ratio

was 80/101. The age of onset ranged from 8 to 45 years old, with a median of 25 years old. One hundred and thirty-three (73.5%) cases had an onset age ≤30 years old. Eighteen patients were diagnosed with pituitary gigantism and the other 163 with acromegaly (Table 1).

180 patients received transsphenoidal surgery (TSS) by experienced neurosurgeons. One patient didn't receive TSS but accepted only SSA treatment due to his worries about surgical risk. The pathological types in the study population consisted of 136 somatotropinomas, 32 GH+ prolactin (PRL) adenomas, 7 GH+ thyroid stimulating hormone (TSH) adenomas, 2 GH+ luteinizing hormone (LH) adenomas, 2 GH + PRL + TSH adenomas, and 1 GH + PRL+ adrenocorticotropic hormone (ACTH) adenomas (Table 1).

Baseline GH and IGF-1 levels were measured in the morning after overnight fasting, during the initial diagnostic evaluation before any therapeutic intervention was given. Biochemical parameters were re-evaluated 3 months after TSS. Biochemical remission is defined as GH nadir <1 ng/ml in OGTT and an IGF-1 level within the age- and gender-matched reference range.

Biochemical measurements

GH measurement was conducted by a two-site chemiluminescent immunometric assay AutoDELFIA® hGH(PerkinElmer Life and Analytical Sciences, Wallac Oy, Finland). IGF-1 was quantified using the Immulite 2000 solid-phase, enzyme-labeled chemiluminescent immuno-metric assay (Siemens Healthcare Diagnostic Products Limited, UK). IGF-1 index = IGF-1/the upper limit of the normal range.

Acute octreotide suppression test (OST)

A standard acute 6-hour OST was performed in the initial diagnostic evaluation before any treatment was given. The procedure was described previously [14]. After an overnight fast, blood samples were collected for baseline GH (GH_{0h}) assessment. Then hourly GH measurements were conducted for 6 consecutive ` following the 100 μg subcutaneous octreotide injection. The GH suppression rate was calculated as [GH_{0h}-nadir GH]/GH_{0h}. The results were available in 139 patients.

Long-acting SSA medication

Fifty-three cases received long-acting SSA (either Sandostatin LAR 20 mg per month or Somatuline 40 mg per 2 weeks) therapy for 3 months before surgery, to improve surgery outcomes or decrease surgery risks. Patients' biochemical parameters were measured at the end of the treatment period.

Total treatment score

The total treatment score was used to estimate treatment burdens, which was calculated as previously described [6]. This evaluation utilizes a precise scoring system, assigning a value of 1 to each distinct instance of surgery, radiotherapy, SSA treatment, Pegvisomant administration, and dopamine agonist treatments.

Genetic analysis

AIP gene analysis was performed using standardized Sanger sequencing protocol as described previously [5, 15] or Next Generation Sequencing (NGS). Genomic DNA was extracted from peripheral blood leukocytes by standard procedures (TIANamp Blood DNA Kit (DP348)). Because of the high cost of NGS, patients in the early years were tested by Sanger sequencing. For Sanger sequencing, the 6 exons and their flanking regions of *AIP* were amplificated by PCR, and the products were then sequenced using ABI3730XL and BigDye Terminator v3.1 technology (Applied Biosystems, Foster City, CA). The sequences of the primers are listed in Supplementary Table 1. As NGS became accessible and affordable, 96 recent patients whose genetic samples were preserved in our center were tested using an NGS panel. The gene screened included *AIP, CDKN1B, GNAS, GPR101, MEN1, PRKACB, PRKAR1A, SDHA, SDHAF2, SDHB, SDHC and SDHD*. *AIP* variants were compared with ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), the human gene mutation database (HGMD), human single nucleotide polymorphism databases (dbSNP, http://www.ncbi.nlm.nih.gov/snp), 1000 Genomes, the exome aggregation consortium (ExAC), and Genome Aggregation Database (gnomAD). SIFT (http://sift.jcvi.org), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2), and Mutation Taster (http://www.mutationtaster.org) were used to predict how the mutations affected the functions of AIP protein. The pathogenicity was ranked by Intervar, following the guidelines of the American College of Medical Genetics and Genomics and the Association (ACMG) for Molecular Pathology [16].

Ethical approval

The Ethic Committees of Huashan Hospital approved the study (KY2018-002). Written informed consent was obtained from all the patients or the legal guardians.

Statistics

Statistical analyses were performed using SPSS software (version 21.0, SPSS Inc). Either the T-test (for normally distributed continuous variables) or the Mann-Whitney U

Table 1 An overview of the clinical characteristics of the study population and comparative analyses

	Overall study population	*AIP*neg	*AIP*var (P/LP variants)	*AIPvar* (SNPs excluded)	*AIP*var (Total)	P^a	P^b	P^c
N	181	166	10	12	15	/	/	/
Onset age (years old)	25.0 (22.0, 31.0)	25.0 (22.0, 31.0)	20.5 (13.0, 24.3)	20.5 (13.0, 24.8)	23.0 (13.5, 27.5)	0.010*	0.012*	0.062
Gender (M/F)	80/101	72/94	5/5	5/7	8/7	0.750	0.908	0.637
Pituitary gigantism (%)	9.9 (18/181)	7.83 (13/166)	30.0 (3/10)	41.7 (5/12)	33.3 (5/15)	0.018*	0.003**	0.009**
Baseline GH level (ng/ml)	19.0 (8.2, 43.5)	18.8 (8.1, 39.3)	49.8 (10.9, 92.4)	49.8 (8.2, 70.5)	50.0 (19.4, 63.9)	0.052	0.090	0.016*
Baseline IGF-1 (ug/ml)	775.5 (674.0, 948.5)	791.0 (682.3, 963.5)	757.0 (659.5, 847.5)	739.0 (657.0, 768.0)	739.0 (658.3, 769.5)	0.585	0.335	0.357
Baseline IGF-1 index	2.42±0.85	2.46±0.84	1.69±0.58	1.75±0.59	1.96±0.81	0.007**	0.006**	0.041*
Tumor volume (cm^3)	3.9 (0.9, 7.9)	3.1 (0.8, 7.9)	7.8 (4.9, 19.6)	12.2 (5.5, 21.3)	7.8 (4.2, 19.0)	0.008**	0.002**	0.010*
Tumor maximum diameter (cm)	2.2 (1.5, 3.0)	2.0 (1.5, 3.0)	3.0 (2.5, 4.0)	3.3 (2.5, 4.0)	3.0 (2.4, 3.8)	0.004**	0.002**	0.010*
Invasion of the cavernous sinus (%)	62.6 (102/163)	61.1 (91/148)	88.9 (8/9)	81.8 (9/11)	78.6 (11/14)	0.156	0.214	0.329
Remission rate after TSS (%)	39.1 (61/156)	40.4 (57/141)	30.0 (3/10)	25.0 (3/12)	26.7 (4/15)	0.740	0.368	0.447
GH suppression rate in OST	78.9 (56.3, 89.3)	80.5 (63.9, 90.2)	17.7 (0, 65.0)	17.7 (2.1, 65.0)	23.7 (4.1, 78.9)	0.001**	0.001**	0.002**
Reduction of GH after pre-operative SSAs (%)	55.5 (10.5, 69.6)	55.7 (13.4, 69.8)	49.8 (10.9, 66.2)	49.8 (10.9, 66.2)	43.8 (0, 56.2)	0.605	0.605	0.169
Reduction of IGF-1 after pre-operative SSAs (%)	20.6 (3.4, 36.2)	20.0 (3.2, 34.7)	30.2 (21.1, 40.1)	30.2 (21.1, 40.1)	24.3 (20.1, 38.7)	0.268	0.268	0.382
Normalized IGF-1 after pre-operative SSAs (%)	15.0 (9/60)	13.2 (7/53)	25.0 (1/4)	25.0 (1/4)	28.6 (2/7)	0.464	0.464	0.281
GHoma	136 (75.6%)	125 (75.8%)	7(70.0%)	8 (66.7%)	11 (73.3%)	/	/	/
GH+PRL adenoma	32 (17.8%)	28 (17.0%)	3(30.0%)	4 (33.3%)	4 (26.7%)			
GH+TSH adenoma	7 (3.9%)	7 (4.2%)	0	0	0 (0)			
GH+LH adenoma	2 (1.1%)	2 (1.2%)	0	0	0 (0)			
GH+PRL+TSH adenoma	2 (1.1%)	2 (1.2%)	0	0	0 (0)			
GH+PRL+ACTH adenoma	1 (0.5%)	1 (0.6%)	0	0	0 (0)			
Ki-67 (%)	2.0 (1.0, 3.0)	2.0 (1.0, 3.0)	4.0 (3.0, 8.0)	4.5 (3.0, 8.0)	4.0 (2.5, 8.0)	0.003**	0.005**	0.024*
p53 (-/weak+/+)	41/39/20	38/37/17	2/1/2	3/1/3	3/2/3	0.586	0.566	0.473
SSTR2 (-/weak+/+)	4/10/77	4/7/73	0/2/4	0/2/5	0/3/4	0.218	0.225	0.053
Total treatment score	2.0 (1.0, 3.0)	2.0 (1.0, 3.0)	2.5 (1.0, 3.0)	2.0 (1.0, 3.0)	3.0 (1.0, 3.0)	0.833	0.908	0.462

AIPvar patients with *AIP* mutational variations, *AIP*neg patients without *AIP* mutational variations, *SNPs* single nucleotide polymorphisms, *P/LP* pathogenic and likely pathogenic, *GH* growth hormone, *IGF-1* insulin-like growth factor 1, *IGF-1* index the ratio of IGF-1 level to the upper limit value of the age- and gender-match IGF-1 reference range, *TSS* transsphenoidal surgery, *OST* octreotide suppression test, *SSAs* somatostatin analogues, *GHoma* GH-secreting adenoma or somatotropinoma, *PRL* prolactin, *TSH* thyroid stimulating hormone, *LH* luteinizing hormone, *ACTH* adrenocorticotropic hormone, *SSTR2* somatostatin receptor 2

*$P < 0.05$; **$P < 0.01$

[a]Comparison between *AIP*var (P/LP variants) and *AIP*neg

[b]Comparison between *AIP*var (SNPs excluded) and *AIP*neg

[c]Comparison between *AIP*var (Total) and *AIP*neg

test (for abnormally distributed continuous variables) was used to compare continuous variables between groups. Differences in the categorical variables between groups were estimated using the $\chi 2$ test. Ordered categorical variables were compared using the Wilcoxon rank sum test. A two-tailed P value < 0.05 was considered statistically significant.

Results

AIP sequencing

In the whole cohort, the prevalence of *AIP* variants was 15/181 (8.29%). Among those with an onset age≤30 years old (n = 133), *AIP* variants were identified in 12 (9.02%) patients. A summary of the detected variants is presented in Table 2. Overall, 13 variants were detected, including 4 synonymous variants, 6 nonsense variants, 3 frameshift variants, 1 nonsynonymous variants of exons, and 1 transversion variants of introns. The variants of p.R304X and p.R81X have been commonly identified as pathogenic (P) before. The prevalence of p.G83G, p.G21G, and p.T48T in the general population ranged between 4×10^{-6} and 4×10^{-4} according to the databases of 1000 Genomes and gnomAD, and they were considered as single nucleotide polymorphisms (SNPs) [p.G83G (dbSNP: rs104895072), p.G21G (dbSNP: rs199913396), p.T48T (dbSNP: rs772658134), respectively]. The clinical significance of p.A299A remained unclear. Six exon variants (p.C254R, p.K103fs, p.Q228fs, p.Y38X, p.Q213*, and p.1115 fs) have been rarely reported before. They were predicted to be likely pathogenic (LP). For the variant in intron1: c.99+2T>A, almost no population record has been reported and the clinical significance remains unknown.

In the 96 patients who were tested by NGS, one male patient of 33 years old was found to carry heterozygous *GNAS* variants associated with McCune Albright Syndrome (MAS): *GNAS*: NM_016592.3:exon1:c.166G>A:p.A56T and *GNAS*: NM_016592.3:exon1:c.167C>T:p.A56V. ACMG category is a variant of uncertain significance (VUS). No *AIP* variant was detected in this patient. He did not present the hallmark clinical features of MAS, such as uneven skin pigmentation, fibrous dysplasia, or precocious puberty, alongside his acromegaly, so he was still included in the study. No other gene variants were identified in these patients.

Clinical characteristics of subjects with *AIP* variants

The 15 patients with *AIP* variants included 8 males and 7 females. They had a median onset age of 23 years old, ranging from 13 to 45 (Tables 1 and 2). Five (33.3%) of them were diagnosed with pituitary gigantism. Among the 14 cases with available imaging data, the median tumor volume was 7.8 cm^3, ranging from 4.2 to 19.0 cm^3. 78.6% (11/14) showed invasion of the cavernous sinus. Of the 7 patients who received pre-operative 3-month SSA treatment, only 2 achieved normal IGF-1 levels at the end of the medical treatment period. All the 15 patients received transsphenoidal surgery. Only 4 (26.7%) patients achieved biochemical remission 3 months after surgery. Histopathological analyses revealed 11 somatotropinomas and 4 GH + PRL adenomas. Among the somatotropinomas, 81.8% (9/11) exhibited a sparse granulation (SG) pattern.

Comparisons between cases with and without *AIP* variants

To better examine the clinical features of patients with *AIP* variants, we conducted a comparative analysis between the group with (*AIP*var) and without (*AIP*neg) *AIP* variants. Specifically, to elucidate the impact of irrelevant variants on clinical phenotypes, we further focused on comparing the P/LP variants group with the *AIP*neg group. Additionally, considering that the vast majority of SNPs are not associated with disease, we also conducted the comparative analysis between the SNPs-excluded group and the *AIP*neg group (Table 1).

The age of onset was significantly younger in the P/LP variants group than in the *AIP*neg group, [20.5 years (13.0, 24.3) vs. 25.0 years (22.0, 31.0), $P = 0.010$]. The difference persisted when excluding SNPs from the total *AIP*var group, yet it became insignificant for the overall group of variants. In contrast to the *AIP*neg group, the *AIPvar* (Total), P/LP variants, and SNPs-excluded group all had a higher prevalence of pituitary gigantism. Interestingly, although the *P/LP variants* group showed a trend towards higher baseline random GH levels [49.8 ng/ml (10.9, 92.4) *vs*. 18.8 ng/ml (8.1, 39.3), $P = 0.052$], IGF-1 levels were comparable between the two groups [757.0 ug/ml (659.5, 847.5) vs. 739.0ug/ml (658.3, 769.5)], and IGF-1 indexes were even lower in the *P/LP variants* group (1.69 ± 0.58 *vs*. 2.46 ± 0.84, $P = 0.007$). Tumor diameters and volumes were markedly larger across all three methods of grouping. Invasion of the cavernous sinus, although not significantly different across the groups, exhibited a higher trend in variant groups.

As for treatment efficacy, the biochemical remission rate after TSS was relatively lower in variant groups although with no statistical significance. Data on acute OSTs were available in 9/10 for the P/LP variants group, 12/12 for the SNPs-excluded group,13/15 for the total *AIP*var group, and 126/166 for the *AIP*neg group. We found that the GH suppression rates in all three variant groups were markedly lower than those in the *AIP*neg

Table 2 A summary of the detected *AIP* mutational variations and the patient clinical characteristics

	No	Gender	Onset age	*AIP* sequence variation	Mutation type	Random GH level (ng/ml)	IGF-1 (ug/ml)	IGF-1 index	Tumor volume (cm^3)	Invasion of the cavernous sinus	Histopathological findings	Granulation pattern	Remission after TSS	Pituitary gigantism	Reduction of GH after SSAs (%)	Reduction of IGF-1 after SSAs (%)	Normalized IGF-1 after SSAs
	ACMG rating																
1	F	18	exon6: c.910C>T: p.R304X	nonsense	6.88	760.0	2.12	3.74	Yes	GHoma	SG	No	Yes	NA	NA	NA	P
2	M	13			49.69	757.0	1.04	25.66	Yes	GHoma	SG	No	Yes	0	41.37	No	P
3	F	23	exon2: c.241C>T: p.R81X	nonsense	77.31	662.0	1.85	21.99	Yes	GHoma	DG	No	No	55.84	20.09	No	P
4	F	24			50	657.0	1.84	16.64	Yes	GHoma	SG	Yes	No	NA	NA	NA	P
5	M	22	exon2: c.249G>T: p.G83G	synonymous	107.69	1348-.0	3.77	9.82	Yes	GHoma	DG	No	No	0	0	No	VUS, possible SNP
6	M	30	exon1: c.63C>T: p.G21G	synonymous	84.23	687.0	2.42	1.6	No	GHoma	SG	No	No	56.58	62.22	Yes	VUS, possible SNP
7	F	13	exon3: c.307_308del: p.K103fs	frameshift	166.92	1208-.0	1.21	5.89	Yes	GHoma	SG	No	Yes	43.78	36.09	Yes	LP
8	M	24	exon5: c.682_683del: p.Q228fs	frameshift	12.23	717.0	2	9.33	No	GHoma	SG	No	No	NA	NA	NA	LP
9	F	25	exon5: c.760T>C: p.C254R	nonsynonymous	28.23	927.0	2.59	6.28	Yes	GH + PRL adenoma	/	No	No	69.59	24.32	No	LP
10	M	42	exon2: c.144C>T: p.T48T	synonymous	50	739.0	2.60	0.39	Yes	GH + PRL adenoma	/	No	No	NA	NA	NA	VUS, possible SNP
11	F	33	exon6: c.897G>T: p.A299A	synonymous	3.04	739.0	2.60	19.24	Yes	GHoma	SG	Yes	No	0	20.16	No	VUS
12	M	34	exon2:c.114C>-A: p.Y38X	nonsense	5.99	643.0	1.80	5.24	Yes	GH + PRL adenoma	/	Yes	No	NA	NA	NA	LP
13	F	13		nonsense	137.80	N/A	N/A	3.13	N/A	GHoma	SG	No	No	NA	NA	NA	LP

Table 2 (continued)

No	Gender	Onset age	*AIP* sequence variation	Mutation type	Random GH level (ng/ml)	IGF-1 (ug/ml)	IGF-1 index	Tumor volume (cm^3)	Invasion of the cavernous sinus	Histopathological findings	Granulation pattern	Remission after TSS	Pituitary gigantism	Reduction of GH after SSAs (%)	Reduction of IGF-1 after SSAs (%)	Normalized IGF-1 after SSAs	ACMG rating
			exon4: c.637 c>t: p.Q213*														
14	M	13	exon3: c.344delt: p.I115fs	frameshift	50	768.0	0.77	18.85	Yes	GH + PRL adenoma	/	Yes	No	NA	NA	NA	LP
15	M	14	intro-n1:c.99+2T>A	transversion	50	582.0	1.41	142.94	Yes	GHoma	SG	No	Yes	NA	NA	NA	VUS

GH growth hormone, *IGF-1* insulin-like growth factor 1, *SSTR2* somatostatin receptor 2, *SG* sparse granulation pattern, *DG* dense granulation pattern, *GHoma* GH-secreting adenoma or somatotropinoma, *PRL* prolactin, *N/A* not available, *TSS* transsphenoidal surgery, *LP* Likely pathogenic, *P* pathogenic, *VUS* variant of uncertain significance

group. In the respective groups—P/LP variants, SNPs-excluded, total AIPvar, and AIPneg—4 out of 10, 4 out of 12, 7 out of 15, and 46 out of 166 patients received a 3-month pre-operative SSA medication. Reductions in GH after SSA therapy reached a median of 49.8% in the P/LP variants group and SNPs-excluded group, and 43.8% in the total *AIP*var group. These reductions were less marked compared to the 55.7% reduction seen in the *AIP*neg group, although the differences were not statistically significant ($P = 0.605$, 0.605, and 0.169 respectively). However, the decrease in IGF-1 levels tended to be greater in the P/LP variants group (30.2% *vs*. 20.0%, $P = 0.268$), and normalization of IGF-1 also showed a higher trend in this group (25.0% *vs*. 13.2%, $P = 0.464$). Total treatment scores showed no significant differences across the groups, with a median of 2.5 for the P/LP variants group, 2.0 for the SNPs excluded group, and 3.0 for the total *AIP*var group, compared to 2.0 in the *AIP*neg group.

The most common histopathological types were somatotropinomas for all groups, accounting for no less than 70%. Besides, the *AIPvar* group had GH + PRL adenoma. While the *AIPneg* group had other histopathological types including GH + PRL adenomas (n = 28), GH + TSH adenomas (n = 7), GH + LH adenomas (n = 2), GH + PRL + TSH adenomas (n = 2), GH + PRL + ACTH adenoma (n = 1). Ki-67 proliferation index was higher in the total *AIP*var, P/LP variants, and SNPs-excluded group ($P = 0.024$, 0.003, and 0.005 respectively). There were no significant differences in p53 and somatostatin receptor 2 (SSTR2) expression across groups.

Discussion

The association between *AIP* variants and both familial and sporadic pituitary adenomas is well-documented in Western countries. In FIPA, the prevalence of *AIP* variants has been reported to be 15-35% [6, 7]. In sporadic somatotropinomas, the prevalence ranged from 2.5% to 20.5%, depending on the selected subjects [7]. In cases of pituitary gigantism, the proportion was even higher. Rostomyan et al. reported that 29% of affected individuals had *AIP* variants in an international collaborative study of 208 patients with pituitary gigantism [6]. And patients under 30 years old had a higher mutation rate [17–19]. However, similar studies in China were scarce. In one of the few Chinese studies, *AIP* pathogenic variants were found in 9.3% of 86 sporadic somatotropinomas [11]. In our cohort, *AIP* variants were identified in 8.29% of the patients with somatotropinomas, with a higher incidence of 9.02% among those with an onset age of ≤30 years. These results are similar to previous studies.

In the genetic analysis of the cohort, Cases No.1 and No.2, both harboring the p.R304X variant, exhibited aggressive clinical features including early onset (≤20 years), pituitary gigantism, macroadenoma, cavernous sinus involvement, a sparse granulation pattern in somatotropinomas, reduced SSA responsiveness (No.2), and failure to achieve biochemical remission post-TSS. This was in accordance with previous reports [10, 17, 18]. The variant of p.R304X was first found in an Italian cohort and was then proven one of the most common pathogenic variants [10, 17, 18]. The p.R81X variant, identified in patients No.3 and No.4, was previously reported in FIPA families [5]. The pathogenic variant led to functional inactivation and could block the AIP protein from interacting with phosphodiesterase 4A (PDE4A) [19]. Consequently, patients No.3 and No.4 experienced early onset, large tumors with cavernous sinus invasion, lack of biochemical remission, and poor response to SSA treatment (in No.3). Eleven novel *AIP* variants were detected. The variations of p.K103fs, p.Q228fs, p.C254R, p.Y38X, p.Q213*, and p.1115 fs (detected in No.7, No.8, No.9, No.12, No.13, and No.14 respectively) were predicted to be likely pathogenic. The 6 patients presented aggressive clinical characteristics, such as young onset age, macroadenoma, and cavernous sinus invasion (in No.7, 9, 12, 14). The clinical significance of p.G83G, p.G21G, and p.T48T (detected in No.5, No.6, and No.10) are uncertain. Based on gene database searches and amino acid alterations, they are considered SNPs. However, 2 of them (No.5, and No.10) had cavernous sinus invasion and none of them achieved remission after TSS. The variant in exon6: c.897G>T: p.A299A in patient No.11 and intron1: c.99+2T>A in patient No.15 have never been reported. Their clinical significance is uncertain. However, patient No.11 had cavernous sinus invasion and poor response to SSAs, and patient No.15 had an onset age of only 14 years old, presented as gigantism, and an extremely large tumor. Therefore these two variations might be pathogenic. Further research is required to ascertain the pathogenicity of these variants.

Comparing the clinical characteristics of patients with and without *AIP* variants, we found that the P/LP variants group had younger onset ages, a higher prevalence of pituitary gigantism, higher baseline GH levels, larger tumor sizes, and higher Ki-67 positive cells. However, the variations in onset ages ceased to be statistically significant for the total *AIP*var group. The analyses of P/LP variants instead of the total AIP variants are more likely to offer a more accurate reflection of the true differences. Overall, the aggressive clinical phenotypes observed in the variants group align with the findings reported by Daly et al. [8]. Furthermore, the incidence of cavernous sinus invasion was relatively higher among *AIP*var subjects, consistent with observations made by Marques et al. [20]. The differences in the aggressiveness of clinical phenotypes led to a trend of higher total treatment scores in *AIP*var patients, but statistical significance was not achieved. This may attributed to the elusive accessibility of pegvisomant and cabergoline in China, which decreased the score levels and dissimulated the difference between groups.

In our cohort, the AIP variant groups exhibited higher baseline GH levels. However, the difference in baseline IGF-1 levels was less pronounced. This phenomenon has been previously reported. In the research of Marques P et al., *AIP*-mutated somatotropinomas had notably lower IGF-1 indexes than the non-mutated ones. Daly AF et al. found significantly higher GH levels in *AIP*-mutated somatotropinomas than the control group while the distinction of IGF-1 levels was insignificant [8]. The mechanism underlying the disproportion remained unclear. GH resistance in the liver resulting in decreased IGF-1 production could be an explanation [21]. However, a direct link between *AIP* variants and liver GH resistance has not been reported. Hanson et al. found that in rainbow trout in vitro, the expression of IGF mRNAs is directly inhibited by environmental estrogens, which disrupt GH post-receptor signaling pathways in an estrogen receptor (ER)-dependent manner [22]. Additionally, a study examining the connection between AIP and ERα has been reported. This study revealed that AIP served as a negative regulator of ERα transcriptional activity, potentially mitigating ERα-dependent cellular processess [23]. Consequently, it can be speculated that mutation of *AIP* may decrease its inhibitory effect on ERα and enhance the interference effect of estrogens on post-receptor signaling of GH receptor, leading to decreased IGF-1 production. Further research is crucial to confirm this hypothesis and explore other potential mechanisms.

In our study, *AIP*var groups showed a marginal decrease in random GH levels after three months of long-acting SSA therapy. Acute OST, which was suggested to be a valid tool in predicting responses to long-acting SSAs in GH-secreting adenomas in our previous study [14], was also compared, and a strikingly reduced suppression of GH levels in all the variant groups during acute OST was observed. These findings were aligned with those reported by Daly et al. They observed a significantly lesser reduction in GH and IGF-1 levels following SSA treatment in patients with *AIP* variants [8]. Reduced immunohistochemical staining for AIP has been associated with a diminished response to SSAs [24–26]. Studies have delved into the molecular mechanisms underlying AIP's role in conferring resistance to SSAs. It was reported that *AIP* knock-out in mouse embryonic fibroblast cell lines and murine pituitary adenoma cell lines led to elevated cAMP concentrations through defective Gαi-2 and Gαi-3 [27], which diminished the efficacy of SSAs. In addition, higher

immunohistochemical expression of ZAC1 was found to correlate with a greater likelihood of IGF-1 normalization and tumor shrinkage following SSA treatment in patients with somatotropinomas [28]. And ZAC1 expression decreased following *AIP* knockout in GH3 cells [29]. This may also be a potential explanation. The precise mechanism warrants further investigation. The reduction in IGF-1 levels between the *AIP*var and *AIP*neg groups was marginally different in our study. In Daly's report, both GH and IGF-1 levels exhibited consistently smaller reductions following treatment with SSAs in the mutation group [8]. A potential reason for the discrepancy between our results and those of previous research may be the small sample size, as well as the bias introduced by the younger age of our cohort, which enrolled patients who were under the age of 45. Previous reports indicate that younger patients often exhibit a lower response to SSA therapy [30, 31]. In addition, the proportion of SSTR2-positive tumors was relatively higher in the *AIP*neg group, which may also contribute to our result. Another explanation might be attributed to the inherent stability of IGF-1, as compared to GH. Given our shorter treatment duration relative to Daly's study (3 months vs. 12 months or more), it's plausible that our timeframe was inadequate for substantial changes in IGF-1 levels to emerge.

There are some limitations in our study. First, the sample size of the *AIP*var group is small, bringing in inevitable bias. Besides, NGS wasn't conducted in all cases, so rare deletions of the *AIP* gene could be missed. Also, we have not conducted tests to determine the carrier status of the offspring of the AIP-affected individuals included in our research. Despite these limitations, our findings lay the groundwork for future studies. Addressing these issues in subsequent research will be vital for refining our understanding of the *AIP* gene's role in pituitary adenomas.

Conclusion

Germline *AIP* variants were observed in young Chinese Han patients with sporadic acromegaly and pituitary gigantism. These variants correlated with more aggressive tumor phenotypes and diminished responsiveness to SSA therapy.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethics approval and consent to participate The study was approved by the Ethic Committees of Huashan Hospital (KY2018-002). Written informed consent was obtained from all the patients or the legal guardians.

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