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Review

The Genetic Pathophysiology and Clinical Management of the TADopathy, X-Linked Acrogigantism

Adrian F. Daly[1] **and Albert Beckers**[1]

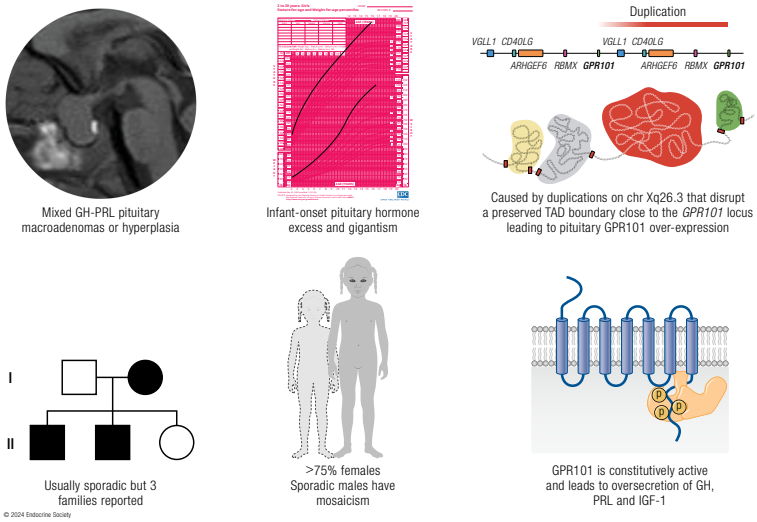
[1]Department of Endocrinology, Centre Hospitalier Universitaire (CHU) de Liège, University of Liège, Domaine Universitaire Sart Tilman, 4000 Liège, Belgium

Correspondence: Adrian F. Daly, MD, PhD, Department of Endocrinology, Centre Hospitalier Universitaire (CHU) de Liège (B35), University of Liège, Domaine Universitaire Sart Tilman, 4000 Liège, Belgium. Email: adrian.daly@chuliege.be; or Albert Beckers, MD, PhD, Department of Endocrinology, Centre Hospitalier Universitaire (CHU) de Liège (B35), University of Liège, Domaine Universitaire Sart Tilman, 4000 Liège, Belgium. Email: albert.beckers@chuliege.be.

Abstract

Pituitary gigantism is a rare manifestation of chronic growth hormone (GH) excess that begins before closure of the growth plates. Nearly half of patients with pituitary gigantism have an identifiable genetic cause. X-linked acrogigantism (X-LAG; 10% of pituitary gigantism) typically begins during infancy and can lead to the tallest individuals described. In the 10 years since its discovery, about 40 patients have been identified. Patients with X-LAG usually develop mixed GH and prolactin macroadenomas with occasional hyperplasia that secrete copious amounts of GH, and frequently prolactin. Circulating GH-releasing hormone is also elevated in a proportion of patients. X-LAG is caused by constitutive or sporadic mosaic duplications at chromosome Xq26.3 that disrupt the normal chromatin architecture of a topologically associating domain (TAD) around the orphan G-protein–coupled receptor, GPR101. This leads to the formation of a neo-TAD in which *GPR101* overexpression is driven by ectopic enhancers ("TADopathy"). X-LAG has been seen in 3 families due to transmission of the duplication from affected mothers to sons. GPR101 is a constitutively active receptor with an unknown natural ligand that signals via multiple G proteins and protein kinases A and C to promote GH/prolactin hypersecretion. Treatment of X-LAG is challenging due to the young patient population and resistance to somatostatin analogs; the GH receptor antagonist pegvisomant is often an effective option. GH, insulin-like growth factor 1, and prolactin hypersecretion and physical overgrowth can be controlled before definitive adult gigantism occurs, often at the cost of permanent hypopituitarism.

Graphical Abstract



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Key Words: X-linked acrogigantism, *GPR101*, gigantism, familial isolated pituitary adenoma, topologically associating domain

Abbreviations: cAMP, cyclic adenosine monophosphate; CGH, comparative genomic hybridization; FIPA, familial isolated pituitary adenomas; GH, growth hormone; GHRH, GH-releasing hormone; GHRHR, GHRH receptor; GnRH, gonadotropin-releasing hormone; GPCR, G-protein–coupled receptor; IGF, insulin-like growth factor; IP1, inositol monophosphate; MEN1, multiple endocrine neoplasia type 1; MRI, magnetic resonance imaging; PKA, protein kinase A; SRO, shared region of overlap; SSA, somatostatin analog; SSTR2, somatostatin receptor subtype 2; TAD, topologically associating domain; TM, transmembrane; ULN, upper limit of normal; X-LAG, X-linked acrogigantism.

ESSENTIAL POINTS

1. X-linked acrogigantism (X-LAG) is a rare and early-onset form of pituitary gigantism that has median ages at onset and diagnosis of 18 months and 4 years, respectively
2. In X-LAG, patients develop pituitary gigantism due to mixed growth hormone (GH) and prolactin pituitary adenomas and/or hyperplasia that lead to exuberant GH/insulin-like growth factor-1 excess, usually accompanied by hyperprolactinemia
3. Treatment of X-LAG is typically complex and multimodal due to the young age of the patients and the resistance to somatostatin analogs, but the GH receptor antagonist pegvisomant has proven effective
4. In X-LAG, like in pituitary gigantism overall, the effective limitation of height excess before the onset of the pubertal growth spurt is a key management goal
5. X-LAG is due to duplications on chromosome Xq26.3 that disrupt a topologically associating domain (TAD) containing *GPR101*, thereby placing the *GPR101* promoter under the control of ectopic enhancers and driving massive pituitary overexpression of *GPR101* (TADopathy)
6. GPR101 is an orphan G-protein–coupled receptor that is constitutively active and stimulates GH and prolactin secretion (using G_s, $G_{q/11}$, and $G_{12/13}$) via protein kinase A and C pathways in transgenic mice and in the tumors of patients with X-LAG.
7. The circulating GH-releasing hormone (GHRH) excess identified in a proportion of patients with X-LAG may play a role in pituitary tumorigenesis via activation of GHRH receptors.

Introduction

Height, Acromegaly, and Pituitary Gigantism

Acromegaly is a rare but clinically well-characterized disease that has a prevalence in the general population of approximately 10.5 cases per 100 000 (or 1 in 9523) individuals (1). Somatotropinomas (pituitary adenomas secreting growth hormone [GH]) are the most frequent cause of acromegaly. They can occur at any age but there is a clear distribution that favors the clinical presentation as acromegaly in adult patients, typically in the fourth decade of life (2, 3). Pituitary gigantism is an early manifestation of acromegaly that begins before growth plates have fused (4). Historically, the differences in physical manifestations between pituitary gigantism and acromegaly fueled active debate about whether they constituted separate diseases or were a single entity (5). Although nowadays we know that they exist as part of the same spectrum of disease, the underlying etiologies of pituitary gigantism and acromegaly do differ in an important way. Pituitary gigantism is caused by a known germline genetic factor in nearly half of all cases, whereas in typical adult-onset acromegaly, known germline causes are very rare indeed (4, 6, 7). Such enrichment of known germline genetic drivers in young-onset somatotropinomas is not yet evident in other sporadic, young-onset pituitary adenomas, such as prolactinomas, nonfunctioning adenomas, and Cushing disease (6-10). This makes genetic studies a central consideration in the management of patients with pituitary gigantism, whereas for other pituitary adenomas genetics currently plays a more restricted role.

The "giant" is a familiar figure from legends and folk tales across almost all human cultures, and is often encountered in tales of valor or in association with heroic exploits (4, 11). This association of immense size with great physical strength has traditionally stimulated public interest in people with pituitary gigantism, as traced by de Herder and others (12-16). The publicity and fame accorded to giants in the past, and even today, belies the heavy toll of the disease from which they suffered. Pituitary gigantism, if untreated or undertreated is one of the most physically deforming diseases known to medical science. Chronic action of elevated GH and insulin-like growth factor (IGF)-1 on the growing skeleton and organs has the ability to induce widespread changes that limit the ability of patients to lead a normal life. Historical data about well-known individuals with gigantism provide an important lesson about the negative outcomes of unopposed tumoral GH secretion in terms of extreme final heights, the consequent curtailment of normal work and living, and the major morbidity and early mortality suffered by patients (17-24). Modern research has also changed the context of historical individuals with severe pituitary gigantism, who are now better understood as patients with rare genetic diseases following the identification of likely causative factors including *AIP* pathogenic variants and Xq26.3 duplications (25-27).

Tall stature is usually considered to be present in individuals who are >2 SD above the mean height for their sex in their relevant population height statistics. This corresponds to those above the 97.7th percentile for height, and will encompass all of those individuals who fall into that category, irrespective of pituitary or other etiology (28). Height varies dramatically in humans depending on regional and ethnic factors, so country-specific and ethnically accurate data should be used (29). In the growing child, there are further factors to be considered when diagnosing gigantism. In children and adolescents, assessments of target height and trends in growth velocity are key metrics (28-30). The midparental height is an important measure that it is easily calculated and incorporates the familial height background. Arriving at a diagnosis of pituitary gigantism can be a long process due to a number of factors. Many causes of tall stature have to be considered and discarded, and tall stature is often familial or constitutional and there is no identifiable pathology (4, 29, 31). From a societal point of view, there is also a greater tolerance of tall

stature as being a positive characteristic, traditionally more so in boys than in girls (29, 31-34). Also, in the community, child growth assessments usually encounter the more common presentation of short stature, so this can potentially delay referring those with clinically significant overgrowth.

Once a patient with tall stature or increased growth is suspected of having a pituitary pathology, the diagnosis of pituitary gigantism should be explored urgently (29, 30). As pituitary gigantism usually overlaps periods of rapid normal growth in childhood and adolescence, prompt referral for hormonal testing is needed. GH suppression should be measured following an oral glucose load and IGF-1 should be assessed vs age and sex-appropriate normal ranges. There should be a low threshold for performing radiological imaging (pituitary magnetic resonance imaging [MRI]) in individuals with evidence of elevated IGF-1 or nonsuppressed GH as identifying a pituitary mass is a priority. In the setting of a new case of pituitary gigantism there are a number of clinical patterns that should be considered as they can point to an underlying genetic etiology. The most common causes of pituitary gigantism are pathogenic variants ("mutations") or deletions in the aryl hydrocarbon receptor interacting protein (*AIP*) gene (29%), duplications on chromosome Xq26.3 affecting *GPR101* gene expression in X-linked acrogigantism (X-LAG; 10%); other rarer causes include activating mutations of the *GNAS* gene causing McCune–Albright syndrome (5%), multiple endocrine neoplasia type 1 (MEN1), and Carney complex (35).

The Emergence of X-LAG

The identification and characterization of X-LAG emerged from a collaborative project with the Stratakis group at the National Institutes of Health into the study of the genetic causes of pediatric and familial pituitary adenomas. Previous work together had focused on germline genetic causes, such as sequence variants in *AIP*, *MEN1*, *PRKAR1A*, and *CDKN1B* (36). In parallel, based on the high rate of pituitary gigantism seen in our international cohort of patients with pathogenic *AIP* variants, we conducted a collaborative study in Liège to explore existing and novel genetic causes of pituitary gigantism (35, 37). Up to then our methods had focused on the sequencing of known endocrine cancer risk genes and performing whole exome/whole genome sequencing. Using array comparative genomic hybridization (CGH), the National Institutes of Health group noted various duplications and deletions in a small group of patients with pediatric-onset pituitary tumors. To explore these further, we applied this methodology to unexplained cases in our wider pituitary gigantism cohort, which by then included >200 patients (35). One of these novel duplications that involved chromosome Xq26.3 initially included about 11 coding genes, none of which were implicated in GH secretion or overgrowth. Together, we identified multiple new cases of which 13 comprised the initially reported X-LAG cohort in late 2014 (38). The clinical, pathological, and genetic profiles of the first cohort of patients with X-LAG have been confirmed and expanded subsequently. These characteristics of X-LAG are detailed comprehensively below in "Chromatin dysregulation in the etiology of X-LAG." In brief, X-LAG causes a specific form of pituitary gigantism that begins in early childhood, leads to very marked GH and IGF-1 hypersecretion from large mixed GH-prolactin pituitary macroadenomas with or without accompanying hyperplasia (Fig. 1). Analysis of the original patients permitted a significant narrowing of the number of potentially involved genes. The microduplications involve shared regions of overlap (SROs). In the discovery cohort all patients shared 2 SROs, the larger SRO1 that included the genes from part of *VGLL1* through *CD40L*, *ARHGEF6*, and *RBMX* and a smaller SRO2 that contained the gene *GPR101* alone. Using fluorescent in situ hybridization these were visualized as tandem duplications (38). There were also 2 familial cases involving a mother and 2 sons in 1 kindred and a mother–son pair in the other, all of whom were affected by familial isolated pituitary adenomas (FIPA; kindreds with at least 2 related members with isolated pituitary adenomas in the absence of MEN1/Carney complex, etc. (39)). These cases revealed that microduplications on chromosome Xq26.3 could be transmitted in an X-linked dominant manner. Initial studies on potential causative genes within the SROs showed that only *GPR101* was highly upregulated in the resected pituitary tissue from affected patients. Together with the Lupski group at Baylor College of Medicine, we showed that microduplications usually arise from errors during DNA replication caused by microhomology-mediated break-induced replication or fork stalling and template switching (38, 40-42). Iacovazzo et al identified 1 further case in which the duplication occurred at an *Alu-Alu* repeat element (43). Each individual sporadic patient has their "own" mutation with unique breakpoints, whereas in familial cases the mother's mutation is transmitted as an identical change in her sons. When studying males and females separately we used high definition (HD)-CGH and digital droplet polymerase chain reaction on peripheral blood leukocyte DNA to show that sporadic males with X-LAG were somatic mosaics for Xq26.3 duplications, whereas all females are constitutively duplicated (42). In males, the somatic mosaic state means that varying levels of duplication can be found in different tissues. Rodd et al reported a sporadic male patient with X-LAG in whom no duplication was found on array CGH of DNA from peripheral blood leukocytes, saliva, and buccal sources, but in whom duplications involving *GPR101* could be identified on digital droplet polymerase chain reaction performed on DNA from pituitary tissue and skin (44). The first studies on surgically resected pituitary tissue showed that of the different genes involved in the original SRO1 and SRO2 regions on chromosome Xq26.3, only *GPR101* was overexpressed at the RNA and protein level (38). Therefore, we directed our research towards characterizing the physiology and pharmacology of this orphan G-protein–coupled receptor (GPCR) and its potential role in the hypothalamo–pituitary axis and elsewhere.

GPR101: Physiological and Pharmacological Profile

Structure and Expression of GPR101

GPR101 is a 7 transmembrane (TM) domain GPCR. Owing to the lack of a proven endogenous ligand, GPR101 is termed an orphan GPCR. GPR101 is a member of the Class A (rhodopsin-like) family of GPCRs, and consists of an extracellular N-terminal and 7 TM domains that are connected via 3 extracellular loops and 3 intracellular loops. The third intracellular loop in GPR101 is very long, although the functional significance of this remains unclear (Fig. 2). GPR101 was first identified in humans by Lee et al in 2001 and was described

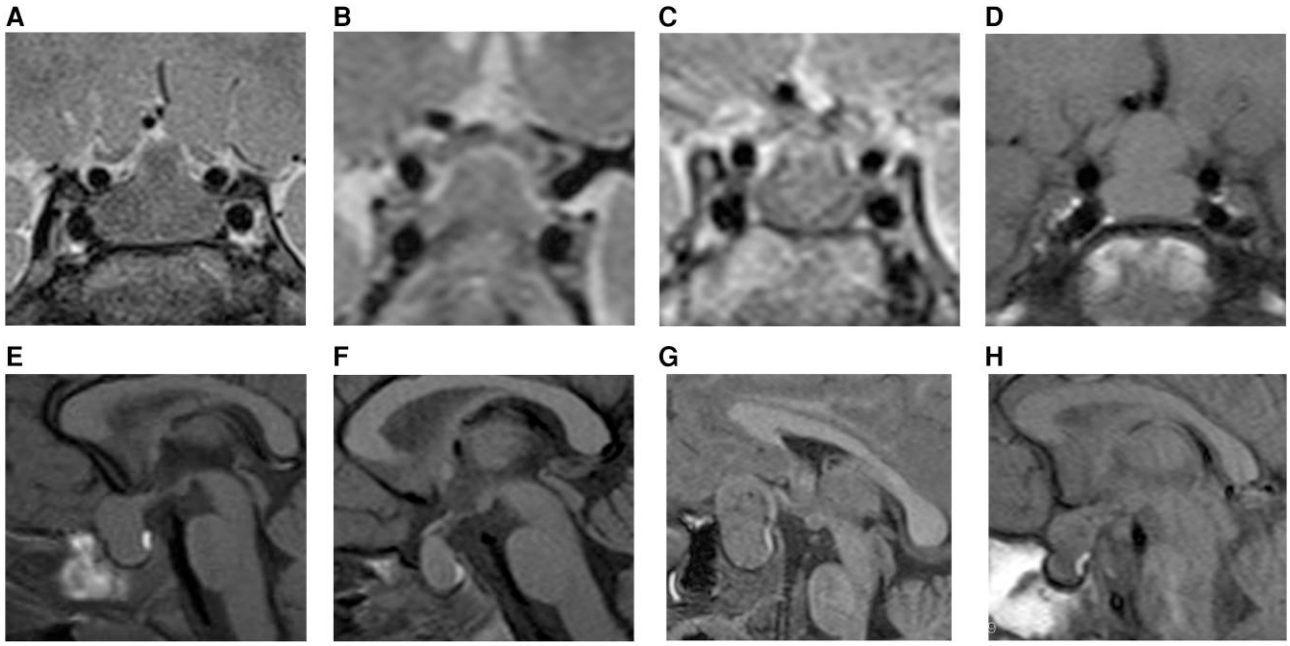


Figure 1. MRI appearance of pituitary adenomas at diagnosis in patients with X-LAG. The upper row shows coronal sections from 3 patients that were later found to have pituitary adenomas (T2 weighted; A-C) or hyperplasia alone (T1-weighted; D). Below are sagittal T1 weighted sections from 4 other patients with pituitary adenomas (E-G) or hyperplasia alone (H).

subsequently in the mouse by Bates et al (46, 47). As originally described, GPR101 shared some sequence homology with adrenergic, muscarinic, serotoninergic, and dopaminergic receptors (46, 47). Classification of GPR101 by TM sequence, general structure, specific regional sequences, and other methods provides divergent results regarding its homology with other GPCRs. Recently, Costanzi et al assessed various strands of data using molecular modeling and artificial intelligence tools including AlphaFold (48). They confirmed the divergent, method-dependent grouping of GPR101 with other GPCRs, such as GPR161, and they developed an in silico model of GPR101 that was based on the α_{1b} adrenergic receptor (48). Concurrently, Yang and colleagues reported a solved cryo-electron microscopy structure (2.89 Å resolution) for GPR101 in its bound state to G_s, one of its G-protein partners (49). This conformation was reported to resemble that of another GPCR, GPR52, when bound to G_s.

In humans, GPR101 is a 508 amino acid protein that is encoded on chromosome Xq26.3. There is a high degree of sequence conservation between humans and Great Apes (>98.6% amino acid identity), which decreases to about 90% to 95% for other primates like Gibbons and Mandrils, and is about 85% for other species like bats, horses, and seals. Homology is modest between humans and mice (70%) and zebrafish (52%). Despite the different homologies and even chromosomal locations, there is notable synteny, or preservation of gene identity and order, in the genetic block containing *GPR101* and surrounding genes that is maintained across humans, mice, and zebrafish (47, 50). This synteny shows the conservation of certain gene regulatory networks between coding and noncoding DNA elements in the region of *GPR101*. In humans, 4 GPR101 transcripts exist, which are generated by alternative splicing at different transcription start sites in the 5′ untranslated region, with isoform 1 being the major transcript overall (Fig. 2) (50). The 3′ untranslated region is approximately 6.1 kilobases (kb) in length. The promoter of *GPR101* probably lacks a TATA box and there is a predicted CpG island 2 kb upstream of the coding sequence that overlaps this promoter (50).

Studies on expression patterns of GPR101 in human and animals show that it is quite restricted in nature. Data from immunohistochemistry are relatively scarce and systematic histology studies across a wide range of normal and diseased tissues have not yet been performed. Much of the available expression data come from RNA datasets, which show expression to be limited largely to the brain. In mice, *Gpr101* is expressed in the hypothalamus (arcuate, medial preoptic, suprachiasmatic, supraoptic, paraventricular nuclei, and the anterior, dorsomedial, ventromedial, lateral, and posterior hypothalamic areas), the amygdala, hippocampus, the lateral parabrachial nucleus, and the medial part of the nucleus of the tractus solitarius (47, 51-53). Similar expression patterns have been reported in the rat, while physiological stimuli such as fasting, obesity, pregnancy, and lactation in rodents led to changes in Gpr101 expression in discrete hypothalamic regions (51, 54). Localization of Gpr101 in inhibitory neurons of the murine basal ganglia has also been described (55). RNA data from adult samples in the Human Protein Atlas overlap these findings, showing consistent brain expression, highest in the hypothalamus and basal ganglia, although there was a low number of samples and marked intersubject variability (56, 57). Detectable levels of *GPR101* RNA are also seen in the human pons, thalamus, medulla, cerebral cortex, and spinal cord (56, 57). Among other normal tissues, GPR101 expression is absent or sparse, apart from in fat, lymphocytes, uvula, and optic nerve (50). In normal adult human anterior pituitary, GPR101 is either absent or expressed at very low levels (38, 50). This is in contrast to the adult rhesus monkey in which Gpr101 immunoreactivity was seen in gonadotrophs, or in adult rats, where Gpr101 staining was positive in somatotropes (50). In cancers, altered GPR101 expression has not been tied as yet to any specific tumor (apart from the

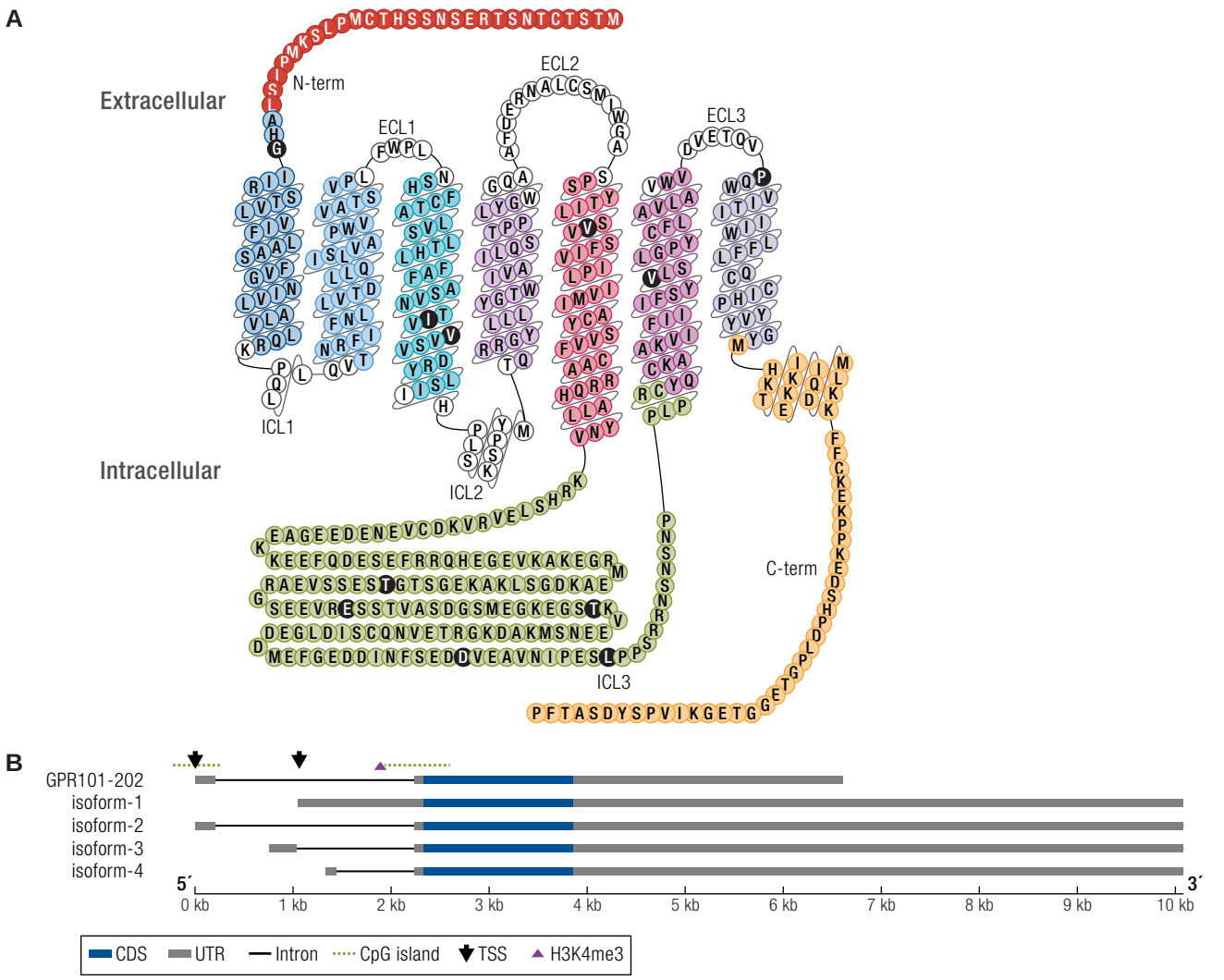


Figure 2. Predicted 2D structure of GPR101 (A), with domains identified as follows: N-terminus domain (red), C-terminus domain (yellow); the 7 transmembrane (TM) domains (blue, cyan, pink, purple shades); intracellular loop 3 (ICL3; green); other domains (white); naturally occurring single nucleotide variants (black). Structural data were retrieved from GPCRdb (https://gpcrdb.org/). (B) The 5 reported GPR101 isoforms. These were drawn using Gene Structure Display Server (GSDS 2.0, http://gsds.cbi.pku.edu.cn/). CpG islands, coding sequences (CDSs), transcription start sites (TSSs) and a H3K4me3 mark are indicated. Adapted and reproduced by the authors from their work in (45).

anterior pituitary) or tumoral outcome. Hypermethylation of the promoter of *GPR101* was, however, reported to be increased in patients with colorectal cancer and correlated with a significantly longer time to disease progression (58).

Most of the published data on GPR101 expression concerns adult tissues. As the phenotype of X-LAG is one of very early childhood onset, we hypothesized that GPR101 might play a role in the development of the anterior pituitary, or at least of certain cell populations like somatotropes. We studied GPR101 staining in a series of human fetal, pediatric, and adult anterior pituitaries from autopsy specimens that were free of pituitary pathologies (50). In the human anterior pituitary, few if any GPR101-positive cells were present up to the end of the 18th week of gestation. There was a progressive rise in GPR101 through to 25 weeks of gestation at which time about one-quarter of cells were positive. Subsequently, GPR101 staining increased greatly to reach about two-thirds of cells by term (38 weeks) (50). GPR101 staining favored the lateral regions of the pituitary gland. In pediatric samples there were very few GPR101-positive cells, while in samples from 2 adolescents positivity was seen although those cells were negative for GH staining. By adulthood GPR101 positivity again disappears. In the fetal rat forebrain (E20), Gpr101 immunoreactivity was readily appreciated in neurons of the accumbens subventricular zone, the rhinencephalic differential field, the striatal neuroepithelium, and striatal subventricular zones around the lateral ventricle, and further into the hippocampus, the lateral third ventricle, anterior hypothalamus, and the amygdala. The developing pituitary demonstrated *Gpr101* expression (RNA) predominantly during the prepubertal period (higher in females than males).

Two studies have examined the role of gpr101 in embryonic growth in zebrafish (50, 59). Using a *danio rerio gpr101* mRNA probe to perform whole mount in situ hybridization, there was rising expression in the brain (approximate to the pituitary and hypothalamic regions) up to day 2 postfertilization that remained strong and constant thereafter. Subsequently Trivellin et al performed knockout and overexpression experiments in zebrafish. First, they showed that overexpression of *gpr101* RNA by direct injection led to developmental

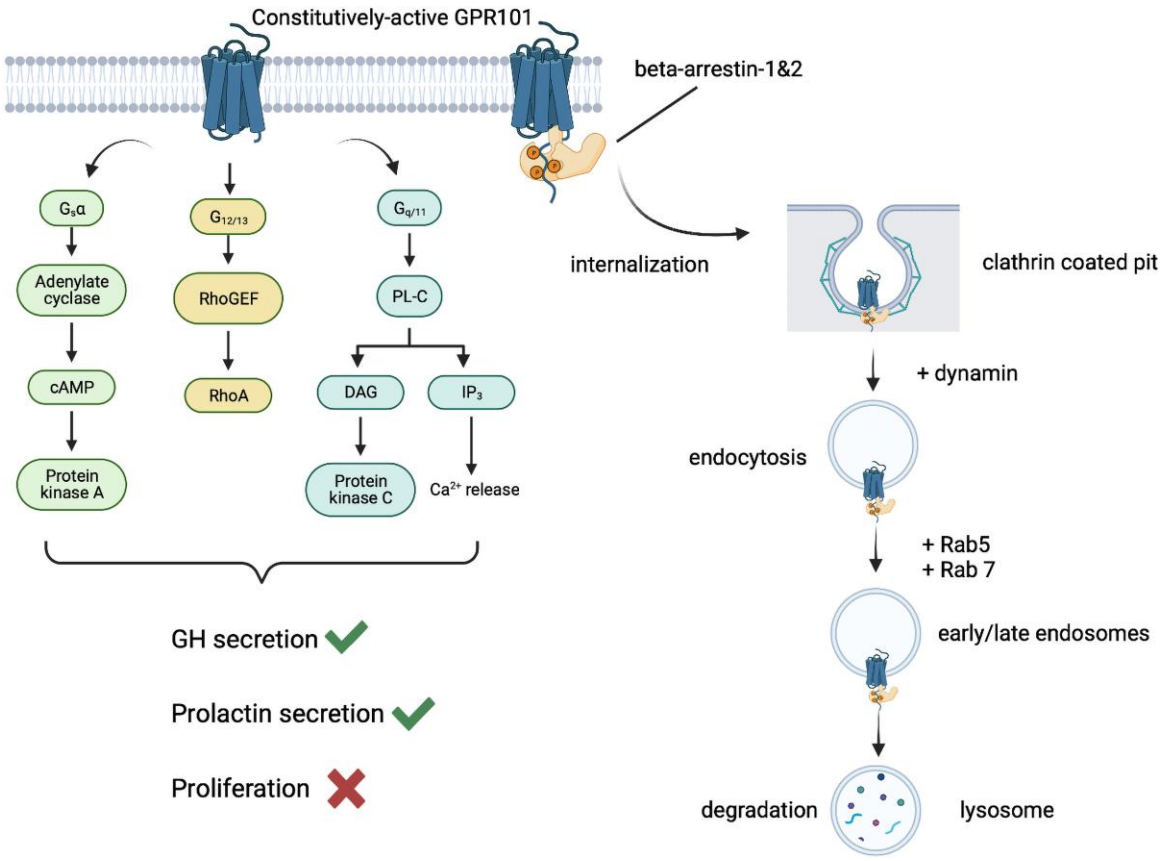


Figure 3. Diagram of the pharmacological characteristics of GPR101-related constitutive signaling. GPR101 was found to constitutively signal through G_s, $G_{q/11}$, and $G_{12/13}$, thereby increasing cAMP, inositol triphosphate, and Rho, respectively. Also, GPR101 constitutively binds to beta-arrestin 1 and 2, thereby triggering internalization via clathrin-coated pits, endocytosis, and eventually lysosomal degradation. DAG, diacylglycerol; IP3, inositol triphosphate; PL-C, phospholipase-C; RhoGEF, Rho-activated guanine nucleotide exchange factor (created with BioRender.com).

abnormalities related to disordered axis pattern (59). Transient low level *gpr101* overexpression did not lead to lasting changes in zebrafish growth. A CRISPR/Cas9 full knockout of *gpr101* led to an undergrowth phenotype with lower girth and length than wild-type controls. Surprisingly, maternal *gpr101* mutants led to greater diminution of body size than the knockouts and this occurred at an early stage and was associated with pathological effects on the hypothalamic–pituitary axis gene expression and hypothalamic morphology. Furthermore, the maternal zygotic *gpr101* model was associated with impaired fertility and a temperature-sensitive phenotype that led to embryonic lethality. Together these results suggest a significant role for *gpr101* in the regulation of very early embryogenic events possibly via dysregulated deposition of maternal mRNAs (59).

Pharmacology of GPR101

Following the initial identification of GPR101, it was predicted that it would couple to G_s and this was confirmed in studies on cyclic adenosine monophosphate (cAMP) generation in GPR101 transfected cells (38, 47). In Abboud et al, we studied the pharmacology of human and mouse GPR101 and determined that it is coupled constitutively not only to G_s but also to $G_{q/11}$ and $G_{12/13}$ (60). Via these different G proteins, GPR101 led to measurable accumulation of cAMP and inositol monophosphate (IP1) and activation of Rho (Fig. 3). In cells transfected with GPR101, these activities spontaneously produce an activation of the mitogen activated protein kinase pathways via phospho-ERK1/2. The contributions of $G_{q/11}$ and $G_{12/13}$ to this process was confirmed using alkaline phosphatase–transforming growth factor beta shedding assays. Upon transfection of rat somatomammotrope GH3 cells with GPR101, increased cAMP and IP1 was accompanied by GH synthesis and release. Blocking G_s and $G_{q/11}$ with small interfering RNAs (siRNA) led to decreased cAMP/IP1 accumulation and a marked reduction in GH release. The downstream targets of GPR101 via G_s and $G_{q/11}$ coupling were found to be protein kinase A (PKA), which was stimulated by G_s and $G_{q/11}$ and PKC, which was the target of only G_s. This dual targeting of PKC and PKA is a specific signature of GPR101 overexpression in the tumors of patients with X-LAG and differentiated them in clinical tumor samples from other aggressive genetic forms of somatotropinomas due to *AIP* mutations (60). Interestingly this dual pattern of signaling exists in immature neonatal somatotropes, which might point to a physiological role for the high GPR101 expression we noted in the developing pituitary gland (50, 61, 62). Very recently Abboud et al extended the findings on constitutive activity of GPR101 to its interactions with beta arrestins (63). These are scaffolding proteins that play a key role in receptor desensitization, trafficking, and also in specific downstream signaling (64). GPR101 constitutively recruits arrestin 2 and 3, which permit its internalization via clathrin-coated pits and its sorting to early endosomes (Fig. 3). The authors suggest that given the high level of constitutive activity

of GPR101, the corresponding constitutive recruitment of arrestins might represent an innate form of regulation of GPR101-mediated actions (eg, hormone secretion), as has been suggested for other GPCRs (63).

Physiology of GPR101 and Putative Ligands

As noted above, GPR101 is mainly found in the brain, particularly the hypothalamus, amygdala, and the basal ganglia, whereas its physiological expression in the pituitary appears to be temporally limited to the fetal period, at least in humans. There are relatively few data in the literature concerning what the physiological role(s) of GPR101 might be. GPR101 remains an orphan GPCR due to the lack of an innate ligand with proven activity across all cells in which it is expressed. Given its strong constitutive activity in pituitary cells, for instance, it might be that no endogenous ligand exists and receptor activity is modulated by altering expression levels alone. That being said, a number of putative ligands have been proposed, and recent advances have led to various potential pharmacological receptor modulators.

The group of T.J. Wu identified a potential role for GPR101 in modulating signaling by a fragment of the gonadotropin-releasing hormone (GnRH), $GnRH_{1\text{-}5}$ (65). The biological activity of $GnRH_{1\text{-}5}$ mediating neuroendocrine function was suggested 30 years ago by Bourguignon et al (66, 67). $GnRH_{1\text{-}5}$ has been shown to regulate GnRH levels in a GnRH-expressing neuron model and to stimulate reproductive behavior in ovariectomized rats independently of the GnRH receptor (68, 69). $GnRH_{1\text{-}5}$ also appears to be capable of influencing GnRH neuronal migration (70, 71). Some actions may occur via GPR101 and another GPCR, GPR173 (65, 72). Bauman et al reported that in an aging ovariectomized rat model, Gpr101 expression was mainly in the medial preoptic area and the arcuate nucleus compared with low pituitary expression (73). In the aging rat with low estrogen, there was a rise in Gpr101 expression in the arcuate nucleus (but not the medial preoptic area), while estrogen treatment in young rats also led to a rise in arcuate Gpr101. Evidence from a Japanese group has recently pointed out that central $GnRH_{1\text{-}5}$ effects stimulating LH release may occur via kisspeptin neurons, while GPR101 can contribute to this process via glutamatergic neurons in the anteroventral periventricular nucleus and kisspeptin neurons in the arcuate nucleus (74).

Outside of the brain, in the Ishikawa endometrial cancer cell model, $GnRH_{1\text{-}5}$ was also shown to induce proliferation via release of epithelial growth factor that was matrix metallopeptidase-9–dependent, leading to transactivation of the epithelial growth factor receptor (65, 75, 76). The effects of $GnRH_{1\text{-}5}$ could be blocked by siRNA or antisense oligonucleotides to silence GPR101 (65). The effects of $GnRH_{1\text{-}5}$ on GPR101 appear to be specific to the neuronal and endothelial models studied, as we noted no effect of $GnRH_{1\text{-}5}$ on the production of GH and prolactin in tumor cells from a patient with X-LAG (77).

Two other potential roles of GPR101 in non-neuroendocrine systems have been proposed. Flak and colleagues studied a novel class of inflammatory mediators derived from leukocytes, the resolvins, whose biological role appears to be protective in the setting of inflammatory cell damage (78). Resolvin D5 ($RvD5_{n\text{-}3DPA}$) has a putative role in limiting inflammation in arthritis models (79). Knockdown of GPR101 had a negative impact on the anti-inflammatory effects of $RvD5_{n\text{-}3DPA}$ by blunting resolvin-mediated reductions in inflammatory eicosanoids in the intestine and joints (78). Macrophages derived from Gpr101 knockout mice displayed a range of pathological changes that led to increased inflammatory responses (80). Qiu et al proposed a role for GPR101 in mediating responses of endothelial cells to shear forces via krüppel-like factor 2, which was modulated by GPR101 in an experimental endothelial model (81). The authors noted that resolvin $RvD5_{n\text{-}3DPA}$ had no effect on krüppel-like factor 2 expression in an endothelial cell model (81). Similarly, we found no evidence to support a role for $RvD5_{n\text{-}3DPA}$ as a ligand of GPR101 in a relevant pituitary model of GH3 cells (Adrian Daly and Albert Beckers, unpublished data).

Very recently, Yang and colleagues reported the structure of GPR101 bound to G_s and the structural basis of its constitutive activity (49). They proposed that while the structure of constitutively active GPR101 might not permit room for a traditional ligand, there existed a side pocket via which a ligand could modulate receptor function. One ligand, termed AA-14, was identified using a screening library and was able to interact with GPR101 to increase G_s signaling. In vitro studies demonstrated that AA-14 could increase Gpr101-mediated hormone production (GH and prolactin) in GH3 cells, while chronic in vivo treatment (2 months) with AA-14 in mice raised GH, IGF-1, prolactin, and thyroid-stimulating hormone. Knocking down/out the activity of Gpr101 in these models abolished the hormonal effects of AA-14. The authors proposed using AA-14 based GPR101 agonism to modulate anti-aging and to improve metabolic profiles. Engineering of an inverse agonist with activity via the side pocket on GPR101 would hold promise for treatment of GH excess in X-LAG, but to date none has been reported in the peer reviewed literature.

Chromatin Dysregulation in the Etiology of X-LAG

Introduction to Topologically Associating Domains

Normal cell function is an immensely intricate process that involves basal activity and dynamic responses to multiple internal and external inputs. At its core is the genome, in which the information for producing RNAs, proteins and other molecules is encoded. The genome itself is broadly the same across somatic cells in each individual, but permits the varied functions of cell types from neurons, epithelium, glands, bone, muscle, etc. These are functions that must occur reliably and automatically in response to cellular requirements, and usually occur as orchestrated patterns of gene upregulation and downregulation. Not only do the nucleotide sequences of genes and other elements have to be conserved, the genome is also organized architecturally. For vital genomic processes to occur appropriately and effectively, the genome has evolved to have a highly regulated, multilevel, and efficient 3D structure. At the highest level there are familiar organizational elements such as chromosomes, or epigenetically active (A) and inactive (B) chromatin compartments. Gene transcription is controlled by cis-regulatory elements, in which transcription factors, enhancers, and other elements interact with the promoters of genes. However, cis-regulatory elements can be physically distant from their gene target on the same DNA strand, thereby necessitating a mechanism to bring them into close proximity. This is explained by loop extrusion in which the promoter and the enhancer are brought into

physical proximity by cohesin, a ring-shaped molecular complex that draws the DNA strand through its center, expending energy by cleaving adenosine triphosphate. This can bring together distant genomic elements so that they can interact directly. Loop formation is halted when cohesin encounters directionally orientated CCCTC binding factor (CTCF) sites. At a local mega-base level there is a high degree of structural–functional organization that has been revealed by chromatin conformation capture techniques like 3C, 4C, and HiC (82-85). Simply put, these methods showed contact maps in which there were increased levels of interactions between local regions of DNA compared with regions lying further apart. At this scale local regions of chromatin are organized into topologically associating domains (TADs) (83, 86). These are regions that are delimited by boundaries containing CTCF (and other elements like transcription factors) that act to increase the likelihood of interactions within the TAD boundaries (intra-TAD) and to decrease interactions between genes in the TAD and regulatory elements outside the TAD boundary (ectopic enhancers). TADs and many of their boundaries are conserved within different cell types in humans and other animals and there is a degree of conservation seen between species. Disruption of TADs by structural or copy number variation is a disease-causing mechanism that produces pathological effects by formation of new interactions between gene promoters and hijacked ectopic enhancers (84, 85). The result of the rewiring of interactions within the neo-TAD can lead to temporal or spatial misexpression of a gene and its product and this can be a disease-causing process. A growing number of diseases caused by neo-TAD formation (TADopathies) have been described in recent years, explaining diverse conditions, such as limb abnormalities, developmental diseases, clotting disorders, retinal diseases, and cancer (87-97).

X-LAG as a Novel TADopathy

In collaboration with Martin Franke's and Giampaolo Trivellin's groups, we recently delineated X-LAG as the first TADopathy of the endocrine system (Fig. 4) (98). As noted above, patients with X-LAG carry small duplications that affect chromosome Xq26.3. All sporadic patients have a different duplication, while familial cases share an identical duplication between affected mother and affected son(s). These duplications all involve *GPR101*, its promoter, and limited regions around the gene. Our earliest studies on X-LAG had revealed a massive upregulation of only *GPR101* (hundreds-fold) in the tumors of patients; such overexpression was not present in non-X-LAG–related somatotropinomas (38). We confirmed and extended these findings using RNAseq data of multiple X-LAG pituitary adenomas and control tumors, showing that *GPR101* is by far the highest overexpressed gene (12 $\log_2$ fold) in somatotropinomas in X-LAG. Such levels of misexpression can be driven by fusions between genes and abnormal promoters; we ruled this out as an explanation for X-LAG. On studying HiC-seq data from normal human (lymphoblastoid) and mouse DNA, and querying chromatin conformation capture databases involving multiple human cell types, we derived a map of the normal TAD structure around *GPR101* (Fig. 4) (98). This showed a consistent pattern in both mice and humans that places *GPR101* alone in its own TAD, with insulation from nearby genes, enhancers, and other elements that were in a separate TAD. We confirmed these data with 4C seq, underlining a lack of interactions between the promoter of *GPR101* and the genes in the centromeric direction (Fig. 4A). When we analyzed the corresponding patterns in DNA from 6 unrelated patients with X-LAG, this showed that despite the different individual duplications, all led to a disruption of the normally invariant TAD border and the formation of new ectopic chromatin interactions (neo-TAD; Fig. 4B). These results confirmed that the addition of a single extra copy of *GPR101* that forms ectopic interactions as part of a neo-TAD can drive the overexpression of *GPR101* seen in X-LAG. There was also a series of potential ectopic enhancers that could -either singly or together- drive the promoter of *GPR101* (98). Ongoing work is addressing the identification and characterization of enhancer–promoter interactions both in the normal physiological state and following enhancer rewiring in X-LAG.

Clinical Characteristics of X-LAG

The Presentation of X-LAG

In X-LAG most patients are born at full term following normal pregnancies. Apart from familial cases, patients with X-LAG reported to date do not have a history of growth disorders in the family and their siblings are of normal size. Pregnancy complications have been reported in individual cases, including vaginal bleeding, small for gestational age, abnormal skeletal development, and maternal drug/alcohol excess (77, 99, 100). Individual cases have shown established overgrowth in the perinatal period or the first months of life (38, 41, 43, 101, 102). For instance, Wise-Oringer et al provided a unique insight on the pregnancy and perinatal presentation in 1 familial X-LAG case (99). The mother, whose diagnosis and treatment of pituitary gigantism had been described in the 1980s, became pregnant following assisted reproduction (103, 104). She had been diagnosed genetically with X-LAG as an adult. During her pregnancy, microarray genetic analysis of a chorionic villus sample revealed a duplication including *GPR101* in the male fetus. Ultrasound demonstrated that the limbs were short due to concomitant rhizomelia, which is not a clinical feature in other X-LAG cases. At birth, the height and weight were unremarkable and the head circumference was +1.96 SD. He had a constellation of facial, thoracic, and limb abnormalities, suggesting a separate skeletal dysplasia, and he required nutritional support from birth to 11 months of life. Elevated levels of GH and prolactin were present soon after birth and the first MRI at 3 weeks of age showed a mass that extended supraselarly (>10 mm in maximum diameter). The mass grew and by 13 months of age measured 18 mm at its largest diameter and abutted the optic chiasm; and despite bromocriptine therapy, physical overgrowth and hormonal excess of GH, IGF-1, and prolactin worsened. Shortly after, he underwent a gross total resection of the mass via a frontotemporal craniotomy, which showed a pituitary adenoma of mixed prolactin and GH positivity, containing separate sparsely and densely granulated populations of lactotropes and somatotropes (99).

The clinical presentation of X-LAG is quite consistent across reported cases and in terms of differential diagnosis is distinct from other major causes of pituitary gigantism, like pathogenic *AIP* variants. Patients are usually female and sporadic; only 3 familial cases of X-LAG have been identified (38, 99, 101, 103, 104). The initial sign is overgrowth, with an increase in height and weight that has a median age of onset of

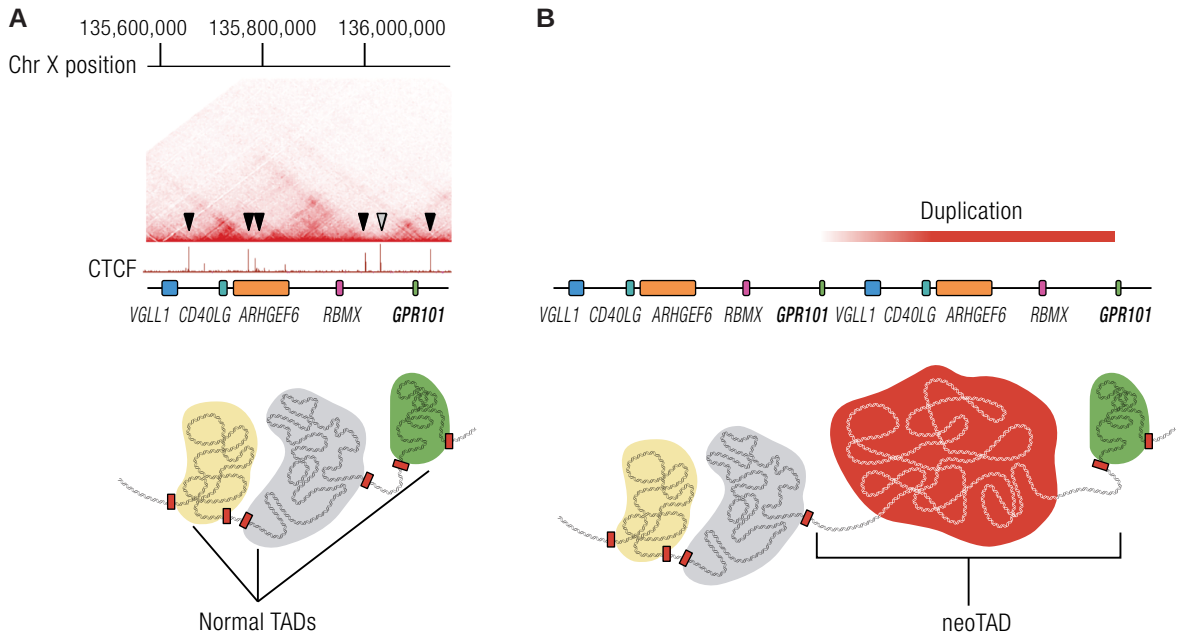


Figure 4. (A). Representation of the normal human TAD structure of the X-LAG locus on chromosome Xq26.3 including *GPR101*. (A) The location on chromosome X is shown on the horizontal line at the top, while below in red are depicted the DNA interactions forming TADs (pyramids). Note *GPR101* sits alone it its own TAD on the right of the figure. The CTCF binding sites that identify TAD boundaries are depicted by vertical black arrowheads, with the normally invariant proximal border of the *GPR101* TAD that is disrupted by duplications in X-LAG identified by a grey arrowhead. At the bottom of A, the normal TADs are represented as colored DNA regions. (B) The typical extent of the tandem duplications that are typically identified in X-LAG. Below, the effect of new ectopic enhancer-promoter interactions are depicted in red as a neo-TAD. CTCF, CCCTC binding factor.

18 months (Table 1). The age at onset of rapid growth is subjective and can present when the child becomes noticeably larger than siblings or age peers. This overgrowth is accompanied by frequent increases of clothes and shoe sizes to those of much older children. During the first 2 to 3 years of life the height and weight increase relatively proportionately so that the child appears large and seems years older than their chronological age but patients are not markedly obese (Fig. 5). At diagnosis the height in reported patients ranged between +1.9 and +11 SD compared with age-corrected means. Examination of growth charts shows that once increased growth begins, the pattern is one of rapidly accelerating height velocity that increasingly diverges from normal curves until effective therapy to reduce IGF-1 is introduced. For weight there is a similar range from +4.1 to +9.3 SD over age-related means, although the gain in weight does not consistently track with height gain as the child with X-LAG ages.

The most frequent physical changes that accompany increased overall body size include large hands and feet and a general coarsening of the facial features with growth of the nose and mandible (Table 2). These acromegaly-like features are also reflected in other signs like increased perspiration or body odor, widening of the interdental spaces, and snoring. Headache and visual signs/symptoms are less frequently reported, but the young age of the patient can make these more difficult to elicit. Particularly during early childhood, some patients with X-LAG can experience increased appetite. The molecular driver of this appetite increase is not yet known, but increased caloric intake is inevitably required to support the characteristic increases in height and weight in X-LAG. Signs of sexual precocity are infrequent in patients with X-LAG, and the bone age is usually normal or slightly advanced.

Table 1. Summary of key features of reported patients with X-LAG

Total population (n)	39
Sex (n, %)	Female: 30 (76.9%) Male 9 (23.1%)
Presentation (%)	Sporadic: 82% Familial (FIPA): 18%
Median age at onset (months, range)	18 months (0-108[a])
Median age at diagnosis (years, range)	4 years (0-22[a])
Median IGF-1 (×ULN, range)	3.1 (1.2-15.9)
Hyperprolactinemia (n, %)	Yes: 31 (79.5%) No: 4 (10.3%) Not available: 4 (10.3%)
Histological pituitary lesion type (n, %)	Adenoma alone: 28 (71.8%) Hyperplasia alone: 4 (10.3%) Adenoma plus hyperplasia: 3 (7.7%) Not available: 4 (10.3%)
Median adenoma diameter (mm, range)	18.2 mm (8-39 mm)
Radiological appearance (n, %)	Macroadenoma: 31 (82.1%) Microadenoma: 1 (2.6%) Diffuse enlargement: 6 (15.4%) Not available: 1 (2.6%)

Abbreviations: FIPA, familial isolated pituitary adenoma; IGF, insulin-like growth factor; ULN, upper limit of normal; X-LAG, X-linked acrogigantism.
[a]Advanced ages at first symptoms and diagnosis occurred in individuals with very limited access to health services.

An intriguing aspect of the pathophysiology of X-LAG is the finding of moderately raised levels of GH-releasing hormone (GHRH) in the circulation of patients at the time of diagnosis (38, 41, 43, 77, 100). Elevated GHRH is not

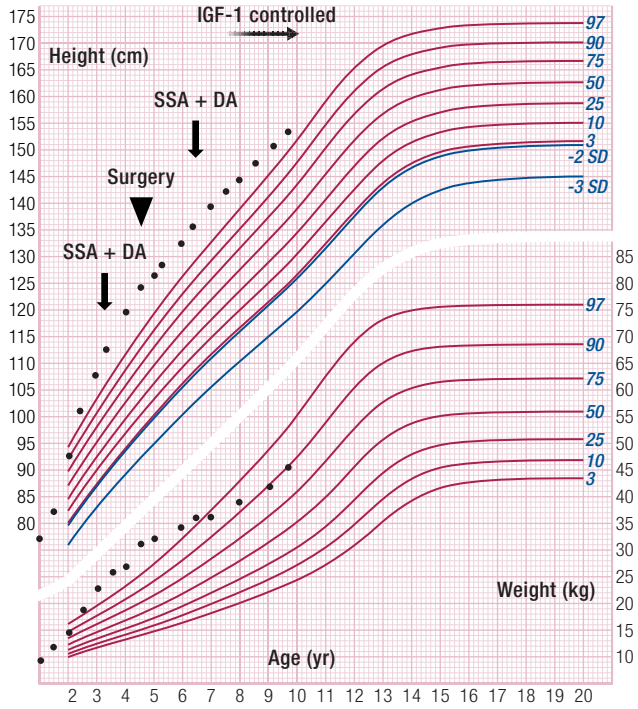


Figure 5. Growth chart from a patient with X-LAG from onset of rapid growth to the achievement of IGF-1 control (105). Early data before the age of 2 are plotted to indicate the onset period for overgrowth. Treatment periods with somatostatin analogs (octreotide and lanreotide) and dopamine agonists (cabergoline) are indicated (arrows) and the date of neurosurgical resection is shown with a black arrowhead. A brief period of treatment with pegvisomant took place after surgery but was halted due to adverse events. Control of IGF-1 was achieved with a combination of surgical gross total resection of a mixed GH and prolactin secreting pituitary adenoma and adult doses of first-generation somatostatin analogs. DA, dopamine agonist; SSA, somatostatin analog; IGF-1, insulin-like growth factor-1.

Table 2. Signs and symptoms of X-linked acrogigantism

Always present	Increased height/length for age Increased weight for age Large hands and feet Coarsening of facial features
Often present	Increased interdental spaces Increased appetite
Occasionally present	Precocious sexual development Increased perspiration or body odor Acanthosis nigricans Small café-au-lait macules Headache Visual field impairment Strabismus
Rarely present	Mild cognitive impairment Acne Ataxia Skeletal developmental disorders

universally found in X-LAG as not all patients were tested, but even in those that are assessed, GHRH may be normal. In historical patients who were subsequently diagnosed with X-LAG, GHRH was measured to rule out ectopic acromegaly due to a peripheral neuroendocrine tumor source. GHRH is not expressed in pituitary tumors in X-LAG (98); as no ectopic source exists, the GHRH appears to arise directly from the hypothalamus, thereby potentially making X-LAG a form of hypothalamic acromegaly. In support of this are the findings of somatotrope, lactotrope, and occasionally somatomammotrope hyperplasia with adenomatous transformation that is seen in some resected X-LAG tumors. This histopathological picture echoes that seen in transgenic mice with endogenous GHRH excess or in patients with hyperplasia due to ectopic GHRH secretion (106-110). Tumors in X-LAG also express the GHRH receptor (GHRHR), which gives credence to a functioning stimulatory role for GHRH in the etiology of the disease (38, 41). In a transgenic mouse model in which Gpr101 was overexpressed only in the pituitary under the control of the GHRHR promoter, we found a surprising dichotomy between the hormonal effects of Gpr101 expression and proliferative effects (60). In these mice overexpressing *Gpr101* only in somatotropes, animals showed a phenotype of excess GH, prolactin and IGF-1 secretion, and physical overgrowth. Unlike in humans with X-LAG, however, the excess hormonal secretion in the mice was not caused by pituitary adenoma or hyperplasia, as the mice had histologically normal pituitaries. In the $Ghrhr^{Gpr101}$ transgenic mice, no abnormal circulating levels of GHRH were detected. Stimulation of pituitary cultures from these mice showed an augmented basal GH secretion and GHRH-induced stimulation was markedly elevated. Together, these data indicate that while *Gpr101* overexpression in somatotropes can induce pituitary gigantism via GH excess in this model, the adenoma and hyperplasia seen in X-LAG likely has another source, such as excess GHRH secretion (Fig. 6). More support comes from in vitro studies of primary cultures of X-LAG tumors (77, 100, 102). Under basal conditions these cells readily secrete GH and prolactin into the medium, while in 2 studies coincubation with GHRH led to augmented secretion GH and more modest effects on prolactin (77, 102). Dopamine agonists had inhibitory effects on both GH and prolactin secretion (77, 100, 102). There were variable effects of octreotide, with studies showing moderate inhibition or no effect, while pasireotide significantly reduced GH but not prolactin in 1 study. Basal secretion of GH and prolactin can be blocked with a GHRH antagonist (77). The precise source of GHRH and the role GPR101 dysregulation plays in its secretion in many cases of X-LAG remain to be determined. In an early case later confirmed as having X-LAG, Zimmerman et al perceptively noted that generation of GHRH in the hypothalamus could provide extremely high local GHRH concentrations to the pituitary, thereby stimulating tumorigenesis (100). The ontogeny of the tumoral/hyperplastic cells in X-LAG might be significant in addressing these issues. There is long-established evidence that fetal/immature somatotropes differ significantly from adult somatotropes in terms of the responses to stimulatory and feedback inhibitory signals like IGF-1 (61, 111-118). Furthermore, the desensitization of somatotropes to GHRH that is seen in adults is not present in the immature state in rats and other models, and other data point to a role for GHRH in upregulating its own receptor in immature somatotropes (61, 62, 111, 119). The increased GPR101 expression seen in fetal human somatotropes could play a role in maintaining high GH secretion in late fetal life (50). Hypothetically, the pathological overexpression of GPR101 in tumoral tissue in X-LAG could represent the persistence of a normally repressed fetal somatotrope phenotype. Possibly GPR101 overexpression could permit constitutive

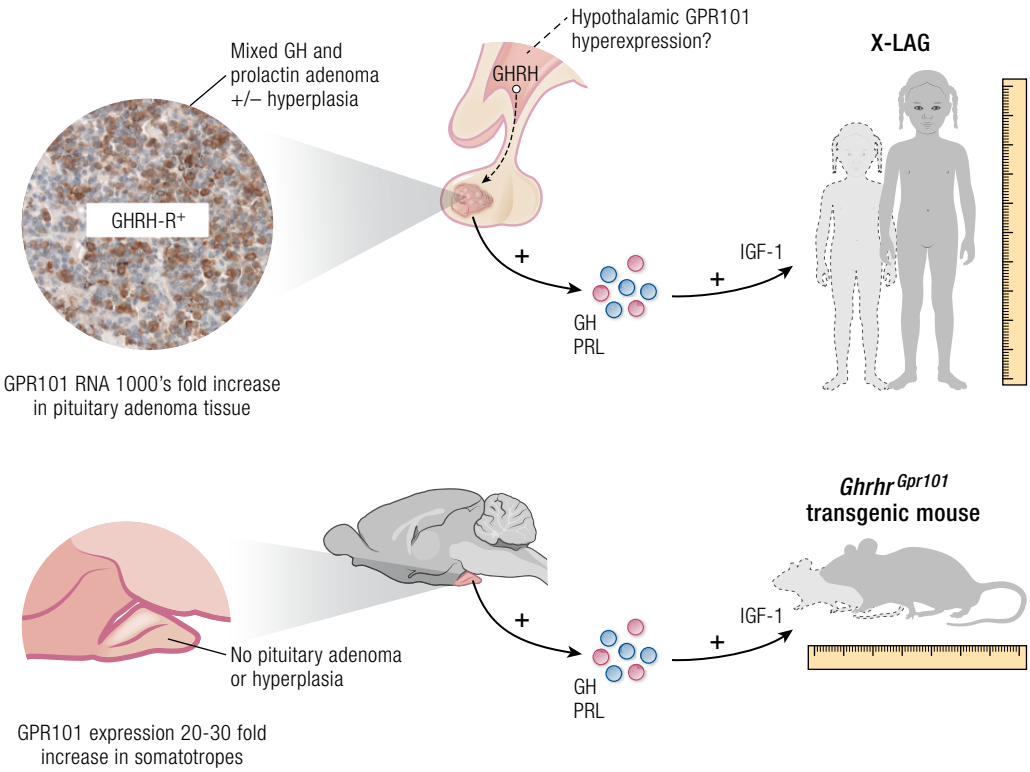


Figure 6. Potential model of gigantism due to GPR101 overexpression in X-LAG and in *Ghrhr*^{Gpr101} somatotrope-specific transgenic mice. In the upper panel, a disease pathway is proposed where the neo-TAD formation is associated with massive GPR101 overexpression in the pituitary causing hyperplasia/adenomas, GH, prolactin, and IGF-1 excess, and gigantism. In patients with X-LAG a proportion have had significant circulating GHRH levels detected and adenomas have been shown to express GHRHR. This leads to the possibility of hypothalamic GHRH hypersecretion driving the proliferative phenotype due to misexpression of *GPR101* in the hypothalamus. In contrast, in *Ghrhr*^{Gpr101} transgenic mice with *Gpr101* overexpression limited only to somatotropes, there is GH, prolactin, and IGF-1 excess, but no pituitary adenoma/hyperplasia and normal circulating GHRH.

hormonal secretion lacking feedback inhibition via raised IGF-1, while maintaining an active GHRH secretion and pituitary GHRHR expression loop (Fig. 6).

Investigation of X-LAG

As physical overgrowth progresses and signs and symptoms become more prominent, a referral for investigation is generally sought. This period from first symptoms to a definitive diagnosis of a pituitary disorder can sometimes take from 2 to 3 years. Typically, the median age at diagnosis is 4 years (Table 1), but can be prolonged in regions with poor health infrastructure and limited access to modern diagnostics. Hormonal testing usually reveals a pattern of greatly elevated GH and a raised IGF-1. The median reported IGF-1 is 3.1 times the upper limit of normal for age and sex, but it can vary, with only slightly abnormal values in the youngest patients to levels as high as >10-fold the upper limit of normal in those diagnosed later in childhood with large tumors. Hyperprolactinemia accompanies GH/IGF-1 excess in nearly 80% of cases of X-LAG, and can be very high at presentation. Despite markedly raised prolactin levels, galactorrhea is not a feature of the disease at presentation. It is now clear that X-LAG can occur with GH/IGF-1 excess alone (41, 43, 120-122). Pathology results were available on only 2 cases with normal circulating prolactin (the other 2 were not operated) and showed prolactin staining to be present in 1 tumor.

MRI of the brain is a key step in making a diagnosis of pituitary gigantism during the initial investigation of a child with overgrowth. In X-LAG the MRI at diagnosis is usually consistent with a large pituitary adenoma or a uniform enlargement suggesting hyperplasia (Fig. 1). Overall, macroadenomas predominate (82.1%), whereas only 1 microadenoma (2.6%) was reported; diffuse enlargement suggesting hyperplasia was seen in 15.4%. The median diameter of adenomas at diagnosis was 18.2 mm, with a range of 8.0 to 39.0 mm. One case of limited pituitary changes due to likely hyperplasia was reported by Burren et al, which led to a lack of a defined pituitary mass on MRI (43, 120). While the largest tumors tend to occur in patients with X-LAG that had a later diagnosis, most patients present with a macroadenoma, even as infants and toddlers. Pituitary lesions typically exhibit suprasellar extension, whereas carotid sinus invasion is infrequent.

Histopathology of Pituitary Lesions

As with the clinical presentation of the disease, the histopathological studies relating to pituitary tumors and hyperplasia in X-LAG have some broadly similar characteristics across different patients (38, 41-43, 99, 100, 102, 105, 123). The most frequent finding is one of a mixed-type GH and prolactin pituitary adenoma/pituitary neuroendocrine tumor. Distinct somatotrope and lactotrope cell populations can be discerned by immunohistochemistry. Positivity for other pituitary hormones is infrequent and scattered. On hematoxylin and eosin staining, the tissue has an enlarged or hyperplastic acinar structure with trabeculae and a sinusoidal and lobular growth pattern. The normal reticulin fiber in hyperplastic acini can

break down into confluent acinar transformation zones. Within areas of transformation, small regions with absent reticulin structure can form nodules like microadenomas in some cases. Evidence of extensive mammosomatotropic hyperplasia (GH and prolactin in the granules of the same cell) is present in individual specimens (confirmed on electron microscopy) (43). There is no clear relationship between patient age at time of surgery and the presence/absence of hyperplasia, so it is not definitively known if hyperplasia can be considered a precursor to adenoma formation in X-LAG. Hemorrhage and necrosis are not generally seen, colloid collections are common and some cases have psammomas and microcalcifications. Among the adenomatous and hyperplastic GH and prolactin positive cells there is evidence of intermingled acidophilic and chromophobic cell types. The acidophils (GH positive, densely granulated, low mitotic activity) make up the majority of cells in most but not all cases (38, 41, 43, 99, 105); they are large with a round central nucleus and eosinophilic cytoplasm. The chromophobic cells were sparsely granulated and were positive for GH or prolactin. Iacovazzo et al reported 1 case that had radiotherapy before surgery in which the nuclei were more hyperchromic, with associated perivascular fibrosis and 1 atypical mitosis was seen in that case (43). Ki-67 is usually <3%, although levels higher than 5% were seen in the cases from 1 family (99); weak nuclear p53 positivity is typical. Between 90% and 100% of cells are Pit-1 positive. Other transcription factors were reported to be positive in cases from 1 kindred: frequent SF1 (gonadotrope lineage) and rare TPIT positivity and abundant cells that were positive for stem cell markers OCT4 or SOX2 (99). Cells have variable somatostatin receptor subtype 2 (SSTR2) positivity (low, moderate, or high) and have a membranous and cytoplasmic staining pattern. Positivity for SSTR1, and SSTR5 is low to moderate and SSTR3 moderate to high. In specimens studied for GHRHR immunoreactivity was higher than that seen in normal pituitary. Pituitary GHRH staining has not been identified. Iacovazzo et al reported electron microscopy results in a group of tumors which showed the predominant cell type to be densely granulated, likely somatotrope cells that had well developed Golgi and rough endoplasmic reticulum with granules of 250 to 600 nm. The sparsely granulated cells were either probable lactototropes (granules of 150-300 nm) or somatotropes (fibrous bodies and granules 200-450 nm in diameter).

The Management of X-LAG

Aims of Treatment

International consensus guidelines for the management of acromegaly are regularly updated and provide detailed, evidence-driven recommendations on the diagnosis, treatment, and outcomes of the disease (124-127). Owing to its rarity, there are few specific management recommendations relating to pituitary gigantism as a whole, or for genetic diseases like X-LAG in particular. In the pituitary gigantism population, there is an added consideration that is not present in adults, namely the need to halt GH/IGF-1–driven overgrowth during childhood and adolescence and to limit the excess final height. In very young–onset disease like X-LAG, prompt control of GH/IGF-1 excess should be sought, in order to achieve as close to normal height as possible before the onset of the pubertal growth spurt.

Neurosurgery

The management of X-LAG is complicated by a series of factors relating to the patient population and the underlying molecular pathology of disease. This is a very young patient population (median age at diagnosis: 4 years) that presents with a macroadenoma or extensive hyperplasia that exuberantly secretes excess GH and usually prolactin. Effective disease control requires timely access to and consistent follow up by pediatric neurosurgery, endocrinology, medical radiology, and neuroradiology teams. From a surgical anatomy perspective, the skull base in the young, growing child raises a number of important challenges for safe access. It is well established that at the typical age at which patients with X-LAG are diagnosed, the usual channels for access are narrow, the sella and other skull base structures are incompletely pneumatized and distances between landmarks and vital structures are altered compared with adults (128-131). These factors combine to make access to and safe resection of extensive macroadenomas challenging. Also, the extent of interventions to resect pituitary lesions in X-LAG need to be balanced against the real risks of provoking permanent hypopituitarism.

Extensive gross total, and focused tumor resections have been performed in patients with X-LAG. Overall, nearly 90% of patients have had neurosurgery, and 23.1% went on to have subsequent operations (maximum: 4). In cases of X-LAG where much of the gland is affected by hyperplasia, patients have been treated surgically with anterior hypophysectomy (38, 101). This has the effect of immediately controlling GH excess and inducing definitive panhypopituitarism. More frequently surgery is undertaken to grossly resect all visible and accessible tumor. While this debulking is often extensive and reduces GH and IGF-1 secretion, it is not curative and also has a high rate of hypopituitarism. A particular difficulty caused by the tumor biology in X-LAG is that the very high GH secretion induced by the constitutively active GPR101 means that even tiny remnants can lead to chronic pathological IGF-1 excess (41, 132-134). Unusually, these highly hormonally active surgical remnants do not have a propensity to regrow. We hypothesize that surgery itself might interfere with tumor behavior by physically interrupting the flow of GHRH to activate GHRHR on remnant tumor cells.

Medical Therapy and Radiotherapy

Medical therapy with first-generation somatostatin analogs (SSAs), such as octreotide and lanreotide, can be used as a primary medical therapy or as an adjunct to neurosurgery. In X-LAG, primary SSA therapy alone has been unsuccessful in controlling GH/IGF-1 and excess growth (38, 41, 43, 100, 102, 120, 134). Even with adult doses in young children, SSAs have a small effect on IGF-1 despite tumor tissue expressing SSTR2 (41, 43, 122, 134, 135). No clinically relevant effects of SSA monotherapy on tumor size in X-LAG have been reported. The full explanation for this resistance is unknown, but the very high expression of constitutively active GPR101 may overwhelm the inhibitory effect derived from SSAs via SSTR2. Debulking of tumor mass can improve responses to postoperative SSA therapy in acromegaly generally (136). Likewise, in 2 children with X-LAG that were resistant to primary SSA therapy and who had active disease following gross total tumor resection, postoperative SSA at 30 to 40 mg/month of octreotide LAR led to IGF-1 control (41, 134). There is little information on the efficacy of the

second-generation SSA, pasireotide, in the management of X-LAG, but it appears to be similar to that of older SSAs (121). Dopamine agonist therapy also has little impact on GH/IGF-1 secretion and growth, but is very effective in controlling concomitant hyperprolactinemia (38, 41, 43, 134). Cabergoline and bromocriptine have been widely used in patients with X-LAG at normally prescribed adult doses and appear to be well-tolerated. A key tool in the effective and rapid control of IGF-1 excess in X-LAG is the GH receptor antagonist, pegvisomant. Like in other forms of aggressive and SSA-resistant pituitary gigantism, IGF-1 excess in X-LAG can be readily blocked with pegvisomant (35). This can take the form of adjuvant treatment in combination with adult doses of a SSA, with the pegvisomant injections titrated in dose and timing (daily, every other day) to IGF-1 levels (35, 38, 41, 43). Also, Burren et al reported the safe and effective use of pegvisomant alone for an extended period of time through to final adult height in a patient with X-LAG due to probable pituitary hyperplasia (120). Various forms of radiotherapy have been used as an adjuvant to surgery in X-LAG (yttrium seeds, conventional, gamma knife, etc.). The onset of effect for radiotherapy is generally prolonged (years), so it is not a major option for achieving rapid hormonal and growth control in patients with X-LAG. As the slow onset of effect of radiotherapy on GH is associated with long-term insufficiency of other hormonal axes, this further underlines its third-line role when other options have failed.

Outcomes of Treatment

Multimodal therapy is the norm in X-LAG with a median of 4 cumulative treatments received by patients (Table 3). For patients with inoperable or difficult to locate tumor remnants, the options include chronic medical therapy for decades, or potentially the use of gamma knife. On the other hand, tumor regrowth or re-expansion during long-term follow-up rarely, if ever, occurs. Despite the hurdles to treatment outlined above, control of IGF-1 is achieved in two-thirds of patients (Table 3). This comes at the cost of hypopituitarism, particularly in those having more radical neurosurgical resections.

Table 3. Overview of treatments and outcomes in X-LAG

Hormonal control at last follow-up (n, %)	Yes: 31 (79.5%) No: 8 (20.5%)
Median cumulative number of treatments[a] (n, range)	4 (0-9)
Neurosurgery performed (n, %)	Yes: 35 (89.7%) No: 4 (10.3%)
Radiotherapy administered (n, %)	15 (38.5%)
Hypopituitarism (n, %)	Any axis: 26 (66.7%)
	0 axes n = 9
	1 axis n = 4
	2 axes n = 5
	3 axes n = 10
	4 axes + AVD n = 7
	N/A n = 4
Control achieved without hypopituitarism (n, %)	8 (20.5%)

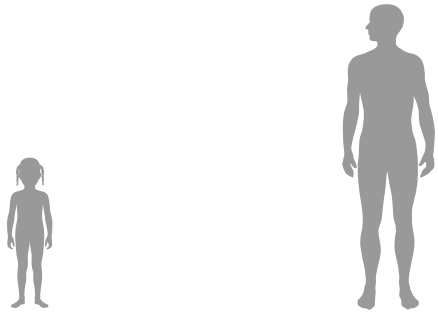
Abbreviations: AVD, arginine vasopressin deficiency (diabetes insipidus); X-LAG, X-linked acrogigantism;.
[a]Each individual surgical intervention or separate radiotherapeutic modality is counted as 1; use of somatostatin analogs, dopamine agonists, or growth hormone receptor antagonist each counted as 1.

Only a minority of patients (20.5%) achieved disease control without hypopituitarism. Pituitary hormone replacement therapy can include induction of puberty and, if needed, GH therapy to reach a normal final height in patients with X-LAG that had complete tumoral resection in early childhood (29, 38, 41, 43, 99, 103, 104). Sexual maturation and fertility are affected in those treated for X-LAG, with 2 of the 3 familial cases needing assisted reproduction to achieve pregnancies (38, 99). In summary, there are multiple routes by which GH/IGF-1 and overgrowth can be controlled in X-LAG, and even with significant postoperative hypopituitarism, positive outcomes in terms of final height and pituitary function can be achieved.

X-LAG: Conclusions and Perspectives

In the nearly 10 years since its first discovery, subsequent research on X-LAG has highlighted a novel pathway of GH regulation and identified GPR101 as a new player in somatotrope function. Study of the patients reported to date confirms that X-LAG is a rare and potentially severe form of pituitary gigantism that usually begins from birth to the first year of life. Its presentation generally occurs in a choreographed fashion, with a previously healthy infant developing marked overgrowth in terms of height and weight, accompanied by acromegalic-like signs. This is due to a mixed GH and prolactin positive macroadenoma with suprasellar extension in the majority of cases, and treatment is complex due to the large tumors, the young patient age and the relative resistance to treatment with somatostatin analogs. Under exceptional circumstances, Xq26.3 duplications can present in families, representing a second genetic cause of FIPA (after *AIP* pathogenic variants). Together X-LAG and *AIP* pathogenic variants are the 2 most common genetic causes of pituitary gigantism (nearly 40% of cases). Extensive international programs have helped to delineate the characteristics of pituitary gigantism due to these 2 causes, which can guide the differential diagnosis (Fig. 7) (35, 137, 138).

The identification of X-LAG has provided a diagnosis for various previously unexplained cases of extreme early-onset gigantism (24, 26, 100-102, 104, 139). Indeed, X-LAG is the most likely disease mechanism behind many of the tallest humans in history (eg, Robert Wadlow, Julius Koch, Sandy Allen) who suffered from pituitary gigantism associated with inexorable overgrowth due to aggressive pituitary adenomas that began in very early childhood (5, 13, 140, 141). Severe outcomes in terms of final height and GH-driven morbidities are faced by patients with X-LAG when early and effective treatment is not available (41, 43, 123). For children and adolescents with GH-driven overgrowth due to X-LAG, *AIP* pathogenic variants, or otherwise, avoidance of an extremely elevated final height must be a priority of treatment. In X-LAG, control of GH/IGF-1 and prolactin excess is achievable, but often requires early access to pediatric neurosurgery, while medical therapy with pegvisomant offers an effective means of IGF-1 control. Multimodal therapy is common in X-LAG, but unfortunately the cumulative effect of treatment, particularly extensive surgery and radiotherapy, leads to a high rate of hypopituitarism. With careful management of pituitary hormone function, normal pubertal development and final height can be achieved. In women affected by X-LAG there is a 50% chance of passing the illness on to their children, which has occurred in 3 kindreds to date in an



	X-LAG (Xq26.3 duplications)	*AIP* pathogenic variants
Cause of pituitary gigantism (%)	10%	29%
Sex (%)	77% female	95% male
Age at onset (yr)	1.5	13.3
Age at diagnosis (yr)	4	16.8
Other associated tumors/features?	No	No
Familial (%)	18%	42.9%
Pituitary lesion	Mixed GH-prolactin adenoma; adenoma (72%, 1 microadenoma), hyperplasia (10.3%), adenoma + hyperplasia (7.7%)	GH or mixed GH-prolactin adenoma; macroadenoma (90.2%)
Prolactin co-secretion present (%)	77%	33.3%
SSA resistance	Y	Y

Figure 7. Summary of the different characteristics of pituitary gigantism due to X-LAG and *AIP* pathogenic variants, the 2 most common and best-characterized genetic forms of the disease. Data on *AIP* associated disease taken from Rostomyan et al (35).

X-linked dominant pattern. As 2 familial cases occurred following assisted reproduction, preimplantation genetic testing might be considered in women affected with X-LAG trying to conceive. No males with X-LAG have, to our knowledge, fathered children, but males with X-LAG should receive genetic counseling regarding the risk of disease transmission to daughters. Deletions or sequence variants involving *GPR101* have not been strongly associated with clinical disease. While some missense variants can alter GPR101 function in vitro, a compelling phenotype associated with GPR101 loss or inactivation remains to be described (38, 142-144).

Nearly 10 years of research on X-LAG has clarified many aspects of the disease and its cause, but a number of important issues remain. The developmental and physiological role of GPR101 in the anterior pituitary, the hypothalamus, and elsewhere remains poorly understood. Studies of the pituitary and brain during development and maturation are needed to reveal the identity and function of cells normally expressing GPR101. Data regarding the expression of GPR101 in the pituitary and hypothalamus in animals varies by species and potentially by sex and age, while data in humans are very limited. Insights could be derived from animal models, such as mice expressing a duplication that alters the TAD structure at GPR101 and promotes its overexpression. That information could throw some light on unexplained aspects of X-LAG such as the source and mechanism for the GHRH oversecretion seen in a subset of patients. GPR101 is now understood to be a highly constitutively active GPCR that can act through G_s, $G_{q/11}$, and $G_{12/13}$, and PKA/PKC to promote GH hypersecretion in vitro and in vivo. Recent data have shown that GPR101 could be modulated by ligands acting by binding at a side pocket in the receptor to increase constitutive activity and promote hormone release in mice. In X-LAG an inverse agonist of GPR101 that could lower constitutive activity and blunt exuberant GH secretion would be of clinical interest.

The very high levels of overexpression of GPR101 in the pituitary of patients with X-LAG is explained by the recent discovery that the chromosome Xq26.3 duplications alter the chromatin structure at the GPR101 locus and create a neo-TAD in which ectopic enhancers drive the GPR101 promoter. This TADopathy is a genomic mechanism that is a growing cause of previously unexplained diseases. We showed that *GPR101* exists normally in its own TAD. There are other developmentally important genes that sit alone in a TAD, and these are good candidates for disruption by duplication and deletion. As chromosome capture techniques become more widely used in multiomics-based clinical research, it is possible that other as yet unexplained neuroendocrine disorders, like some of the 50% of pituitary gigantism, will be unmasked as TADopathies.

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Disclosures

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