REVIEW

Physiological functions of the imprinted *Gnas* locus and its protein variants $G\alpha_s$ and $XL\alpha_s$ in human and mouse

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Abstract

The stimulatory α-subunit of trimeric G-proteins $G\alpha_s$, which upon ligand binding to seven-transmembrane receptors activates adenylyl cyclases to produce the second messenger cAMP, constitutes one of the archetypal signal transduction molecules that have been studied in much detail. Over the past few years, however, genetic as well as biochemical approaches have led to a range of novel insights into the $G\alpha_s$ encoding guanine nucleotide binding protein, α-stimulating (*Gnas*) locus, its alternative protein products and its regulation by genomic imprinting, which leads to monoallelic, parental origin-dependent expression of the various transcripts. Here, we summarise the major characteristics of this complex gene locus and describe the physiological roles of $G\alpha_s$ and its 'extra large' variant $XL\alpha_s$ at post-natal and adult stages as defined by genetic mutations. Opposite and potentially antagonistic functions of the two proteins in the regulation of energy homeostasis and metabolism have been identified in *Gnas*- and *Gnasxl* ($XL\alpha_s$)-deficient mice, which are characterised by obesity and leanness respectively. A comparison of findings in mice with symptoms of the corresponding human genetic disease 'Albright's hereditary osteodystrophy'/'pseudohypoparathyroidism' indicates highly conserved functions as well as unresolved phenotypic differences.

Journal of Endocrinology (2008) **196,** 193–214

The stimulatory G-protein signalling cycle

Heterotrimeric G-proteins that are composed of α, β and γ-subunits, mediate signal transduction from a large number of activated seven-transmembrane receptors to diverse intracellular effector pathways. Many general aspects of G-protein signalling have been covered in recent excellent reviews (Cabrera-Vera *et al.* 2003, Wettschureck & Offermanns 2005). The G_s class of α-subunits is characterised by its ability to stimulate adenylyl cyclases (ACs) to produce the second messenger molecule cAMP. It comprises two genes, *Gnas* (*GNAS* in human) and guanine nucleotide binding protein, α stimulating, olfactory type (*Gnal*), which encode $G\alpha_s$ and $G\alpha_{olf}$ respectively. While *Gnas* is generally regarded as a ubiquitously expressed gene, *Gnal* expression is limited to the olfactory epithelium and a few brain regions, in which it largely replaces *Gnas* expression with very little overlap of the two α-subunits (Belluscio *et al.* 1998, Zhuang *et al.* 2000, Herve *et al.* 2001). We will focus here on novel findings related to the $G\alpha_s$-subunit, its gene locus, variant protein isoforms and physiological functions.

G-proteins undergo a cycle of active and inactive states during the signal transduction process as summarised for $G\alpha_s$ in Fig. 1. The inactive form of the G-protein consists of a trimer comprising $G\alpha_s$ in association with β- and γ-subunit complexes at the plasma membrane, whereby $G\alpha_s$ occupies the GDP nucleotide-bound conformation. Membrane anchorage of the α- and γ-subunits is achieved via lipid modifications, in the case of $G\alpha_s$ palmitoylation of the NH_2-terminus (Kleuss & Krause 2003). β- and γ-subunits form a very tight and stable complex (Wettschureck & Offermanns 2005). A ligand-bound G-protein coupled receptor (GPCR) activates the G_s-protein through promoting the exchange of GDP for GTP on the α-subunit, which results in its dissociation from the receptor and the β- and γ-complexes. The free $G\alpha_s$ subunit can now interact with and stimulate its effector AC until the intrinsic GTPase activity (hydrolysis of GTP) of the α-subunit returns it into the inactive GDP-bound form, which reassociates with the β- and γ-complexes, to enter a new cycle (Sunahara *et al.* 1997, Cabrera-Vera *et al.* 2003). Very little is known about specificities in the interactions between $G\alpha_s$ and the 5 different β-subunits and 12 γ-subunits that have been identified, nor whether specific combinations of these subunits preferentially interact with certain GPCRs.

The $G\alpha_s$ effector AC comprises a family of proteins encoded by nine different genes in mammalian genomes, termed type

DOI: 10.1677/JOE-07-0544
0022–0795/08/0196–193
Online version via http://www.endocrinology-journals.org

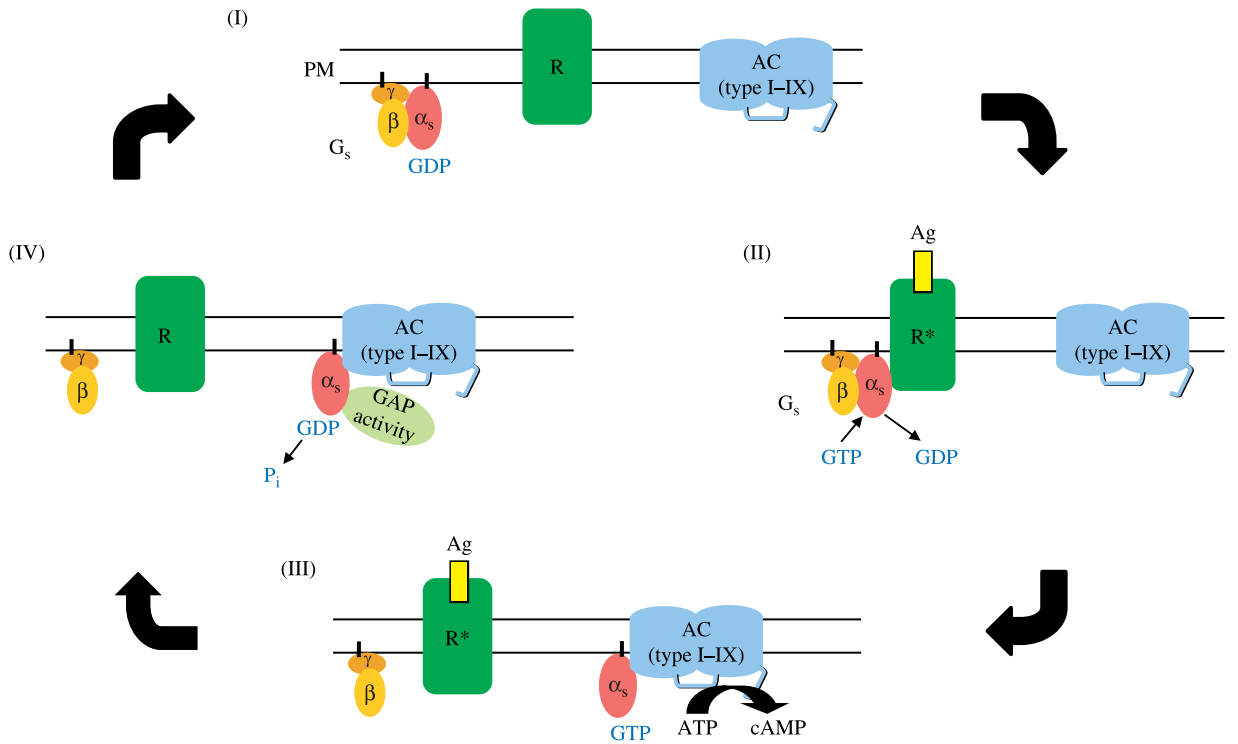


Figure 1 Scheme of the signalling cycle of the trimeric G_s-protein. (I) The inactive, trimeric G_s-protein, consisting of α-, β- and γ-subunits, is associated with the plasma membrane via lipid modifications. The α_s-subunit, e.g. $G\alpha_s$ or $XL\alpha_s$, is in its GDP-bound conformation. (II) Agonist binding to a G_s-coupled seven-transmembrane receptor (GPCR) causes a conformational switch in the α-subunit, which also involves an exchange of GDP for GTP, leading to its activation and dissociation from β- and γ-subunits. (III) The active, GTP-bound form of $G\alpha_s/XL\alpha_s$ interacts with and activates transmembrane adenylyl cyclases type I–IX, resulting in increased formation of the second messenger cAMP. (IV) The intrinsic GTP hydrolysis activity of $G\alpha_s/XL\alpha_s$, which can be stimulated by GTPase-activating enzymes (GAPs), results in its inactivation and reassociation with β- and γ-subunits.

I–IX, all of which are large transmembrane proteins with a bipartite catalytic domain (Kamenetsky *et al.* 2006, Willoughby & Cooper 2007). Although all transmembrane ACs can be stimulated by $G\alpha_s$, they vary in their responsiveness to additional regulators, e.g. $G\alpha_i$, G β- and γ-subunits, Ca^{2+} and protein kinases (Kamenetsky *et al.* 2006, Willoughby & Cooper 2007). Most cell types express several AC genes, but certain isoforms dominate in specific tissues (Hanoune & Defer 2001, Krumins & Gilman 2006, Willoughby & Cooper 2007). In the context of some of the physiological functions of $G\alpha_s$ discussed below, it is noteworthy, for example, that AC III exerts a specific role in brown adipose tissue (BAT). In rodents, AC III expression and AC activity in BAT is transiently increased during the neonatal period, when offspring are especially sensitive to environmental conditions and maintenance of body temperature (Chaudhry *et al.* 1996). Stimulation of this signalling pathway results in increased lipolysis and heat production in mitochondria. AC III is strongly upregulated upon stimulation by the sympathetic nervous system, e.g. adrenergic receptor stimulation (Granneman 1995).

The last step of the G-protein cycle (Fig. 1), the inactivation of the $G\alpha_s$ subunit and re-association with β- and γ-subunits into the trimeric complex, is triggered by the intrinsic GTPase activity of $G\alpha_s$ (Cabrera-Vera *et al.* 2003). Generally, the hydrolysis of GTP by α-subunits is stimulated *in vivo* by GTPase-activating proteins (GAPs). In the case of $G\alpha_s$, several proteins have been demonstrated to exert a GAP function, including regulator of G-protein signalling 2 (RGS2; Abramow-Newerly *et al.* 2006, Roy *et al.* 2006), AC V itself (Scholich *et al.* 1999), RGS-PX1 (Zheng *et al.* 2001) and cysteine string protein (Natochin *et al.* 2005). Their importance in $G\alpha_s$ signalling *in vivo* remains to be confirmed.

The $G\alpha_s$ variant $XL\alpha_s$ also stimulates cAMP signalling from activated receptors

The identification in PC12 cells of an alternative 'extra large' form of the α_s subunit, $XL\alpha_s$, brought novel aspects to this signalling pathway (Kehlenbach *et al.* 1994). The $XL\alpha_s$ protein was found to be mostly identical in sequence to $G\alpha_s$, apart from the NH_2-terminal domain, which was replaced by a different (~370 amino acid) sequence. As detailed below, the two variants are transcribed from alternative promoters/first exons of the *Gnas* gene and spliced onto shared downstream exons from exon 2 onwards. The novel, XL-specific NH_2-terminus consists

of a repeated, alanine-rich motif, a proline-rich domain, a highly charged and cysteine-containing region and a sequence motif that includes a stretch of leucines and is highly conserved among all α-subunits (Fig. 2A; Kehlenbach *et al.* 1994). While the repeat motif varies among mammals (Hayward *et al.* 1998*a*, Freson *et al.* 2003), the other XL-specific domains are well conserved. The function of the proline-rich domain is uncertain; however, the cysteine residues serve for lipid anchorage (palmitoylation) to the plasma membrane similar to $G\alpha_s$ (Ugur & Jones 2000), while the leucine-containing motif participates in the binding of G-protein β- and γ-subunits (Kehlenbach *et al.* 1994, Lambright *et al.* 1996, Klemke *et al.* 2000). The ability of $XL\alpha_s$ to act as a fully functional G_s-protein, i.e. binding of β- and γ-subunits, activation of AC and coupling of activated receptors, was established in biochemical assays (Klemke *et al.* 2000) and in transfections of fibroblasts that lack endogenous G_s-proteins (Bastepe *et al.* 2002, Linglart *et al.* 2006); the characteristics of cAMP signalling were identical for $XL\alpha_s$ and $G\alpha_s$ (for rat and human versions) in these transfection studies (Bastepe *et al.* 2002, Linglart *et al.* 2006). Neuroendocrine cell lines that express both proteins endogenously have not yet been analysed (see also Klemke *et al.* 2000).

While $G\alpha_s$ is regarded as being more or less ubiquitously expressed, $XL\alpha_s$ shows a much restricted expression pattern,

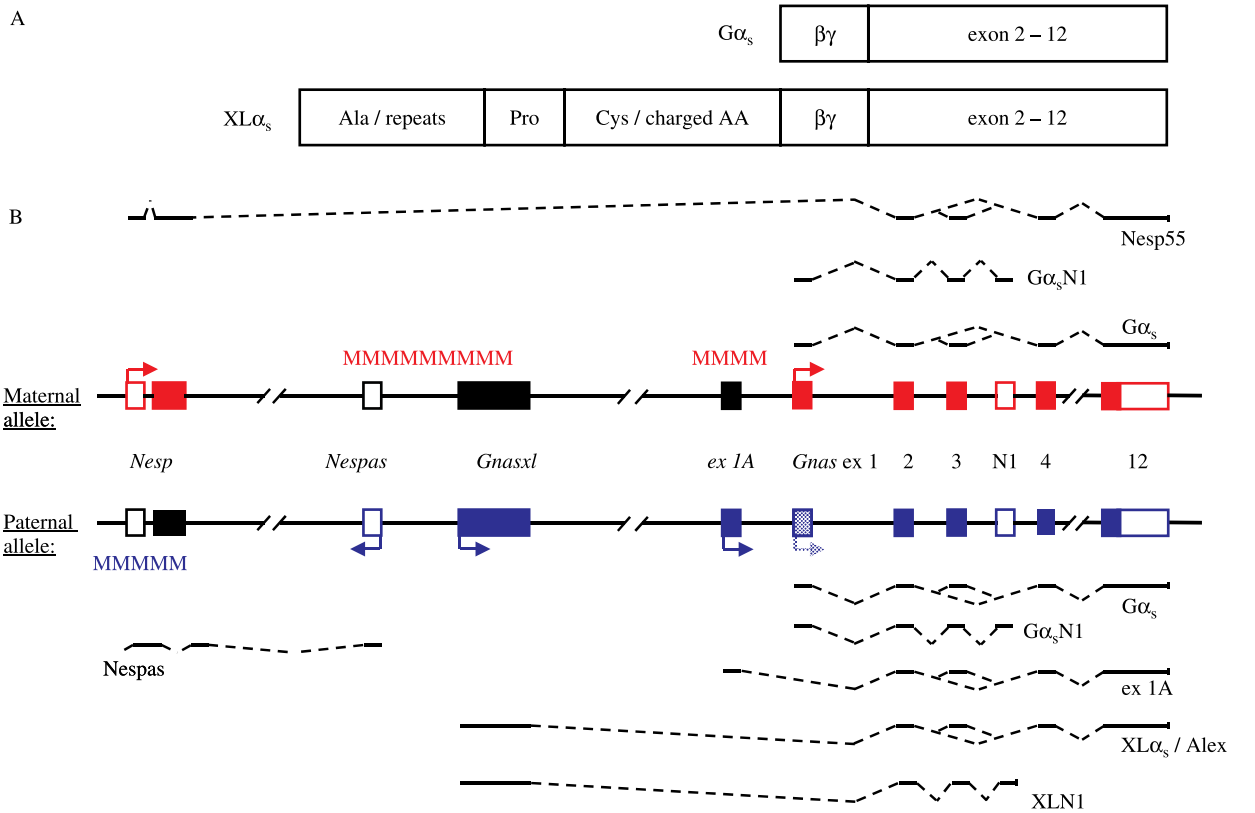


Figure 2 Scheme of the protein domains of $G\alpha_s$ and $XL\alpha_s$ encoded by their first exons and of the imprinted *Gnas* locus. (A) Conserved protein regions encoded by *Gnas* ($G\alpha_s$) and *Gnasxl* ($XL\alpha_s$) first exons. The first exons encode conserved amino acids (βγ) that contribute to the binding of β- and γ-subunits. The *Gnasxl* specific exon contains further protein regions that are conserved among mammals, e.g. a region with cysteines and charged amino acids (Cys/charged AA) that mediates lipid membrane anchorage, a proline-rich domain (Pro) and a domain containing an alanine-rich repetitive motif. The C-terminus of the two proteins, encoded by exons 2–12 (exons 2–13 in human), is identical. (B) The exon–intron structure (coding exons filled), promoter activities and alternative splicing of the murine imprinted *Gnas* locus are depicted. The maternally and paternally inherited alleles are indicated in red and blue respectively. Arrows indicate the promoters and transcriptional direction of the individual RNAs. Regions of differential DNA methylation (DMRs) are marked by MMM; DMRs at *Nespas*/*Gnasxl* and *exon 1A* represent imprinting control regions (ICRs). Splicing patterns of the transcripts and encoded proteins are shown above and below the genomic locus. *Gnas* is expressed biallelically in most tissues, but is silenced on the paternal allele in some cell types (hatched blue box). *Gnasxl* shows exclusive paternal allele-specific expression and is spliced onto exons 2–12 of *Gnas* (exons 2–13 in human *GNAS*). The *Gnasxl*-specific first exon also contains a second potential open reading frame (ORF) for a protein termed Alex. *Nesp* is expressed exclusively from the maternal allele. The Nesp55 ORF is contained within the second *Nesp*-specific exon. Only a single, uninterrupted *Nesp*-specific exon is found in human. *Exon 1A* (exon A/B in human) and *Nespas* produce non-coding, regulatory RNAs; *Nespas* transcripts exist in multiple spliced and unspliced forms that extend beyond the *Nesp* exons. Tissue-specific splicing onto exon N1 exclusively in neural tissues leads to premature transcription termination and expression of a truncated XLN1 protein (existence of a corresponding $G\alpha_s N1$ protein is uncertain).

being mostly confined to neural and endocrine tissues (Pasolli *et al.* 2000, Pasolli & Huttner 2001, Plagge *et al.* 2004). At embryonic stages, XLα_s is already detectable from mid-gestation onwards in regions of neurogenesis and in early differentiating neurons, mainly in areas of the midbrain, hindbrain and spinal cord, including the sympathetic trunk and ganglia (Pasolli & Huttner 2001). At later embryonic stages expression was also found in the hypothalamus and the pituitary (adenohypophysis and pars intermedia). In the neonatal brain, XLα_s expression is confined to distinct regions of the midbrain and hindbrain, e.g. the centre of the noradrenergic system of the brain (locus coeruleus), laterodorsal tegmental nucleus, motor nuclei that innervate orofacial muscles (hypoglossal, motor-trigeminal and facial nuclei), as well as scattered cells in the medulla oblongata (Plagge *et al.* 2004). Further, sites of expression include the neuroendocrine pituitary (pars anterior and intermedia), the catecholaminergic adrenal medulla and some peripheral tissues, e.g. white adipose tissue (WAT) and BAT, pancreas, heart, kidney and stomach (Plagge *et al.* 2004). There are indications that this expression pattern changes towards adulthood, as no XLα_s was detected in adult adipose tissues, kidney and heart, but expression persists in brain, pancreatic islets, the pituitary and adrenal glands (Pasolli *et al.* 2000, Xie *et al.* 2006).

The *Gnas* locus: alternative promoters, splicing and regulation by genomic imprinting

Although the location of the Gα_s encoding *Gnas* gene on mouse distal chromosome 2/human chromosome 20q13.2–q13.3 and its exon–intron structure had been known for some time (Blatt *et al.* 1988, Kozasa *et al.* 1988, Gejman *et al.* 1991, Levine *et al.* 1991, Rao *et al.* 1991, Peters *et al.* 1994), and despite some early indications for alternative upstream promoters (Ishikawa *et al.* 1990, Swaroop *et al.* 1991), the full complexity of the *Gnas* locus was only discovered through work in a different field, i.e. genomic imprinting. Imprinting affects a small number of genes in the mammalian genome, currently comprising ~90 identified transcription units (see databases: http://igc.otago.ac.nz/home.html and http://www.mgu.har.mrc.ac.uk/research/imprinting/index.html). It describes a phenomenon of gene regulation in mammals, whereby one of the two chromosomal alleles is silenced depending on its parental origin. Thus, an imprinted gene is expressed from either the paternally or the maternally inherited chromosome, and this monoallelic, parent of origin-dependent transcription is achieved through mechanisms of DNA methylation, as well as chromatin modifications (Reik & Walter 2001, Morison *et al.* 2005, Edwards & Ferguson-Smith 2007).

Separate screens for imprinted genes in human and mouse resulted in the identification of the XLα_s-specific first exon of the *Gnas* locus and an additional exon and promoter, which initiates a transcript that also splices onto downstream *Gnas* exons but encodes an unrelated, previously identified protein termed Nesp55 (Fig. 2B; Hayward *et al.* 1998*a*,*b*, Kelsey *et al.* 1999, Peters *et al.* 1999). The *Gnas* locus is now known to comprise a complex arrangement of three protein-coding and two non-coding transcripts regulated by imprinting mechanisms. We will describe the murine locus here, but most features are conserved in humans. As the mechanisms of regulation of the locus by genomic imprinting are currently under much investigation, we will only focus on the main characteristics here, but see Peters *et al.* (2006) for a recent review.

The protein coding transcripts

The three protein transcripts *Gnas*, *Gnasxl* and *Nesp* each initiate at separate promoters/first exons, but share most of the downstream exons (Fig. 2B; Plagge & Kelsey 2006, Weinstein *et al.* 2007). The Gα_s encoding *Gnas* transcript is composed of 12 exons (13 in human, due to an additional intron interrupting exon 9). Most cell types express two variants of the Gα_s protein, a small (45 kDa) and a long 52 (kDa) version, which are functionally equivalent (Graziano *et al.* 1989, Levis & Bourne 1992) and are generated through alternative splicing of the 15 codons comprising exon 3. Both Gα_s versions can vary further by the inclusion of a single serine residue, added through usage of an alternative splice acceptor site at exon 4 (Bray *et al.* 1986, Kozasa *et al.* 1988). The *Gnas* promoter and exon 1, which encodes amino acids 1–45 of Gα_s, do not carry primary marks of genomic imprinting (Liu *et al.* 2000*b*) and in most tissues transcription occurs equally from both alleles. In a subset of tissues or cell types, however, expression is monoallelic and restricted to the maternal allele, e.g. in proximal renal tubules, anterior pituitary, thyroid gland and ovary (Yu *et al.* 1998, Hayward *et al.* 2001, Germain-Lee *et al.* 2002, 2005, Mantovani *et al.* 2002, 2004, Liu *et al.* 2003); this is relevant to human inherited disorders that are associated with hormone resistance symptoms, as discussed below. Imprinting of *Gnas* in adipose tissue is still contentious, as some studies showed predominant maternal allele-specific expression (Yu *et al.* 1998, Williamson *et al.* 2004), while others found no such preference (Mantovani *et al.* 2004, Chen *et al.* 2005, Germain-Lee *et al.* 2005). It remains to be clarified whether these discrepant data reflect the analysis of different developmental stages, implying a change in the imprinting status of the *Gnas* transcript in adipose tissue during the lifetime. In general, tissue-specific imprinting of *Gnas* has been difficult to demonstrate, since a small amount of transcripts derived from the paternal allele is often detected among the majority that stems from the maternally inherited allele. Whether this is due to incomplete silencing of the paternal allele or a mixture of cell types with imprinted and non-imprinted expression in the tissue samples analysed is unresolved.

A second promoter and first exon are located ~30 kb upstream of *Gnas* exon 1 and initiates the *Gnasxl* transcript (Fig. 2B; Hayward *et al.* 1998*a*, Kelsey *et al.* 1999, Peters *et al.* 1999), which is spliced onto exon 2–12 of *Gnas*. This splice form retains the *Gnas* open reading frame (ORF) and translates into the XLα_s protein as a NH_2-terminal variant of

$G\alpha_s$ (Kehlenbach *et al.* 1994). In contrast to *Gnas*, the *Gnasxl* promoter is silenced on the maternal chromosome and activates transcription exclusively from the paternal allele. Apart from the full-length *Gnasxl* transcript, a prominent truncated form, encoding the protein XLN1, is found in neuroendocrine tissues only (brain, pituitary, adrenal medulla; Klemke *et al.* 2000, Plagge *et al.* 2004). This truncation is due to alternative, neural tissue-specific splicing of exon N1, which is located between exons 3 and 4 and contains a termination codon and polyadenylation signal. Originally, exon N1 was described as causing neural-specific truncation of the *Gnas* transcript (Crawford *et al.* 1993) but, in contrast to XLN1 (Klemke *et al.* 2000), it remains uncertain whether a corresponding $G\alpha_s$N1 protein is stably expressed. The neural N1 proteins retain the residues for membrane anchorage and part of the domain interacting with β- and γ-subunits (Klemke *et al.* 2000), but lack the major functional domains that are encoded by the downstream exons as well as further residues for interaction with β- and γ-complexes (Lambright *et al.* 1996). The significance of the exon N1 splice forms, if any, remains to be determined.

The complexity of the *Gnasxl* transcript is further increased through the highly unusual feature in mammalian mRNAs of a second potential ORF, which is shifted by +1 nucleotide, begins a short distance downstream of the $XL\alpha_s$ start codon and terminates at the end of the *Gnasxl*-specific exon (Klemke *et al.* 2001). This ORF encodes a protein termed Alex, which is conserved, but unrelated to G-proteins (Klemke *et al.* 2001, Nekrutenko *et al.* 2005). Although Alex was detected in PC12 cells and human platelets (Klemke *et al.* 2001, Freson *et al.* 2003), its abundance, expression level and significance *in vivo* remain unclarified.

As a third promoter for a protein-coding transcript within the *Gnas* locus, the *Nesp* promoter and first exon are located ~15 kb upstream of the *Gnasxl* exon (Hayward *et al.* 1998*b*, Kelsey *et al.* 1999, Peters *et al.* 1999). Although the single human *NESP*-specific exon is interrupted by a short intron in the mouse genome, the downstream splicing onto exons 2–12 of *Gnas* is conserved and occurs similarly to *Gnasxl* and *Gnas* itself. *Nesp* is imprinted in an opposite way to *Gnasxl* being expressed only from the maternally derived allele (Hayward *et al.* 1998*b*, Kelsey *et al.* 1999, Peters *et al.* 1999). The ORF, which encodes the neuroendocrine secretory protein of M_r 55 000 (Ischia *et al.* 1997), is confined to the *Nesp*-specific exon, and the shared downstream exons function as 3′-untranslated sequence. The Nesp55 protein has similarities with the chromogranin family, is associated with secretory vesicles in neuroendocrine cells and is regarded as a marker for the constitutive secretory pathway (Fischer-Colbrie *et al.* 2002). Little is known about its molecular function, but the protein is processed into peptides to variable extent in different cell types (Lovisetti-Scamihorn *et al.* 1999). In agreement with its predominant expression in the nervous system and endocrine tissues (Bauer *et al.* 1999*a*,*b*), mice deficient for Nesp55 show a behavioural phenotype, specifically an altered response to novel environments (Plagge *et al.* 2005, Isles *et al.* manuscript in preparation) but, in contrast to $G\alpha_s$- and $XL\alpha_s$-deficient mice (see below), they exhibit no major effects on development, growth or metabolism.

Non-coding transcripts and imprinting marks

The complexity of the *Gnas* locus is not limited to the protein-coding transcripts, but is increased by the occurrence of non-coding transcripts and differentially methylated regions of DNA (DMRs). As noted above, we will only briefly describe how these features relate to how imprinting in the locus is controlled (see also Peters *et al.* 2006).

Two untranslated transcripts are produced from separate promoters within the locus (Fig. 2B). The paternal allele-specific exon 1A transcript (exon A/B in human) is initiated ~2·4 kb upstream of *Gnas* exon 1 (Ishikawa *et al.* 1990, Swaroop *et al.* 1991, Liu *et al.* 2000*b*, Peters *et al.* 2006) within a CpG dinucleotide-rich cis-regulatory region that is methylated on the maternal allele (exon 1A DMR). This transcript also splices onto exon 2 of *Gnas*. The second non-coding RNA, *Nespas*, begins ~2·1 kb upstream of the *Gnasxl*-specific exon, but it is transcribed in the opposite direction, i.e. antisense to *Nesp* (Hayward & Bonthron 2000, Wroe *et al.* 2000, Williamson *et al.* 2006), and is transcribed solely from the paternal allele from within a CpG-rich DMR (methylated on the maternal allele; Coombes *et al.* 2003). An increasing evidence points towards a role for such non-coding RNA in the regulation of the imprinted, monoallelic expression of the coding transcripts (Pauler *et al.* 2007).

The DMRs at exon 1A and *Nespas* have been shown to be of central importance for the imprinting of the locus (Williamson *et al.* 2004, 2006, Liu *et al.* 2005). At both sites, differential methylation of the maternal allele is established in oocytes and maintained after fertilisation and into adulthood in all somatic tissues (Liu *et al.* 2000*b*, Coombes *et al.* 2003). Such germline differences in DNA methylation are characteristic of imprinting control regions (ICRs; Spahn & Barlow 2003). A third DMR located at the *Nesp* promoter is unmethylated in oocytes and sperm, but acquires methylation on the paternal allele during embryonic development (Liu *et al.* 2000*b*, Coombes *et al.* 2003).

The roles of the exon 1A and *Nespas* DMRs have been demonstrated through targeted deletion in mice (Williamson *et al.* 2004, 2006, Liu *et al.* 2005). These studies show that the exon 1A region controls the tissue-specific imprinting of *Gnas* without affecting the upstream transcription units (Williamson *et al.* 2004, Liu *et al.* 2005). Deletion of the exon 1A DMR and promoter on the paternal (normally unmethylated) allele leads to upregulation in cis of the usually silenced expression of *Gnas* in imprinted tissues. The exact nature of the silencing mechanism exerted by the paternal exon 1A region on *Gnas* transcription is unknown at present (Peters *et al.* 2006).

Deletion of the *Nespas* promoter, in contrast, affects the imprinting status of all transcripts of the locus (Williamson *et al.* 2006), such that the *Nespas* DMR can be regarded as the principal ICR for the locus. Thus, when *Nespas* transcription

is ablated on the paternal allele, *Nesp* and *Gnas* become derepressed, while *Gnasxl* and the exon 1A transcript are downregulated. Furthermore, the *Nesp* DMR loses and the exon 1A DMR gains methylation on the paternal allele (Williamson *et al.* 2006). The molecular mechanisms through which this ICR controls the imprinted expression of all transcripts of the *Gnas* locus remain to be elucidated.

Physiological functions of the gene products as revealed by mutations in mice and humans

It has been known for some time that inactivating mutations in the human *GNAS* gene are associated with the inherited disorder 'Albright's hereditary osteodystrophy' (AHO)/'pseudohypoparathyroidism' (PHP; Levine *et al.* 1980, 1983*a*, Patten *et al.* 1990, Weinstein *et al.* 1990, Davies & Hughes 1993). Fuller Albright and his colleagues originally described a disorder characterised by hypocalcaemia, hyperphosphataemia and end organ resistance (in proximal renal tubules) to the main plasma Ca^{2+} regulator parathyroid hormone (PTH), and therefore named the disease PHP (Albright *et al.* 1942). As PTH levels are not reduced, but typically elevated, and since *GNAS* is biallelically expressed in the calcium-reabsorbing thick ascending limb of the kidney, hypercalciuria does usually not occur in these patients. They also described other specific somatic and developmental abnormalities in these patients and the disorder is now known to include the following additional symptoms: a round face with a 'short, thickset figure', early closure of the epiphyses with resultant shortening of one or more metacarpals or metatarsals (brachydactyly), s.c. ectopic ossifications, dental hypoplasia, obesity and cognitive abnormalities of varying degrees from learning disabilities to severe retardation (Albright *et al.* 1942, 1952, Weinstein *et al.* 2001, Levine 2002). Albright and colleagues also noticed patients who showed many of the latter physical features, but had normal calcium, phosphate and PTH levels (Albright *et al.* 1952). They termed this combination of symptoms, which was not associated with hormone resistance, 'pseudopseudohypoparathyroidism' (PPHP). Both conditions are also referred to as AHO, and identical mutations in *GNAS* that affect the protein coding sequence can cause AHO with or without hormone resistance. It was Davies & Hughes (1993) who described for the first time the association of the syndromes with the parental origin of the mutation. Thus, paternal inheritance of a *GNAS* exon mutation results in (AHO-)PPHP, while maternal inheritance is associated with additional resistance to PTH (and other hormones, see below; Levine *et al.* 1983*a*) and is now termed 'PHP type Ia' (PHP-Ia; Weinstein *et al.* 2001). Some of the typical features of AHO are shown in Fig. 3A–F and are summarised in Table 1; however, not all features are present in all patients.

The recent analysis of several mouse models with deficiencies of the individual protein products has deepened our understanding of the associated physiological and endocrine functions (Plagge & Kelsey 2006, Weinstein *et al.* 2007). Not surprisingly, homozygous deficiency of $G\alpha_s$ is incompatible with life as embryos die soon after implantation (Yu *et al.* 1998, Chen *et al.* 2005, Germain-Lee *et al.* 2005). Heterozygous mutations of the different proteins of the *Gnas* locus cause distinct dysfunctions (Table 1). In the case of $G\alpha_s$ some aspects of the phenotype vary with the parental origin of the mutation, reflecting its imprinted expression, while other dysfunctions occur after both maternal and paternal transmission, indicating haploinsufficiency of $G\alpha_s$ in some tissues. Heterozygous loss of $G\alpha_s$ in mice recapitulates many aspects of the human disorders, but haploinsufficiency effects seem to be more prevalent in human than in mice. Furthermore, the consequences of loss of $XL\alpha_s$ in mice differ and are in several respects opposite to those of specific loss of $G\alpha_s$, despite their similar capability to activate the cAMP signalling pathway.

Before discussing the physiological and endocrine roles of the different proteins and evaluating the (in some aspects limited) extent of functional conservation between the two species (Table 1), it should be noted that activating or gain of function mutations of *Gnas* have also been identified. These are beyond the scope of this review, but have been summarised elsewhere recently (Hayward *et al.* 2001, Weinstein *et al.* 2006, 2007). Furthermore, a separate human disorder associated with the *GNAS* locus is not due to mutations affecting the protein-coding sequences, but is caused by deregulated imprinting and gene expression control. Originally, it has been characterised by PTH resistance only without clear AHO symptoms and was therefore termed 'PHP type Ib' (PHP-Ib; Bastepe & Jüppner 2005). Our current understanding of PHP-Ib is briefly summarised towards the end of this review.

Post-natal physiological functions

All manipulations in mice that lead to lack of maternal allele-specific $G\alpha_s$ or $XL\alpha_s$ show an impaired neonatal phenotype with reduced survival (Cattanach & Kirk 1985, Yu *et al.* 1998, Cattanach *et al.* 2000, Plagge *et al.* 2004, Chen *et al.* 2005, Germain-Lee *et al.* 2005).

Heterozygous deficiency of $G\alpha_s$ in mice, generated through deletion of *Gnas* exon 1, results in a neonatal phenotype on maternal transmission (Chen *et al.* 2005, Germain-Lee *et al.* 2005). The paternally inherited deletion has few consequences at this developmental stage, although some mortality was observed in an inbred strain background (Germain-Lee *et al.* 2005). For $exon1^{m-/p+}$ mice a survival rate to weaning age of 34–51% was observed, again varying with the genetic background used. Most of the losses occur within 3 days after birth, and may result from a severe s.c. oedema, which has been described in several mouse models lacking maternal allele-specific $G\alpha_s$ protein (Cattanach & Kirk 1985, Yu *et al.* 1998, Cattanach *et al.* 2000, Chen *et al.* 2005). The physiological cause of the oedema, which resolves within a few days after birth, is currently unclear, although a placental dysfunction has been suggested (Chen *et al.* 2005, Weinstein *et al.* 2007). Another consequence of loss of $G\alpha_s$ expression from the maternal allele is the development of

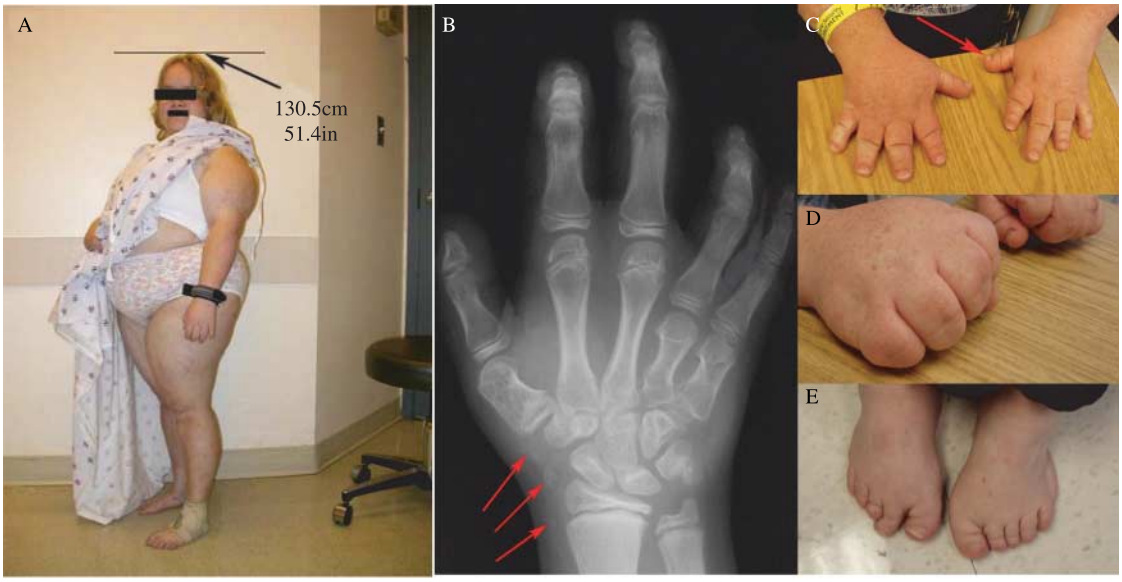
profound obesity in adulthood (discussed in detail below). The increase in adiposity arises already during the post-natal stage, as has been documented in mice with maternally inherited mutations of exons 2 and 6 (Cattanach *et al.* 2000, Yu *et al.* 2000, Plagge & Kelsey 2006). Despite their increased lipid accumulation and adipose tissue mass, these mice remain underweight until after weaning.

Comparatively little information on post-natal symptoms is available from case studies of AHO/PHP-Ia patients who carry mutations in *GNAS* exons on the maternal chromosome. An s.c. oedema has not been documented. However, a few reports describe an early onset of some symptoms characteristic of PHP-Ia at later juvenile or adult stages (see also below; Levine *et al.* 1985, Weisman *et al.* 1985, Yokoro *et al.* 1990, Scott & Hung 1995, Yu *et al.* 1999, Riepe *et al.* 2005, Gelfand *et al.* 2006). From these studies a pattern seems to emerge in which abnormal thyroid function and resistance to thyroid-stimulating hormone (TSH), due to deficient receptor signalling via $G\alpha_s$, are among the first symptoms detectable: typically, TSH levels are elevated in PHP-Ia at birth (Levine *et al.* 1985, Weisman *et al.* 1985, Yokoro *et al.* 1990, Yu *et al.* 1999). The s.c. ossifications can also develop from the first few months onwards, while resistance to PTH, hypocalcaemia and hyperphosphataemia are usually detected only at later stages of infancy or juvenile age (Eddy *et al.* 2000, Riepe *et al.* 2005, Gelfand *et al.* 2006, 2007). Progressive osseous heteroplasia (POH), a more severe form of extraskeletal ossification with invasion into deeper tissues, can also begin early on, and has been described in association with paternally inherited as well as spontaneously occurring *GNAS* mutations (Eddy *et al.* 2000, Shore *et al.* 2002, Faust *et al.* 2003, Gelfand *et al.* 2007). In general, ossification symptoms are a classical AHO feature, as they can occur upon mutations of the maternal or paternal allele.

Loss of paternally expressed $XL\alpha_s$ (through gene targeting of the *Gnasxl*-specific exon) causes lethality in inbred mouse strains, but 15–20% of mutants survive into adulthood if maintained on an outbred genetic background (Cattanach *et al.* 2000, Plagge *et al.* 2004, Xie *et al.* 2006). Deficient pups become distinguishable from wild-type littermates within 1 or 2 days after birth, due to a failure to thrive, characterised by severe growth retardation, poor suckling, hypoglycaemia, hypoinsulinaemia, lack of adipose reserves and inertia (Plagge *et al.* 2004). This phenotype is most likely related to pleiotropic functions of $XL\alpha_s$ in the central nervous system (CNS, e.g. orofacial motornuclei in the context of suckling activity), as well as peripheral tissues that are involved in the maintenance of energy homeostasis (e.g. adipose tissues, pancreas; Plagge *et al.* 2004). Impairment in neonatal feeding, growth and maintenance of energy balance is found not only in mice with a specific mutation of the *Gnasxl* exon but also in other mutants that lack $XL\alpha_s$ (Plagge & Kelsey 2006, Weinstein *et al.* 2007). Thus, mice that carry two copies of the maternally inherited gene locus and no paternal copy (MatDp.dist2) show narrow, flat-sided bodies with reduced adiposity in BAT, hypoactivity, failure to suckle and lethality within a day after birth (Cattanach & Kirk 1985, Williamson *et al.* 1998). Two further mutations, a deletion of exon 2 and a point mutation in exon 6 (termed Oed-Sml), affect both $G\alpha_s$ and $XL\alpha_s$ upon paternal transmission (Yu *et al.* 1998, 2000, Cattanach *et al.* 2000, Skinner *et al.* 2002); however, the phenotypes of $exon2^{m+/p-}$ mice and Sml mice are identical in many respects to *Gnasxl* deficiency (Yu *et al.* 1998, 2000, Cattanach *et al.* 2000, Plagge & Kelsey 2006, Weinstein *et al.* 2007). The similarity of the phenotypes of these latter two mutations to the *Gnasxl* mutation indicates that in mice the loss of $XL\alpha_s$ is dominant over the simultaneous loss of paternal allele-derived $G\alpha_s$. Furthermore, as the paternally inherited exon 6 point mutation does not affect the other two proteins expressed from the *Gnasxl* exon (XLN1 and Alex), this indicates that loss of $XL\alpha_s$ is the main cause for the lack of paternal function phenotypes (Plagge & Kelsey 2006, Weinstein *et al.* 2007).

The post-natal phenotype of $XL\alpha_s$ deficiency improves at around weaning age; no further premature mortality occurs from this stage onwards, although adults remain lean (see below). It is not unlikely that changes in $XL\alpha_s$ expression underlie these phenotype changes, since it has been shown for adipose tissue that *Gnasxl* expression becomes downregulated during the second half of the post-natal period (Xie *et al.* 2006).

It is currently uncertain whether $XL\alpha_s$ has a similar role in human neonatal physiology. The classical descriptions of patients with AHO/PPHP do not include comparable symptoms. As PPHP patients carry paternally inherited mutations in *GNAS*-coding exons, similar to $exon2^{m+/p-}$ and Sml mice, $XL\alpha_s$ function would be expected to be impaired and dominant over loss of paternally expressed $G\alpha_s$. However, these mutations cause the same common AHO features as in maternally inherited PHP-Ia (plus additional hormone resistances). A conclusive human case study, which could distinguish between $XL\alpha_s$ functions and paternal haploinsufficiency of $G\alpha_s$ by analysing paternally inherited *GNAS* exon 1 mutations, has not yet been published (Patten *et al.* 1990, Fischer *et al.* 1998, Aldred & Trembath 2000, Mantovani *et al.* 2000, Long *et al.* 2007). However, other rare genetic anomalies that disrupt the *GNAS* locus and $XL\alpha_s$ expression, e.g. large chromosomal deletions and maternal uniparental disomies (UPD) of chromosome 20q13.2–q13.3, have been associated with neonatal impairments. Patients with maternal UPD20q13.2–q13.3, who lack a corresponding paternal allele and can be compared with MatDp.dist2 mice described above, show pre- and post-natal growth retardation (Chudoba *et al.* 1999, Eggermann *et al.* 2001, Salafsky *et al.* 2001, Velissariou *et al.* 2002). The 20q13.2–q13.3 deletions that include the *GNAS* locus on the paternal allele, also lead to growth retardation, failure to thrive, feeding difficulties requiring artificial feeding, hypotonia and adipose tissue abnormalities (Aldred *et al.* 2002, Genevieve *et al.* 2005), reminiscent of *Gnasxl* knockout mice. Although these cases of chromosomal deletions and UPD20s require careful interpretation, as other potentially contributing genes might also be affected, they nevertheless encourage an investigation of PPHP patients for post-natal symptoms, as far as this is feasible and records are available.



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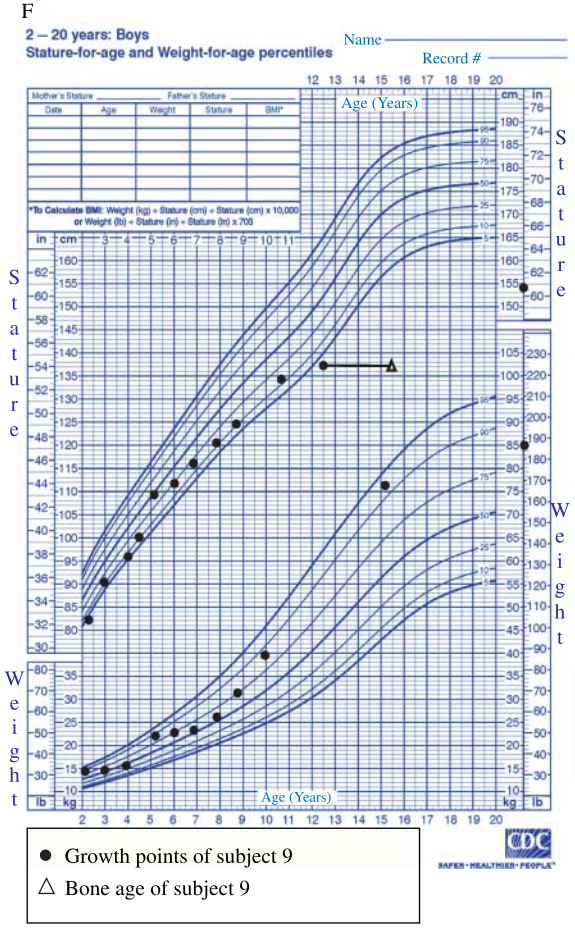

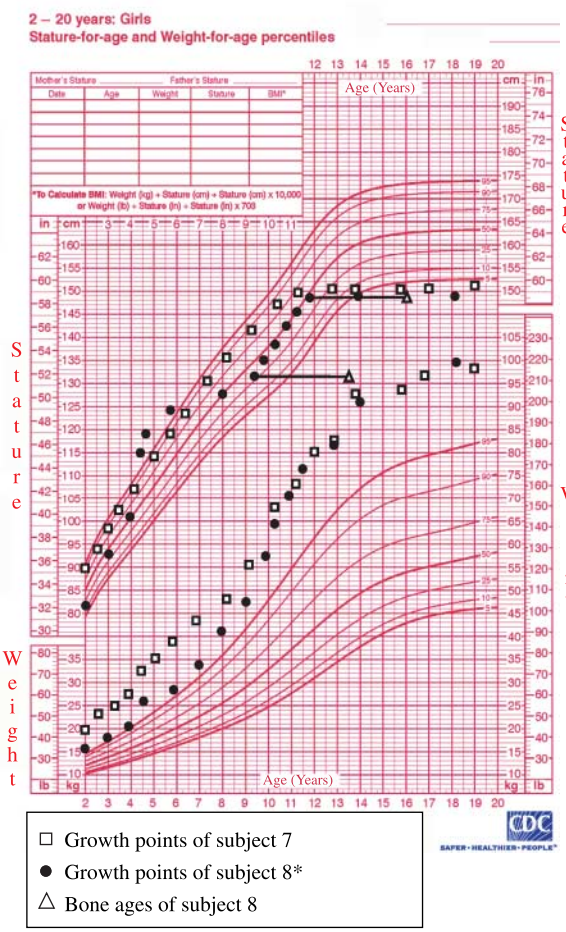

*Subject 8 treated with GH from~9.5–12yrs

No null mutations for the *Gnasxl*-specific exon have been reported in humans, but a polymorphism in the XLα_s domain, which results in varied numbers of a 12 amino acid NH_2-terminal repeat unit, has been associated with symptoms such as growth retardation, unexplained mental retardation and brachydactyly (Freson *et al.* 2001, 2003). Further characterisation of the patients as well as the biochemical functionality of the XLα_s repeat variants is required.

Physiological functions in adulthood

The roles of the proteins of the *Gnas*-locus at adult stages have been characterised in more detail, both in human and mouse (Table 1). The symptoms common to PHP-Ia and PPHP, which occur independently of parental origin and are due to haploinsufficiency of Gα_s in cells with biallelic expression of *GNAS*, as well as the hormone resistances associated with PHP-Ia upon maternal inheritance of mutations, fully develop towards adulthood. With regard to Gα_s, many parallels have now been described between the human diseases and corresponding mouse models, although a role of XLα_s in humans remains uncertain.

Hormone resistances

TSH resistance Mild TSH resistance occurs in most adult patients with PHP-Ia in addition to the usually pronounced PTH resistance described below (Levine *et al.* 1983*a*, Weinstein *et al.* 2001, Levine 2002). A study using thyroid membranes isolated from a patient with PHP-Ia demonstrated that the defect lies in the signal transduction pathway for TSH, consistent with a defect in Gα_s (Mallet *et al.* 1982).

Three studies confirmed with strikingly similar results that *GNAS* is expressed preferentially from the maternal allele in normal human thyroid tissue (mean contribution of the maternal allele: 71·3–75·7%; Germain-Lee *et al.* 2002, Mantovani *et al.* 2002, Liu *et al.* 2003). The fact that the imprinting in the thyroid is partial, e.g. incomplete silencing of the paternal *GNAS* allele, may provide an explanation for the mild TSH resistance and hypothyroidism found in patients with PHP-Ia. Partial imprinting probably accounts for incomplete hormonal resistance in other tissues as well.

Concurrent studies in *Gnas* knockout mice with a targeted disruption of exon 1 revealed Gα_s imprinting in the thyroid, accompanied by TSH resistance and normal to elevated TSH plasma levels in mice inheriting a disrupted maternal allele, but not in mice with a disrupted paternal allele, similar to humans (Yu *et al.* 2000, Chen *et al.* 2005, Germain-Lee *et al.* 2005). Although TSH resistance could contribute to other symptoms observed in AHO/PHP-Ia, e.g. short stature and obesity (see below), it seems unlikely that this would be the sole cause in light of the mild degree of hypothyroidism that occurs, as well as the fact that even when patients are successfully treated throughout their lifetime, they are short as adults and also obese (Long *et al.* 2007). In addition, mice with maternal Gα_s deficiency were obese with normal thyroxine levels, thus implicating other factors in the development of the obesity (Yu *et al.* 2000, Chen *et al.* 2005, Germain-Lee *et al.* 2005).

PTH resistance PTH resistance in PHP-Ia patients typically develops over the first several years of life with an elevated PTH usually preceding the hypocalcaemia and hyperphosphataemia (Werder *et al.* 1978, Barr *et al.* 1994, Yu *et al.* 1999), although there are some patients who do not develop hypocalcaemia until late in adulthood (Hamilton 1980) and others who maintain normal calcium levels throughout their lifespan (Balachandar *et al.* 1975, Drezner & Haussler 1979). Abnormalities in calcium levels most likely result from lack of PTH signalling in the kidney, where it acts on proximal renal tubules as well as distal portions of the nephron. *GNAS* imprinting and preferential expression from the maternal allele in the kidney occur only in proximal renal tubules, but not in the thick ascending limb or in the collecting ducts, as based on PHP-Ia patients (Moses *et al.* 1986, Faull *et al.* 1991) as well as on results in knockout mouse models (Yu *et al.* 1998, Ecelbarger *et al.* 1999, Weinstein *et al.* 2000, Germain-Lee *et al.* 2005). Loss of Gα_s from the maternal allele, therefore, disturbs the PTH-mediated inhibition of phosphate reabsorption and its stimulation of 1,25-dihydroxycholecalciferol (activated Vitamin D3) synthesis in the proximal tubules more than the calcium reabsorption in distal parts of the nephron. This combination of effects leads to an imbalance characterised by reduced excretion of phosphate and reduced 1,25-dihydroxycholecalciferol-mediated uptake of calcium via the intestines, as well as reduced mobilisation of calcium from bone, whereas calcium reabsorption in the distal parts of the kidney remains normal and hypercalciuria is rarely observed in PHP-Ia patients (Weinstein *et al.* 2000). In some

Figure 3 Typical features of AHO. (A) Typical round face and short, obese body habitus (although extreme obesity has been found to be specific for PHP-Ia). (B) X-ray of the hand of an AHO patient showing the striking shortening of the fourth and fifth metacarpals. Arrows are pointing to multiple s.c. ossifications in the hand. (C) Brachydactyly of the hands with marked shortening of the fourth phalanx and metacarpal. (Not the same patient shown in B). The asymmetry in appearance of the hands is common. The arrow points to the very short left thumb referred to as 'potter's thumb' or 'Murder's thumb.' (D) As a result of the brachymetacarpia, the knuckles are absent and are replaced by dimples when the fist is clenched. This is referred to as 'Archibald's sign.' (E) Shortening of the toes is found commonly in AHO. (F) Growth curves of three GH-deficient patients with PHP-Ia (one male (left) and two females (right)) showing the frequent absence of short stature in childhood with resulting short final adult heights. In addition, the pubertal growth spurts are absent. One patient was treated with GH from approximately age 9·5–12 years (referred to as Subject 8) prior to referral. Triangles refer to bone age (no bone age data for Subject 7). Reproduced with permission from Germain-Lee EL, Groman J, Crane JL, Jan de Beur SM & Levine MA 2003 Growth hormone deficiency in pseudohypoparathyroidism type 1a: another manifestation of multihormone resistance. (see comment). *Journal of Clinical Endocrinology and Metabolism* **88** 4059–4069. Copyright 2003, (The Endocrine Society). Signed informed consents were obtained for the patient photographs.

Table 1 Physiological functions affected by mutations at the *GNAS/Gnas* imprinted locus in human and mouse

		Disorder or type of physiological dysfunction		
	Type of mutation	Human	Mouse	**References**
Protein				
$G\alpha_s$ (biallelic expression)	Mouse: homozygous *Gnas* exon 1 deletion or exon 2 disruption	Unknown	Embryonic lethality	Mouse: Yu *et al.* (1998), Chen *et al.* (2005) and Germain-Lee *et al.* (2005)
		(a) Post-natal stage		
$G\alpha_s$ (maternal allele-specific expression)	Human: missense or nonsense mutations in *GNAS* exons 1–13; (point mutations, small deletions, splice site mutations) Mouse: deletion of *Gnas* exon 1, disruption of exon 2, missense point mutation in exon 6, paternal uniparental duplication of distal chr. 2	AHO/PHP-Ia •Mild hypothyroidism, early onset TSH resistance in thyroid cells and elevated TSH levels •Early onset s.c. ossifications •Brachydactyly	49–66% preweaning lethality •S.c. oedema, resolving during first few days •Increased adiposity •Reduced pre-weaning body weight. •Also reported in some mouse models: tremor, imbalance, hyperactivity, square shaped body, microcardia	Human: Levine *et al.* (1985), Weisman *et al.* (1985), Yokoro *et al.* (1990), Scott & Hung (1995), Yu *et al.* (1999), Eddy *et al.* (2000), Faust *et al.* (2003), Chan *et al.* (2004), Riepe *et al.* (2005) and Gelfand *et al.* (2006, 2007) Mouse: Cattanach & Kirk (1985), Williamson *et al.* (1998), Yu *et al.* (1998, 2000), Cattanach *et al.* (2000), Skinner *et al.* (2002), Chen *et al.* (2005) and Germain-Lee *et al.* (2005)
		(b) Adult stage		
		AHO/PHP-Ia •Resistance to TSH in thyroid cells, elevated TSH levels, mild hypothyroidism •Resistance to PTH in proximal renal tubules, elevated PTH levels, hypocalcaemia, hyperphosphataemia •GHRH resistance in pituitary somatotroph cells, GH deficiency variable •Reduced sensitivity to gonadotrophins LH and FSH, hypogonadism •Short stature, brachydactyly •S.c. ossification, progressive osseous heteroplasia (POH)	•Mild and variable TSH resistance and elevated TSH levels •Resistance to PTH in proximal renal tubules, elevated PTH levels, hypocalcaemia, hyperphosphataemia •Reduced fertility •Reduced body length •S.c. ossification	Human: see text; also reviewed in: Aldred & Trembath (2000), Weinstein *et al.* (2001, 2006), Bastepe & Jüppner (2005), Germain-Lee (2006) and Mantovani & Spada (2006) Mouse: Yu *et al.* (1998, 2000, 2001), Cattanach *et al.* (2000), Skinner *et al.* (2002), Chen *et al.* (2005) and Germain-Lee *et al.* (2005)

(continued)

Table 1 *Continued*

	Type of mutation	Disorder or type of physiological dysfunction: Human	Disorder or type of physiological dysfunction: Mouse	References
		• Severe obesity	• Severe obesity, increased body weight, hyperlipidaemia, hyperglycaemia, glucose intolerance, hyperinsulinaemia, insulin resistance, reduced energy expenditure (hypometabolic)	
		• Variable mental retardation and neurological symptoms	• Reduced SNS activity, reduced mothering behaviour towards offspring • Reduced locomotor activity	
	Human: imprinting defects affecting *GNAS* expression; e.g. loss of methylation at exon A/B; *STX16* deletions; *Nesp* deletions	PHP-Ib • Resistance to PTH, elevated PTH levels, hypocalcaemia, hyperphosphataemia • Mild TSH resistance • Brachydactyly, short stature, round face • Obesity • Abnormal ossifications		Human: Liu *et al.* (2003), Bastepe & Jüppner (2005), Linglart *et al.* (2007), Mantovani *et al.* (2007) and de Nanclares *et al.* (2007) see also text)
		(a) Post-natal stage		
$G\alpha_s$ (paternal allele-specific expression)	Human: missense or nonsense mutations in *GNAS* exons 1–13; (point mutations, small deletions, splice site mutations) Mouse: deletion of *Gnas* exon 1	AHO/PPHP • S.c. ossifications • Brachydactyly	• Normal development (but 31–40% lethality on 129/Sv strain background)	Human: Eddy *et al.* (2000), Shore *et al.* (2002), Faust *et al.* (2003), Chan *et al.* (2004), Riepe *et al.* (2005) and Gelfand *et al.* (2006, 2007) Mouse: Chen *et al.* (2005) and Germain-Lee *et al.* (2005)
		(b) Adult stage		
		AHO/PPHP • Short stature, brachydactyly • S.c. ossification, progressive osseous heteroplasia (POH)	• Reduced body length • S.c. ossification	Human: see text; also reviewed in: Aldred & Trembath (2000), Weinstein *et al.* (2001, 2006), Bastepe & Jüppner (2005), Germain-Lee (2006) and Mantovani & Spada (2006) Mouse: Chen *et al.* (2005) and Germain-Lee *et al.* (2005)
		• Mild obesity	• Mild forms of obesity, glucose intolerance, hyperinsulinaemia, insulin resistance	

(continued)

Table 1 *Continued*

	Type of mutation	Disorder or type of physiological dysfunction		References
		Human	Mouse	
		• Variable mental retardation and neurological symptoms		
		(a) Post-natal stage		
XLα_s	Human: chromosomal abnormalities of the 20q13.2–13.3 region, which affect XLα_s among other genes (maternal uniparental disomies, paternally inherited deletions); Repeat length polymorphism in *Gnasxl* exon Mouse: *Gnasxl* exon mutation; paternally inherited *Gnas* exon 2 and exon 6 mutations; maternal duplication of distal chromosome 2 (matDp.dist2)	• Growth retardation • Hypotonia • Feeding difficulties • Adipose tissue abnormalities • Mental retardation (but no *GNASXL* specific null mutations available for confirmation)	• Growth retardation • Hypotonia, hypoactivity • Poor suckling • Lack of lipid reserves in adipose tissue • Hypoglycaemia • Hypoinsulinaemia • ~80% mortality	Human: Chudoba *et al.* (1999), Eggermann *et al.* (2001), Salafsky *et al.* (2001), Aldred *et al.* (2002), Velissariou *et al.* (2002) and Genevieve *et al.* (2005) Mouse: Cattanach & Kirk (1985), Williamson *et al.* (1998), Yu *et al.* (1998, 2000), Cattanach *et al.* (2000), Skinner *et al.* (2002) and Plagge *et al.* (2004)
			(b) Adult stage • Reduced body weight and length • Reduced BAT and WAT mass and lipid content, stimulated lipolysis • Increased food intake • Increased metabolic rate • Hypoglycaemia • Hypoinsulinaemia • Hypolipidaemia • Increased glucose tolerance and glucose uptake in muscle and adipose tissue • Increased insulin sensitivity and signalling • Increased SNS activity	Mouse: Cattanach *et al.* (2000), Yu *et al.* (2000, 2001), Skinner *et al.* (2002), Chen *et al.* (2004) and Xie *et al.* (2006).

cases of PHP-Ia hypercalcitoninaemia has been reported, which seems to be contradictory at first sight to the indication of hypocalcaemia in these patients (Wagar *et al.* 1980, Fujii *et al.* 1984, Kageyama *et al.* 1988, Vlaeminck-Guillem *et al.* 2001, Zwermann *et al.* 2002). However, resistance to calcitonin signalling (via its $G\alpha_s$-coupled receptor) and reduced levels of 1,25-dihydroxycholecalciferol, which normally downregulate calcitonin production, have been implicated in causing this symptom (Vlaeminck-Guillem *et al.* 2001).

The PTH resistance was also apparent in *Gnas* knockout mouse models after maternal inheritance of the mutations. On a normal diet, PTH levels were significantly higher (two- to threefold) in m−/p+ mice when compared with wild-type littermates (Yu *et al.* 1998, Germain-Lee *et al.* 2005). On a high phosphate diet, the PTH levels were increased by approximately sixfold over levels in mice fed a standard diet, and the m−/p+ mice showed again significantly elevated levels (2·9-fold) of PTH compared with wild types. The m+/p− mice had PTH levels that were intermediate, trending approximately twofold higher than in wild types, but lower than in m−/p+ mice (Germain-Lee *et al.* 2005), indicating that a low level of $G\alpha_s$ expression might normally occur from the paternal allele in renal proximal tubules.

Growth hormone-releasing hormone (GHRH) resistance GHRH is a hypothalamic hormone, whose receptor on pituitary somatotroph cells is G_s-coupled, leading to stimulation of GH release. It was demonstrated that $G\alpha_s$ is expressed predominantly from the maternal allele in normal pituitary tissue (Hayward *et al.* 2001), thereby strengthening the hypothesis that subjects with a defective maternal *GNAS* allele could have $G\alpha_s$ deficiency in somatotrophs and a reduced GH response to GHRH. Previous scattered case reports of patients with PHP-Ia indicated a broad range of GH status from deficiency to sufficiency (Urdanivia *et al.* 1975, Wagar *et al.* 1980, Faull *et al.* 1991, Scott & Hung 1995, Marguet *et al.* 1997). A recent systematic analysis confirmed a markedly increased prevalence of GH deficiency in patients with PHP-Ia due to resistance to GHRH, thus expanding the range of multi-hormone resistances in PHP-Ia (Germain-Lee *et al.* 2003, Mantovani *et al.* 2003). The penetrance of GH deficiency is not 100% though, e.g. ∼68% of PHP-Ia patients (Germain-Lee *et al.* 2003, Mantovani *et al.* 2003), which is in agreement with partial imprinting and incomplete silencing of the paternal allele of *GNAS* (Hayward *et al.* 2001) similar to thyroid and ovary tissues. Structural abnormalities in the pituitary or hypothalamus were not detected, but insulin-like growth factor-I (IGF-I) levels in these patients were subnormal and therefore consistent with GH deficiency. The markedly increased prevalence of GH deficiency has now been confirmed in a much larger group of PHP-Ia patients (Germain-Lee unpublished results); these data argue strongly for the evaluation of GH status in all PHP-Ia patients, since it may be a contributing factor to the other symptoms of short stature and obesity (see below).

Luteinising hormone and follicle-stimulating hormone (LH/FSH) resistance Patients with PHP-Ia, especially females, usually have evidence of hypogonadism and incomplete sexual maturation (Namnoum *et al.* 1998). The features are less noticeable in men, being limited to lack of full pubertal development in some (Levine 2000). Amenorrhoea or oligomenorrhoea is common (Wolfsdorf *et al.* 1978, Levine *et al.* 1983*a*, Namnoum *et al.* 1998), but occasionally there are women with normal menstrual cycles and full-term pregnancies (Namnoum *et al.* 1998, Levine 2000). Women show low oestrogen and progesterone levels similar to those in the normal early follicular phase. Elevated LH and FSH levels would be expected in the face of gonadotropin resistance as found in several studies (Wolfsdorf *et al.* 1978, Shapiro *et al.* 1980, Kageyama *et al.* 1988), but this is not a consistent observation (Faull *et al.* 1991, Namnoum *et al.* 1998). It has been proposed that PHP-Ia patients have a partial sensitivity to gonadotropins that is sufficient for normal follicular development, and also have adequate oestrogen production for appropriate negative feedback, but not enough for normal ovulation. Therefore, resistance to gonadotropins in women with PHP-Ia is more subtle than the other hormonal resistances described above (Namnoum *et al.* 1998). This partial gonadotropin resistance is consistent with the majority of *GNAS* transcripts being derived from the maternal allele in normal ovarian granulosa cells, with a small contribution of transcripts from the paternal allele (Mantovani *et al.* 2002).

While it is difficult to assess the true reproductive fitness of PHP-Ia patients (Namnoum *et al.* 1998), studies in *Gnas* exon 1 knockout mice have revealed reduced fertility. Whenever a male or female inherited the disrupted allele from a female (analogous to mother or father having PHP-Ia), the number of progeny born was dramatically decreased (Germain-Lee *et al.* 2005). There was no significant effect on the number of offspring born, however, when either parent had inherited a disrupted paternal allele (analogous to mother or father having PPHP).

Common characteristics of PHP-Ia and PPHP

Short stature and brachydactyly These two somewhat related AHO characteristics are described together in this section, as they are most likely due to common causes. Brachydactyly (brachymetacarpia/brachymetatarsia) are the most reliable signs for diagnosing AHO. The pattern of shortening is usually most notable in the distal phalanx of the thumb and the third through fifth metacarpals (Fig. 3; Graudal *et al.* 1988, Levine 2002). Striking bone age advancement also occurs, as described below.

The short stature in PHP-Ia and PPHP most likely results from a combination of multiple factors including GH deficiency, premature bone fusion and absence of a pubertal growth spurt. Of note is that patients are often not short as children (de Wijn & Steendijk 1982, Germain-Lee *et al.* 2003, Germain-Lee 2006), but the incidence of short stature in

adults with AHO is ~80% (Nagant de Deuxchaisnes & Krane 1978). An extensive search of the literature and of historical controls from patients (Germain-Lee *et al.* 2003 and unpublished) has revealed that the mean height is ~5 ft 0·5 in ±0·7 in (153·4 cm ±1·8 cm) in adult males and 4 ft 8·7 in ±0·7 in (144 cm ±1·8 cm) in females.

During childhood PHP-Ia patients with GH deficiency follow the same pattern as other patients with AHO/PPHP, i.e. they are usually not short at this stage (Fig. 3F). In most GH-deficient PHP-Ia children IGF-I levels were slightly below the normal range, but seemed adequate enough to maintain normal growth velocities. The growth curves of GH-deficient PHP-Ia patients revealed normal stature until approximately early adolescence, at which time there is a cessation in growth and an apparent lack of pubertal growth spurt (Fig. 3F; Germain-Lee *et al.* 2003). This is consistent with a premature epiphyseal closure in bones as an important factor causing short stature and brachydactyly in PHP-Ia and PPHP. Both are also characterised by markedly advanced hand-wrist bone ages, thought to be secondary to premature epiphyseal fusion (Albright *et al.* 1952, Steinbach & Young 1966, Germain-Lee *et al.* 2003, Germain-Lee 2006). Several studies have implicated haploinsufficiency of $G\alpha_s$ as being responsible for the premature epiphyseal fusion (Kobayashi *et al.* 2002, Bastepe *et al.* 2004, Tavella *et al.* 2004, Sakamoto *et al.* 2005*a*,*b*). Biallelic expression of *GNAS* has been demonstrated in human bone (Mantovani *et al.* 2004) and in mouse chondrocytes (Bastepe *et al.* 2004). A 50% reduction of $G\alpha_s$ levels in PHP-Ia and PPHP could impair signalling via the PTH/PTH-related peptide receptor, which mediates chondrocyte proliferation and inhibits differentiation. Bone mineral density does not seem to be affected (Long *et al.* 2006).

Although GH deficiency cannot fully explain short stature, as both PHP-Ia and PPHP patients have reduced heights, it seems to be playing a supplementary role to that of premature epiphyseal fusion. In support of this notion, adults with PHP-Ia and GH deficiency have a lower height SDS than GH-sufficient PHP-Ia patients (Germain-Lee *et al.* 2003). Studies are currently underway to evaluate whether recombinant GH treatment in GH-deficient PHP-Ia children can increase growth velocity and final adult height (Germain-Lee 2006 and unpublished results). GH treatment could potentially augment linear growth and permit an increased growth velocity prior to the premature fusion of the epiphyses not only in GH-deficient PHP-Ia children, but also in GH-sufficient PHP-Ia and PPHP cases. Also, further comparative investigation of adult patients with PHP-Ia and PPHP is required, to examine the GH status and its influence on short stature in AHO (Germain-Lee *et al.* unpublished).

The symptom of short stature is reproduced in *Gnas* knockout mice, as body length of heterozygotes with either a maternally (m−/p+) or a paternally (m+/p−) inherited $G\alpha_s$ mutation is significantly reduced (Yu *et al.* 2000, Germain-Lee *et al.* 2005). Of note is that the m−/p+ females are significantly shorter than their m+/p− counterparts (Germain-Lee *et al.* 2005), which raises the possibility that patients with PHP-Ia may be shorter than PPHP patients, due to their additional hormone resistance.

Several further studies using *Gnas* mouse models have provided evidence that $G\alpha_s$ is important for the control of both the chondrocyte and osteoblast differentiation. In one study, chimeric mice consisting of wild-type and $G\alpha_s$-deficient cells were generated (Bastepe *et al.* 2004). Analysis of the growth plates of chimeric bones revealed that the $G\alpha_s$-null chondrocytes undergo premature hypertrophic differentiation. This was also detected, although to a lesser extent, in chimaeras with heterozygous mutations (Bastepe *et al.* 2004), mimicking the $G\alpha_s$ haploinsufficiency of AHO patients. In a second mouse model, a chondrocyte-specific $G\alpha_s$ knockout, similar premature differentiation of chondrocytes, shortened growth plates, markedly shortened limbs and ectopic cartilage formation were described (Sakamoto *et al.* 2005*a*). In a third study, an osteoblast-specific $G\alpha_s$ knockout, Sakamoto *et al.* (2005*b*) described shortened long bones, reduced trabecular and thickened cortical bone and an overall reduced bone turnover. In contrast to the chimaera study, however, heterozygotes with 50% reduced levels of $G\alpha_s$ specifically in chondrocytes or osteoblasts did not show any phenotypic changes. Heterozygous mice with a general *Gnas* deletion were also reported to be normal with regards to bone length, histomorphology and mineral density (bone volume, osteoblast surface, trabecular thickness, trabecular separation, trabecular number, mineralizing surface and mineral apposition rate; Germain-Lee *et al.* 2005).

In summary, although $G\alpha_s$ haploinsufficiency causes short adult height and brachydactyly in humans, most likely via ineffective PTH/PTH-related peptide receptor signal transduction resulting in accelerated differentiation of chondrocytes and osteoblasts and premature fusion of the growth plates, clear changes in bone morphology of mice are only observed upon complete loss of $G\alpha_s$ in relevant cells.

S.c. ossifications S.c. heterotopic ossifications, also known as osteoma cutis, develop in patients with both PHP-Ia and PPHP. AHO is the only monogenic condition, in which de novo ossifications form subcutaneously and remain limited to the skin, causing pain and morbidity for the patients and requiring recurrent surgeries. The aetiology of the ossifications is as yet unknown and is unrelated to abnormalities in serum calcium or phosphorus levels. They can occur spontaneously or in response to minor trauma and are sometimes the presenting sign of AHO (Izraeli *et al.* 1992, Prendiville *et al.* 1992). Patients with *GNAS* mutations can also develop POH, a more limited disorder, in which severe heterotopic ossifications invade from s.c. tissue into deep connective tissue and skeletal muscle (Kaplan & Shore 2000, Shore *et al.* 2002, Gelfand *et al.* 2007).

Extensive s.c. heterotopic ossifications were found recently in the *Gnas* exon 1 knockout mouse model of Germain-Lee *et al.* (Huso *et al.* 2007). There are no s.c. ossifications in 3-month-old mice as reported previously (Germain-Lee *et al.* 2005); however, because of the increased frequency and size of

s.c. ossifications in ageing AHO patients (Germain-Lee unpublished), 12-month-old heterozygous mutants were analysed and revealed extensive heterotopic s.c. bone formation in the dermis (Huso *et al.* 2007). Mineral deposits in the areas surrounding hair follicles were detected, and many of these areas contained bone marrow elements, consistent with true s.c. bone formation, which was confirmed by X-ray and computed tomography imaging. There were no differences in the frequency or histology of the s.c. ossifications in mice with either a maternally or paternally inherited mutation, which is analogous to its occurrence in AHO (PHP-Ia and PPHP) patients and consistent with haploinsufficiency/lack of imprinting of $G\alpha_s$ in the relevant cell types (Levine *et al.* 1983*b*, Mantovani *et al.* 2004).

Cognitive and other CNS abnormalities AHO is often, but not always, accompanied by cognitive deficits ranging from learning disabilities to severe retardation (Marguet *et al.* 1997, Rutter & Smith 1998, Levine *et al.* 2000, 2002, Weinstein *et al.* 2001). Reductions in $G\alpha_s$ levels have been associated with cognitive deficiency (Farfel & Friedman 1986). Patients with medically well-controlled hypocalcaemia and hypothyroidism still present with cognitive deficits, thus excluding these symptoms as potential causes for the neurological findings. Patients with PHP-Ia frequently have seizures, and these may occur before hypocalcaemia is recognised (Bonadio 1989, Faig *et al.* 1992). Basal ganglia calcifications can be extensive in PHP-Ia, as they are in regular hypoparathyroidism, and can sometimes lead to movement disorders (Blin *et al.* 1991, Dure & Mussell 1998). Abnormalities in olfaction and hearing have also been reported in PHP-Ia and are not present in PPHP (Henkin 1968, Weinstock *et al.* 1986, Koch *et al.* 1990, Doty *et al.* 1997), suggesting the involvement of *GNAS* imprinting in the CNS. In addition, abnormalities in taste sensation have been identified in an early study of PHP (Henkin 1968). In most of the above studies it has not been determined conclusively, however, whether differences occur between PHP-Ia and PPHP, i.e. whether these CNS-related abnormalities are related to imprinting of *GNAS* or $G\alpha_s$ haploinsufficiency.

Mouse models of $G\alpha_s$ deficiency have provided some evidence for neural functions, although a detailed characterisation is still required (Yu *et al.* 1998, Chen *et al.* 2005). The key question of whether *Gnas* is imprinted and monoallelically expressed in subregions of the mouse brain remains unclarified for the time being. A first indication that this might be the case was reported in *Gnas* exon1 knockout mice (Germain-Lee *et al.* 2005). Females with a maternally inherited mutation (m−/p+ mothers) neglected their young, resulting in a very high (~80%) mortality among their pups before weaning. In contrast, females with a paternally inherited mutation (m+/p− females) showed normal mothering behaviour, leading to much less mortality among their offspring (~27%; Germain-Lee *et al.* 2005). The poor mothering skills of the m−/p+ females may be reflective of cognitive/sensory defects or hormonal dysfunctions involving the hypothalamus. The behavioural differences between m−/p+ and m+/p− mothers argue against simple haploinsufficiency and in favour of a predominant maternal-allele specific expression of *Gnas* in some CNS regions.

A role of the maternal allele-derived Nesp55 protein in neural symptoms of AHO/PHP-Ia can be excluded, as mutations in exons 2–13, which often occur in these patients, would only affect the 3′-untranslated sequence of the *Nesp* transcript without impacting on its coding region. Nevertheless, a mouse knockout of Nesp55 showed a behavioural phenotype, as noted above (Plagge *et al.* 2005).

Metabolic deregulation Obesity is commonly found in AHO subjects and altered metabolic phenotypes are amongst the most interesting effects in *Gnas* knockout mice. The original knockout in mice revealed an intriguing difference in metabolic phenotype amongst adult mice heterozygous for a disruption of exon 2, depending on parental inheritance. Thus, $exon2^{m-/p+}$ mice were described as showing accelerated weight gain from around weaning, with increased weights of gonadal white adipose tissue (WAT) and interscapular BAT, whereas $exon2^{m+/p-}$ mice remained underweight with reduced WAT and BAT weights (Yu *et al.* 2000). Further examination revealed that $exon2^{m-/p+}$ mice did not, paradoxically, have increased food intake, but reduced ambulatory activity and resting metabolic rate, whereas $exon2^{m+/p-}$ mice had increased activity and metabolic rate, and a tendency towards hyperphagia.

With more recent, transcript-specific knockouts, the basis for these opposing phenotypes has become clearer. The lean, hypermetabolic phenotype can be attributed to loss of paternally expressed $XL\alpha_s$ (or other translation products of the *Gnasxl* transcript), as it is also present in $Gnasxl^{m+/p-}$ mice, but not in $Gnas\ exon1^{m+/p-}$ mice, which are deficient only for paternally expressed $G\alpha_s$ (Chen *et al.* 2005, Xie *et al.* 2006). And the obese, hypometabolic phenotype can be put down to loss of $G\alpha_s$ from the maternal allele, as an essentially similar phenotype occurs in $Gnas\ exon1^{m-/p+}$ (Chen *et al.* 2005). Interestingly, mice heterozygous for the exon 1 disruption on the paternal allele ($Gnas\ exon1^{m+/p-}$) have a far milder obesity, without significant effects on metabolic rate.

These observations prompt two conclusions. First, mild obesity reflects haploinsufficiency for $G\alpha_s$, whilst severe obesity reflects the additional and more profound loss of $G\alpha_s$ function in specific sites caused by its imprinted expression. This leads to the conclusion that $G\alpha_s$ expression is imprinted in hypothalamic or hindbrain nuclei regulating metabolic rate; imprinted expression of $G\alpha_s$ in adipose tissues (see below) appears not to be a factor (Yu *et al.* 2000). Second, from a comparison of the $Gnas\ exon1^{m+/p-}$ and $Gnasxl^{m+/p-}$ phenotypes, the physiological effects of $XL\alpha_s$ predominate over those of $G\alpha_s$ expressed from the paternal allele.

The physiological basis of the lean/obese phenotypes is not entirely clear and is likely to be complex, but a primary defect in adipose tissues appears to be ruled out. Maternal monoallelic expression of $G\alpha_s$ in adipose tissues could give rise to resistance to the lipolytic activity of sympathetic innervation or circulating catecholamines, however, as discussed earlier, there is

disagreement over whether *Gnas* is imprinted in adipose tissues. In addition, *Gnasxl* is abundantly expressed in adipose tissues in neonatal mice, but is strongly downregulated around weaning (Plagge *et al*. 2004, Xie *et al*. 2006), implying that the enhanced metabolic rate in adults is not caused by increased sensitivity intrinsic to the tissue. An explicit test of the sensitivity of adipose tissues in the mutants is the metabolic response to an agonist of the adipose-specific β3-adrenoreceptor: such studies have revealed essentially normal responsiveness in *Gnas exon2*$^{m-/p+}$ and *Gnasxl*$^{m+/p-}$ mice (Yu *et al*. 2000, Xie *et al*. 2006). These results rather suggest a differential effect of maternal $G\alpha_s$ and $XL\alpha_s$ on sympathetic activity towards adipose tissues, and support for this proposition comes from the finding of reduced urinary excretion of noradrenalin in *exon2*$^{m-/p+}$ and increased excretion in *Gnasxl*$^{m+/p-}$ mice (Yu *et al*. 2000, Xie *et al*. 2006).

In keeping with their lean phenotype, *Gnasxl*$^{m+/p-}$ and *Gnas exon2*$^{m+/p-}$ mice have strongly increased insulin sensitivity, as evidenced by improved glucose tolerance and an exaggerated hypoglycaemic response to injected insulin. Euglycaemic–hyperinsulinaemic clamp studies demonstrated increased glucose uptake into skeletal muscle, WAT and BAT. The mutants also respond to an oral triglyceride load with an increased clearance rate (Yu *et al*. 2001, Chen *et al*. 2004, Xie *et al*. 2006). Gene expression analysis in *Gnasxl*$^{m+/p-}$ mice reveals a profile in adipose tissues consistent with increased sympathetic activation and induction of genes associated with triglyceride uptake and hydrolysis, lipid oxidation and the adipogenic pathway (Xie *et al*. 2006). In contrast, the paucity of expression changes in skeletal muscle of genes associated with energy metabolism suggests that increased energy dissipation in adipose tissues is the principal cause of the elevated metabolic rate of these mutants.

Glucose homeostasis in mice lacking maternal $G\alpha_s$ is somewhat more confusing: there are differences in phenotypes of *Gnas* exon1 and *Gnas exon2*$^{m-/p+}$ mice, which are unexpected, as both are deficient presumably only in $G\alpha_s$ produced from the maternal allele. *Gnas exon1*$^{m-/p+}$ mice have insulin resistance and associated serum abnormalities classically associated with obesity, whereas obese *Gnas exon2*$^{m-/p+}$ mice are described as having increased insulin sensitivity, coupled to increased insulin-stimulated glucose uptake into skeletal muscle (Yu *et al*. 2001, Chen *et al*. 2005). Part of the reason for these and other discrepancies between the reports on the various *Gnas* mutants could be put down to variation in experimental design and environment (i.e. age or gender of experimental groups, husbandry) or genetic background. Studies have used outbred CD1 mice or combinations of inbred strains, which has been done because of the poor viability of the mutants on pure backgrounds. It is also possible that the genetic manipulations themselves may have had unforeseen consequences on the expression of other, relevant transcripts in the locus that could modify sensitive phenotypes such as metabolism.

Until recently, there was no recognition that imprinting of *GNAS* was relevant to the presentation of obesity in AHO. Obesity is described in both PHP-Ia and PPHP, and irrespective of whether inactivating mutations involve exon 1 (specific for $G\alpha_s$) or the downstream exons common to all protein-coding transcripts; there was certainly no metabolic phenotype reminiscent of mice lacking $XL\alpha_s$. The demonstration of strikingly opposite effects on metabolism in knockout mice has stimulated a re-evaluation of the clinical data and one recent study has concluded that severe obesity is characteristic of PHP-Ia specifically and not PPHP with the mean BMI *z*-score (±S.E.M.) in PHP-Ia versus PPHP being 2·31 (±0·18) and 0·65 (±0·31) respectively (Long *et al*. 2007). This finding is consistent with $G\alpha_s$ imprinting in a pathway leading to obesity in humans as well as in mice.

PHP-Ib, a disorder due to deregulated imprinting of *GNAS* PHP-Ib was initially thought to be a distinct disease entity, because it was presented with isolated PTH resistance without the other endocrine anomalies commonly associated with PHP-Ia or the clinical signs typical of AHO. However, mapping studies in four PHP-Ib kindreds located the disease locus in the 20q region containing *GNAS*, and also found maternal transmission of disease-associated haplotypes consistent with the presumed imprinting of *GNAS* (Jüppner *et al*. 1998). Although a structural defect in $G\alpha_s$ that selectively affects coupling with the PTH/PTHrP-receptor has been found in one PHP-Ib family (Wu *et al*. 2001), the great majority of cases appear to arise from defects in *GNAS* imprinting, and recent clinical investigations have in fact found mild TSH resistance and even AHO-like symptoms in PHP-Ib patients (Liu *et al*. 2003, Mantovani *et al*. 2007, de Nanclares *et al*. 2007). The most consistent molecular finding in PHP-Ib is loss of methylation of the exon A/B DMR, which has been detected in the majority of familial cases (Liu *et al*. 2000*a*, Bastepe *et al*. 2001, Linglart *et al*. 2007). Studies in mice have shown that the equivalent DMR is required for the tissue-specific imprinting of *Gnas* (Williamson *et al*. 2004, Liu *et al*. 2005). Although the mechanism of action of the exon A/B DMR is unclear, loss of methylation is predicted to cause silencing of the *GNAS* promoter on the maternal allele specifically in those tissues in which expression is normally monoallelic, thereby resulting in PTH resistance, without the accompanying symptoms of AHO (Jüppner *et al*. 2006). One of the original reports was able to map the genetic defect causing the methylation loss >56 kb upstream of the DMR (Bastepe *et al*. 2001), indicating the action of a long-range, cis-acting element. Subsequently, a recurrent 3-kb microdeletion in the neighbouring syntaxin-16 (*STX16*) gene 220 kb upstream of the DMR was identified in PHP-Ib families (Bastepe *et al*. 2003), and has now been documented in over 20 unrelated kindreds (Linglart *et al*. 2007, Mantovani *et al*. 2007). Identification of an overlapping deletion has refined the critical region to 1286 bp containing exon 4 of *STX16* (Linglart *et al*. 2005). *STX16* expression appears not to be imprinted and the mechanism by which these microdeletions result in loss of exon A/B methylation is obscure, particularly as mice engineered to carry a deletion of *Stx16* exons 4–6 do not have equivalent methylation

abnormalities or develop a PHP-Ib-like phenotype (Fröhlich *et al.* 2007). Whilst in most PHP-Ib cases methylation loss is limited to exon A/B, in others there are additional methylation changes across the *GNAS* locus, and these do not have *STX16* deletions (Bastepe *et al.* 2001, 2003, Linglart *et al.* 2007). Instead, two families with loss of methylation of the exon A/B, *GNASXL* and *NESPAS* DMRs have been found to have deletions and/or rearrangements spanning the *NESP* exon (Bastepe *et al.* 2005). Again, the mechanism by which these deletions result in failure to establish or maintain methylation of the maternal allele is currently unclear. In contrast to these familial forms, most PHP-Ib cases with more extensive methylation defects present as sporadics with no evidence of *STX16* or *NESP* deletions. In some such cases, unaffected sibs have the same maternal 20q13 haplotype, suggesting the presence of a newly acquired mutation in cis or that the defect is not linked to the 20q13 region (Linglart *et al.* 2007). It is interesting to note that a 'maternal hypomethylation syndrome' has been described in which affected individuals have loss of methylation at more than one maternal DMR, so that some sporadic PHP-Ib cases may be a manifestation of a more global imprinting defect (Mackay *et al.* 2006). An intriguing difference between the various forms of PHP-Ib is that sporadics appear to be more severely affected, while as many as 40% of individuals identified with maternally inherited *STX16* deletions are asymptomatic (Linglart *et al.* 2007). It is not possible at present to exclude ascertainment bias as the basis for this observation, but it might relate to different molecular events in the establishment of the abnormal methylation patterns or how they impact on the regulation of *GNAS* imprinting.

Concluding remarks

Since the discovery of the complexity of the *Gnas* locus and its regulation by genomic imprinting, a number of different mouse models with targeted mutations have greatly contributed to our understanding of the physiological functions of the different protein products. Many parallels between phenotypes in mice and human disease symptoms in AHO/PPHP and AHO/PHP-Ia have become apparent (Table 1), although some differences are unresolved and might be confirmed as species-specific functions. A role of XLα_s in humans remains uncertain. Furthermore, the explanation for the opposite metabolic phenotypes in mice with deficiency of maternally expressed Gα_s and paternally expressed XLα_s respectively which is likely due to their distinct roles in the CNS regulation of homeostasis, constitutes a major task. A detailed description of the mechanisms of genomic imprinting and regulation of monoallelic expression of this locus are beyond the scope of the review, but progress in this field will be exciting and relevant for the human disorder PHP-Ib, since it is associated with defects in the imprinting mechanisms of *GNAS*.

Acknowledgements

Work in AP's group is funded by The Royal Society and the Medical Research Council of the UK. Work in GK's group is funded by the UK Biotechnology and Biological Sciences Research Council, Medical Research Council and the European Union. Work in ELG-L's group is funded by the US. Food and Drug Administration Orphan Products Development Grant R01 FD-R-002568, Thrasher Research Foundation Grant 02818-8, the National Institutes of Health/National Center for Research Resources Grant M01RR00052 (to Johns Hopkins University School of Medicine General Clinical Research Center), and The Bosworth Family and Friedman Family Funds. Signed informed consents were obtained for the patient photographs which appear in this publication. All human subjects research referenced as 'Germain-Lee, unpublished' was approved by the Internal Review Board of the Joint Committee on Clinical Investigation of the Johns Hopkins University School of Medicine, and informed consent was obtained from all subjects, or parent of each subject, before participation. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received in final form 3 December 2007
Accepted 5 December 2007
Made available online as an Accepted Preprint
5 December 2007