Klotz communications 2017: From the shortest to the tallest

X-LAG: How did they grow so tall?

X-LAG ou comment sont-ils devenus si grands ?

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Abstract

X-linked acrogigantism (XLAG) is a new, pediatric-onset genetic syndrome, due to Xq26.3 microduplications encompassing the GPR101 gene. XLAG has a remarkably distinct phenotype with disease onset occurring before the age of 5 in all cases described to date, which is significantly younger than in other forms of pituitary gigantism. These patients have mixed GH and prolactin positive adenomas and/or mixed-cell hyperplasia and highly elevated levels of GH/IGF-1 and prolactin. Given their particularly young age of onset, the significant GH hyperscretion can lead to a phenotype of severe gigantism with very advanced age-specific height Z-scores. If not adequately treated in childhood, this condition results in extreme final adult height. XLAG has a clinical course that is highly similar to some of the tallest people with gigantism in history.

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Keywords: Gigantism; Pituitary adenoma; X-linked acrogigantism (X-LAG); GPR101 gene; Familial isolated pituitary adenoma (FIPA)

Résumé

« X-linked acrogigantism » (XLAG) est un syndrome pédiatrique récemment décrit, lié à des microduplications du chromosome Xq26.3, englobant le gène GPR101, responsable de l’affection. Les patients XLAG présentent un phénotype remarquablement distinct des autres cas de gigantisme hypophysaire. Dans tous les cas décrits, la maladie s’exprime avant 5 ans soit beaucoup plus tôt que dans les autres formes. Les patients ont habituellement un gros adénome ou une hyperplasie mixte pour la GH et la prolactine et des taux très élevés de GH/IGF1 et prolactine. En raison de son début très précoce, l’hypersécration importante de GH peut conduire à un gigantisme extrêmement sévère avec un Z-score très important pour l’âge. Si cette condition n’est pas traitée pendant l’enfance, elle peut conduire à une taille finale extrême. XLAG montre une évolution clinique similaire à celle observée chez les géants les plus grands de l’histoire.

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Mots clés : Le gigantisme ; L’adénome hypophysaire ; L’acro-gigantisme lié au chromosome X (X-LAG) ; Le gène GPR101 ; L’adénome hypophysaire familial isolé (FIPA)

1. Introduction

Acromegaly and gigantism result from chronic excessive production and secretion of growth hormone (GH), usually by a pituitary adenoma, and are considered very rare conditions. GH-secreting pituitary tumors are predominantly sporadic lesions that occur in adults, although rare pediatric or familial forms can occur and these can have aggressive characteristics. The molecular genetics of these aggressive forms of somatotropinomas is of interest for research and clinical practice. Known genetic syndromes associated with the occurrence of somatotropinomas include multiple endocrine neoplasia (MEN) type 1 and MEN 4, Carney complex, McCune–Albright syndrome (MAS), and pheochromocytoma/paraganglioma and pituitary adenoma syndrome (3PAs). The most frequent familial form of pituitary adenoma is familial isolated pituitary adenoma

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(FIPA) syndrome [1]. Approximatively 15–20% of general FIPA kindreds and about 50% of homogeneous FIPA families with acromegaly-gigantism are caused by mutations/deletions in the *aryl hydrocarbon receptor interacting protein (AIP)* gene [2,3].

In Trevellin et al., we recently described an international cohort of patients with a new genetic form of pituitary gigantism beginning in early childhood due to a microduplication on Xq26.3, that we termed X-linked acrogigantism (XLAG) [4]. In a large pituitary gigantism-specific cohort study of 208 patients, we found that *AIP* mutations and XLAG accounted for 29% and 10%, respectively, of genetically tested patients, making them the two most common causes of pituitary gigantism. The previously known genetic causes of somatotropinomas are responsible for a much lower percentage of pituitary gigantism cases: MAS (5%), MEN1 (1%), and Carney complex (1%) [5]. To date, 32 patients with XLAG duplications have been published in the scientific literature [4,6–11]. Notably, XLAG explains the majority of prepubertal cases (80%) and almost all of the tallest cases in our 208-patient cohort; these XLAG cases manifested with either exceedingly excessive growth rate or a very increased final adult height (height Z-score > +4.5 SD).

2. Clinical profile

XLAG is a pediatric syndrome of pituitary gigantism with a very young age of disease onset. Usually increased length and weight are seen before the age of 1 year and most patients are diagnosed by the age of three with already substantially advanced overgrowth [4,9,12]. This young age at first manifestations renders this particular form of gigantism different from other forms of gigantism and acromegaly. Genetic forms of somatotropinomas, particularly *AIP*-related, are more frequent in younger patients than in the general sporadic acromegaly population [5]. *AIP*-related cases, however, usually occur in adolescents and young adults, whereas children with XLAG, who are usually born with normal anthropometric parameters after uncomplicated pregnancies, begin their abnormal growth in infancy (Fig. 1A) [4]. This means that patients with XLAG can have had a long duration of overgrowth by the time they reach puberty, which translates into increased height Z-scores as compared with other forms of pituitary gigantism [5].

Besides the early excessive acceleration of linear growth and body size, these young patients frequently present some clinical signs and symptoms of GH/IGF-1 excess that are more typical of adult acromegaly (Fig. 1B). They may develop soft tissue swelling and coarse facial features and an increased interdental space, as well as marked enlargement of hands and feet [12]. In some cases, increased appetite (25%) and signs of insulin resistance (such as *acanthosis nigricans*) are also noticed at diagnosis [12]. XLAG has a female predominance (71%); this feature differentiates it from *AIP*-mutation related gigantism cases and gigantism patients without a known genetic cause in which cases most of the affected subjects are males (95% and 78%, respectively) [5].

In both male and female XLAG cases, the extraordinary growth velocity and prolonged linear growth is underpinned by the aggressive behavior of the pituitary lesion [4,12]. Children with XLAG develop relatively large pituitary adenomas for their young age (Fig. 2), which can be accompanied by pituitary hyperplasia. In some cases, diffuse hyperplasia can be present alone and it is unknown whether the mixed GH-prolactin...
secretion macroadenomas that are seen in most XLAG patients begin as hyperplasia before transforming into adenoma. Pathology studies have demonstrated foci of transformation from hyperplasia to adenoma, which further supports the concept that the adenomas in XLAG develop against a background of hyperplasia [4,7,12,13] (Table 1).

We recently showed that modestly elevated circulating GH releasing hormone (GHRH) levels can occur in XLAG [13]. GHRH levels in XLAG are not as high as those usually encountered in ectopic GHRH secretion from neuroendocrine carcinomas. Moreover, intense GHRH-receptor (GHRH-R) staining was found in resected pituitary tumor tissue in XLAG.

![Fig. 2. Coronal and sagittal MRI images of patients with XLAG. Panels A and B. A large pituitary mass with important suprasellar extension in a female patient was revealed by gadolinium-enhanced MR imaging at diagnosis (2 years 11 months). Panels C and D. Gadolinium-enhanced MR images of a female patient, who was diagnosed at age 3 years with a large pituitary mass with marked suprasellar and posterior extension and areas of degenerative changes. Panels E and F show postoperative images from the same patient with resected adenoma. However, the administration of SSA and pegvisomant were required after surgery in this patient for hormonal and growth control. Panels G and H show MR images of a female patient at diagnosis (aged 3 years) presenting with a large pituitary mass with extrasellar expansion [12].](image-url)

**Table 1**

<table>
<thead>
<tr>
<th>Name</th>
<th>Country</th>
<th>Sex</th>
<th>Year of birth</th>
<th>Birth weight (kg)</th>
<th>Normal family history</th>
<th>Age at which abnormal growth noted (years)</th>
<th>Final height (cm)</th>
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<td>F</td>
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<td>Y</td>
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<tr>
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<td>USA</td>
<td>F</td>
<td>1872</td>
<td>3.4</td>
<td>Y</td>
<td>7</td>
<td>225</td>
</tr>
<tr>
<td>Fedor Andreevich Machnow</td>
<td>Russia</td>
<td>M</td>
<td>1878</td>
<td>NA</td>
<td>Y</td>
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</tr>
<tr>
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<td>&lt;3</td>
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<td>1920</td>
<td>Normal</td>
<td>Y</td>
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<tr>
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<td>1922</td>
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<td>&lt;3</td>
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<td>F</td>
<td>1946</td>
<td>Normal</td>
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<td>1955</td>
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<td>3</td>
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<tr>
<td>Yao Defen</td>
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* a Swan and Bates were married and had two pregnancies; one female child was stillborn (weighed 8.1 kg) and later a son who died in early infancy was 10.8 kg and 76 cm in length when born.

b Cases for which pituitary disease was reliably diagnosed/reported.

which suggests that the cell population involved in hyperplasia and adenomatous transformation may be sensitive to GHRH. Furthermore, in vitro cell culture studies of an XLAG pituitary lesion revealed that a GHRH-R antagonist inhibited both GH and prolactin and antagonised the effect of GHRH stimulation. These data support hypothalamic involvement via GHRH and GHRH-R dysregulation in the pathogenesis of XLAG. The GHRH-related GH hypersecretion in XLAG could potentially be targeted by therapeutic blockade of the GHRH-R [13].

In terms of treatment, XLAG patients have particularly difficult to treat pituitary lesions, not only due to their young age and relatively large tumor size. They often require multi-modal treatment, including pituitary surgery, medical treatment and radiotherapy. Almost all XLAG cases have partial or complete resistance to octreotide/lanreotide, despite a relatively high expression of somatostatin receptor type 2 [12]. Prolactin hypersecretion demonstrates variable responsiveness to dopamine agonist therapy. In order to control GH hypersecretion, some patients have required aggressive surgical resection (including anterior hypophysectomy when extensive hyperplasia has been present), in combination with radiotherapy. The consequences of these multiple lines of treatment is the frequent occurrence of partial or complete hypopituitarism, requiring lifelong replacement therapy starting from a very young age. Adjuvant use of the GH receptor antagonist, pegvisomant, has proven successful in controlling GH secretion in some XLAG patients [12]. Prompt and effective hormonal control is a key requisite in halting linear growth before patients reach a markedly increased adult height [5]. In the absence of effective measures to control tumor growth, marked tumor progression can continue in XLAG, with exceptionally severe phenotypes of pituitary gigantism being established before puberty [7]. A number of patients with extremely gigantism described in historical and medical records had clinical presentations that corresponded to the growth pattern of XLAG patients [4,12]. In particular, the tallest man, Robert Wadlow, and the tallest woman, Zeng Jinlian, in recorded human history presented the characteristics of XLAG patients with rapid overgrowth in early childhood (Fig. 3).

3. Genetics

XLAG has a specific genetic etiology. Unlike the other pituitary adenoma-associated genetic syndromes usually caused by inactivating mutations or deletions in tumor suppressor genes or activating mutations in an oncogene, XLAG is due to a duplication on chromosome Xq26.3. Array comparative genomic hybridization (aCGH) is usually used for detecting such copy number variation (CNV) abnormalities. All 18 XLAG patients in our initial cohort were found to have microduplications with two smallest regions of overlap, encompassing four genes (CD40LG, ARHGEF6, RBMX and GPR101) [4]. Among the initial four candidate genes, GPR101, an orphan G-protein coupled receptor gene, was shown to be the only one significantly overexpressed in the pituitary tissue of patients with Xq26.3 microduplications. The causative pathogenic role of the GPR101 gene in XLAG was thereafter supported by a XLAG patient with duplication of GPR101 alone [8].

GPR101 is an orphan Gs protein-coupled receptor, whose endogenous ligand and exact functions are unknown. GPR101 is normally expressed at particularly high levels in the mouse hypothalamus and in the pituitary of the rhesus macaque and the rat. In humans, GPR101 mRNA is predominant in the nucleus accumbens and its expression is low in adult pituitary gland. However, GPR101 might have a role during pituitary ontogenesis. GPR101 expression was found in the pituitary gland of human embryos starting from 19 weeks of gestational age with the highest levels of GPR101 staining (65%) at 38 weeks [4,14]. GPR101 duplication might support mixed GH and prolactin positive cellular hyperplasia in XLAG starting from the fetal period with further adenoma formation in the early period after birth. However, the role of GPR101 in pituitary development, tumorigenesis and stimulation of GH/prolactin hypersecretion remains unclear. So far, inactivating mutations of the GPR101 gene do not seem to be commonly involved in GH deficiency [15].
Most XLAG cases are sporadic and duplications in all sporadic cases appear to be non-recurrent with unique boundaries. These are generally due to a DNA replication error because of microhomologies between proximate fragments at Xq26.3 [4]. In familial cases, a transmission of the same XLAG microduplication from affected mother to affected son with 100% penetrance has been reported in three homogeneous FIPA kindreds with early onset acrogigantism [4,11]. Along with AIP mutation/deletion, XLAG is the second known genetic cause of FIPA in AIP-negative kindreds with familial acrogigantism.

While the GPR101 duplication has been found in all the XLAG cases reported so far, in sporadic males the aCGH result demonstrates the presence of somatic mosaicism that leads to a variable proportion of affected cells (Fig. 4) [9]. Relatively low levels of GPR101 duplicated cells (as low as 16–17%) have been confirmed using digital droplet PCR (ddPCR), which allows the detection of quite small rates of mutations or CNV–deletions or duplications (Fig. 5). The clinical presentation of XLAG in all mosaic cases is similar to that of patients with constitutional duplication, in terms of overgrowth and pituitary disease severity. Thus, sporadic males could exhibit dramatic forms of pituitary gigantism even when only a low proportion of cells carry the GPR101 duplication. We screened a large group of patients with acromegaly and gigantism using quantitative ddPCR for GPR101 duplications. This method allows the identification of new XLAG cases by means of increased GPR101 copy number, which can later be confirmed on aCGH [9].

Recently, we further applied this screening ddPCR method to ancient DNA retrieved from skeletal remains of a historical case of extreme acrogigantism (giant Constantin; 259 cm). The individual died of septicemia following surgery for gangrene as a young adult in 1902, and his autopsy demonstrated a massively enlarged pituitary gland and pituitary fossa [10]. According to published sources and original medical documentation available to us, the abnormal growth started early and extremely tall stature (1.94 m) was already present at the age of 14. Paleontological DNA extraction techniques permitted the extraction of DNA from bone powder and subsequent ddPCR revealed elevated copy number of GPR101 indicating a probable diagnosis of XLAG (Fig. 6).
4. Conclusion

XLAG syndrome is characterized by a very specific phenotype of infant-onset gigantism with markedly increased height and weight z-scores. It is caused by mixed GH and prolactin secreting pituitary adenomas and/or hyperplasia that can produce exceptionally high levels of GH and prolactin. This new genetic form of pituitary gigantism is due to the duplication of the GPR101 gene and can present either sporadically (as constitutional or mosaic duplication) or in a FIPA setting. To date, 32 XLAG patients have been reported, most of whom are females. XLAG is a clinical entity that, while rare, leads to an exceptionally severe pituitary gigantism phenotype that requires early intervention to control overgrowth.

Disclosure of interest

The authors declare that they have no competing interest.

References
