Pituitary gigantism due to a novel AIP germline splice-site variant

Elisa Lamback1,2,3, Renan Lyra Miranda2, Leila Chimelli2, Felipe Andreiuolo2, Leandro Kasuki1,3,4, Luiz Eduardo Wildemberg1,3, Mônica R. Gadelha1,2,3

1 Neuroendocrinology Research Center, Endocrinology Section, Medical School and Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Brazil; 2 Neuropathology and Molecular Genetics Laboratory, Instituto Estadual do Cérebro Paulo Niemeyer, Secretaria Estadual de Saúde, Rio de Janeiro, Brazil; 3 Neuroendocrine Unit, Instituto Estadual do Cérebro Paulo Niemeyer, Secretaria Estadual de Saúde, Rio de Janeiro, Brazil; 4 Endocrinology Division, Hospital Federal de Bonsucesso, Rio de Janeiro, Brazil

Corresponding author: Elisa Lamback, MD, MSc
ORCID: 0000-0002-6026-4329
Email: elisalamback@gmail.com

Summary

Pituitary gigantism is a rare pediatric disorder caused by excess growth hormone (GH) secretion. In almost 50% of cases, a genetic cause can be identified, with pathogenic variants in the aryl hydrocarbon receptor interacting protein (AIP) gene being the most common. We present a case of a 11-year-old boy who exhibited progressive vision loss, associated with accelerated linear growth and weight gain. On physical examination, he had enlarged hands, right eye amaurosis, and was already above his target height. Increased GH and IGF-I concentrations confirmed the diagnosis of pituitary gigantism. A magnetic resonance imaging showed a giant sellar lesion with supra- and para-sellar extensions. He underwent two surgeries which did not achieve cure or visual improvement. Histopathological analysis revealed a sparsely granulated tumor, negative for somatostatin receptor type 2 (SST2) and immunoreactivity score of 6 for somatostatin receptor type 5 (SST5). Our published artificial intelligence prediction model predicted an 83% chance of not responding to first-generation somatostatin receptor ligands. Pasireotide was therefore prescribed and afterwards cabergoline was added on. IGF-I concentrations decreased, but did not normalize. We discovered a novel germline single nucleotide variant in the splicing donor region of intron 2 of AIP gene (NM_003977.4:c.279+1 G>A), classified as likely pathogenic according to the American College of Medical Genetics and Genomics guidelines.

Key Words: gigantism, AIP, SST2, SST5, pasireotide

Learning points

• Young-onset AIP-related pituitary tumors are associated with invasive, large and treatment-resistant somatotroph tumors.
• Intronic splice variants can lead to truncated protein possibly explaining the clinical findings.
• Pasireotide biochemical response depends upon the presence of SST (SST2 and SST5) and possibly post-receptor mechanisms, as AIP.

Background

Gigantism is a rare pediatric disorder caused by excess growth hormone (GH). In almost 50% of patients, a genetic cause can be identified, mainly familial isolated pituitary adenoma caused by the aryl hydrocarbon receptor interacting protein (AIP) variants and X-linked acro-gigantism (X-LAG), or McCune Albright syndrome (Rostomyan L et al, 2015). Over 100 germline variants have been reported in AIP gene, with less than 20 splice site variants described (Caimari F et al, 2018). We report a novel germline splice-site variant in AIP in a young boy with gigantism.

Case presentation
An 11-year-old boy presented with progressive vision loss for at least 8 months, accelerated linear growth and weight gain. On physical examination, he was above his target height [154.5cm (p85-97), target height of 171.7 cm/p23.6], weight 66kg (p10-p25), with enlarged hands, Tanner stage G2P3 (Figure 1).

Increased GH of 32.7 ng/mL and insulin-like growth factor I (IGF-I) concentrations of 901 ng/mL (reference range 69-316) confirmed the diagnosis of gigantism.

A visual field revealed right eye amaurosis and loss of superior quadrants in the left eye. Sellar magnetic resonance imaging showed a large and invasive tumor (Figure 2).

**Investigation**

All exons and splicing sites of AIP were sequenced using Sanger sequencing, in blood leukocytes. We encountered a novel single nucleotide variant (SNV) in the splicing donor region of intron 2: NM_003977.4:c.279+1 G>A (Figure 3). Sequencing of the patient’s tumor DNA showed loss of heterozygosity (LOH).

The parents’ sequencing showed that the 48-year-old asymptomatic mother also had the same variant. The patient’s mother has normal IGF-I and monomeric prolactin serum concentrations. The family does not have any known history of pituitary disease, tall stature or infertility. The patient has no siblings from the mother’s part of the family.

Using FATHMM-XF (Rogers MF et al. 2018) and Eigen bioinformatics tools, the variant was considered pathogenic (non-coding score of 0.991 and 0.913, respectively). Considering the American College of Medical Genetics and Genomics (ACMG) guidelines, it is considered likely pathogenic [one very strong and one moderate pathogenic criteria (PVS1 and PM2)].

We performed *in silico* prediction using SpliceAI (Jaganathan K et al. 2019). The SNV results in a loss of the canonical splice-donor site of exon 2 (SpliceAI ΔScore= -0.99) and can possibly activate one of two nearby cryptic splice-sites (CSS) (Figure 4). The first CSS occurs at position NM_003977.4:c.279+11 (ΔScore= 0.27) and leads to an insertion of 11 bases and a frameshift. The second CSS occurs at position NM_003977.4:c.279+24 (ΔScore= 0.28) and results in an insertion of 24 bases from intron 2 (8 amino acids inserted in the final protein). A ΔScore 0.20-0.35 has a validation rate of 20 to 40%; and a ΔScore 0.80-1.00 a validation rate >80%, indicating that changes with higher scores are more likely to occur and cause impact. Polymerase chain reaction from complementary DNA of the AIP RNA from tumor sample was performed and showed several different sequences: 1- we were still able to identify an AIP mRNA equivalent to NM_003977.4 reference sequence; 2- we confirmed the predicted NM_003977.4:c.279+24 CSS; 3- we identified a 2 bases insertion from intron 2 that results in a frameshift and would produce a truncated protein; 4- a smaller sequence that is more expressed than all others that lacks exon 2 and 3; 5- a sequence that lacks exon 2 – Supplementary Materials.

**Treatment**

He underwent transcranial and transsphenoidal surgeries which did not achieve cure or visual improvement. Gross total resection was not expected as the patient had a very invasive tumor (Knosp 4). Unfortunately, not even vision was improved. Histopathological report is shown in Figure 5. The diagnosis of a sparsely granulated tumor was made, with negative SST2, moderate expression in around 30% for SST3 (immunoreactive score; IRS 4) and moderate expression in around 60% for SST5 (IRS 6). Prolactin was positive in only sparse cells (around 3%) and was considered as negative. This percentage of positive cells was not sufficient to consider the adenoma as a co-secreting tumor (Dottermusch M et al, 2024).

Our artificial intelligence model predicted an 83% chance of not responding to first-generation somatostatin receptor ligand (fg-SRL) (Wildemberg LE et al, 2021).

Intramuscular pasireotide was started at a dose of 60mg every 28 days, with IGF-I concentrations falling >20%, but not normalizing [before: GH 5.0ng/mL, IGF-I 736ng/mL (69-316); four months after pasireotide: GH 12.9ng/mL, IGF-I 575ng/mL (143-506)]. Oral cabergoline was added-on at a dose of 0.5mg 3x/week, without IGF-I normalization [one month after cabergoline: GH 12.3ng/mL; IGF-I 595ng/mL (143-506)].

**Outcome and follow-up**

Patient developed pre-diabetes with pasireotide [glucose 121 mg/dL, glycated hemoglobin A1C 6.1%]. He gained 9.0 cm in the last 13 months (present height 163.5cm at the age of 12 years and five months – Figure 1) and the tumor did not exhibit signal intensity change or shrinkage – Figure 2. The patient is...
undergoing radiotherapy and will receive pegvisomant. His treatment will be pasireotide combined with pegvisomant.

**Discussion**

The *AIP* variant reported has no registry on Clinical Genome Resource or ClinVar database. SNV in splice-sites can cause different effects on mRNA, and in our case, we could predict two possible outcomes: a frameshift that would result in a stop codon in codon 159 at the end of exon 3 and a truncated protein or an insertion of 24 bases. Splice variants leading to truncating variations in *AIP* have been considered as disease-causing (Boguslawska A & Korbonits M, 2021). Also, since the predicted scores of the new splice-donor sites were weak (< 0.40) we have to consider that the SNV could lead to more complex alterations during splicing and whole or multiple exons skipping.

Patients with pathogenic variants in *AIP* present early onset of symptoms, are more often males, with aggressive tumors, requiring multimodal therapy (Marques P et al, 2020), as seen in our patient. Poor response to fg-SRL has been described (Daly AF et al, 2010). In our case, since the response to fg-SRL was very unlikely because of negative SST2 expression and isointensity on T2, we chose not to use it. Pasireotide was our initial choice. In three cases, pasireotide normalized IGF-I in patients resistant to fg-SRL with *AIP*-mutations (Daly AF et al, 2019; Rostomyan L et al, 2017). However, in ours and in another case, normalization of IGF-I was not seen (van Santen SS et al, 2021), illustrating that *AIP* variants might be involved in treatment resistance. Pegvisomant may be an alternative, however, *AIP*-mutated tumors are often more aggressive and pegvisomant has no tumoral effect (Giustina A et al, 2017). However, in cases like ours, both IGF-I control and tumor size control can be achieved with combined pegvisomant and SRL (Coopmans EC et al, 2022).

In conclusion, we describe a novel germline splice variant in *AIP* leading to gigantism. The patient had a sparsely granulated tumor, with a modest response to pasireotide.
<table>
<thead>
<tr>
<th>Funding statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>This research received a grant from Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq) to MRG.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Declaration of interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL has received speaker fees from Ipsen. LK has received speaker fees from Ipsen and Novo Nordisk. LEW has received speaker fees from Ipsen and Recordati, and is sub-investigator in clinical trials from Recordati and Crinetics. MRG has received speaker fees from Recordati, Ipsen and Novo Nordisk, has served as a member of the advisory board of Recordati, Ipsen, Novo Nordisk and Crinetics, and as principal investigator in clinical trials from Recordati and Crinetics. The other authors report no conflict of interest.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient consent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Written informed consent was obtained from the patient’s mother for publication of the submitted article and accompanying images.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author contributions and acknowledgements</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL gathered the data and wrote the initial draft, RLM did the Sanger sequencing and pathogenic variant analysis, FA and LC were responsible for the histological slides, LEW is the patient’s main physician and with LK and MRG critically reviewed the draft. All authors accepted the final version of the draft.</td>
</tr>
</tbody>
</table>
References


Legends to figures

**Figure 1 – Physical findings**
A-Photograph of the patient’s enlarged left hand compared to Dr. Lamback’s hand (adult female) that measures 17cm (arrow).
B-Growth chart showing the patient’s height (dot) and target height.

**Figure 2.** Magnetic resonance imaging
Sagittal T1 post-contrast (A), coronal T1 post-contrast (B) and coronal T2 (C) imaging prior to surgery demonstrates a 5.6 x 4.7 x 5.8 cm (transverse x anterior-posterior x craniocaudal) invasive sellar lesion (Knosp 4) with suprasellar extension, compressing the optic chiasm, isointense on T2 and with heterogeneous contrast enhancement, suggestive of cystic or necrotic degeneration.
Sagittal T1 post-contrast (D), coronal T1 post-contrast (E) and coronal T2 (F) imaging after the two surgeries exhibiting partial tumor resection.
Sagittal T1 post-contrast (G), coronal T1 post-contrast (H) and coronal T2 (I) after 5 months of pasireotide and 2 months of cabergoline showing no change in signal intensities or tumor shrinkage. The fat graft in the middle of the tumor reabsorbed partially.

**Figure 3. Germline AIP sequencing**
A- Reference sequence NG_008969
B- The patient’s blood sample sequencing exhibiting a single nucleotide variant in the splicing donor region of intron 2 with a nucleotide substitution of G>A in position 279+1.
C- The mother’s sequencing demonstrating the same variant.
D- The father was wild type.
E- The patient’s tumor sample sequencing demonstrating LOH.

**Figure 4.** Effects of variant NM_003977.4:c.279+1 G>A on splice-donor region
The presence of the variant results in loss of the canonical splice-site (red column, SpliceAI ΔScore= -0.99) and increases the chance of activating two possible cryptic splice-sites (green columns, ΔScore= 0.27 and ΔScore= 0.28).

**Figure 5.** Main histological findings
A: Hematoxylin and eosin-stained section showing monomorphic tumor cells, displaying round to oval nuclei with conspicuous nucleoli and eosinophilic cytoplasm. A mitotic figure is shown in the insert in upper right of the panel (up to three mitoses were found in ten 0.237 square mm high-power fields).
B: Immunohistochemistry for growth hormone depicts cytoplasmic positivity in part of tumor cells.
C: CAM5.2 immunostaining highlights cytoplasmic fibrous bodies in tumor cells, characterizing a sparsely granulated tumor.
D: The proliferation index assessed semi-quantitatively (Ki-67 immunostaining; MIB-1 clone) was overall around 4%.
E: Somatostatin receptor type 2 (SST2; UMB1 clone) immunostaining showing no expression in tumor cells.

Scale bars equal 30 micrometers in A and B, and 60 micrometers in D to F.
Figure 1 – Physical findings
A-Photograph of the patient’s enlarged left hand compared to Dr. Lamback’s hand (adult female) that measures 17cm (arrow).
B-Growth chart showing the patient’s height (dot) and target height.

158x60mm (220 x 220 DPI)
Figure 2. Magnetic resonance imaging

Sagittal T1 post-contrast (A), coronal T1 post-contrast (B) and coronal T2 (C) imaging prior to surgery demonstrates a 5.6 x 4.7 x 5.8 cm (transverse x anterior-posterior x craniocaudal) invasive sellar lesion (Knosp 4) with suprasellar extension, compressing the optic chiasm, isointense on T2 and with heterogeneous contrast enhancement, suggestive of cystic or necrotic degeneration.

Sagittal T1 post-contrast (D), coronal T1 post-contrast (E) and coronal T2 (F) imaging after the two surgeries exhibiting partial tumor resection.

Sagittal T1 post-contrast (G), coronal T1 post-contrast (H) and coronal T2 (I) after 5 months of pasireotide and 2 months of cabergoline showing no change in signal intensities or tumor shrinkage. The fat graft in the middle of the tumor reabsorbed partially.
Figure 3. Germline AIP sequencing

A- Reference sequence NG_008969

B- The patient's blood sample sequencing exhibiting a single nucleotide variant in the splicing donor region of intron 2 with a nucleotide substitution of G>A in position 279+1.

C- The mother's sequencing demonstrating the same variant.

D- The father was wild type.

E- The patient's tumor sample sequencing demonstrating LOH.

254x142mm (96 x 96 DPI)
Figure 4. Effects of variant NM_003977.4:c.279+1 G>A on splice-donor region
The presence of the variant results in loss of the canonical splice-site (red column, SpliceAI ΔScore= -0.99) and increases the chance of activating two possible cryptic splice-sites (green columns, ΔScore= 0.27 and ΔScore= 0.28).
Figure 5. Main histological findings

A: Hematoxylin and eosin-stained section showing monomorphic tumor cells, displaying round to oval nuclei with conspicuous nucleoli and eosinophilic cytoplasm. A mitotic figure is shown in the insert in upper right of the panel (up to three mitoses were found in ten 0.237 square mm high-power fields).

B: Immunohistochemistry for growth hormone depicts cytoplasmic positivity in part of tumor cells.

C: CAM5.2 immunostaining highlights cytoplasmic fibrous bodies in tumor cells, characterizing a sparsely granulated tumor.

D: The proliferation index assessed semi-quantitatively (Ki-67 immunostaining; MIB-1 clone) was overall around 4%.

E: Somatostatin receptor type 2 (SST2; UMB1 clone) immunostaining showing no expression in tumor cells.


Scale bars equal 30 micrometers in A and B, and 60 micrometers in D to F.
Supplementary Materials

Methods

For DNA sequencing, we used five primer pairs to cover the six exons of AIP (supplementary table 1). To sequence the cDNA and have a broader view of possible effects of the splice variant, we used two different primers pairs. One primer pair comprised a forward primer on exon 1 and a reverse primer on exon 3, the other primer pair comprised a forward primer on exon 1 and a reverse primer on exon 5 (supplementary table 2). All PCR products were evaluated on 3% agarose gel before preparing sequencing reactions, and for cDNA products we purified bands with different sizes with the kit Wizard® SV Gel and PCR Clean-Up System (Promega). It is important to note that since all primers have M13 universal sequences added to them, the final products have additional 36bp. All alignments were performed using Benchling 2024 (https://benchling.com), DNA reference NG_008969 and for RNA (cDNA) NM_003977.4

Supplementary table 1: Primer pairs used for AIP DNA sequencing

<table>
<thead>
<tr>
<th>Target Region</th>
<th>Amplicon Size (no M13)</th>
<th>Sense</th>
<th>M13+ Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>495bp</td>
<td>Forward</td>
<td>TGTAAAACGACGGCCAGTCCGAGACATTCTAGGCTCCG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>CAGGAAACAGCTATGACCCGAGGGTGATGGGAGGAG</td>
</tr>
<tr>
<td>Exon 2</td>
<td>381bp</td>
<td>Forward</td>
<td>TGTAAAACGACGGCCAGTGGAGGGTGATGGGAGGAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>CAGGAAACAGCTATGACCCGAGGGTGATGGGAGGAG</td>
</tr>
<tr>
<td>Exon 3</td>
<td>492bp</td>
<td>Forward</td>
<td>TGTAAAACGACGGCCAGTGGAGGGTGATGGGAGGAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>CAGGAAACAGCTATGACCCGAGGGTGATGGGAGGAG</td>
</tr>
<tr>
<td>Exon 4 and 5</td>
<td>763bp</td>
<td>Forward</td>
<td>TGTAAAACGACGGCCAGTGGAGGGTGATGGGAGGAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>CAGGAAACAGCTATGACCCGAGGGTGATGGGAGGAG</td>
</tr>
<tr>
<td>Exon 6</td>
<td>509bp</td>
<td>Forward</td>
<td>TGTAAAACGACGGCCAGTGGAGGGTGATGGGAGGAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>CAGGAAACAGCTATGACCCGAGGGTGATGGGAGGAG</td>
</tr>
</tbody>
</table>
Supplementary table 2: Primer pairs used for *AIP* cDNA sequencing

<table>
<thead>
<tr>
<th>Target Region</th>
<th>Amplicon Size</th>
<th>Sense</th>
<th>M13 + Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon1-Exon5</td>
<td>689bp</td>
<td>Forward</td>
<td>TGTAAAACGACGGCCAGTCTGCCGTGGGG AGTACAGCCTTTCA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>CAGGAAACAGCTATGACC GGTCCACACCCTCCTGGAGTATGA</td>
</tr>
<tr>
<td>Exon1-Exon3</td>
<td>300bp</td>
<td>Forward</td>
<td>TGTAAAACGACGGCCAGTCTGCCGTGGGG AGTACAGCCTTTCA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>CAGGAAACAGCTATGACC GGTCCACACCCTCCTGGAGTATGA</td>
</tr>
</tbody>
</table>

Results

We observed that the exon 1-5 pair generated products of different sizes and we were able to identify: 1- had a size similar to the intended product (689bp + 36bp of M13); 2- around 650bp; 3- around 550bp; 4- around 350bp (Supplementary figure 1).

Since the exon 1-5 primer pair generated a 725bp product when considering M13 sequence, we preferred using the exon 1-3 primer pair to investigate intronic insertions between exons 2 and 3. We found that the electropherogram had double peaks in the beginning of exon 2 in a 66bp region indicating there were 2 sequences in that region, one of them matched the start of exon 3 and the reverse primer with M13 sequence (Supplementary figure 2).
Supplementary Figure 2 Alignment of AIP cDNA sequence around exon 1 and 2 junction from patient. (A) In the start of exon 2 of AIP, there is the presence of double peaks on the electropherogram that lasts for 66 bases before going back to single peaks. (B) This 66 bp region is compatible with the start of AIP’s exon 3 and we are able to identify M13 complement of the reverse primer, which indicates the presence an AIP cDNA that lacks exon 2. Highlighted sequences: Blue= complement of reverse primer; Gray= complement of reverse M13 sequence.

We found that the electropherogram had double and triple peaks in the cDNA region after exon 2, as expected. Further analysis of the sequencing revealed that the electropherogram had double and triple peaks in the cDNA region after exon 2, as expected by having different products caused by the SNV in the splice donor region. We had the reference sequence present, even with the loss of the canonical splice site, a sequence that had a 24bp intronic insertion and another with a 2bp insertion that lead to frameshift and an early stop codon (Supplementary figure 3).
Supplementary Figure 3 Alignment of AIP cDNA sequence around exon 2 and 3 junction from patient. (A) In the start of exon 3 of AIP, there is the presence of double and triple peaks on the electropherogram. (B) Comparing the peaks to AIP reference cDNA NM_003977.4 we identified 3 different sequences: the reference sequence; a 2bp intronic insertion; a 24bp intronic insertion. Highlighted sequences: Blue= complement of reverse primer; Gray= complement of reverse M13 sequence.

We then purified the smaller PCR band from the agarose gel and sequenced it to obtain a sequence of AIP cDNA that lacked exon 2 and 3. For alignment, we used the reference ENST00000682659 from Ensembl database (https://www.ensembl.org/), that is an AIP transcript that lacks exons 2 and 3.

Supplementary Figure 4 Alignment of AIP cDNA product that lacks exon 2 and 3. The alignment was performed between the purified product of ~350bp with the reference ENST00000682659, that is and AIP transcript that misses exon 2 and 3.