RESISTANT PEDIATRIC SOMATOTROPINOMAS DUE TO *AIP* MUTATIONS: ROLE OF PEGVISOMANT

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Abstract –

Background: Somatotropinomas are rare in childhood and frequently associated with genetic mutations. *AIP* mutations are found in 20-25% cases and cause aggressive somatotropinomas, often resistant to somatostatin analogues.

Aims: To assess responses to multimodal therapy including pegvisomant, in two children with sporadic somatotropinomas due to *AIP* mutations

Case description: We report two children; a boy aged 13 and a girl aged 10, with rapid growth, visual impairment and GH hypersecretion. MRI confirmed a pituitary macroadenoma with parasellar extension in both. Despite multiple surgical attempts to debulk tumour mass, residual tumour persisted. Genetic analysis showed two different *AIP* mutations (patient 1: c.562delC (p.Arg188Glyfs*8); patient 2: c.140_163del24 (p.Gly47_Arg54del8)). They were initially treated with a long acting somatostatin analogue [Octreotide LAR 30mg/month]and cabergoline, as a dopamine agonist , with later addition of pegvisomant titrated up to 20mg/day, with radiotherapy for long term control. Somatostatin analogue was ceased, due to patient intolerance and lack of control

Patient 1 had normalisation of IGF1 after 5 months of combined therapy with pegvisomant and cabergoline. For patient 2, normalisation of IGF1 was achieved after 2 months of cabergoline and pegvisomant.

Conclusion: *AIP* associated tumors can be resistant to management with somatostatin analogues. Pegvisomant can safely be used, to normalise IGF1 levels and help disease control

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Established facts:

Pediatric somatotropinomas are rare tumors and frequently occur in the context of known genetic tumor syndromes

Novel insights

Recent recognition of *AIP* mutations leading to aggressive pediatric somatotropinomas which exhibit somatostatin receptor resistance, has facilitated new management strategies in this condition.

Introduction

Pituitary adenomas constitute 3% of all pediatric brain tumours [1]. Of these, somatotropinomas account for 5-15%, with frequent underlying genetic causes, including known multi-organ syndromic associations of McCune Albright syndrome, Carney complex and multiple endocrine neoplasia type 1 (MEN1) [1]. Isolated GH secreting pituitary adenomas can also occur in patients with germline mutations of the *aryl hydrocarbon receptor interacting protein* (*AIP*) gene, or due to X-linked acrogigantism (X-LAG) syndrome (*GPR101* duplications). Somatotropinomas cause chronic GH and insulin-like growth factor -1 (IGF-1) hypersecretion with soft tissue swelling, metabolic changes and acral overgrowth. In adults, this causes acromegaly; in pediatric somatotropinoma, open epiphyses allow excessive linear growth and gigantism. *AIP* mutations underlie about 30% of cases [2]. The phenotype of patients with *AIP* mutations is of aggressive disease at a young age, with relative resistance to somatostatin analogs [2]. Hence, effective management of pediatric somatotropinomas requires early recognition and effective therapy to halt GH hypersecretion and arrest excessive linear growth before gigantism becomes established. Here we describe our experience with two pediatric somatotropinoma cases due to novel *AIP* mutations that illustrate particular clinical and therapeutic challenges encountered.

Patients and Methods

Case Presentations

Patient 1. A 13-year-old boy presented elsewhere with excessive height (185 cm; >97th centile) and weight (85 kg; >97the centile), headaches and progressive visual loss (Figure 1A). He was blind in his left eye with right sided hemianopia and acral enlargement. . Family history was negative for tall stature, tumors or other endocrine conditions. Baseline investigations demonstrated high random growth hormone (GH) (>312 mIU/L) and prolactin (1240 mIU/L; NR: 0-500), central hypothyroidism (thyroid stimulating hormone (TSH): 1.33 mIU/L (NR: 0.1-4), free T4: 7.7 pmol/L; NR: 10-25). Growth hormone suppression was not undertaken. Magnetic resonance imaging (MRI) confirmed a macroadenoma 34x42 mm with suprasellar extension, optic chiasm distortion and left sided parasellar invasion (Figure 1B). Unsuccessful transphenoidal surgical debulking, confirmed acidophilic pituitary adenoma. Elevated GH persisted (533 mIU/L) postoperatively with raised IGF-1 (164 nmol/L; NR: 14.6- 69.1). Short-term subcutaneous octreotide and oral bromocriptine did not alter hormonal hypersecretion and were ceased due to cost constraints.

At age 15, he was referred to our care, at 199 cm (>97th centile) and 86 kg with Tanner stage 2 pubic hair development and 10 ml testes bilaterally. Bone age was 12 years, with a calculated potential for 40 cm of further statural growth. Random GH was >120 mIU/L (NR: 1-30), IGF1 126 nmol/L (NR: 20-72), with central hypothyroidism (TSH 1.16 mIU/L; free T4 8.6 pmol/l) and hypogonadism (LH) 0.3 IU/L; NR: 3-10 IU/L, testosterone: 0.4 nmol/L; NR 7.6-27.7 nmol/L). Prolactin (500 mIU/L) and morning cortisol were normal (262 nmol/l; NR 100-500). Thyroxine was commenced. Despite

pre-operative normal morning cortisol, corticosteroid cover for stress was used during and after surgery.

MRI showed a large macroadenoma. Two trans-sphenoidal surgical procedures debulked tumor mass. Post–operative replacement thyroxine continued. He developed evidence of secondary hypocortisolism with an 8 am cortisol of 61 nmol/L and was started on corticosteroid replacement Post-operative imaging showed inoperable residual parasellar tumor (Figure 1B). Histopathology demonstrated a sparsely granulated pituitary adenoma with positive staining for GH, negative for Prolactin, Ki67 index was <3%. Hormonal hypersecretion continued (GH>120mIU/l, IGF-1 130 nmol/l). Depot somatostatin analog (octreotide LAR 20 mg/month) was commenced, then increased to 30 mg/month (table 1). GH and IGF-1 remained uncontrolled. Stereotactic radiotherapy was administered to active tumor remnant. Post radiation severe headache was thought to relate to tumour apoplexy, with response to short course dexamethasone for 2 weeks. To control ongoing GH excess, prior to expectation of radiation benefit, the GH receptor antagonist, pegvisomant was commenced at 10 mg/day s.c [2]. Octreotide LAR was ceased. IGF1 levels remained elevated after 2 months of pegvisomant monotherapy. Pegvisomant dose was increased to 20mg/day . Cabergoline, as dopamine agonist was added, at 0.5 mg twice weekly as combination therapy. After six months of combined therapy, IGF1 normalised to 64.8 nmol/L with >50% reduction in residual tumor size on MRI (Figure 1B & table 1). Genetic testing demonstrated a novel heterozygous indel mutation of the AIP gene c.562delC (p.Arg188Glyfs*8), predicted to be pathogenic. Analysis of resected tumor tissue demonstrated loss of heterozygosity at the *AIP* locus, indicating a somatic "second-hit" at the non-mutated allele to have occurred.

Due to hypogonadism with pubertal arrest, intramuscular testosterone undecanoate 1000 mg every 6 weeks was commenced. With a predicted final adult height of 240 cm, a short course of oestradiol valerate 4mg/day was simultaneously used, to accelerate epiphyseal maturation and fusion, thereby limiting growth. Marked reduction in growth velocity occurred, with height 204 cm (Figure 1A) and bone age advanced to 14 years after 10 months of combined treatment, together with continuing virilization. Minimal gynaecomastia <4cm resolved when oestrogen was ceased. Since return to his home country, he remains well and asymptomatic, with modest improvement in left visual acuity. Growth has ceased (4 cm since treatment). Requirement for hydrocortisone, thyroxine and androgen replacement is ongoing. Pegvisomant and cabergoline were continued for 6 months after return to his home country but continuing supplies were not available due to cost constraints.

Patient 2: A 10-year-old girl presented with rapid growth over one year, lethargy and recent blurred vision. Height was 149.5 cm (95^{th} centile), weight 56.6 kg (>95^{th} centile; Figure 2A), very tall for her family with midparental height at the 10th centile. There was no family history of tumors or tall stature. Examination revealed bitemporal hemianopia, puffy hands and feet but no acromegaloid facial features. She was prepubertal.

Endocrine evaluation revealed high IGF1, 121 nmol/L (NR: 10-52), mild central hypothyroidism, (TSH: 1.53 mIU/L; free T4: 8.8 pmol/L), prolactin 418 mIU/L (NR: 40-204). Morning cortisol (220 nmol/l) was normal. Oral glucose tolerance test (OGTT) demonstrated a non-suppressed nadir GH of 380 mIU/L (<3). MRI confirmed a large pituitary macroadenoma with parasellar invasion and optic chiasmal distortion (Figure 2B).

She underwent tumor resection via fronto-temporal craniotomy then subsequent trans-sphenoidal approach. Residual tumour remained in cavernous sinus and parasellar areas. Histopathology showed a sparsely granulated GH positive pituitary adenoma with a small cluster of cells staining positive for prolactin, topoisomerase index was 5% indicating increased risk of local recurrences. Thyroxine and hydrocortisone were commenced post operatively. Adjuvant octreotide LAR was instituted at 20 mg/month, increasing to 60 mg/month, with cabergoline to 2 mg/week (table 2); hormonal hypersecretion remained uncontrolled (IGF-1: 90-120 nmol/L); GH unsuppressed on OGTT and no tumor shrinkage on MRI (Figure 2B). She therefore underwent stereotactic radiotherapy for long term tumour control. Pegvisomant was commenced, at 10 mg/day, Within two months, IGF1 normalised to 51.5 nmol/L (10-52 nmol/L) and octreotide was weaned and ceased (table 2). On further follow up IGF1 normalised further and Cabergoline could be stopped as well. Prolactin remained normal. Clinical course was complicated by cholelithiasis, related to octreotide. Cholecystectomy was performed. Post cholecystectomy and octreotide cessation the liver functions normalised despite continuation of Pegvisomant. They have remained normal on follow up and therefore are unlikely to be related to pegvisomant but can be attributed to a side effect of octreotide.

Due to failure of pubertal progression, with a bone age of 12 years, undetectable gonadotropins (FSH<0.7 IU/L, LH <0.2 IU/L) and an oestradiol of <20 pmol/L (NR: 39-631), hormone replacement therapy was started at age 12. She is currently 13.5 years and continues to tolerate pegvisomant 10mg/day (with normal IGF1 levels). Her MRI shows evidence of almost complete resolution of tumor and only a small sellar remnant (Figure 2B). She grew 1.5 cm over 12 months with a final height of 164 cm, and a bone age of 15 indicating completion of linear growth.

Genetic testing revealed a heterozygous in frame deletion of the *AIP* gene c.140_163del24 (p.Gly47_Arg54del8). Two of her siblings were negative for the *AIP* mutation.

Discussion

Somatotropinomas are rare during childhood and adolescence but can be large and aggressive. In addition to soft tissue, metabolic and musculoskeletal effects seen in adults with acromegaly, pediatric somatotropinoma patients have open epiphyses, with potential for gigantism. Management is extrapolated from adult protocols with no specific treatment recommendations for affected children [1]. In young pituitary adenoma patients genetic causes are more common, those most frequent being *AIP* and *MEN1* mutations [3].

Germline inactivating mutations or deletions of *AIP* increase risk of pituitary tumorigenesis, commonly presenting as familial isolated pituitary adenoma (FIPA) kindreds. *AIP* mutations are rare (0-4%) in sporadic pituitary adenomas, are associated with all types but more than 80% are somatotropinomas, mixed GH and prolactin secreting adenomas or prolactinomas. They underlie 12-23% of pediatric pituitary adenomas [4]. Awareness of *AIP* mutations among pediatricians could help direct management.

The cases described here exemplify challenges faced with the clinically aggressive characteristics of *AIP* mutation related pituitary adenomas. *De novo AIP* mutations can occur rarely [3] as in our cases but are commonly familial, with pituitary tumours in 20% of mutation carriers [5]. This can lead to skipping of generations in a familial *AIP* mutation with occurrence of apparent simplex cases. Very limited family screening was possible in our first case. Both mutations are, to our knowledge, novel in the setting of pediatric somatotropinomas. The *AIP* mutation in patient 2 leads to a 24 nucleotide deletion and an in frame deletion of 8 amino acids. A similar nucleotide change with an in frame amino acid deletion was reported in a FIPA family with somatotropinomas [6].

Our patients had large, invasive tumours, with poor treatment response to surgery or somatostatin analogues, typical of *AIP* associated somatotropinomas [2], with currently unexplained etiology. AIP staining is an important predictor of hormonal response to somatostatin receptor subtype 2 (SSTR2) specific analogues like octreotide and lanreotide [7]. Loss of AIP function in tumors from patients with *AIP* mutations provides a phenotype of aggressive tumor growth and poor control with SSTR2 related somatostatin analogues. Lower SSTR2 levels are seen in tumors with decreased AIP expression. Effects of multi-receptor somatostatin analogues (pasireotide) are unclear to date. AIP may act via pathways related to ZAC or Gai-2 to determine hormonal or proliferative responses to somatostatin analogues [8].

Pegvisomant as a GH receptor antagonist, blocks endogenous GH binding at its receptor and inhibits IGF-1 production. GH production is not blocked so IGF1 testing best monitors treatment efficacy. GH levels may be persistently high [9]. Pegvisomant is effective in adult trials of acromegaly with control rates of 63.1% after 5 years use reported in a large cohort [10]. Data in pediatric populations is limited. A 2011 review of pediatric somatotropinomas reported 7 children, of whom 4 showed clinical and biochemical response [11]. Combined pegvisomant with somatostatin analogues and dopamine agonists in pituitary gigantism has reported control in 53.5% cases [12], as seen in our case . Potential concern with pegvisomant includes risk of tumor expansion, not seen in our patients. Derangement of liver enzymes can occur with pegvisomant and somatostatin analogues.

Radiotherapy can be used as adjuvant therapy for uncontrolled acromegaly [13]. In children it incurs a risk of neurocognitive dysfunction but was deemed necessary for our patients [14]. Therapeutic effect of radiotherapy usually takes 5-10 years but in our patients, reduction in tumor size was surprisingly rapid, within 1- 2 years. Cabergoline

may have contributed to tumor shrinkage and tumor apoplexy might have played a role, in patient 1.

Hypopituitarism is reported in 64% of pituitary gigantism, the gonadal axis being most commonly affected [12], with delayed epiphyseal fusion increasing time for growth, as in our patients. Epiphyseal fusion was successfully accelerated, with growth truncation in patient 1 who had an estimated final height of 240cm, by use of adult doses of testosterone, for aromatization to oestrogen, plus a short course of oestrogen. This option has not been reported in males, except those with LH receptor defects.

In conclusion, pediatric somatotropinomas due to *AIP* mutations are difficult to treat and usually resistant to somatostatin analogues. Management requires a combination of surgery, radiotherapy and adjuvant medical therapy. Pegvisomant is effective as a growth hormone blocking agent while resistance to somatostatin analogues is a major obstacle to effective hormonal control.

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Figure legends:

Figure 1: A. Growth chart of patient 1 from first presentation at another center to definitive treatment. B. MRI images of the tumor of patient 1 at baseline, before surgery and while on pegvisomant therapy.

Figure 2: Growth chart of patient 2 from first presentation at our center to definitive treatment. B. MRI images of the tumor of patient 2 at baseline, before surgery and while on pegvisomant therapy.