THEMATIC REVIEW

HEREDITARY ENDOCRINE TUMOURS: CURRENT STATE-OF-THE-ART AND RESEARCH OPPORTUNITIES

GPR101, an orphan GPCR with roles in growth and pituitary tumorigenesis

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Abstract

We recently described X-linked acrogigantism (X-LAG) in sporadic cases of infantile gigantism and a few familial cases of pituitary gigantism in the context of the disorder known as familial isolated pituitary adenomas. X-LAG cases with early onset gigantism (in infants or toddlers) shared copy number gains (CNG) of the distal long arm of chromosome X (Xq26.3). In all patients described to date with Xq26.3 CNG and acrogigantism, the only coding gene sequence shared by all chromosomal defects was that of *GPR101*. GPR101 is a class A, rhodopsin-like orphan guanine nucleotide-binding protein (G protein)-coupled receptor (GPCR) with no known endogenous ligand. We review what is known about GPR101, specifically its expression profile in human and animal models, the evidence supporting causation of X-LAG and possibly other roles, including its function in growth, puberty and appetite regulation, as well as efforts to identify putative ligands.

Key Words

- hypothalamus
- pituitary
- gigantism
- cyclic AMP
- protein kinase A
- growth hormone

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Introduction

In 2014 (Trivellin *et al.* 2014), we described X-linked acrogigantism (X-LAG, MIM #300942), a disorder that presents with infant-onset overgrowth and gigantism due to growth hormone (GH) over secretion (Beckers *et al.* 2015, Hannah-Shmouni *et al.* 2016, Trivellin *et al.* 2018*a*). The clinical characteristics of patients with X-LAG are described in the accompanying review by Vasilev *et al.* (2020).

In this review, we will focus on the identification of what strongly appears to be the causative gene in X-LAG, *GPR101*, a class A, rhodopsin-like orphan guanine nucleotide-binding protein (G protein)-coupled receptor (GPCR) (https://www.guidetopharmacology.org/GRAC/GPCRListForward?class=A) with an as yet unknown endogenous ligand (Alexander *et al.* 2019), the delineation of its expression and ongoing work in animal models.

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Identification of *GPR101* in X-LAG

To date, 36 patients have been reported to have X-LAG due to Xq26.3 copy number gains (CNG) (Trivellin *et al.* 2018*a*, Trivellin & Stratakis 2019). In more than 30 of these cases, array comparative genomic hybridization (aCGH) has been performed and confirmed that the smallest region of overlap (SRO) by these non-recurrent genomic rearrangements contains a single fully coded gene within a 73-kb sequence that is known to harbor *GPR101* (SRO2) (Trivellin *et al.* 2018*a*). Patients also share an 8-kb sequence that includes the last two exons of the *VGLL1* gene and the miRNA *miR-934* (SRO1). All CNG described so far are duplications that involve just the Xq26.3 locus with no other genomic regions consistently affected (e.g., no insertions of the Xq26.3 genes to distant loci were reported).

The originally reported SRO contained at least four protein-coding genes and a number of other sequences, but subsequent work narrowed the X-LAG-linked region down to SRO1 and SRO2. Two subsequent cases helped to confirm the importance of *GPR101*: one subject with X-LAG in whom the SRO contained only *GPR101* (Iacovazzo *et al.* 2016) and another boy with various developmental defects, who carried a Xq26.3 CNG that stopped short of including *GPR101* and did not have overgrowth or other X-LAG related abnormalities (Trivellin *et al.* 2018*b*).

The duplications are on average about 600-kb long and are generated by replication errors at regions of microhomology which, in all but one case analyzed so far, can be explained by the mitotic DNA replication-based mechanism of fork stalling and template switching/microhomology-mediated break-induced replication (FoSTeS/MMBIR) (Trivellin *et al.* 2014, Beckers *et al.* 2015, Iacovazzo *et al.* 2016). An Alu-Alu mediated rearrangement was reported in the remaining case (Iacovazzo *et al.* 2016). So far, the CNG were germline in females and somatic in sporadic males, with mosaicism levels varying from 15% to 60% (Daly *et al.* 2016*a*, Iacovazzo *et al.* 2016, Rodd *et al.* 2016). However, the clinical characteristics of X-LAG patients are similar in both sexes (Beckers *et al.* 2015, Daly *et al.* 2016*a*). This suggests that even a small percentage of cells harboring the duplication in specific tissues is sufficient to cause the phenotype, as we have reviewed elsewhere (Trivellin *et al.* 2018*a*).

Moreover, the contribution of X chromosome inactivation (XCI) in females should be taken into consideration, as this could alter the expression of the duplicated genes. To test this hypothesis, we conducted an XCI analysis in 12 X-LAG patients. We observed skewed XCI in just two patients (17%) (Trivellin *et al.* 2014, Trivellin & Stratakis 2019). However, no significant differences in clinical phenotypes were observed in these two patients compared with the rest. This observation resembles what happens in mosaic males, in whom just a small fraction of cells with the duplicated X-chromosome leads to acrogigantism. While we cannot completely rule out an effect of age on the rate of skewing (these two patients were tested for XCI using blood that was collected, on average, 26 years later than the others), these data suggest that X-LAG patients commonly undergo random XCI. Most recently, it was reported that patients with autoimmunity and *CD40LG* duplications preferentially inactivate their duplicated X-chromosome (Le Coz *et al.* 2018). Although *CD40LG*, located in the Xq26.3 region about 400 kb upstream of *GPR101*, is commonly duplicated alongside *GPR101* in patients with X-LAG, no autoimmune phenotypes have been observed to date (Beckers *et al.* 2015, Iacovazzo *et al.* 2016). This likely suggests that Xq26.3 CNG associated with X-LAG have a different impact on local chromatin domains and consequently on gene regulation, therefore causing unrelated phenotypes.

There are several results that support a crucial role for *GPR101* as being the causative gene in X-LAG. Besides being the only entire protein-coding gene that is always duplicated in all X-LAG patients analyzed so far, as stated previously, it is also strongly over-expressed in their pituitary tumors/hyperplasia (up to 1000 times the expression levels detected in non-duplicated somatotroph tumors or the normal pituitary gland) (Trivellin *et al.* 2014, 2016), and it activates the cyclic AMP (cAMP) signaling pathway (Bates *et al.* 2006, Trivellin *et al.* 2014), which is one of the major stimuli of proliferation and GH and prolactin secretion in pituitary cells (Hernandez-Ramirez *et al.* 2018). *GPR101* seems thus to behave as a putative oncogene in X-LAG, but it remains to be fully elucidated how this GPCR is involved in the pathogenesis of this disease. To address this aspect, our groups are currently characterizing GPR101 transgenic and knockout mouse and zebrafish models.

Moreover, there is no published data as yet that explain how *GPR101* expression is so massively enhanced in X-LAG lesions. Simplistically, by duplicating the genomic content of a DNA segment, one can expect to find double the expression of the affected genes. Clearly, what happens in X-LAG is more complicated than that. We hypothesize that Xq26.3 CNG affects local interactions of non-coding regulatory sequences;

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this, in turn, leads to the abnormal expression of *GPR101* in specific tissues, such as the pituitary (Trivellin *et al.* 2014). Other tissues might be involved as well, but this will be difficult to prove since X-LAG patients develop gross lesions affecting only the pituitary gland; finer cellular and subcellular abnormalities in other tissues such as the brain are difficult to assess in humans. The animal models under study can also help to shed light on this still obscure aspect of X-LAG molecular pathogenesis.

Although GPR101 was identified as a GPCR linked to Gs and is constitutively active, as shown by increased cAMP production following transient over-expression in HEK293 and GH3 cells (Bates *et al.* 2006, Trivellin *et al.* 2014), one recent study reported conflicting results (Martin *et al.* 2015). In the experimental setting used by the authors (CHO-K1 cells), GPR101 did not meet their criteria for constitutive activity (200% elevation over baseline cAMP-dependent response element (CRE) reporter activity). Further studies will be needed to address these different observations about the pharmacology of GPR101 in different cellular settings.

The *GPR101* gene and protein: structure and expression

Structure

The human *GPR101* gene was identified and found to be located on the X chromosome in 2001 (Lee *et al.* 2001), while its murine orthologue was identified 5 years later (Bates *et al.* 2006). Until we reported in late 2014 that *GPR101* was associated with a pathological condition, X-LAG, only a handful of studies had investigated this orphan receptor, mainly in rodents. In the following 5 years, more than 30 studies on *GPR101* and/or X-LAG came out, greatly contributing to expand our understanding of this disease and the biology of this receptor (Fig. 1). Indeed, until recently, just the coding sequence (CDS) of *GPR101* was reported in human genome databases (NM_054021.1). *GPR101* encodes a 508 amino acid-long GPCR; its CDS is composed of a single protein-coding exon that is about 80% and 55% identical to the rodents and zebrafish orthologues, respectively (http://www.ncbi.nlm.nih.gov/homologene/). Interestingly, some domains seem to have diverged substantially more than others during evolution, especially the intracellular loop 3 (ICL3) (Trivellin *et al.* 2018*a*, Hou & Tao 2019).

We contributed to the characterization of the structure of *GPR101* by investigating its repertoire of transcripts (Trivellin *et al.* 2016*a*). We identified four isoforms generated by alternative splicing involving the 5′UTR; RNA-Seq data from X-LAG tumors allowed to discover that the 3′UTR is 6.1-kb long (Fig. 2). Isoform-1 is expressed at levels much higher than what is seen for the other isoforms in the pituitary cells of X-LAG patients; this finding suggests that this represents the main transcript, at least in the pituitary gland (Trivellin *et al.* 2016*a*). Supporting our findings, in the most recent assembly of the human genome (hg38), a novel transcript (ENST00000651716.1, GPR101–202) has been manually annotated from the Havana project (https://www.sanger.ac.uk/science/groups/vertebrate-annotation). The structure of this transcript closely resembles that of isoform-2 (Fig. 2), and it may be that the two isoforms actually represent the same transcript species.

We also predicted using bioinformatics that the *GPR101* promoter is TATA-less and overlaps with a CpG island located about 2 kb upstream of the CDS, thus including the first non-coding exon of isoform-2 (Trivellin *et al.* 2016*a*).

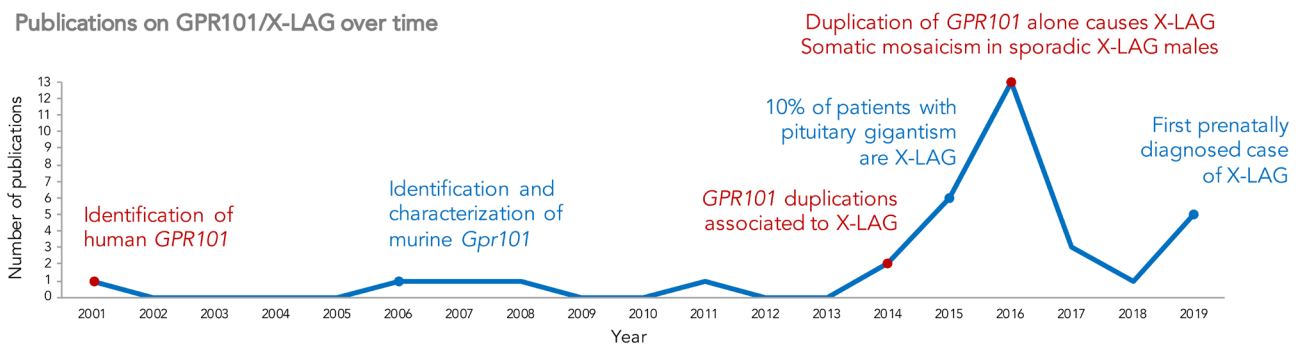

Figure 1
Number of publications studying GPR101 and X-LAG from 2001 to 2019. The following parameters were used for the search: source, PubMed; search terms, 'GPR101', 'GPCR101', 'Xq26.3 microduplication', 'X-LAG', 'XLAG', 'X-linked acrogigantism'. Only relevant publications encompassing original studies or correspondence letters were included, while literature and systematic reviews were excluded.

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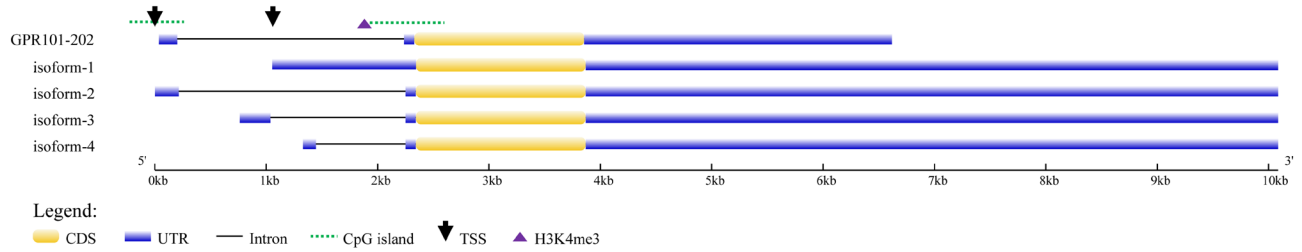

Figure 2
The five different GPR101 isoforms reported in the literature (isoform-1 to isoform-4, (Trivellin *et al.* 2016*a*)) and in the UCSC Genome Browser (GPR101-202, http://genome.ucsc.edu/) were drawn with the Gene Structure Display Server (GSDS 2.0, http://gsds.cbi.pku.edu.cn/) (Hu *et al.* 2015). The location of two CpG islands, the transcription start sites (TSS) and a H3K4me3 mark (promoter-specific histone modification) are also indicated.

Interestingly, another CpG island overlaps with the ATG, indicating that *GPR101* transcription may be regulated by two promoter sequences, but this prediction requires further confirmation. Estradiol was recently described to stimulate *Gpr101* expression in the arcuate nucleus (ARC) in rats, but no classical estrogen response elements (EREs) were detected in *Gpr101* promoter (Bauman *et al.* 2017).

GPCRs are integral membrane proteins with a common architecture: an extracellular N-terminal domain, seven transmembrane domains (TM1-TM7) linked by three extracellular (ECL1-ECL3) and three intracellular loops (ICL1-ICL3) and an intracellular C-terminal domain. While the TM domains show great similarity in overall architecture and secondary structure across different GPCRs, there is considerable variation among ICL and ECL regions, in particular the ICL3 (Moreira 2014). According to most recent estimates, the human genome contains about 800 GPCRs (Lv *et al.* 2016, Sriram & Insel 2018, Alexander *et al.* 2019), phylogenetically categorized into five classes: class A (rhodopsin-like), class B1 (secretin-like), class B2 (adhesion-like), class C (glutamate-like) and the Frizzled/Taste2 family. About half of the GPCRs have sensory functions, the rest are non-sensory GPCRs that mediate signaling, usually following ligand binding. Class A comprises the majority of GPCRs, 719, of which around 300 are non-sensory; about 90 class A GPCRs are categorized as 'orphan' (no known endogenous ligand) (Alexander *et al.* 2019). GPCRs vary greatly in length, with some consisting of more than 1000 residues; most, however, are around 200–400 amino acid in length (Tao & Conn 2014, Lv *et al.* 2016, Alexander *et al.* 2019).

Phylogenetic analyses revealed that GPR101 belongs to class A (Kakarala & Jamil 2014, Alexander *et al.* 2019), which includes the prototypical rhodopsin and β2-adrenergic receptors. Within class A, GPR101 belongs to the family of aminergic receptors (adrenergic, serotonin (5-HT), dopamine and histamine receptors). In particular, the 5-HT_{2C} receptor shows the highest identity with GPR101 in the transmembrane domains (24%), while the 5-HT_{2B} shows the highest identity in the putative binding cavity (28%) (analysis performed through the structure-based alignment tool of GPCRdb) (Munk *et al.* 2019). GPR101 was also shown to be related to GPR161, another orphan GPCR that is associated with primary cilia (Mukhopadhyay *et al.* 2013, Bachmann *et al.* 2016). Interestingly, GPR161 defects were associated to a pituitary developmental disorder: a homozygous loss-of-function (LOF) missense variant was observed in a consanguineous family with pituitary stalk interruption syndrome (Karaca *et al.* 2015).

A transmembrane topology and signal peptide analysis of human GPR101 predicted the absence of a N-terminal signal peptide for endoplasmic reticulum targeting and insertion (analysis performed with Phobius, http://phobius.sbc.su.se/, (Kall *et al.* 2004) and further confirmed by SignalP 4.1, http://www.cbs.dtu.dk/services/SignalP-4.1/, (Petersen *et al.* 2011)). This is not surprising, since the vast majority of GPCRs (90–95%) rather contain signal anchor sequences that are not cleaved-off but are inherent parts of the mature GPCRs. This function is usually exerted by TM1 (Rutz *et al.* 2015). Moreover, out of three highly conserved structural motifs involved in G protein coupling/recognition and activation, GPR101 was found to harbor only an intact E/DRY motif at the ECL2-TM3 interface (DRY residues 128–130), while it lacks a complete BBXXB motif (B represents a basic residue and X a non-basic residue) at the ICL3-TM6 interface (CKAAK residues 395–399) and a complete D/NPXXY motif within TM7 (HPYVY residues 450–454). As in the majority of GPCRs, GPR101 contains also two conserved cysteines in TM3 and ECL2 that form a disulphide bond (residues 104 and 182) (Trivellin *et al.* 2018*a*). Different kinases usually phosphorylate GPCRs at serine/threonine residues located in the ICL3 and C-terminus after activation of the receptor. This event promotes β-arrestin binding, which, in turn, leads to receptor desensitization and

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internalization (Yang *et al.* 2017). Four phosphorylation sites at serine 15 (N-terminus), 27 (TM1) and 309–310 (ICL3) have been functionally annotated for GPR101 (Hornbeck *et al.* 2015). It is interesting to note that the p.E308D variant precedes the ICL3 phosphorylation site and might therefore interfere with this post-translational modification, an hypothesis that needs to be tested.

Out of the more than 800 diverse GPCRs, about 60 have been crystalized to date (https://zhanglab.ccmb.med.umich.edu/GPCR-EXP/); the vast majority belongs to class A (Munk *et al.* 2019). The modest number of crystalized 3D structures depends mainly by challenges in GPCRs expression, purification, stability and crystallization (Salon *et al.* 2011). Using the crystallized structure of the activated β2 adrenergic receptor (β2 AR) as a template, we have previously generated an *in silico* predicted structural model of GPR101 in complex with the Gs heterotrimer (Trivellin *et al.* 2014). Of note, the long ICL3 connecting TM5 and TM6 (163 amino acids, about 30% of the total protein length) could not be modeled because of the lack of a template. ICL3 is indeed one of the least conserved GPCR domains, being very heterogeneous in sequence and length. In particular, the difference in length (which can range from five to up to hundreds of residues) has been proposed to account for selectivity on G proteins coupling (Katritch *et al.* 2012, Bouvier 2013).

Tissue expression

Different labs have extensively studied GPR101 mRNA and protein expression in vertebrate tissues. Several studies revealed a strong expression in the CNS in different species (zebrafish, rodents and humans), suggesting its function has been conserved during evolution. In particular, GPR101 is highly expressed in the amygdala and hypothalamus, especially the ARC, and in the nucleus accumbens (NAc) (Bates *et al.* 2006, Nilaweera *et al.* 2007, 2008, Regard *et al.* 2008, GTEx Consortium 2013, Ronnekleiv *et al.* 2014, Trivellin *et al.* 2014, 2016*a*, Ehrlich *et al.* 2018, LaRese *et al.* 2019, Ieda *et al.* 2020). Other human tissues showing moderate mRNA expression are fat, optic nerve and lymphocytes (Trivellin *et al.* 2016*a*). The detection of GPR101 in the NAc is an interesting finding, especially in the context of X-LAG. The NAc is the main component of ventral striatum and is involved in food reward by coding for motivated appetite behavior (Uribe-Cerda *et al.* 2018). About one-third of X-LAG patients show increased food seeking, a behavior that we speculate might be controlled by GPR101 (Beckers *et al.* 2015). Supporting the findings in humans, *Gpr101* expression in mouse brain regions was found to positively correlate with *GPR101* expression in human brain. In mice, *Gpr101* was found to be restricted in the shell of the NAc, enriched in the central amygdala and densely localized throughout several hypothalamic nuclei (Ehrlich *et al.* 2018). Moreover, Gpr101 was reported as a marker of γ-Aminobutyric acid (GABA) neurons and also expressed by a small subgroup of dopamine neurons within the ventral tegmental area (VTA) (Paul *et al.* 2019). Starvation was also found to increase *Gpr101* expression in the posterior hypothalamus, while ob/ob obese mice showed decreased expression. Within the ARC, Gpr101 is expressed in about 50% of the neurons expressing the anorexigenic peptide proopiomelanocortin (POMC) (Nilaweera *et al.* 2007) and in glutamatergic neurons (Ieda *et al.* 2020), while thyrotropin-releasing hormone (TRH) positive neurons expressed Gpr101 in the lateral hypothalamus (Mickelsen *et al.* 2019). All these brain regions are implicated in various aspects of feeding and reward (Uribe-Cerda *et al.* 2018). Interestingly, several GPCRs endowed with constitutive activity play a crucial role in modulating dopamine signaling in the mesolimbic dopamine system, a neural network critical in processing rewards and their cues (Meye *et al.* 2014). GPR101 might well be one of these. Altogether, these findings suggest a possible role for GPR101 in the control of energy balance and showed that mice may represent a good model to test this hypothesis.

The expression of *Gpr101* in the medial preoptic (mPOA) area in mice suggests also a possible role for this GPCR in reproduction. Gonadotrophin-releasing hormone (GnRH) is the master regulator of reproduction through its activity on the hypothalamus–pituitary–gonadal axis, and the mPOA is a major site for GnRH cell bodies. In the ARC, kisspeptin neurons control the pulsatile release of GnRH. Very few kisspeptin neurons express Gpr101, while Gpr101 is found in the glutamatergic neurons (Ieda *et al.* 2020). Since glutamate stimulates luteinizing hormone (LH) release from the pituitary by affecting kisspeptin neurons (Uenoyama *et al.* 2015), these findings raise the possibility that GPR101 signaling may facilitate LH release via indirect activation of kisspeptin neurons (Ieda *et al.* 2020). Further studies are therefore necessary to shed light on the precise role played by GPR101 in reproduction.

The elevated circulating growth hormone releasing hormone (GHRH) levels detected in some X-LAG patients (Daly *et al.* 2016*b*) pointed toward the possible regulation of hypothalamic GHRH secretion by GPR101. The high levels of GPR101 in the ARC, where GHRH neurons are localized, support this hypothesis. However, no studies

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have yet specifically addressed whether GPR101 indeed co-localizes with GHRH in the ARC. Whether GPR101 has a direct or indirect effect on GHRH secretion is still an answered question.

In the normal human anterior pituitary gland, GPR101 seems to show an age-dependent pattern of expression. GPR101 was indeed found to be strongly expressed during fetal development (starting at around the gestational age of 19 weeks), while it appears only moderately so during the so-called adolescent growth spurt; on the contrary, GPR101 is expressed at very low levels in adult life. This expression pattern points toward an important physiological role for GPR101 during pituitary maturation. Moreover, the strong expression restricted to the lateral wings of the fetal anterior lobe, where most GH- and prolactin-secreting cells are located, suggests that GPR101 might regulate, directly or indirectly, the differentiation of mammosomatotroph cells. In the adult pituitary gland of rat and rhesus macaque, GPR101 is expressed in different cell types: gonadotroph cells in monkey and somatotrophs in rat. These results imply that GPR101 might have different functions in the pituitary gland of different species (Trivellin *et al.* 2016*a*, 2018*a*).

As mentioned in the previous section, GPR101 was found to be strongly over-expressed both at the mRNA and protein level in the pituitary lesions of X-LAG patients (Trivellin *et al.* 2014, 2016*a*). However, GPR101 rarely co-localized with GH-expressing cells, as assessed by immunostaining (Trivellin *et al.* 2014). The tumors expressed relatively high levels of somatostatin receptor type 2 and GHRH receptor (Beckers *et al.* 2015), as well as stem cell/progenitor cell markers such as SOX2 and OCT4 and multiple lineage-specific transcription factors such as PIT1, suggesting that the tumors are multipotential (Wise-Oringer *et al.* 2019).

GnRH-(1–5) and RvD5$_{n\text{-}3\ DPA}$: putative ligands?

GPCRs can be activated by an extremely diverse repertoire of ligands (Alexander *et al.* 2019). In 2014, the same year we described X-LAG, GnRH-(1–5), a processed pentapeptide cleaved from GnRH, was reported as a putative GPR101 ligand (Cho-Clark *et al.* 2014). GnRH-(1–5) is involved in reproduction by regulating GnRH and LH levels (Wu *et al.* 2005, Ieda *et al.* 2020). In Ishikawa cells (endometrial cancer), following GPR101 binding, GnRH-(1–5) induced EGF release, followed by EGF receptor (EGFR) phosphorylation and activation of downstream signaling pathways; this ultimately led to increased proliferation, migration and invasion (Cho-Clark *et al.* 2014, 2015). However, this ligand does not seem to be effective in pituitary cells secreting GH and prolactin. Indeed, no significant effects on cAMP pathway activation and hormone secretion were observed in *in vitro* studies using GH3 cells (rat pituitary tumor cells) and primary X-LAG tumor cells with high GPR101 expression (Naves *et al.* 2016, Trivellin *et al.* 2018*a*). Since the treatment of Ishikawa cells with GnRH-(1–5) had no effect on cAMP accumulation (Baldwin *et al.* 2007), it is possible that this molecule activates a different signal transduction pathway in a cell type-dependent fashion. Further studies employing different and relevant cell types using the same experimental settings are needed to determine whether this is indeed the case.

Most recently, N-3 docosapentaenoic acid-derived resolvin D5 (RvD5$_{n\text{-}3\ DPA}$), an autacoid, pro-resolving mediator, regulating inflammatory responses like arachidonic acid was reported as a putative ligand of GPR101 in macrophages and other monocytes (Flak *et al.* 2020). Interestingly, knockdown of *GPR101* reversed the protective actions of RvD5$_{n\text{-}3\ DPA}$ in limiting inflammatory arthritis, suggesting a potential role for GPR101 in the regulation of inflammation in leucocytes (Flak *et al.* 2020). It remains unclear how these findings translate to the pituitary gland and hypothalamic GPR101 functions and require characterization in these tissues.

GPCRs are considered the largest family of targets for approved pharmaceutical drugs, with estimates of around 30–50% of them targeting these proteins (Fang *et al.* 2015). We are currently performing a high-throughput screening of small molecule libraries to identify additional GPR101-binding molecules. Clearly, the discovery of inhibitors, especially inverse agonists, can be very beneficial to illuminate the physiology of GPR101 and hopefully can lead to a specific treatment for X-LAG patients.

Is *GPR101* involved in other diseases?

Like we and others have done elsewhere (Trivellin *et al.* 2018*a*, Hou & Tao 2019), we will discuss here several rare *GPR101* single nucleotide variants (SNVs) with a global minor allele frequency (MAF) <1% as reported in the Genome Aggregation Database (gnomAD). These variants have been reported in patients with X-LAG and other pathologies, such as other types of pituitary tumors, GH deficiency and heterotaxy. With the exception of c.370G>T (p.V124L, MAF=36.7%), more common SNVs

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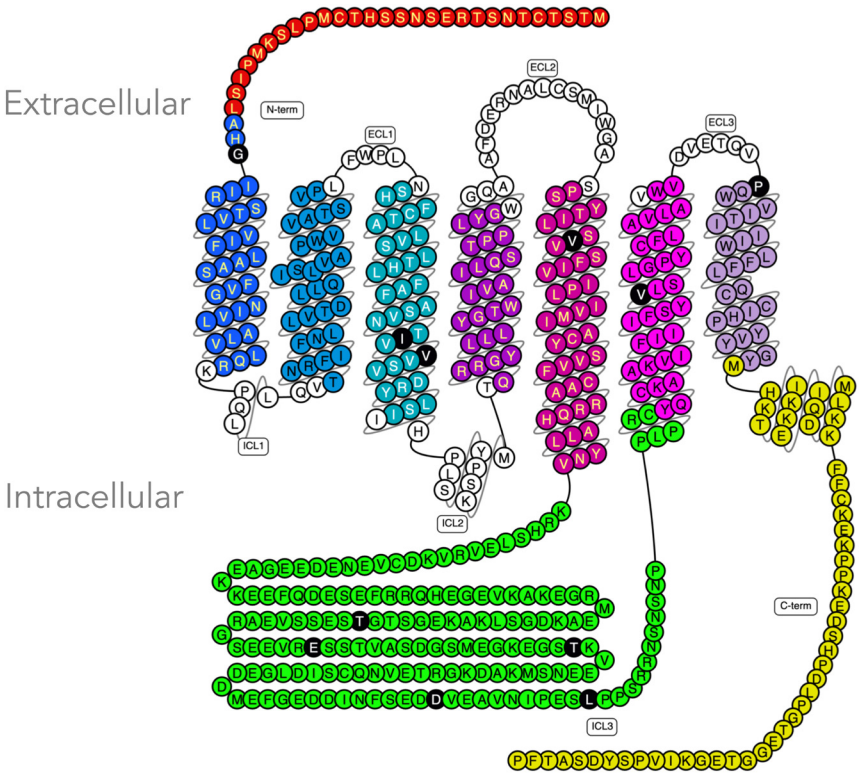

Figure 3
The 2D-predicted structure of GPR101 was retrieved from GPCRdb (https://gpcrdb.org/) and modified to accurately demarcate the transmembrane domains. The N-terminal domain is given in red, while the C-terminus is given in yellow; the seven TM domains are highlighted with a different saturation of blue and purple; ICL3 is in green; all other domains are in white; the naturally occurring SNVs are given in black.

such as c.878C>T (p.T293I, MAF=2.2%) and c.1127T>C (p.L376P, MAF=16.8%) will not be discussed. The location of all naturally occurring SNVs is shown in Fig. 3.

A heterozygous missense SNV affecting residue 308 (c.924G>C, p.E308D), located within ICL3, was identified in about 4% of patients with sporadic acromegaly, in one case occurring *de novo* in the pituitary tumor (Trivellin *et al.* 2014). This variant was described in controls with a MAF of 0.36%. A synonymous SNV affecting the same residue (c.924G>A, p.E308D) was also observed with a MAF of 0.18% (Lek *et al.* 2016). p.E308D moderately increased GH secretion and cell proliferation when transiently over-expressed in GH- and prolactin-secreting cells (Trivellin *et al.* 2014). However, subsequent studies in separate cohorts of acromegaly patients did not find an increased prevalence, suggesting that it probably does not contribute to the pathogenesis of this disease (Ferrau *et al.* 2016, Iacovazzo *et al.* 2016, Lecoq *et al.* 2016, Matsumoto *et al.* 2016). Interestingly, p.E308D was identified by whole exome sequencing (WES) as a secondary finding in two out of six patients with premature ovarian failure primarily caused by hemizygous deletions in genes essential for meiosis or folliculogenesis (Tsuiko *et al.* 2016). Another heterozygous missense, SNV located in ICL3, c.1098C>A (p.D366E), was also reported in a patient with sporadic acromegaly (Lecoq *et al.* 2016). This variant has been submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) with accession number RCV000172847.6. p.D366E was not found in controls or in a series of almost 400 patients with sporadic acromegaly (Iacovazzo *et al.* 2016) but has not been functionally tested yet. A structural hypothesis on the effect of all SNVs located on ICL3 cannot be proposed at the moment giving the absence of a properly modeled domain. Solving the structure of GPR101 would constitute a great step toward a better understanding of the effects of its variants, especially since ICL3, where many of the variants are located, appears to be a hotspot for gain-of-function SNVs (Fukami *et al.* 2018).

Other nonsynonymous SNVs were also detected in other types of pituitary tumors, such as prolactinomas and corticotropinomas. Two heterozygous missense SNVs affecting highly conserved amino acids, c.974C>T (p.T325I) and c.1294C>T (p.P432S), were found in one prolactinoma patient each (cohort of 256 patients). p.T325I is a novel SNV located in ICL3, while p.P432S in ECL3 and is rarely seen in controls (MAF=0.04%) (Lecoq *et al.* 2016). The impact of these SNVs on the structure/function of GPR101 is not known. Two heterozygous missense SNVs were also described in patients with Cushing disease (CD). A novel SNV in TM3, c.365T>C (p.I122T), was reported in a female patient (cohort of 68 patients) (Lecoq *et al.* 2016).

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This SNV affects a highly conserved residue and is predicted to be deleterious, but no *in vitro* studies have been done. A rare SNV located in TM1, c.91G>A (p.G31S, MAF=0.11%), was also reported in a separate cohort (36 CD patients). This SNV was evaluated *in vitro* in a mouse corticotroph tumor cell line (AtT-20 cells) but did not affect ACTH secretion or cell proliferation (Trivellin *et al.* 2016*b*).

GPR101 has also been screened for LOF SNVs and deletions in patients with congenital isolated GH deficiency (GHD) of unknown genetic etiology (cohort of 41 patients). A novel heterozygous missense SNV in TM5, c.589G>T (p.V197L), was found in one female patient. Although predicted to be deleterious, when functionally tested in GH3 cells, it did not significantly decrease GH secretion or intracellular cAMP levels (Castinetti *et al.* 2016).

Heterotaxy syndrome is a condition arising from defects of the correct specification of left–right patterning established during embryonic development. Interestingly, *GPR101* is located next to *ZIC3*, a gene causing X-linked heterotaxy (Paulussen *et al.* 2016). Moreover, GPR161, a GPCR related to GPR101 (Mukhopadhyay *et al.* 2013), was also shown to be required for left–right patterning (Leung *et al.* 2008). A rare (MAF=0.07%) missense SNV in *GPR101*, c.1225G>A (p.V409M), was detected by WES in one family with X-linked heterotaxy. The pedigree consisted of four affected males. This SNV is located in TM6, was predicted to be damaging, and functional studies in animal models supported a causative role (Tariq *et al.* 2013). However, peer-reviewed studies are necessary to confirm these preliminary findings published as a conference proceeding.

Most recently, the common p.V124L *GPR101* SNV was reported in a meta-analysis of up to 622,409 individuals as being associated with smoking behavior traits, specifically cigarettes per day and pack-years (Erzurumluoglu *et al.* 2019). This finding is intriguing in light of the expression of GPR101 in brain regions associated with addictive behaviors.

In addition to variants within the CDS, the promoter region of *GPR101* was found to be hypermethylated in about 40% of patients with colorectal cancer (Kober *et al.* 2011); this epigenetic modification was found to be of prognostic value in stage-IV male patients, as it correlated with a longer time to disease progression.

Conclusions and future perspectives

Since the discovery of *GPR101* almost 20 years ago, some progress, mainly concentrated during the last 6 years, has been made. This was mainly due to a strong boost in research that took place after the characterization of X-LAG and was aided by the rapid progress in the development of new high-throughput screening technologies. We have now started to shed light on the expression pattern of this orphan GPCR in different vertebrates, its regulatory regions and isoforms and the intracellular signaling pathways engaged when active; new putative ligands have been reported, as well as naturally occurring SNVs.

Clearly, much more needs to be done. Concerning physiological functions, we need to dissect the potential roles played by GPR101 in regulating appetite, energy homeostatis, reproduction and human growth. How GPR101 affects GH release and pituitary function in physiological and pathological states needs also to be investigated. Many questions concerning the biology of GPR101 remain still unanswered. For example, the role of the two reported putative ligands needs to be further investigated in physiologically representative models as well as the constitutive activity and activation of G proteins in different cell types. Finding new ligands, especially inhibitors, would also aid in the treatment of X-LAG. From a genetic perspective, functional studies for many SNVs are still lacking. How GPR101 expression is regulated and what are the pituitary cell types expressing this receptor in the context of X-LAG are also two important translational aspects that have to be addressed. Clarifying these and many other gaps in knowledge using the most recent technological tools at our disposal as well as novel animal models will ultimately benefit not only our basic understanding of GPR101 but also, more importantly, the patients that harbor defects in this orphan GPCR.

Declaration of interest

Dr Trivellin, Dr Faucz, Dr Stratakis, Dr Daly and Dr Beckers hold patents on technologies involving *GPR101* causing pituitary tumors. Both laboratories have received research funding support by Pfizer Inc. for investigations on growth hormone-producing pituitary adenomas.

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References

Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Mathie A, Peters JA, Veale EL, Armstrong JF, Faccenda E, Harding SD, *et al.*

https://erc.bioscientifica.com
https://doi.org/10.1530/ERC-20-0025

2019 THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: G protein-coupled receptors. *British Journal of Pharmacology* **176** (Supplement 1) S21–S141. (https://doi.org/10.1111/bph.14748)

Bachmann VA, Mayrhofer JE, Ilouz R, Tschaikner P, Raffeiner P, Rock R, Courcelles M, Apelt F, Lu TW, Baillie GS, *et al*. 2016 Gpr161 anchoring of PKA consolidates GPCR and cAMP signaling. *PNAS* **113** 7786–7791. (https://doi.org/10.1073/pnas.1608061113)

Baldwin EL, Wegorzewska IN, Flora M & Wu TJ 2007 Regulation of type II luteinizing hormone-releasing hormone (LHRH-II) gene expression by the processed peptide of LHRH-I, LHRH-(1–5) in endometrial cells. *Experimental Biology and Medicine* **232** 146–155. (https://doi.org/10.3181/00379727-207-2320146)

Bates B, Zhang L, Nawoschik S, Kodangattil S, Tseng E, Kopsco D, Kramer A, Shan Q, Taylor N, Johnson J, *et al*. 2006 Characterization of Gpr101 expression and G-protein coupling selectivity. *Brain Research* **1087** 1–14. (https://doi.org/10.1016/j.brainres.2006.02.123)

Bauman BM, Yin W, Gore AC & Wu TJ 2017 Regulation of gonadotropin-releasing hormone-(1–5) signaling genes by estradiol is age dependent. *Frontiers in Endocrinology* **8** 282. (https://doi.org/10.3389/fendo.2017.00282)

Beckers A, Lodish MB, Trivellin G, Rostomyan L, Lee M, Faucz FR, Yuan B, Choong CS, Caberg JH, Verrua E, *et al*. 2015 X-linked acrogigantism syndrome: clinical profile and therapeutic responses. *Endocrine-Related Cancer* **22** 353–367. (https://doi.org/10.1530/ERC-15-0038)

Bouvier M 2013 Unraveling the structural basis of GPCR activation and inactivation. *Nature Structural and Molecular Biology* **20** 539–541. (https://doi.org/10.1038/nsmb.2584)

Castinetti F, Daly AF, Stratakis CA, Caberg JH, Castermans E, Trivellin G, Rostomyan L, Saveanu A, Jullien N, Reynaud R, *et al*. 2016 GPR101 mutations are not a frequent cause of congenital isolated growth hormone deficiency. *Hormone and Metabolic Research* **48** 389–393. (https://doi.org/10.1055/s-0042-100733)

Cho-Clark M, Larco DO, Semsarzadeh NN, Vasta F, Mani SK & Wu TJ 2014 GnRH-(1–5) transactivates EGFR in Ishikawa human endometrial cells via an orphan G protein-coupled receptor. *Molecular Endocrinology* **28** 80–98. (https://doi.org/10.1210/me.2013-1203)

Cho-Clark M, Larco DO, Zahn BR, Mani SK & Wu TJ 2015 GnRH-(1–5) activates matrix metallopeptidase-9 to release epidermal growth factor and promote cellular invasion. *Molecular and Cellular Endocrinology* **415** 114–125. (https://doi.org/10.1016/j.mce.2015.08.010)

Daly AF, Yuan B, Fina F, Caberg JH, Trivellin G, Rostomyan L, de Herder WW, Naves LA, Metzger D, Cuny T, *et al*. 2016*a* Somatic mosaicism underlies X-linked acrogigantism syndrome in sporadic male subjects. *Endocrine-Related Cancer* **23** 221–233. (https://doi.org/10.1530/ERC-16-0082)

Daly AF, Lysy PA, Desfilles C, Rostomyan L, Mohamed A, Caberg JH, Raverot V, Castermans E, Marbaix E, Maiter D, *et al*. 2016*b* GHRH excess and blockade in X-LAG syndrome. *Endocrine-Related Cancer* **23** 161-170. (https://doi.org/10.1530/ERC-15-0478)

Ehrlich AT, Maroteaux G, Robe A, Venteo L, Nasseef MT, van Kempen LC, Mechawar N, Turecki G, Darcq E & Kieffer BL 2018 Expression map of 78 brain-expressed mouse orphan GPCRs provides a translational resource for neuropsychiatric research. *Communications Biology* **1** 102. (https://doi.org/10.1038/s42003-018-0106-7)

Erzurumluoglu AM, Liu M, Jackson VE, Barnes DR, Datta G, Melbourne CA, Young R, Batini C, Surendran P, Jiang T, *et al*. 2019 Meta-analysis of up to 622,409 individuals identifies 40 novel smoking behaviour associated genetic loci. *Molecular Psychiatry* [epub]. (https://doi.org/10.1038/s41380-018-0313-0)

Fang Y, Kenakin T & Liu C 2015 Editorial: Orphan GPCRs as emerging drug targets. *Frontiers in Pharmacology* **6** 295. (https://doi.org/10.3389/fphar.2015.00295)

Ferrau F, Romeo PD, Puglisi S, Ragonese M, Torre ML, Scaroni C, Occhi G, De Menis E, Arnaldi G, Trimarchi F, *et al*. 2016 Analysis of GPR101 and AIP genes mutations in acromegaly: a multicentric study. *Endocrine* **54** 762–767. (https://doi.org/10.1007/s12020-016-0862-4)

Flak MB, Koenis DS, Sobrino A, Smith J, Pistorius K, Palmas F & Dalli J 2020 GPR101 mediates the pro-resolving actions of RvD5n-3 DPA in arthritis and infections. *Journal of Clinical Investigation* **130** 359–373. (https://doi.org/10.1172/JCI131609)

Fukami M, Suzuki E, Igarashi M, Miyado M & Ogata T 2018 Gain-of-function mutations in G-protein-coupled receptor genes associated with human endocrine disorders. *Clinical Endocrinology* **88** 351–359. (https://doi.org/10.1111/cen.13496)

GTEx Consortium 2013 The genotype-tissue expression (GTEx) project. *Nature Genetics* **45** 580–585. (https://doi.org/10.1038/ng.2653)

Hannah-Shmouni F, Trivellin G & Stratakis CA 2016 Genetics of gigantism and acromegaly. *Growth Hormone and IGF Research* **30–31** 37–41. (https://doi.org/10.1016/j.ghir.2016.08.002)

Hernandez-Ramirez LC, Trivellin G & Stratakis CA 2018 Cyclic 3′,5′-adenosine monophosphate (cAMP) signaling in the anterior pituitary gland in health and disease. *Molecular and Cellular Endocrinology* **463** 72–86. (https://doi.org/10.1016/j.mce.2017.08.006)

Hornbeck PV, Zhang B, Murray B, Kornhauser JM, Latham V & Skrzypek E 2015 PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids Research* **43** D512–D520. (https://doi.org/10.1093/nar/gku1267)

Hou ZS & Tao YX 2019 Mutations in GPR101 as a potential cause of X-linked acrogigantism and acromegaly. *Progress in Molecular Biology and Translational Science* **161** 47–67. (https://doi.org/10.1016/bs.pmbts.2018.10.003)

Hu B, Jin J, Guo AY, Zhang H, Luo J & Gao G 2015 GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics* **31** 1296–1297. (https://doi.org/10.1093/bioinformatics/btu817)

Iacovazzo D, Caswell R, Bunce B, Jose S, Yuan B, Hernandez-Ramirez LC, Kapur S, Caimari F, Evanson J, Ferrau F, *et al*. 2016 Germline or somatic GPR101 duplication leads to X-linked acrogigantism: a clinico-pathological and genetic study. *Acta Neuropathologica Communications* **4** 56. (https://doi.org/10.1186/s40478-016-0328-1)

Ieda N, Assadullah MS, Ikegami K, Watanabe Y, Sugimoto Y, Sugimoto A, Kawai N, Ishii H, Inoue N, *et al*. 2020 GnRH(1-5), a metabolite of gonadotropin-releasing hormone, enhances luteinizing hormone release via activation of kisspeptin neurons in female rats. *Endocrine Journal* **67** 409–418. (https://doi.org/10.1507/endocrj.EJ19-0444)

Kakarala KK & Jamil K 2014 Sequence-structure based phylogeny of GPCR class A rhodopsin receptors. *Molecular Phylogenetics and Evolution* **74** 66–96. (https://doi.org/10.1016/j.ympev.2014.01.022)

Kall L, Krogh A & Sonnhammer EL 2004 A combined transmembrane topology and signal peptide prediction method. *Journal of Molecular Biology* **338** 1027–1036. (https://doi.org/10.1016/j.jmb.2004.03.016)

Karaca E, Buyukkaya R, Pehlivan D, Charng WL, Yaykasli KO, Bayram Y, Gambin T, Withers M, Atik MM, Arslanoglu I, *et al*. 2015 Whole-exome sequencing identifies homozygous GPR161 mutation in a family with pituitary stalk interruption syndrome. *Journal of Clinical Endocrinology and Metabolism* **100** E140–E147. (https://doi.org/10.1210/jc.2014-1984)

Katritch V, Cherezov V & Stevens RC 2012 Diversity and modularity of G protein-coupled receptor structures. *Trends in Pharmacological Sciences* **33** 17–27. (https://doi.org/10.1016/j.tips.2011.09.003)

Kober P, Bujko M, Oledzki J, Tysarowski A & Siedlecki JA 2011 Methyl-CpG binding column-based identification of nine genes hypermethylated in colorectal cancer. *Molecular Carcinogenesis* **50** 846–856. (https://doi.org/10.1002/mc.20763)

LaRese TP, Rheaume BA, Abraham R, Eipper BA & Mains RE 2019 Sex-specific gene expression in the mouse nucleus accumbens before and

after cocaine exposure. *Journal of the Endocrine Society* **3** 468–487. (https://doi.org/10.1210/js.2018-00313)

Le Coz C, Trofa M, Syrett CM, Martin A, Jyonouchi H, Jyonouchi S, Anguera MC & Romberg N 2018 CD40LG duplication-associated autoimmune disease is silenced by nonrandom X-chromosome inactivation. *Journal of Allergy and Clinical Immunology* **141** 2308.e7–2311.e7. (https://doi.org/10.1016/j.jaci.2018.02.010)

Lecoq AL, Bouligand J, Hage M, Cazabat L, Salenave S, Linglart A, Young J, Guiochon-Mantel A, Chanson P & Kamenicky P 2016 Very low frequency of germline GPR101 genetic variation and no biallelic defects with AIP in a large cohort of patients with sporadic pituitary adenomas. *European Journal of Endocrinology* **174** 523–530. (https://doi.org/10.1530/EJE-15-1044)

Lee DK, Nguyen T, Lynch KR, Cheng R, Vanti WB, Arkhitko O, Lewis T, Evans JF, George SR & O'Dowd BF 2001 Discovery and mapping of ten novel G protein-coupled receptor genes. *Gene* **275** 83–91. (https://doi.org/10.1016/s0378-1119(01)00651-5)

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, *et al*. 2016 Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536** 285–291. (https://doi.org/10.1038/nature19057)

Leung T, Humbert JE, Stauffer AM, Giger KE, Chen H, Tsai HJ, Wang C, Mirshahi T & Robishaw JD 2008 The orphan G protein-coupled receptor 161 is required for left-right patterning. *Developmental Biology* **323** 31–40. (https://doi.org/10.1016/j.ydbio.2008.08.001)

Lv X, Liu J, Shi Q, Tan Q, Wu D, Skinner JJ, Walker AL, Zhao L, Gu X, Chen N, *et al*. 2016 In vitro expression and analysis of the 826 human G protein-coupled receptors. *Protein and Cell* **7** 325–337. (https://doi.org/10.1007/s13238-016-0263-8)

Martin AL, Steurer MA & Aronstam RS 2015 Constitutive activity among orphan class-A G protein coupled receptors. *PLoS ONE* **10** e0138463. (https://doi.org/10.1371/journal.pone.0138463)

Matsumoto R, Izawa M, Fukuoka H, Iguchi G, Odake Y, Yoshida K, Bando H, Suda K, Nishizawa H, Takahashi M, *et al*. 2016 Genetic and clinical characteristics of Japanese patients with sporadic somatotropinoma. *Endocrine Journal* **63** 953–963. (https://doi.org/10.1507/endocrj.EJ16-0075)

Meye FJ, Ramakers GM & Adan RA 2014 The vital role of constitutive GPCR activity in the mesolimbic dopamine system. *Translational Psychiatry* **4** e361. (https://doi.org/10.1038/tp.2013.130)

Mickelsen LE, Bolisetty M, Chimileski BR, Fujita A, Beltrami EJ, Costanzo JT, Naparstek JR, Robson P & Jackson AC 2019 Single-cell transcriptomic analysis of the lateral hypothalamic area reveals molecularly distinct populations of inhibitory and excitatory neurons. *Nature Neuroscience* **22** 642–656. (https://doi.org/10.1038/s41593-019-0349-8)

Moreira IS 2014 Structural features of the G-protein/GPCR interactions. *Biochimica and Biophysica Acta* **1840** 16–33. (https://doi.org/10.1016/j.bbagen.2013.08.027)

Mukhopadhyay S, Wen X, Ratti N, Loktev A, Rangell L, Scales SJ & Jackson PK 2013 The ciliary G-protein-coupled receptor Gpr161 negatively regulates the Sonic hedgehog pathway via cAMP signaling. *Cell* **152** 210–223. (https://doi.org/10.1016/j.cell.2012.12.026)

Munk C, Mutt E, Isberg V, Nikolajsen LF, Bibbe JM, Flock T, Hanson MA, Stevens RC, Deupi X & Gloriam DE 2019 An online resource for GPCR structure determination and analysis. *Nature Methods* **16** 151–162. (https://doi.org/10.1038/s41592-018-0302-x)

Naves LA, Daly AF, Dias LA, Yuan B, Zakir JC, Barra GB, Palmeira L, Villa C, Trivellin G, Junior AJ, *et al*. 2016 Aggressive tumor growth and clinical evolution in a patient with X-linked acro-gigantism syndrome. *Endocrine* **51** 236–244. (https://doi.org/10.1007/s12020-015-0804-6)

Nilaweera KN, Ozanne D, Wilson D, Mercer JG, Morgan PJ & Barrett P 2007 G protein-coupled receptor 101 mRNA expression in the mouse brain: altered expression in the posterior hypothalamus and amygdala by energetic challenges. *Journal of Neuroendocrinology* **19** 34–45. (https://doi.org/10.1111/j.1365-2826.2006.01502.x)

Nilaweera KN, Wilson D, Bell L, Mercer JG, Morgan PJ & Barrett P 2008 G protein-coupled receptor 101 mRNA expression in supraoptic and paraventricular nuclei in rat hypothalamus is altered by pregnancy and lactation. *Brain Research* **1193** 76–83. (https://doi.org/10.1016/j.brainres.2007.11.048)

Paul EJ, Tossell K & Ungless MA 2019 Transcriptional profiling aligned with in situ expression image analysis reveals mosaically expressed molecular markers for GABA neuron sub-groups in the ventral tegmental area. *European Journal of Neuroscience* **50** 3732–3749. (https://doi.org/10.1111/ejn.14534)

Paulussen AD, Steyls A, Vanoevelen J, van Tienen FH, Krapels IP, Claes GR, Chocron S, Velter C, Tan-Sindhunata GM, Lundin C, *et al*. 2016 Rare novel variants in the ZIC3 gene cause X-linked heterotaxy. *European Journal of Human Genetics* **24** 1783–1791. (https://doi.org/10.1038/ejhg.2016.91)

Petersen TN, Brunak S, von Heijne G & Nielsen H 2011 SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nature Methods* **8** 785–786. (https://doi.org/10.1038/nmeth.1701)

Regard JB, Sato IT & Coughlin SR 2008 Anatomical profiling of G protein-coupled receptor expression. *Cell* **135** 561–571. (https://doi.org/10.1016/j.cell.2008.08.040)

Rodd C, Millette M, Iacovazzo D, Stiles CE, Barry S, Evanson J, Albrecht S, Caswell R, Bunce B, Jose S, *et al*. 2016 Somatic GPR101 duplication causing X-linked acrogigantism (XLAG)-diagnosis and management. *Journal of Clinical Endocrinology and Metabolism* **101** 1927–1930. (https://doi.org/10.1210/jc.2015-4366)

Ronnekleiv OK, Fang Y, Zhang C, Nestor CC, Mao P & Kelly MJ 2014 Research resource: gene profiling of G protein-coupled receptors in the arcuate nucleus of the female. *Molecular Endocrinology* **28** 1362–1380. (https://doi.org/10.1210/me.2014-1103)

Rutz C, Klein W & Schulein R 2015 N-terminal signal peptides of G protein-coupled receptors: significance for receptor biosynthesis, trafficking, and signal transduction. *Progress in Molecular Biology and Translational Science* **132** 267–287. (https://doi.org/10.1016/bs.pmbts.2015.03.003)

Salon JA, Lodowski DT & Palczewski K 2011 The significance of G protein-coupled receptor crystallography for drug discovery. *Pharmacological Reviews* **63** 901–937. (https://doi.org/10.1124/pr.110.003350)

Sriram K & Insel PA 2018 G Protein-coupled receptors as targets for approved drugs: how many targets and how many drugs? *Molecular Pharmacology* **93** 251–258. (https://doi.org/10.1124/mol.117.111062)

Tao YX & Conn PM 2014 Chaperoning G protein-coupled receptors: from cell biology to therapeutics. *Endocrine Reviews* **35** 602–647. (https://doi.org/10.1210/er.2013-1121)

Tariq M, Cast A, Belmont J & Ware S 2013 Identification of a novel cause of X-linked heterotaxy. In *ASHG 2013*. Conference abstract.

Trivellin G & Stratakis CA 2019 CD40LG duplications in patients with X-LAG syndrome commonly undergo random X-chromosome inactivation. *Journal of Allergy and Clinical Immunology* **143** 1659. (https://doi.org/10.1016/j.jaci.2018.12.1017)

Trivellin G, Daly AF, Faucz FR, Yuan B, Rostomyan L, Larco DO, Schernthaner-Reiter MH, Szarek E, Leal LF, Caberg JH, *et al*. 2014 Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. *New England Journal of Medicine* **371** 2363–2374. (https://doi.org/10.1056/NEJMoa1408028)

Trivellin G, Bjelobaba I, Daly AF, Larco DO, Palmeira L, Faucz FR, Thiry A, Leal LF, Rostomyan L, Quezado M, *et al*. 2016*a* Characterization of GPR101 transcript structure and expression patterns. *Journal of Molecular Endocrinology* **57** 97–111. (https://doi.org/10.1530/JME-16-0045)

Trivellin G, Correa RR, Batsis M, Faucz FR, Chittiboina P, Bjelobaba I, Larco DO, Quezado M, Daly AF, Stojilkovic SS, *et al*. 2016*b* Screening for GPR101 defects in pediatric pituitary corticotropinomas.

Endocrine-Related Cancer **23** 357–365. (https://doi.org/10.1530/ERC-16-0091)

Trivellin G, Hernandez-Ramirez LC, Swan J & Stratakis CA 2018*a* An orphan G-protein-coupled receptor causes human gigantism and/or acromegaly: molecular biology and clinical correlations. *Best Practice and Research. Clinical Endocrinology and Metabolism* **32** 125–140. (https://doi.org/10.1016/j.beem.2018.02.004)

Trivellin G, Sharwood E, Hijazi H, Carvalho CMB, Yuan B, Tatton-Brown K, Coman D, Lupski JR, Cotterill AM, Lodish MB, *et al*. 2018*b* Xq26.3 duplication in a boy with motor delay and low muscle tone refines the X-linked Acrogigantism genetic locus. *Journal of the Endocrine Society* **2** 1100–1108. (https://doi.org/10.1210/js.2018-00156)

Tsuiko O, Noukas M, Zilina O, Hensen K, Tapanainen JS, Magi R, Kals M, Kivistik PA, Haller-Kikkatalo K, Salumets A, *et al*. 2016 Copy number variation analysis detects novel candidate genes involved in follicular growth and oocyte maturation in a cohort of premature ovarian failure cases. *Human Reproduction* **31** 1913–1925. (https://doi.org/10.1093/humrep/dew142)

Uenoyama Y, Nakamura S, Hayakawa Y, Ikegami K, Watanabe Y, Deura C, Minabe S, Tomikawa J, Goto T, Ieda N, *et al*. 2015 Lack of pulse and surge modes and glutamatergic stimulation of luteinising hormone release in Kiss1 knockout rats. *Journal of Neuroendocrinology* **27** 187–197. (https://doi.org/10.1111/jne.12257)

Uribe-Cerda S, Morselli E & Perez-Leighton C 2018 Updates on the neurobiology of food reward and their relation to the obesogenic environment. *Current Opinion in Endocrinology, Diabetes, and Obesity* **25** 292–297. (https://doi.org/10.1097/MED.0000000000000427)

Vasilev V, Daly AF, Trivellin G, Stratakis CA, Zacharieva S & Beckers A 2020 HEREDITARY ENDOCRINE TUMOURS: CURRENT STATE-OF-THE-ART AND RESEARCH OPPORTUNITIES: The roles of *AIP* and *GPR101* in familial isolated pituitary adenomas (FIPA). *Endocrine-Related Cancer* **27** 77–86. (https://doi.org/10.1530/ERC-20-0015)

Wise-Oringer BK, Zanazzi GJ, Gordon RJ, Wardlaw SL, William C, Anyane-Yeboa K, Chung WK, Kohn B, Wisoff JH, David R, *et al*. 2019 Familial X-linked Acrogigantism: postnatal outcomes and tumor pathology in a prenatally diagnosed infant and his mother. *Journal of Clinical Endocrinology and Metabolism* **104** 4667–4675. (https://doi.org/10.1210/jc.2019-00817)

Wu TJ, Mani SK, Glucksman MJ & Roberts JL 2005 Stimulation of luteinizing hormone-releasing hormone (LHRH) gene expression in GT1-7 cells by its metabolite, LHRH-(1-5). *Endocrinology* **146** 280–286. (https://doi.org/10.1210/en.2004-0560)

Yang Z, Yang F, Zhang D, Liu Z, Lin A, Liu C, Xiao P, Yu X & Sun JP 2017 Phosphorylation of G protein-coupled receptors: from the barcode hypothesis to the flute model. *Molecular Pharmacology* **92** 201–210. (https://doi.org/10.1124/mol.116.107839)

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