Physiological Dysfunctions Associated With Mutations of the Imprinted Gnas Locus

The ubiquitous Gαs 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 Gαs 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 Gαs 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 “s-stimulatory” subunit of the trimeric G-protein, Gαs (70). Although this classical signaling protein has been studied in detail, the significance of an NH2-terminal splice variant, termed XLαs (eXtra 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 maternal/paternal 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, an increasing body of evidence suggests that maternally and paternally imprinted genes have opposite effects on a number of converging/overlapping pathways involved in determining early growth dynamics and energy acquisition. In this light, genomic imprinting is regarded as a mechanism through which conflicting parental influences can affect the growth and development of offspring. Whereas paternally expressed alleles typically stimulate growth and nutrient acquisition, maternally expressed alleles have a limiting or restricting role (28). The characterization of a potential network of imprinted genes regulating the development of these crucial physiological pathways has become the current focus of research in the field of imprinting and epigenetic control of energy homeostasis (9, 65).

The Transcripts and Proteins Encoded by the Gnas Locus and Their Regulation by Genomic Imprinting

Genome-wide screens for novel imprinted genes in the human and mouse have revealed the complex structural arrangement of the Gnas locus (29, 30, 33, 53). In addition to the core exons and promoter of Gnas itself, the locus is now known to contain several alternative upstream promoters that initiate further coding and noncoding transcripts. The gene structure, and pattern of imprinted expression are largely conserved between the murine and human loci on distal chromosome 2 and chromosome 20 (13.2–13.3), respectively, with only some minor differences as mentioned below. At the center of the locus, Gnas itself comprises 12 exons, which encode the Gαs 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, 70). 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, 45, 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 Gaα2 (XLα2).

In neuroendocrine tissues, e.g., the brain stem (magnocellular and paraventricular nuclei), the Gnasxl transcript is generated from the exon 1A variant (48, 49, 54). Expression of the Gnasxl transcript in the hypothalamic paraventricular nucleus (PVN) is essential for the development of pulsatile corticotropin-releasing hormone (CRH) secretion (32). In contrast, the Gnas transcript is maternally inherited and highly restricted to the neonatal stomach, kidneys, adrenal medulla, and brainstem (FIGURE 1) (48, 49, 54). Expression of the Gnas transcript in the adrenal gland is highly tissue specific; the Gnas transcript is almost identical to the Adra2a transcript, which encodes the α1-adrenergic receptor.

In neuroendocrine tissues, e.g., the adenohypophysis, the Gnasxl transcript is expressed almost exclusively from the maternal allele; whereas the Gnas transcript is expressed to a similar extent from both parental alleles (48, 49, 54). Whether these differences in the pattern of expression of the Gnasxl transcript reflect differences in the function is currently unknown.

Approximately 25% of the paternal Gnasxl knockout mice die before birth; these mice exhibit severe intrauterine growth retardation and intractable neonatal hypotonia (62, 63). In humans, homozygous mutations in GNAS lead to congenital hypothyroidism (13, 14, 16, 23, 24, 26, 27, 42, 45, 47, 59, 60, 71). These mutations include point mutations, insertions, deletions, and/or small copy-number variants (22, 23, 25, 27, 44, 45, 64, 71). The genomic alterations in GNAS lead to constitutively active Gaα2/αs signaling, which results in severe obesity, severe growth retardation, and hypothyroidism (13, 14, 16, 23, 24, 26, 27, 42, 45, 47, 59, 60, 71). The Gnasxl knockout mouse is viable and displays reduced growth and obesity (62, 63). The Gnasxl knockout mouse is viable and displays reduced growth and obesity (62, 63).

Table 1. Genetic causes of GNAS-related disorders

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>Human Symptom</th>
<th>Maternal mutation</th>
<th>Paternal mutation</th>
</tr>
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<tbody>
<tr>
<td>Point mutation</td>
<td>Short stature, subcutaneous fat, multiple hormone resistance</td>
<td>Severe obesity, variable mental retardation</td>
<td>Mild obesity, variable mental retardation</td>
</tr>
<tr>
<td>Inactivating mutation</td>
<td>Subcutaneous fat, multiple hormone resistance</td>
<td>Severe obesity, variable mental retardation</td>
<td>Mild obesity, variable mental retardation</td>
</tr>
<tr>
<td>Deletion mutation</td>
<td>Subcutaneous fat, multiple hormone resistance</td>
<td>Severe obesity, variable mental retardation</td>
<td>Mild obesity, variable mental retardation</td>
</tr>
<tr>
<td>Chromosomal translocation</td>
<td>Hypothyroidism, mental retardation</td>
<td>Severe obesity, variable mental retardation</td>
<td>Mild obesity, variable mental retardation</td>
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**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, Gnasxl, 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. Neapas transcripts exist in multiple spliced and unspliced forms. In humans, GNAS consists of 13 exons and NESP only comprises one 5’-exon. The 5’-exon 1A is termed exon A/B. MM1 indicates DNA methylation at differentially methylated regions (DMRs) of the respective alleles; the DMRs at Neapas 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.
The tissue-specific imprinting at the Gnasxl locus is highly relevant to the expression patterns of the genes encoded by this gene. It is a result of the action of two independent promoters, a maternal promoter (~30 kb upstream of exon 1A) and a paternal promoter (~9 kb upstream of exon 1A). The transcription of the Gnasxl gene results in the production of maternal and paternal transcripts, which are then processed into mature RNAs. The maternally derived transcript is expressed only from the maternal chromosome, while the paternally derived transcript is expressed only from the paternal chromosome. This results in the imprinting of the Gnasxl gene, which is a critical factor in the development of various physiological characteristics.

The Gnasxl gene encodes the Nesp55 protein, which is highly expressed in neuroendocrine tissues. The Nesp55 protein is involved in the regulation of multiple physiological functions, including the control of hormone levels and cellular metabolism. The expression of the Nesp55 protein is controlled by the imprinting of the Gnasxl gene, which results in the production of two distinct transcripts, one from each parent.

The Gnasxl gene is also involved in the regulation of various physiological characteristics, such as body weight, adiposity, and hormone levels. Mutations in the Gnasxl gene have been associated with various human disorders, including developmental delays, neurological defects, and hormone resistance.

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

<table>
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<tr>
<th>Human Symptoms</th>
<th>Murine Phenotype</th>
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<tr>
<td>Maternal mutations in GNAS exons (AHO/PHP-1a)</td>
<td>Paternal Gnas-specific knockout (Gnas&lt;sup&gt;m&lt;/sup&gt;)&lt;sup&gt;NA&lt;/sup&gt;</td>
</tr>
<tr>
<td>(Point mutations, small deletions, splice site mutations in exons 1-13)</td>
<td>Maternal Gnas-specific knockout (Gnas&lt;sup&gt;m&lt;/sup&gt;)&lt;sup&gt;NA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Short stature, brachyacdyctyly</td>
<td>50–80% neonatal lethality</td>
</tr>
<tr>
<td>Subcutaneous ossification</td>
<td>Reduced body length</td>
</tr>
<tr>
<td>Multiple hormone resistance (TSH, PTH, GHRH, gonadotropins)</td>
<td>Multiple hormone resistance (PTH)</td>
</tr>
<tr>
<td>Severe obesity</td>
<td>Severe obesity with insulin resistance and glucose intolerance</td>
</tr>
<tr>
<td>Variable mental retardation and neurological defects</td>
<td></td>
</tr>
<tr>
<td>Paternal mutations in GNAS exons (AHO/PHP-P)</td>
<td>Paternal Gnas-specific knockout (Gnas&lt;sup&gt;m&lt;/sup&gt;x&lt;sup&gt;NA&lt;/sup&gt;)</td>
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</tr>
<tr>
<td>Short stature, brachyacdyctyly</td>
<td>0–30% neonatal lethality</td>
</tr>
<tr>
<td>Subcutaneous ossification</td>
<td>Reduced body length</td>
</tr>
<tr>
<td>Mild obesity</td>
<td>Subcutaneous ossification</td>
</tr>
<tr>
<td>Variable mental retardation and neurological defects</td>
<td>Mild form of obesity, insulin resistance and glucose intolerance</td>
</tr>
<tr>
<td>Chromosomal abnormalities of the 20q13.2-13.3 region</td>
<td>Paternal Xnas-specific knockout (Gnas&lt;sup&gt;x&lt;/sup&gt;&lt;sup&gt;NA&lt;/sup&gt;)</td>
</tr>
<tr>
<td>(Paternal deletions affecting Xnas among other genes; maternal uniparental disomy)</td>
<td>Paternal Xnas-specific knockout (Gnas&lt;sup&gt;x&lt;/sup&gt;&lt;sup&gt;NA&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Severe intrauterine growth retardation</td>
<td>~80% neonatal lethality</td>
</tr>
<tr>
<td>Intractable neonatal feeding difficulties</td>
<td>Reduced body length and weight</td>
</tr>
<tr>
<td>Abnormal distribution of adipose tissue, significant reduction in subcutaneous adiposity</td>
<td>Poor suckling at birth</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>Reduced adiposity, hypermetabolism</td>
</tr>
<tr>
<td>Mental retardation and other neurological defects</td>
<td>Hypotonia</td>
</tr>
</tbody>
</table>

Note: <sup>NA</sup> indicates not applicable.

Hormone resistances and several of these other symptoms have also been described in patients with deregulated imprinting of the GNAS locus (termed PHP-Ilb). Most maternal mutations in the GNAS exons have been predicted to affect Xnas as well. In non-specific paternal Gnas knockouts, the Gnas<sup>x</sup><sup>NA</sup> phenotype appears to be dominant. However, this is not the case with human mutations, where the combined disruption of GNAS and GNASXL does not appear to recapitulate a phenotype resembling that of Gnas<sup>x</sup><sup>NA</sup> mice.
The discovery of mutations in the highly conserved human \( G\text{nas} \) locus gave the first indications that the encoded gene products influenced important physiological processes. The genetic disorders Albright’s Hereditary Osteodystrophy (AHO) and pseudopseudohypoparathyroidism (PHP) were found to be due to missense or nonsense mutations in \( G\text{nas} \) exons \( 1, 36, 37, 67 \). AHO is characterized by a range of symptoms, including short stature, brachydactyly, subcutaneous oedema, obesity, and variable mental retardation. In some cases, additional symptoms of hypocalcaemia, hyperphosphataemia, and end-organ resistance to parathyroid hormone (PTH) in the proximal renal tubules are associated with AHO. This observation has led to the condition being termed AHO plus resistance to parathyroid hormone (PTH) (PTHR). Upon Davies and Hughes’ observation, it became clear that AHO plus PTH resistance (as well as other hormone resistances), i.e., PPHP-1a, only occurred after maternal inheritance of a \( G\text{nas} \) mutation (17). By contrast, AHO without hormone resistance (PPHP) is found after paternal transmission of the same \( G\text{nas} \) mutations (Table 1). This pattern of inheritance reflects the tissue-specific imprinting of \( G\text{nas} \), with hormone resistance arising from imprinting tissues, where the paternal allele is naturally silenced and the inheritance of an inactivating mutation in the maternal allele leads to an almost complete absence of \( G\text{nas} \) expression. Thus the common AHO symptoms can be attributed to haploinsufficiency of \( G\text{nas} \) in a subset of cell types demonstrating biallelic expression. It is beyond the scope of this review to describe the human disorders in more detail, but many of those symptoms are reproduced in recently generated mouse models, and we will refer to them in the following discussion of physiological phenotypes of mutant mice. The human disorders have recently been reviewed elsewhere (5, 35, 57, 69).

**Neuroendocrine and Metabolic Phenotypes in Mice Lacking \( G\text{as} \), \( G\text{a}s \), and Their Relevance to Human Disease**

Advances in our understanding of the complexities of the \( G\text{as} \) 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 carrying transcript-specific deletions. In turn, these new mutants have prompted a re-interpretation of earlier findings described in older mouse models, in which carried less specific \( G\text{as} \) mutations. Earlier mutational models, involving mice with uniparental duplications/deficiencies of distal chromosome 2 and the Oed-Soul point mutation, typically affected both \( G\text{as} \) and \( G\text{as} \) transcripts and demonstrated remarkably consistent parent-of-origin effect on neonatal and adult phenotypes (FIGURE 1) (7, 25). A heterozygous mutation of \( G\text{as} \) has been shown to result in a high \( bpk \) mutation rate in both strains, but only the maternal \( G\text{as} \) allele causes the maternal \( G\text{as} \)-deficient phenotype (7, 54). Furthermore, a point mutation in exon 6, termed Oed-Soul, causes symptoms reminiscent of lack of maternal \( G\text{as} \) expression when inherited maternally (FIGURE 1). On the other hand, the same mutation, when inherited paternally, results in a phenotype similar to \( XL\text{as} \) deficiency (7, 63). Further phenotype characterizations of these nonspecific mutants and detailed comparisons with transcript-specific knockouts are currently under way, and, therefore, we will focus on the description of the transcript-specific mutants here, which indicate distinct and possibly opposite physiological roles for \( G\text{as} \) and \( G\text{as} \) in determining postnatal and adult metabolic functions (57, 68).

**\( G\text{as} \) deficiency**

From its global pattern of expression, \( G\text{as} \) has been thought to have an essential and non-redundant function in normal development and adult physiology. This hypothesis has been confirmed by the various \( G\text{as} \) knockout models, which show that homozygous \( G\text{as} \) mutations are invariably embryonically lethal (11, 25, 79). Such mutant embryos fail to develop beyond gestational day 10.5. Significantly, heterozygous knockout mice also demonstrate a high degree of lethality, indicating the critical nature of \( G\text{as} \) in development. A disruption of \( G\text{as} \) exon 2 was used to generate the original \( G\text{as} \) knockout mouse (FIGURE 1) (79). As we now know, this E2 mutation, when maternally inherited, effectively eliminates the expression of \( G\text{as} \) in imprinted tissues, as well as causing a 50% reduction in tissues with biallelic expression. It also has the potential to disrupt receptor signaling in embryonic and postnatal tissues. By contrast, when inherited from the male, it affects cells with bicistronic transcripts (79). Thus mice that carry two copies of a paternally inherited distal chromosome 2 (and no maternal copy), i.e., paternal duplication distal chromosome 2 (PatDp.dist2), show a phenotype that is similar to that of maternally inherited \( G\text{as} \) deficiency. Vice versa, mice that carry two copies of a maternally inherited distal chromosome 2 (no paternal copy, i.e., MatDp.dist2) largely reproduce a \( XL\text{as} \)-deficient phenotype (7, 54). Furthermore, a point mutation in exon 6, termed Oed-Soul, causes symptoms reminiscent of lack of \( G\text{as} \) expression when inherited maternally (FIGURE 1). On the other hand, the same mutation, when inherited paternally, results in a phenotype similar to \( XL\text{as} \) deficiency (7, 63). Further phenotype characterizations of these nonspecific mutants and detailed comparisons with transcript-specific knockouts are currently under way, and, therefore, we will focus on the description of the transcript-specific mutants here, which indicate distinct and possibly opposite physiological roles for \( G\text{as} \) and \( G\text{as} \) in determining postnatal and adult metabolic functions (57, 68).

**FIGURE 1**

Model showing sympathetic nerves innervating the renal medulla and hypothalamus. Sympathetic nerves are indicated by blue arrows, whereas the autonomous nerves are indicated by black arrows. The microvasculature is indicated by red blood vessels. The right panel depicts a schematic of the sympathetic nerve innervation of the renal medulla and hypothalamus. The left panel shows a schematic of the autonomic nerve innervation of the renal medulla and hypothalamus.
complexities of characterize the in products. This at mouse lines in turn, these representation of mouse mutations. Earlier on uniparental or chromosome 2 and affected both demonstrated a effect on maternal, termed Oef- 
also very similar to XLs, a characterization and detailed knockouts will focus on a specific expression of the two pro-
models, including in turn the offspring of lethal (11, 54). The expression of both proteins is indicated within each tissue, and a deficiency is marked by an X.

**A** Maternal allele-specific Gnas knock-out

- Loss of Gnas 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 XLs on this system. Reduced sympathetic output has a major effect on the development of an obesity phenotype. Loss of maternal Gnas expression in peripheral tissues might contribute to the systemic effect, although heterozygous deficiency specifically in liver or pancreatic β-cells did not show the major phenotypical effects that were described in general Gnas-/- 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 Gnas-/- mice and, therefore, unlikely to contribute significantly to the metabolic phenotype. Loss of paternally expressed XLs leads to increased SNS outflow, but a role of XLs in peripheral tissues is currently unclear since no tissue-specific knock-out exists, but it might add to the net phenotypic effect. XLs is downregulated in adult adipose tissue.

**B** Paternal allele-specific XLs knock-out

- Loss of paternally expressed XLs leads to increased SNS outflow, contributing to the development of a lean, hypermetabolic phenotype. As a role of XLs in peripheral tissues is currently unclear, no tissue-specific knock-out exists, but it might add to the net phenotypic effect. XLs is downregulated in adult adipose tissue.

FIGURE 2. Opposite metabolic effects in Gnas-/- and XLs-/- knockout mice

Model showing the main metabolic dysfunctions of Gnas-/- and XLs-/- mice, favoring antagonistic roles of Gnas and XLs 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. A loss of Gnas 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 XLs on this system. Reduced sympathetic output has a major effect on the development of an obesity phenotype. Loss of maternal Gnas expression in peripheral tissues might contribute to the systemic effect, although heterozygous deficiency specifically in liver or pancreatic β-cells did not show the major phenotypical effects that were described in general Gnas-/- 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 Gnas-/- mice and, therefore, unlikely to contribute significantly to the metabolic phenotype. Loss of paternally expressed XLs leads to increased SNS outflow, but a role of XLs in peripheral tissues is currently unclear since no tissue-specific knock-out exists, but it might add to the net phenotypic effect. XLs is downregulated in adult adipose tissue.

Physiological Genes in Maternal Inheritance to...
sympathetic output (78). In contrast to a close similarity
in obesity phenotypes, E1m–/p− and E2m−/− mutants
display marked differences in glucose tolerance and
insulin sensitivity. In E1m−/− mice, obesity develop-
ment is accompanied by hyperglycemia, glucose intoler-
ance, hyperinsulinaemia, and insulin resistance, as
well as hyperlipidemia and a tendency toward reduced
locomotor activity (11). By contrast, obese E2m−/− 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 demon-
strate multiple hormone resistances due to disrupted
receptor signaling in imprinted tissues. This feature
recaptures the defining aspects of PHP-Ia patients,
with Gnas−/− mice exhibiting a resistance to parathy-
roid 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
therefore unlikely to contribute significantly to the
change in metabolic rate (11, 25, 79). There are also
further indications for a resistance to gonadotropins,
since both male and female Gnas−/− mice show
reduced fertility rates (25). Female Gnas−/− mice are also
less successful in rearing offspring to weaning age,
which potentially reflects deficits in hormone-mediat-
ed mothering behavior. These observations are in line
with clinical findings in PHP-Ia patients who variably
present with hypogonadism, delayed sexual develop-
ment, amenorrhea or oligomenorrhea, and occasi-
onally elevated LH and FSH levels (47, 57). Evidence
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ly from the maternal allele in human ovarian granu-
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inherited exon 2 mutation affected both Gnas
and XLN1 proteins (57, 78). The paternally
acting XLN1 protein provides modest additional
enhanced mobilization of insulin sensitive (77).
A greater degree of weight gain is also significantly
greater compared with patients with PHP-Ib who inherit
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-Gnas signaling in E1m−/−
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 mice
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
phenotypes, crucial tissue-specific knockouts (i.e.,
departmental insulins and/or brain-specific) have not yet
been reported but will be very informative for the clas-
ification of Gnas functions in energy homeostasis.
A should be noted, however, that heterozygous Gnas
deficiency (maternal allele), specifically in pancreatic
β-cells, contributes little to the general E1m−/− phenotype
(unchanged first-phase insulin release and initial
insulin resistance only) (FIGURE 2) (75). In addition,
no effects have been described in hypertrophy
or hypothyroidism carrying a liver-specific Gnas knockout (12).

XLN1 deficiency
In the original Gnas exon 2 knockout model, maternal
and paternal transmission of the mutation causes
opposite metabolic phenotypes: maternal transmis-
sion (E2m−/−) led to adult obesity, whereas paternal
transmission (E2m+/−) resulted in a lean, hypermetab-
olic 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
Gnas haploinsufficiency (54, 76). The XLN1-specific
knockout model was generated through a targeted
deletion of a small (60 bp) part of the Gnas exon 2. This
deletion specifically terminates the expression of XLN1,
while preserving the paternal expression of Gnas. As
can be expected, Gnas mutation did not pass through
receiving the paternal
Gnas+/+ maternal
tility (54). This study demonstrated that XLN1
provides a predominant role in the early postnatal
metabolic adaptation (78). The observed paternal
hypoglycemia and hyperinsulinemia, which is typical
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 inher-
inating 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 PHP-II (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 the matched paternal mutation,
but also that XLN1 haploinsufficiency is strongly associ-
ated with clinical findings in PHP-Ia patients who variably
showed the development of obesity, hyperinsulinemia,
and insulin resistance, as well as hyperlipidemia and a
possible tendency toward reduced locomotor activity
(11, 12, 59). In adult life, the XLN1 deficiency is
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review (68). With regard to the metabolic and obesity
phenotypes, crucial tissue-specific knockouts (i.e.,
departmental insulins and/or brain-specific) have not yet
been reported but will be very informative for the clas-
ification of Gnas functions in energy homeostasis.
A should be noted, however, that heterozygous Gnas
deficiency (maternal allele), specifically in pancreatic
β-cells, contributes little to the general E1m−/− phenotype
(unchanged first-phase insulin release and initial
insulin resistance only) (FIGURE 2) (75). In addition,
no effects have been described in hypertrophy
or hypothyroidism carrying a liver-specific Gnas knockout (12).

XLN1 deficiency
In the original Gnas exon 2 knockout model, maternal
and paternal transmission of the mutation causes
opposite metabolic phenotypes: maternal transmis-
sion (E2m−/−) led to adult obesity, whereas paternal
transmission (E2m+/−) resulted in a lean, hypermetab-
olic 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
Gnas haploinsufficiency (54, 76). The XLN1-specific
knockout model was generated through a targeted
deletion of a small (60 bp) part of the Gnas exon 2. This
deletion specifically terminates the expression of XLN1,
while preserving the paternal expression of Gnas. As

Nongenetic maternal effects appear to further influence the phenotypic expression of the gnl mutations. Indeed, significant phenotypic variability is noted in gnl offspring from maternal lineages that are otherwise nearly identical in genetic background. Some maternal lineages are more permissive of the gnl phenotype whereas others result in a more repressing effect, independent from the affected embryonic sex (59). This may be due to epigenetic differences between the maternal lineages, which had been shown to influence the phenotype of gnl knockout mice (60). Given the importance of sex determination in mouse development, it is likely that the maternal inheritance of the gnl mutation affects the establishment of sex-specific differences in gene expression, which may impact the severity of the phenotype. While the gnl knockout mouse model provides a valuable tool for understanding gnl-mediated effects on mouse development, the need for maternal genetic background is important to consider in future studies.

The significance of maternal effects on murine development is further highlighted by recent studies on the role of the maternal genome in X-linked genetic disorders. Specifically, the X-linked gene XLN1, which is involved in X-chromosome inactivation (XCI), has been shown to play a critical role in determining the expression of genes on the X chromosome. The maternal expression of XLN1 results in the preferential inactivation of the paternal X chromosome, leading to a skewed XCI pattern. This skewed XCI can affect the expression of genes on the non-inactivated X chromosome, leading to phenotypic differences between males and females. The maternal expression of XLN1 is thus essential for the establishment of a normal XCI pattern, which is necessary for normal development and fertility in females. Moreover, maternal inheritance of XLN1 mutations can lead to skewed XCI and associated phenotypes, such as reproductive failure and developmental abnormalities. These studies emphasize the importance of maternal influence in determining the phenotype of X-linked genetic disorders, highlighting the need for a comprehensive understanding of the maternal contribution to murine development.

In conclusion, maternal factors play a crucial role in shaping the phenotype of gnl knockout mice. The maternal inheritance of the gnl mutation, along with epigenetic differences between maternal lineages, can influence the severity of the phenotype and the expression of sex-specific gene expression differences. These findings underscore the importance of considering maternal factors in the interpretation of gnl knockout mouse data, which may have implications for the understanding of gnl-mediated effects on human development.
rupture 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 XLoS in human disease remains uncertain and may be confounded by species-specific. The development of further tissue-specific knockouts, of both Gs and XLoS, 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 tissue-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 physiological functions, further work is also necessary to characterize mutations of the Gnas locus that impact its imprinting status and gene regulatory mechanisms that control the various coding and non-coding transcripts.

Our work is supported by grants from the Royal Society and the Medical Research Council of the United Kingdom.

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