Physiological Dysfunctions Associated with Mutations of the Imprinted Gnas Locus

The ubiquitous Gsα subunit of the trimeric, stimulatory G-protein plays a central role in receptor-mediated signal transduction, coupling receptor activation with the production of cAMP. The Gsα encoding locus Gnas is now known to consist of a complex arrangement of several protein-coding and noncoding transcripts. We provide an overview of its genomic organization, its regulation by genomic imprinting, and a summary of the physiological roles of the alternative protein variants Gsα2 and XLαs, as determined from deficient mouse models.

Signal transduction from a large number of activated seven-pass transmembrane receptors to adenyl cyclases and subsequent generation of the second messenger cAMP requires the "α-stimulatory" subunit of the trimeric G-protein, Gsα (70). Although this classical signaling protein has been studied in detail, the significance of an NH2-terminal splice variant, termed XLαs (αMrA Large αs), and the complexity of the genomic locus (Gnas) encoding these proteins has only recently been recognized (4, 32, 34, 49, 56, 68). The identification of alternative promoters and 5' exons within the locus and the discovery that genomic imprinting regulates their activity has played a fundamental role in forming our current understanding of what physiological functions depend on the different proteins of the Gnas locus (29, 30, 33, 53–56). The term genomic imprinting describes a regulatory mechanism, present in mammals, that controls gene dosage through the silencing of one parental allele in a parent-of-origin-dependent manner (46, 58, 73). Thus some imprinted genes are only expressed from the maternally inherited allele, whereas others are exclusively active on the paternally derived chromosome. Epigenetic changes, including DNA methylation and chromatin modifications, mediate the specific silencing of imprinted parental alleles. The establishment of parental/maternal epigenetic and imprinting patterns occurs in the developing germ cells of the parents and is maintained in the embryo after fertilization and into adulthood (18, 62). Although the number of imprinted genes currently identified in the human and mouse genomes is relatively small (~90 transcripts) (databases: http://www.har.mrc.ac.uk/research/genomic_imprinting/; http://igc.otago.ac.nz/home.html), many of them play essential roles in typical mammalian physiological adaptations, e.g., placenta functions, fetal and postnatal growth, resource acquisition, and energy homeostasis (9, 14, 21, 64). Notably, only some minor differences as mentioned below. At the center of the locus, Gnas itself comprises 12 exons, which encode the Gsα protein (13 exons in human, due to an additional intron interrupting exon 9) (Figure 1). However, only exon 1, which contains the 5'-UTR and 46 codons, is specific to Gnas, since the remaining exons are also utilized by several other transcripts. Gnas is widely expressed in most tissues and generally transcribed from both chromosomes, that is, except in a specific subset of tissue types, where it is expressed preferentially from the
maternally inherited allele (69). Silencing of the paternally inherited allele occurs in proximal renal tubule cells, thyroid gland, anterior pituitary, and ovaries (23–25, 27, 40, 44, 45, 79). Reports on Gnas imprinting in adipose tissue are currently contradictory; some studies have shown preferential expression from the maternal allele, whereas others indicated biallelic transcription (11, 25, 71, 79). Regarding the adipose imprinting of Gnas, differences in species and developmental stages need to be carefully considered before solid conclusions can be reached. The tissue-specific silencing of the paternal allele is highly relevant for disorders associated with mutations on the maternally inherited allele as described in more detail below.

Gnasxl, the second protein-coding transcript of the Gnas locus, is initiated at a separate promoter ~30 kb upstream of Gnas exon 1 (FIGURE 1) (29, 33, 53). The transcript splices onto Gnas exons 2–12, whereby the open reading frame remains conserved, resulting in the NH2-extended protein variant of Gs1, termed XL

![Diagram of the imprinted Gnas locus and summary of the main phenotypical features associated with mutant mouse models](image)

**FIGURE 1.** Scheme of the imprinted Gnas locus and summary of the main phenotypical features associated with mutant mouse models.

The maternal and paternal alleles of the locus are depicted in the center, coding exons as filled boxes, and noncoding exons as light gray boxes. Active promoters and transcriptional directions of the RNAs for Gnas, exon 1A, Gnas1, Nespas, and Nesp are marked by arrows. Although Gnas is expressed biallelically in most tissues, silencing of the promoter in some cell types is indicated by the light blue exon 1 box on the paternal allele. The splicing patterns of the various transcripts are shown above and below the maternal and paternal alleles, respectively. Nespas transcripts exist in multiple spliced and unspliced forms. In humans, GNAS consists of 13 exons and NESP only comprises one 5'-exon; exon 1A is termed exon A/B. MMM indicates DNA methylation at differentially methylated regions (DMRs) of the respective alleles; the DMRs at Nespas and exon 1A constitute imprinting control regions (ICRs) of the locus. The locations of various mutations in mice are indicated by X, and the major phenotypical features upon maternal or paternal transmission are mentioned above and below the respective alleles.

<table>
<thead>
<tr>
<th>Table 1. Genotype/Phenotype Summary</th>
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<tbody>
<tr>
<td><strong>Human Syndrome</strong></td>
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<tr>
<td><strong>Maternal mutant</strong></td>
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<tr>
<td>Short stature, subcutaneous obesity, multiple hormone resistance</td>
</tr>
<tr>
<td>Severe obesity</td>
</tr>
<tr>
<td>Variable mental retardation</td>
</tr>
<tr>
<td><strong>Paternal mutant</strong></td>
</tr>
<tr>
<td>Short stature, subcutaneous obesity, mild mental retardation</td>
</tr>
<tr>
<td><strong>Chromosomal abnormalities</strong></td>
</tr>
<tr>
<td>Maternal uniparental disomy</td>
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<tr>
<td>Severe intrauterine growth retardation</td>
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<tr>
<td>Intractable neonatal hypercapnia</td>
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<tr>
<td>Abnormal distal phalanges</td>
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<tr>
<td>Hypotonia</td>
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<tr>
<td>Mental retardation</td>
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1Hormone resistance syndrome (termed XL/H9251s). Gnas knockout mice exhibit combined disrupted growth and testosterone resistance. In contrast, XL/H9251s mice exhibit normal growth and testosterone resistance, but have severe obesity.
The tissue-specific imprinting of the Gnasxl transcript is highly relevant for normal development (15, 41). Functionally, the XLN1 protein, which lacks most of the functional domains of Gsα, subunit-interacting domain and can stimulate, with almost identical characteristics to Gsα, adenyl cyclases on activation of several hormone/neurotransmitter receptors (4, 38).

In neuroendocrine tissues, e.g., brain, pituitary, and adrenal glands, a truncated splice form of the Gnasxl transcript is generated through usage of the alternative exon N1, which is located between exons 3 and 4 (16, 49, 54). Whether the XLN1 protein, which lacks most of the functional domains of Gsα subunits, exerts any function is currently unclear.

Approximately 15 kb upstream of the Gnasxl exon, a third promoter initiates the Nesp transcript, which encodes the "neuroendocrine-specific protein of Mr 55,000" (Nesp55) (30, 31, 33, 53). The Nesp55 transcript is also imprinted, but in contrast to Gnasxl it is expressed from the maternally inherited chromosome only. The Nesp-specific upstream exons (2 exons in mouse, 1 in human) are also spliced onto exons 2-12 of Gnasxl (FIGURE 1). However, in this case, the latter only contains the 3’-UTR, since the Nesp55 open reading frame remains confined to the upstream exon. Little is known about the molecular functions of the Nesp55 protein, although it has been associated with secretory vesicles and can be processed into smaller peptides (20, 43).

Apart from these three protein-coding transcripts, two additional RNAs are expressed from the paternally inherited allele (4, 76). Although its NH2-terminus varies, the XLα protein retains a βγ-subunit-interacting domain and can stimulate, with almost identical characteristics to Gsα, adenyl cyclases on activation of several hormone/neurotransmitter receptors (4, 38).

Table 1. General physiological characteristics affected by mutations at the GNAS/Gnas imprinted locus in human and mouse

<table>
<thead>
<tr>
<th>Human Symptoms</th>
<th>Murine Phenotype</th>
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<tbody>
<tr>
<td>Maternal mutations in GNAS exons1 (AHO/PHP-1a)</td>
<td>Maternal Gsα-specific knockout (Gnas&lt;sup&gt;m/m&lt;/sup&gt;)</td>
</tr>
<tr>
<td>(Point mutations, small deletions, splice site mutations in exons 1-13)</td>
<td>50–80% neonatal lethality</td>
</tr>
<tr>
<td>Short stature, brachydactyly</td>
<td>Reduced body length</td>
</tr>
<tr>
<td>Subcutaneous ossification</td>
<td>Multiple hormone resistance (PTH)</td>
</tr>
<tr>
<td>Multiple hormone resistance (TSH, PTH, GHRH, gonadotropins)</td>
<td>Severe obesity with insulin resistance and glucose intolerance</td>
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<tr>
<td>Severe obesity</td>
<td></td>
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<tr>
<td>Variable mental retardation and neurological defects</td>
<td></td>
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<td>Paternal Gsα-specific knockout (Gnas&lt;sup&gt;m/m&lt;/sup&gt;)</td>
</tr>
<tr>
<td>(Point mutations, small deletions, splice site mutations in exons 1-13)</td>
<td>0.30% neonatal lethality</td>
</tr>
<tr>
<td>Short stature, brachydactyly</td>
<td>Reduced body length</td>
</tr>
<tr>
<td>Subcutaneous ossification</td>
<td>Subcutaneous ossification</td>
</tr>
<tr>
<td>Mild obesity</td>
<td>Mild form of obesity, insulin resistance and glucose intolerance</td>
</tr>
<tr>
<td>Variable mental retardation and neurological defects</td>
<td></td>
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<tr>
<td>Chromosomal abnormalities of the 20q13.2-13.3 region</td>
<td>Paternal XLα-specific knockout (Gnasxl&lt;sup&gt;m/m&lt;/sup&gt;)</td>
</tr>
<tr>
<td>(Paternal deletions affecting XLα among other genes; maternal uniparental disomy)</td>
<td>~80% neonatal lethality</td>
</tr>
<tr>
<td>Severe intrauterine growth retardation</td>
<td>Reduced body length and weight</td>
</tr>
<tr>
<td>Intractable neonatal feeding difficulties</td>
<td>Poor suckling at birth</td>
</tr>
<tr>
<td>Abnormal distribution of adipose tissue; significant reduction in subcutaneous adiposity</td>
<td>Reduced adiposity, hypermetabolism</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>Hypotonia</td>
</tr>
<tr>
<td>Variable mental retardation and other neurological defects</td>
<td></td>
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</tbody>
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**REVIEWS**

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the normally silenced paternal Gnas transcript in
imprinted tissues (39, 71). Similarly, paternal deletion
of the Nejio DMR and transcriptional start site affects
all transcripts of the locus (72). The role of a third DMR
at Nejio remains to be clarified (3). Our current under-
standing of the precise imprinting regulatory mecha-
nisms at the Gnas locus is limited and awaits further
investigation; for a more detailed discussion of these
aspects, the reader is referred to recent excellent
reviews (51, 52).

"Both maternal E1 and E2 Gnas\textsuperscript{+\textendash}3\textendash}mutants demonstrate
multiple hormone resistances due to disrupted
receptor signaling in imprinted tissues."

The discovery of mutations in the highly conserved
human GNAS locus gave the first indications that the
coded gene products influenced important physio-
logical processes. The genetic disorders Albright’s Hereditary Osteodystrophy (AHO) and pseudoby-
parathyroidism (PHP) were found to be due to mis-
sense or nonsense mutations in GNAS exons (1, 36, 37,
67). AHO is characterized by a range of symptoms,
including short stature, brachydactyly, subcutaneous
ossifications, obesity, and variable mental retardation
(Table 1) (57, 69). In some cases, additional symptoms
of hypocalcemia, hyperphosphatemia, and end-organ
resistance to parathyroid hormone (PTH) in the prox-
imal renal tubules are associated with AHO. This
observation has led to the condition being termed
PHP-type 1a (5, 35, 57, 69). AHO symptoms without
hormone resistance and normal calcaemia are
referred to as "pseudopseudohypoparathyroidism"
(PPHP). Upon Davies and Hughes’ observation, it
became clear that AHO plus PTH resistance (as well as
other hormone resistances), i.e., PHP-1a, only
occurred after maternal inheritance of a GNAS muta-
ton (17). By contrast, AHO without hormone resist-
ances (PPHP) is found after paternal transmission of
the same GNAS mutations (Table 1). This pattern of
inheritance reflects the tissue-specific imprinting of
GNAS, with hormone resistance arising from imprint-
ted tissues, where the paternal allele is naturally
silenced and the inheritance of an inactivating muta-
tion reflects the tissue-specific imprinting of
GNAS. This hypothesis has been confirmed by the various
mouse models, which show that homozygous
\textit{Gnas} mutations are invariably embryonically lethal (14, 25, 79). Such mutant embryos fail to develop beyond
gestation day 10.5. Significantly, heterozygous
knockouts also demonstrate a high degree of lethality,
indicating the critical nature of \textit{Gnas} in development.

A disruption of Gnas exon 2 was used to generate
the original Gnas knockout mouse (FIGURE 1) (79). As
we now know, this E2 mutation, when maternally
inherited, effectively eliminates the expression of \textit{Gnas}
in imprinted tissues, as well as causing a 50% reduc-
tion in tissues with biallelic expression. It also has the

Neuroendocrine and Metabolic Phenotypes in Mice Lacking \textit{Gnas}, or XL\textit{s}, and Their Relevance to Human Disease

Advances in our understanding of the complexities of
the Gnas locus have led to a need to characterize the in-
vivo functions of its different protein products. This
has initiated the development of different mouse lines
that carry two copies of a maternally inherited
imprinted distal chromosome 2 (and no maternal copy), i.e.,
paternal duplication distal chromosome 2 (PatDp.dist2), show a phenotype that is similar to the
maternally inherited \textit{Gnas} deficiency. Vice versa, mice
that carry two copies of a maternally inherited distal
chromosome 2 (no maternal copy), i.e., MatDp.dist2,
largely reproduce a XL\textit{s}-deficient phenotype (7, 54).
Furthermore, a point mutation in exon 6, termed
Oed-Sml, causes symptoms reminiscent of lack of maternal
\textit{Gnas} expression when inherited maternally (FIGURE 1). On the other hand, the same mutation, when inherit-
ed paternally, results in a phenotype similar to XL\textit{s}-
deficiency (7, 63). Further phenotype characterizations of these non-specific mutants and detailed
comparisons with transcript-specific knockouts are currently under way, and, therefore, we will focus on a description of the transcript-specific mutants here,
which indicate distinct and possibly opposite physio-
logical roles for \textit{Gnas} and XL\textit{s} in determining postnatal
and adult metabolic functions (57, 68).

\textit{Gnas} deficiency

From its global pattern of expression, \textit{Gnas} had been
thought to have an essential and non-redundant func-
tion in normal development and adult physiology.
This hypothesis has been confirmed by various
\textit{Gnas} knockout models, which show that homozygous
\textit{Gnas} mutations are invariably embryonically lethal (14, 25, 79). Such mutant embryos fail to develop beyond
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in imprinted tissues, as well as causing a 50% reduc-
tion in tissues with biallelic expression. It also has the
potential to disrupt signaling within cells with bis-
ense \textit{Gnas} expression. By contrast, when
\textit{Gnas} is expressed in a high \textit{Gnas} in specifically in late embryonic and newborn mouse strains, the transcript is
localized to the maternal chromosome 2q35 (79) and
is expressed in adult adipose tissues.
potential to disturb the Neup transcript in its 3'-UTR. By contrast, when the E2 mutation is paternally inherited, it affects the transcripts Gnasxl, Gnas (to 50% in cells with biallelic expression), and the noncoding exon-IA. The phenotype of heterozygotes carrying a maternally inherited exon 2 mutation (E2m+/p+) was later confirmed to be comparable to a Gnas exon 1 mutation, specific for Gox (E1p+/p+) (FIGURE 1) (11, 25). A heterozygous, maternal allele-specific deficiency of Gox has severe neonatal consequences, resulting in a high (~50–80%) preweaning mortality rate, which notably varies with the genetic background of different mouse strains (11, 25, 79). Characteristic features of the maternal Gox mutants include a transient subcutaneous edema immediately after birth, an increased level of interscapular brown adipose tissue (BAT), and a wide, square body shape. Despite an increased adiposity (adipocyte hypertrophy), Goxm–/p+ mutants remain underweight in the immediate neonatal period (78). However, toward adulthood, they experience accelerated catch-up growth, increased adiposity, and enhanced obesity development (11, 25, 78). The metabolic phenotype of adult Goxm–/p+ mutants has now been characterized in detail (FIGURE 2). In the absence of changes in food intake, the obesity phenotype is associated with reductions in energy expenditure and metabolic rate (reduced oxygen consumption) (11, 68, 78). A trend toward reduced urinary catecholamine metabolites suggests that this shift in metabolic rate reflects a decrease in overall neural sympathetic nerve activity (SNS).

*FIGURE 2. Opposite metabolic effects in Gox and XL/H9251 knockouts.*

Model showing the main metabolic dysfunctions of Goxm–/p+ and XL/H9251 mice, favoring antagonistic roles of Gox and XL/H9251 in sympathetic nervous system (SNS) outflow as the most important influence on overall phenotypes. The expression of the two proteins is indicated within each tissue, and a deficiency is marked by an X. A: loss of Gox expression from the maternal allele impairs signaling within brain, resulting in reduced SNS activity (light green arrows), which might potentially be exacerbated by inhibitory effects of XL/H9251 on this system. Reduced sympathetic output has a major effect on the development of an obesity phenotype. Loss of maternal Gox expression in peripheral tissues might contribute to the net systemic effect, although heterozygous deficiency specifically in liver or pancreatic β-cells did not show the major phenotypical effects that were described in general Goxm–/p+ mice. Brain or adipose tissue-specific knockouts have not yet been reported. Secondary effects of the knockout are symbolized by arrows between peripheral tissues. Despite mild resistance to TSH, thyroid hormone levels are unchanged in Goxm–/p+ mice, therefore, unlikely to contribute significantly to the metabolic phenotype. B: loss of paternally expressed XL/H9251 leads to an increased SNS outflow, contributing to the development of a lean, hypermetabolic phenotype. A role of XL/H9251 in peripheral tissues is currently unclear since no tissue-specific knockout exists, but it might add to the net phenotypic effect. XL/H9251 is downregulated in adult adipose tissue.
sympathetic output (78). In contrast to a close similarity in obesity phenotypes, E1m+/p− and E2m+/p− mutants display marked differences in glucose tolerance and insulin sensitivity. In E1m+/p− mice, obesity development is accompanied by hyperglycemia, glucose intolerance, hyperinsulinemia, and insulin resistance, as well as hyperlipidemia and a tendency toward reduced locomotor activity (31). By contrast, obese E2m+/p− mice were found to be glucose tolerant and insulin sensitive (77). This apparent discrepancy between the two mouse models remains unresolved for the time being, since there is no other maternal allele-specific Gnas product known that could account for it (68).

Both maternal E1 and E2 Gnas+/− mutants demonstrate multiple hormone resistances due to disrupted receptor signaling in imprinted tissues. This feature recapitulates the defining aspects of PHP-Ia patients, with Gnas+/− mice exhibiting a resistance to parathyroid hormone (PTH) in the renal proximal tubules (Table 1) (25, 79). Consistent with Gnas imprinting in the thyroid, Gnas+/− mutants also demonstrate a mild resistance to thyroid-stimulating hormone (TSH) as indicated by elevated plasma TSH levels, but this does not impact on plasma T4 or T3 levels and is not presently known whether Gnas, inherited from the maternal allele in human ovarian granulosa cells further supports the notion of gonadotropin from the maternal allele in human ovarian granulosa cells. The loss of maternal Gnas expression within imprinted tissues plays a key role in the development of human obesity. Chen et al. propose a specific reduction in hypothalamic melanocortin-4 receptor signaling in E1m+/p− mice as one potential mechanism (11). However, it is not possible to speculate that the loss of maternal Gnas expression is responsible for the specific phenotype, since both male and female Gnas+/− mice show reduced fertility rates (25). Female E1+/− mice are also less successful in rearing offspring to weaning age, which potentially reflects deficits in hormone-mediated mothering behavior. These observations are in line with clinical findings in PHP-Ia patients who variably present with hypogonadism, delayed sexual development, amenorrhea or oligomenorrhea, and occasionally elevated LH and FSH levels (47, 57). Evidence showing Gnas transcripts being derived predominantly from the maternal allele in human ovarian granulosa cells further supports the notion of gonadotropin resistance as an additional symptom of a maternal allele-specific deficiency of Gnas (44).

In contrast to the significant physiological deficiencies described for maternally inherited Gnas mutations, heterozygous mice carrying a paternally derived mutation show a much milder phenotype (11). Postnatal development has been described as normal in a CD1 genetic background, although a 30−40% mortality occurred in the 129Sv strain (11, 25). In adult E1+/− mice, mild metabolic abnormalities can be found, including a moderate increase in adipose tissue, mild hyperglycemia, glucose intolerance, and insulin resistance. The presence of obesity and insulin resistance in both maternal and paternal E1 mutants suggests that the haploinsufficiency of Gnas in biallelic tissues has a significant effect on the regulation of energy homeostasis. In light of Gnas's ubiquitous expression, possible obesogenic mechanisms are likely to involve a number of subtle widespread changes across multiple tissues. However, a greater degree of obesity can be observed in mice inheriting maternal Gnas-specific mutations compared with mice inheriting the matched paternal mutation. On this basis, it is possible to speculate that the loss of maternal Gnas expression within imprinted tissues may act to further amplify obesogenic pathways. In humans, significant obesity has long been viewed as a classic feature of both PHP-Ia and PPHP (57, 69). Consistent with the findings above, a recent clinical study has now found that not only is obesity more common in patients with PHP-Ia, inheriting maternal Gnas deficiency, which has a dominant effect over paternal Gnas deficiency, than in patients with PHP-Ia, inheriting paternal Gnas deficiency (54). This difference holds even when adjusting for maternal mortality (54).

Although obesity has long been viewed as a classic feature of PHP-Ia, inheriting maternal Gnas+/− mutations indicates that not only is obesity more common in patients with PHP-Ia, inheriting maternal Gnas deficiency, but the degree of weight gain is also significantly greater compared with patients with PHP-Ia, inheriting paternal Gnas+/− mutations (Table 1) (42). Together, these findings strongly suggest that the loss of maternal Gnas within imprinted tissues plays a key role in the development of human obesity. Chen et al. propose a specific reduction in hypothalamic melanocortin-4 receptor signaling in E1m+/p− mice as one potential mechanism (11). However, it is not presently known whether Gnas is imprinted within the hypothalamic nuclei or other brain regions involved in regulating energy homeostasis.

Apart from these global heterozygous knockouts, several tissue-specific, homozygous Gnas mouse models have been generated (6, 10, 12, 59, 60, 75). A detailed description of dysfunctions due to complete lack of Gnas in specific cell types is beyond the scope of this article but is summarized in a recent excellent review (68). With regard to the metabolic and obesity-related phenotype, crucial tissue-specific knockouts (i.e., adipose tissue- and/or brain-specific) have not yet been reported but will be very informative for the clarification of Gnas functions in energy homeostasis. It should be noted, however, that heterozygous Gnas deficiency (maternal allele), specifically in pancreatic β-cells, contributes little to the general E1+/− phenotype (impaired first-phase insulin release and initial glucose intolerance only) (Figure 2) (75). In addition, no effects have been described in heterozygous mice carrying a liver-specific Gnas knockout (12).

**XLN1 deficiency**

In the original Gnas exon 2 knockout model, maternal and paternal transmission of the mutation caused opposite metabolic phenotypes: maternal transmission (E2m+/−) led to adult obesity, whereas paternal transmission (E2m+/−) resulted in a lean, hypermetabolic phenotype (Figure 1) (78). The paternally inherited exon 2 mutation affected both Gnas and XLN1, and we now know from the Gnas-specific knockout that this latter phenotype can be attributed to XLN1 deficiency, which has a dominant effect over paternal XRn haploinsufficiency (54, 76). The XLN1-specific knockout model was generated through the targeted deletion of a small (60 bp) part of the Gnas exon. This deletion specifically terminates the expression of XLN1, while preserving the paternal expression of Gnas. As expected, Gnas XLN1 mutants passed through the maternal transmission of XLN1, recapitulates the defining aspects of PHP-Ia patients, indicating that both maternal and paternal Gnas XLN1 deficiency is associated with an increased incidence of congenital heart defects (54). This is consistent with the finding that maternal XLN1 deficiency is associated with a more severe maternal mortality (54). This finding is consistent with the finding that maternal XLN1 deficiency is associated with an increased incidence of congenital heart defects (54). This is consistent with the finding that maternal XLN1 deficiency is associated with a more severe maternal mortality (54). This finding is consistent with the finding that maternal XLN1 deficiency is associated with a more severe maternal mortality (54).
can be expected from a maternally silenced gene, the Gnasxl knockout is without phenotypic effects when passed through the maternal line. However, offspring receiving the paternal mutation (Gnasxl+/p–) demonstrate a phenotype closely comparable to E2m+/p– mice. Gnasxl+/p– mice typically show a high level of neonatal mortality due to an inability to suckle successfully (34). This is immediately apparent within the first few days after birth. On an inbred background, mortality rates reach 100% by P9, whereas on an outbred background there is an 10–20% survival rate, which can be improved by increasing maternal and interlitter competition. The observation of Gnasxl expression within the facial, hypoglossal, and trigeminal brain nuclei, which provide motor innervation to the orofacial muscles, indicates that suckling problems may involve a localized motor deficit. Notably, this characteristic feeding deficiency is confined to the neonatal period, with adult mutants demonstrating a significantly increased food intake (76). Other key features of the Gnasxl+/p– mutation include low birth weight, severe postnatal growth retardation, a narrow body shape, low neonatal blood glucose and insulin levels, reduced levels of BAT and WAT, and a lean, hypermetabolic adult phenotype, featuring increased adipose lipid oxidation (FIGURE 2) (54, 76). Unsurprisingly, reduced adiposity and an enhanced mobilization of lipid stores are associated with improved glucose tolerance and increased insulin sensitivity in adult Gnasxl+/p– mice. Enhanced insulin-stimulated glucose uptake is observed within WAT, BAT, and skeletal muscle. Currently, improvements in skeletal muscle insulin sensitivity are believed to be secondary to a dramatic reduction in tissue triglycerides brought about by the increased oxidative flux within the adipose tissue (76). The reduced adiposity and increased lean body mass of Gnasxl+/p– mice appears to result from an increase in energy expenditure (O2 consumption). Metabolically, this increased energy expenditure appears to be driven by enhanced rates of lipid oxidation within the adipose tissue. Morphological, biochemical, and gene-expression analysis of both BAT and WAT suggest that such an elevation in adipose tissue lipid mobilization is a consequence of enhanced β-adrenergic/Gs/CAMP signaling, stemming from increased basal sympathetic nervous activity (FIGURE 2) (76). The expression pattern of Xlnr within the noradrenergic locus coeruleus, sympathetic trunk, and catecholamine-producing adrenal medulla supports this conclusion and suggests that Xlnr plays a key role as a negative regulator of the sympathetic nervous system (48, 49, 54). Of further note, it is hypothesized that the neural-specific, alternatively spliced and truncated Xlnr protein may also be involved in the inhibition of central sympathetic output. One possible mechanism, potentially mediating this inhibition of sympathetic activity involves the action of Xlnr and/or Xln1 in a negative regulatory pathway, inhibiting Gαs/SNS signaling within the brain (FIGURE 2). An alternative possibility also exists, i.e., a direct effect of Xlnr deficiency within adipose tissue, although this is thought to be unlikely since Xlnr expression is downregulated in adult adipose tissue (76). However, Xlnr is expressed in adipose tissue at fetal and neonatal stages. Consequently, there remains the possibility that an absence of Xlnr during early development may permanently alter adult adipose function.

Although mutational studies have clearly demonstrated a critical biological role for Xlnr in the mouse, it is important to note that a possible role of Xlnr in human disease remains uncertain. AHO/PHP-Ia patients with various paternal GNAS exon 2-13 mutations, predicted to disrupt Xlnr, generally fail to develop the defining hypermetabolic characteristics, which appear to predominate in the knockouts. Such a discrepancy suggests that, in contrast to Gnasxl, the metabolic actions of Xlnr might be species specific. However, some rare human chromosomal abnormalities that lead to a deficiency of Xlnr (e.g., maternal uniparental disomies and paternally inherited deletions) appear to generate a phenotype that shares some of the defining aspects of Gnasxl+/p– knockout mice, such as intractable neonatal feeding difficulties, prenatal and postnatal growth retardation, and abnormal adipose tissue deposition (Table 1) (2, 13, 19, 22, 61, 66). As much as this is feasible, future studies of human mutation carriers at postnatal stages should be encouraged to clarify species differences.

Nesp55 deficiency

A specific mutation of Nesp, which leads to loss of the protein but does not eliminate the transcript, showed that Nesp55 is not required for postnatal development or adult metabolic control (55). Corresponding with the expression of Nesp in the brain and endocrine system, a behavioral phenotype relating to novel environments was identified. However, although Nesp55 is associated with serine vesicles, its molecular functions remain uncertain (20, 43). Since it was recently discovered that larger deletions in the Nesp region, when inherited maternally in human kindreds, lead to changes in the imprinting status of the other downstream Gnas transcripts, it seems likely that the DMR at Nesp and/or the transcript itself has a regulatory function in the control of imprinting of the locus (3). Modeling of these human NESP mutations in mice should provide us with highly valuable information on its potential roles in Gnas imprinting.

Concluding Remarks

The development of Gnas knockout models has demonstrated many parallels between mouse phenotypes and human disease symptoms in AHO/PHP-Ia and PHPH (Table 1). These studies have greatly advanced our understanding of how the specific
ruption of maternal or paternal alleles can lead to opposite phenotypes in knockout mice and clinically distinct disorders in humans. Yet it is important to note that differences have been observed between the phenotype of some knockouts and the presentation of human symptoms. Specifically, a role for XLO in human disease remains uncertain and may be confirmed as species specific. The development of further tissue-specific knockouts, of both Gs and XLO, is necessary to identify the tissues where disruption of different parental alleles leads to the development of parent-of-origin-specific phenotypes. A greater understanding of the species-specific contributions of Gnas mutations to phenotype development will provide a deeper insight into the complex molecular mechanisms that underlie the genetic disorders of AHO/PHP-Ia and PPHP. Apart from the analysis of the deeper insight into the complex molecular mechanisms that underlie the genetic disorders of AHO/PHP-Ia and PPHP.

References
1. Albright F, Burnett CH, Smith PH, Parson W. Pseudohypoparathyroidism: an example of "Seabright-Bantam" phenotype of some knockouts and the presentation of human disease remains uncertain and may be confirmed as species specific. The development of further tissue-specific knockouts, of both Gs and XLO, is necessary to identify the tissues where disruption of different parental alleles leads to the development of parent-of-origin-specific phenotypes. A greater understanding of the species-specific contributions of Gnas mutations to phenotype development will provide a deeper insight into the complex molecular mechanisms that underlie the genetic disorders of AHO/PHP-Ia and PPHP.

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A. Pathological and clinical aspects of GNAS mutations leading to hormone resistance
B. Molecular genetics of GNAS mutations in pseudohypoparathyroidism

C. Non-endocrine manifestations of GNAS mutations
D. Animal models of GNAS mutations
E. Functional consequences of GNAS mutations

III. Conclusions

References