The **GNAS Locus: Quintessential Complex Gene Encoding Gsa, XLαs, and other Imprinted Transcripts**

Murat Bastepe*

**Endocrine Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA**

**Abstract:** The currently estimated number of genes in the human genome is much smaller than previously predicted. As an explanation for this disparity, most individual genes have multiple transcriptional units that represent a variety of biologically important gene products. GNAS exemplifies a gene of such complexity. One of its products is the α-subunit of the stimulatory heterotrimeric G protein (Gsa), a ubiquitous signaling protein essential for numerous different cellular responses. Loss-of-function and gain-of-function mutations within Gsa-coding GNAS exons are found in various human disorders, including Albright’s hereditary osteodystrophy, pseudohypoparathyroidism, fibrous dysplasia of bone, and some tumors of different origin. While Gsa expression in most tissues is biallelic, paternal Gsa expression is silenced in a small number of tissues, playing an important role in the development of phenotypes associated with GNAS mutations. Additional products derived exclusively from the paternal GNAS allele include XLαs, a protein partially identical to Gsa, and two non-coding RNA molecules, the A/B transcript and the antisense transcript. The maternal GNAS allele leads to NESP55, a chromogranin-like neuroendocrine secretory protein. In vivo animal models have demonstrated the importance of each of the exclusively imprinted GNAS products in normal mammalian physiology. However, although one or more of these products are also disrupted by most naturally occurring GNAS mutations, their roles in disease pathogenesis remain unknown. To further our understanding of the significance of this gene in physiology and pathophysiology, it will be important to elucidate the cellular roles and the mechanisms regulating the expression of each GNAS product.

Received on: August 19, 2007 - Revised on: September 22, 2007 - Accepted on: September 28, 2007

**INTRODUCTION**

The first draft of the human genome DNA sequence was reported in 2001 independently by the Human Genome Project and Celera Genomics [1, 2]. Since this important landmark in medical research, the entire research community has had access to a wealth of information that would otherwise have taken many years to gather, resulting in a tremendous acceleration of virtually all research studies that involve human biology or disease. With updates of the project, the sequence is nearly complete [3, 4]. It is interesting, however, that the number of genes in the human genome appears to be much smaller than initially estimated. Although the exact number of genes in the genome still remains unknown, more recent estimate for the number of protein-coding genes is between 20,000 and 25,000 [5], which markedly differs from the previously predicted number of approximately 100,000 [6-9]. The currently estimated gene number, of course, appears quite small, considering the complexity of our species. However, the difference between the older and most recent predictions of the gene number can be accounted for by the presence of multiple transcriptional units associated with many individual genes. In fact, the total number of proteins encoded by the human genome may be similar to, if not higher than, the past estimates of the gene number. This review focuses on the GNAS locus, which exemplifies a complex gene with multiple gene products derived through alternative promoter use and alternative pre-mRNA splicing.

**THE COMPLEX GNAS LOCUS AND Gsa**

The human GNAS locus maps to the telomeric end of the long arm of chromosome 20 (20q13.2-20q13.3) [10-12], while its mouse ortholog is located in distal chromosome 2 [13, 14]. GNAS in humans and mice appear structurally and functionally similar to one and another. This locus has multiple promoters and differentially methylated regions (DMR) and gives rise to non-coding RNA molecules and transcripts that encode functional proteins (Fig. 1). Furthermore, nearly all GNAS products show parent-of-origin specific expression. Among the multiple products derived from GNAS, the best characterized protein is the α-subunit of the heterotrimeric stimulatory G protein (Gsa), which has at least five variants that result from alternative pre-mRNA splicing. Derived from distinct promoters are at least four different additional GNAS products, including the neuroendocrine secretory protein 55 (NESP55), the extra-large variant of Gsa (XLαs), the A/B transcript, and the GNAS antisense transcript. Most of these additional transcripts also undergo alternative splicing and, thus, have different variants.

Gsa is a ubiquitous protein whose activity is essential for the cellular actions of many neurotransmitters, autocrine/paracrine factors, and hormones. As in α-subunits of other heterotrimeric G proteins, activation of Gsa by an agonist-occupied cell surface receptor results in a GDP-GTP exchange on Gsa, causing dissociation of the latter from Gβγ subunits and, thereby, allowing both Gsa and Gβγ to stimulate their respective effectors. GTP-bound, free Gsa can directly activate several different effectors, including Src tyrosine kinase [15] and certain Ca-channels [16, 17]. However,
by far the most ubiquitous and the most extensively investigated effector molecule stimulated by Gsα is adenyl cyclase, an integral membrane protein that catalyzes the synthesis of the ubiquitous second messenger cyclic AMP (cAMP), thereby triggering an intracellular signaling cascade that brings about an agonist- and cell-specific response. The activation of adenylyl cyclase and other effectors by Gsα is tightly regulated. The intrinsic GTP hydrolase (GTPase) activity of Gsa reverts the GTP-bound Gsα to its GDP-bound state and, thereby, results in the re-assembly of the G protein heterotrimer, which can no longer mediate effector stimulation. Mutagenesis experiments often lead to alteration in activity and/or subcellular distribution, indicating that changes are non-tolerable at many of the amino acid positions. Particularly, Arg201 and Gln227 are critical, since modifications of these residues, such as ADP-ribosylation of Arg201 that can be induced by cholera toxin or mutations at Arg201 that can be induced by cholera toxin or mutations at Arg201 or Arg227, respectively, lose GTPase activity and, thereby, results in the re-assembly of the G protein heterotrimer, which can no longer mediate effector stimulation.

Fig. (1). Multiple imprinted sense and antisense transcripts from the complex GNAS locus. Exons 1-13 encode Gsa, which is biallelic in most tissues; however, paternal Gsa allele is silenced in a small number of tissues, including the renal proximal tubule, thyroid, and pituitary. From differentially methylated promoters arise several other transcripts, including the maternally expressed NESP55 and the paternally expressed XLs. Both of these transcripts use individual first exons that splice onto exons 2-13. Nearly all tissues express both of these Gsa variants, which are typically detected as 52- and 45-kDa protein bands on Western blots. In addition, each Gsa form either includes or excludes a CAG tri-nucleotide (encoding serine) at the start of exon 4. Some investigations have revealed small, but potentially important, differences between the activities of Gsa-L and Gsa-S. For example, Gsa-L has been demonstrated to have a greater ability to transmit receptor signaling than Gsa-S when partially purified proteins from rabbit liver were examined [27], although the opposite was suggested in an assay system using cultured pancreatic islet cells [28]. Moreover, Gsa-L appears to release GDP ~2-fold faster than Gsa-S [29], and consistent with that finding, study of fusion proteins involving the β2-adrenergic receptor and either Gsa-L or Gsa-S has shown higher constitutive activity of the receptor when it is associated with Gsa-L [30]. In addition, differences in the subcellular trafficking of these two variants have been reported in response to activation by agonist, forskolin (a direct activator of adenylyl cyclase), or GTPγS (a stable GTP analog) [31-33]. Currently, it remains unclear whether these differences translate into biologically significant effects, such as divergence in the variety of effectors and/or the efficiency of effector activation. An important recent finding regarding the long and short Gsa forms is that, for the first time, an inactivating mutation in exon 3 has been identified in a patient with pseudo-hypoparathyroidism type-Ia [34], a disorder known to be caused by inactivating mutations in Gsa-coding GNAS exons (see below). The patient...
with the exon 3 mutation has an apparently mild form of this disorder, consistent with the disruption of only one of the two main Gsa variants, i.e. Gsa-L [34]. It is conceivable that, depending on the effector selectivity and relative expression levels of Gsa-L and Gsa-S in different tissues, this mutation impairs agonist responses in an effector- and tissue-specific manner. This possibility remains unexplored.

Alternative pre-mRNA splicing leads to another Gsa variant, termed Gsa-N1 (Fig. 2). Identified first in the brain, Gsa-N1 is truncated in the C-terminus due to splicing of exon 3 (or exon 2 in the case of Gsa-S) onto a distinct exon containing an in-frame termination codon [35]. Exon N1 is located between exons 3 and 4. Gsa-N1 lacks most functional domains of Gsa, and therefore, it is probably unable to function in a way similar to the latter. Its cellular role and its biological significance remain unknown.

Alternative pre-mRNA splicing leads to another Gsa variant, termed Gsa-N1 (Fig. 2). Identified first in the brain, Gsa-N1 is truncated in the C-terminus due to splicing of exon 3 (or exon 2 in the case of Gsa-S) onto a distinct exon containing an in-frame termination codon [35]. Exon N1 is located between exons 3 and 4. Gsa-N1 lacks most functional domains of Gsa, and therefore, it is probably unable to function in a way similar to the latter. Its cellular role and its biological significance remain unknown.

Fig. (2). Splice variants of Gsa. Alternative splicing of exon 3 leads to Gsa-L and Gsa-S, each of which has subvariants due to alternative splicing of a serine codon at the start of exon 4. Gsa-N1 is formed through the use of an exon that comprises an in-frame termination codon and is located between exons 3 and 4. Boxes and connecting lines depict exons and introns, respectively. The alternatively spliced serine codon is depicted as a circle. Splicing patterns are indicated by broken lines. Filled boxes indicate 5’ and 3’ untranslated regions.

TISSUE-SPECIFIC Gsa IMPRINTING

Several studies have demonstrated that the expression of Gsa in most fetal and adult tissues is biallelic, consistent with the absence of differential methylation at its promoter [36-39]. However, in some human and mouse tissues, Gsa expression is predominantly maternal, i.e. paternal Gsa expression is silenced. For example, in the renal cortex, mice with paternal disruption of Gnas exon 2 exhibit Gsa mRNA and protein levels that are similar to those seen in wild-type littermates, whereas mice with maternal disruption of Gnas exon 2 are nearly devoid of Gsa mRNA and protein [40], indicating that renal cortical Gsa expression is derived mostly from the maternal allele. This monoallelic, parent-of-origin specific Gsa expression occurs in a tissue-specific manner, as both mice with the maternal disruption and mice with the paternal disruption show Gsa levels in the renal medulla that are about 50% of the levels seen in wild-type littermates [40]. Similar findings have been reported recently in mice heterozygous for either paternal or maternal disruption of Gnas exon 1, and these studies additionally demonstrated that Gsa expression is also predominantly maternal in the thyroid [41]. Consistent with these findings, the active maternal Gsa promoter shows a greater ratio of tri- to dimethylated histone-3 Lys4 compared to the silenced paternal promoter in the proximal tubule, whereas the amount of methylated histones is similar in maternal and paternal Gsa promoters in liver, a tissue in which Gsa is biallelic [42].

Monoallelic, maternal Gsa expression has also been documented in human tissues through the analysis of a single nucleotide polymorphism located in exon 5. Thus, Gsa is predominantly maternal in thyroid, pituitary, and ovaries [43-45]. On the other hand, biallelic Gsa expression has been demonstrated in lymphocytes, adrenal, adipocytes, and bone cells [44, 46, 47]. The tissue-specific, imprinted expression of Gsa has important implications in the pathogenesis of diseases caused by mutations within GNAS, particularly pseudohypoparathyroidism (see below).

Several Gsa knockout mouse models have been generated in order to study the role of Gsa in vivo. The mouse models with universal Gsa disruption will be discussed below in the context of human diseases that are caused by heterozygous inactivating mutations within one of the 13 exons encoding this protein. In addition, Gsa has been conditionally ablated, through the use of the Cre-Flox technology, in specific tissues including liver, cartilage, bone, and glomerulus, confirming the absolute requirement of Gsa in the proper function of these tissues [48-51].

NOVEL IMPRINTED GENE PRODUCTS OF THE GNAS LOCUS

Human and mouse studies over the past several years have identified novel promoters and exons within GNAS, thereby unveiling the previously unrecognized complexity of this gene locus. The sense strand comprises at least three promoters besides the promoter driving the expression of the aforementioned Gsa variants (Fig. 2). In addition, there is at least one promoter that shows activity on the antisense strand. All of the recently described promoters are located within CpG islands that show differential, parent-of-origin specific methylation. Therefore, the GNAS locus harbors a total of four distinct DMRs. Consistent with the differential methylation of its promoter, each of the additional GNAS product shows an imprinted expression profile in nearly all tissues investigated in that regard.

NESP55

The most upstream of the different GNAS promoters with respect to the promoter of Gsa drives the expression of NESP55, a neuroendocrine secretory protein with an apparent molecular mass of 55,000 (Fig. 1). The NESP55 promoter is differentially methylated and active on the non-methylated maternal allele only [37, 52]. In humans, NESP55 protein is encoded by a single exon, while in mice, the ORF consists of two separate exons; however, in both species, these exons splice onto Gsa exons 2-13, which comprise the 3’ untranslated region (UTR) [37, 52]. The
predicted molecular mass of NESP55 is 28 kDa, but it has the same electrophoretic mobility as a protein of 55 kDa [53]. NESP55 is a chomogranin-like protein expressed in neuroendocrine tissues, peripheral and central nervous system, and some endocrine tissues [53-56]. It is associated with the constitutive secretory pathway [57] and can be located in cholinergic, peptidergic, and adrenergic neurons [58]. In AtT20 cells, a corticotroph-like cell line, NESP55 has been shown to be exported out into the medium, a process which can be blocked markedly by lowering the temperature and modestly by treatment with a cAMP analog (8-bromo-cAMP) [59]. In different tissues, differential post-translational processing of NESP55 leads to smaller peptides, which appear to accumulate during antegrade transport of this protein along the axon [58]. One of the putative peptide products, Leu-Ser-Ala-Leu (LSAL), which may be produced by prohormone convertase cleavage, has been identified as an endogenous antagonist of the serotoninergic 5-HT1B receptor subtype [53], although this possibility and its potential biological significance have yet to be investigated.

A knockout of the Nesp55 transcript has been generated in mice through introduction, by homologous recombination in ES cells, of a small deletion at the translation initiation site [60]. This mutation leads to a complete ablation of Nesp55 protein after maternal transmission, without affecting the expression of other Gnas transcripts. Nesp55 knockout mice appear to have no overt phenotypic abnormalities and are fertile. However, behavioral studies of these mice using several distinct tasks have revealed increased reactivity to novel environments, but these mice do not differ from wild-type mice in general locomotion and anxiety. Although the role of this protein at the molecular level remains to be explored, the findings in the Nesp55 deficient mice appear to be consistent with the localization of this protein in specific parts of the central nervous system, particularly noradrenergic locus coeruleus [61]. In humans, loss of NESP55 expression occurs as a result of a gain of methylation at its promoter (observed in some patients with pseudohyoparath smoirdism type-Ib). This defect, however, does not appear to lead to an obvious phenotype, because no significant differences have been identified between patients with the gain of NESP55 methylation and those without this epigenetic alteration [62, 63].

XLαs

Another promoter, located ~11 kb downstream of the NESP55 promoter, drives the expression of XLαs mRNA, which encodes a protein with partial identity to Gsa (Fig. 1). As in NESP55, the XLαs transcript uses a novel first exon (exon XL) that splices onto exons 2-13 of Gsa [36, 52]. Unlike in the case of NESP55, however, the in-frame termination codon for the XLαs transcript is the same as for Gsa, making the encoded XLαs and Gsa proteins identical over a long C-terminal stretch [64]. Exon XL and the XLαs promoter are located in a CpG island methylated on the maternal allele [36, 52]. Consistent with this epigenetic mark, XLαs is derived exclusively from the paternal allele in all investigated tissues [36, 52, 65]; however, variable biallelic expression of XLαs has been recently demonstrated in clonal bone stromal cells [47]. Most abundant expression of XLαs is detected in neuroendocrine tissues, particularly pituitary, but the expression of its mRNA is readily detected by Northern blot or RT-PCR in various tissues, including brain, pancreas, heart, kidney, and adipose tissue [36, 64, 66, 67]. As demonstrated in the rat nervous system, XLαs expression is developmentally regulated [68].

Because XLαs shares significant amino acid identity with Gsa, it comprises most domains of the latter shown to be functionally important. Furthermore, the C-terminal end of the XL domain has significant homology to the exon 1 encoded portion of Gsa. Consistent with the high degree of overall similarity between XLαs and Gsa, various studies have demonstrated that XLαs is able to act in a manner similar to Gsa in vitro. First, XLαs show enhanced ADP ribosylation and an increased sucrose-density sedimentation rate upon addition of the Gβγ subunits, indicating that XLαs is able to form a heterotrimer [69]. Second, expression of an XLαs mutant carrying the homolog of the Gsa Glu227 mutation results in elevated cAMP formation, indicating that XLαs can stimulate adenyl cyclase at least in the basal state [69]. Third, XLαs can mediate receptor-stimulated cAMP formation when overexpressed in opossum kidney cells that show endogenous Gsa expression [70] and in mouse embryonic fibroblasts that endogenously lack Gsa and XLαs due to homozygous disruption of Gnas exon 2 [71]. Finally, mutations that impair Gsa activity have similar effects on XLαs activity when introduced into the backbone of the latter [70]. Interestingly, however, beta-adrenergic stimulation seemingly fails to elicit cAMP generation in XLαs transfected S49 lymphoma cells that are also Gsa deficient (cyc - clone) [69]. Furthermore, attempts to show coupling of XLαs to receptors have failed, using PC12 cells transfected with XLαs cDNA and pituitary membranes [69]. Taken together, these findings suggest that the Gsa-like activity of XLαs may be cell specific.

As illustrated in Fig. (3A), XLαs has several different variants. Similar to Gsa, it uses exon 3 alternatively, thus having at least a long and a short variant. In addition, analogous to Gsa-N1, a C-terminally truncated XLαs variant, termed XLαs-N1, also exists. Human XLαs-N1 appears to have a subvariant produced by inclusion of sequences from two additional exons, A20 and A21, located immediately following exon XL [36]. A similar subvariant has also been demonstrated for rat XLαs-N1 [66]. The introduction of these additional sequences into XLαs-N1 results in a frameshift, leading to a putative protein with partial identity to XLαs but not to Gsa. Functional significance of XLαs-N1 and its variants remains unknown.

There is evidence for the existence of a long XLαs transcript, termed XXLαs, which extends in the 5’ direction (Fig. 3A). The XXLαs transcript is supported by Est databases and a single study that could amplify portions of the XXLαs transcript by RT-PCR [72]. A long XLαs transcript has also been revealed by Northern blot in mouse heart and adipose tissue [67]. In fact, based on existing data, the translation initiation codon for XLαs does not match with that corresponding to the longest open reading frame (ORF), making it likely that an N-terminally extended XLαs variant is expressed at least in some tissues. Est databases also suggest the existence of transcripts that correspond to XXLαs-N1, but neither the role of XXLαs nor the role of XXLαs-N1 is currently known. Interestingly, however, unlike the XL domain in XLαs and
main in XLs and XLs-N1 -- the portion encoded by exon XL -- the N-terminally extended part in XXLs and XXLs-N1 is highly conserved among mammals, suggesting that it may represent a functionally significant domain.

The XLs mRNA has a second ORF that encodes another protein termed ALEX (for alternative protein encoded by XLs mRNA), which uses a termination codon located within exon XL and is, therefore, smaller than XLs (Fig. 3B). In vitro studies have shown that ALEX, which has a predicted molecular mass of 38 kDa, can interact with XLs [73, 74]. An insertion polymorphism within XLs mRNA, affecting both proteins, appears to impair this interaction, a finding that has been offered as an explanation as to why some individuals carrying this insertion polymorphism show elevated agonist-induced cAMP accumulation in platelets [74]. However, this increased agonist responsiveness could also result from elevated Gsα levels, which was demonstrated in several individuals who carried the same insertion polymorphism and had increased bleeding tendency [75]. Hence, the significance of XLs-ALEX interaction on XLs or Gsα function remains to be clarified. Of note, this alternative ORF also extends into the XXLs mRNA, generating a putative N-terminally extended ALEX variant, termed ALEXX [72]; however, there are currently no available experimental data to suggest that this protein actually exists.

Exon XL has been disrupted in mice through introduction of a small deletion into the beginning part of both XLs and ALEX ORF; this disruption appears to preserve the imprinted expression of other Gnas transcripts [67]. The mice with paternal inheritance of the disrupted XLs allele (Gnasxl knockout mice) lack XLs expression, consistent with the exclusive paternal expression of the XLs transcript. The Gnasxl knockout pups show defective suckling, hypoglycemia, growth retardation, and early postnatal mortality. Despite having hypoglycemia, the Gnasxl knockout mice show reduced glucagon levels and inappropriately normal serum catecholamine and cortisol levels, suggesting a possible impairment of the physiological responses to hypoglycemia. When crossed into the CD1 background, about 20% of the Gnasxl knockout animals survive with an apparently normal life-span. However, these mice display increased glucose tolerance and insulin sensitivity, as well as a hypermetabolic state associated with reduced adiposity and hypolipidemia [67]. In addition, circulating levels of norepinephrine is elevated in these animals, indicating increased sympathetic nervous system activity, which explains, at least in part, the defects in the energy metabolism [76]. On the other hand, adipocyte-autonomous factors appear less likely to be involved in these phenotypes, because XLs is not expressed in the adipocytes of adult wild-type mice [76].

The genetic alteration in the Gnasxl knockout mice is predicted to disrupt not only XLs but also XLs-N1, ALEX, XXLs, XXLs-N1 and ALEXX. It thus appears unlikely that the phenotype of Gnasxl knockout mice is due solely to XLs deficiency. Nonetheless, a similar phenotype is present in mice with paternal Gnas exon 2 disruption [77-79] and in mice with a paternally inherited missense mutation in exon 6 [80, 81], thereby ruling out the involvement of ALEX, XLs-N1, and XXLs-N1 deficiency in the phenotypes of the Gnasxl knockout mice. Thus, it is more likely that the deficiency of XLs and/or XXLs underlies the findings observed in the latter mouse model, although this conclusion needs to be verified through further investigation.

**Fig. (3).** XLs and its multiple variants. XLs is derived from a promoter upstream of that which drives the expression of Gsα. Panel A. Alternative splicing leads to different XLs variants that are analogues to Gsα variants. In addition, an N-terminally extended XLs variant, termed XXLs, is made, representing the 5' extension of the ORF. For simplicity, GNAS exons located upstream of exon XL are not shown. Panel B. XLs and XXLs mRNA include two ORFs each. The second ORF leads to ALEX or ALEXX, respectively.
is important to note that the phenotype of the Gnasxl knockout mice differs, by and large, from the phenotype of mice heterozygous for disruption of Gnas exon 1, in which Gsa, but not XLas, is ablated [41, 82]. In fact, Gnasxl knockout mice demonstrate slightly elevated basal and isoproterenol-stimulated cAMP levels in brown adipose tissue at birth [67]. These findings suggest that XLas has a role distinct from the role of Gsa in mammals, and that it may oppose, at least in certain tissues, the actions of Gsa.

Findings that are reminiscent of those observed in Gnasxl knockout mice and the mice with paternal Gnas exon 2 disruption have been reported in two unrelated children with large paternal deletions of chromosome 20q13.3 that comprise the GNAS locus [83]. These findings included perinatal growth retardation, intractable feeding difficulties, and loss of subcutaneous adiposity, as well as dysmorphic facial features. Because the deletion involved, in each case, the entire GNAS locus, it is possible that the phenotype reflects, at least partially, the deficiency of Gsa and/or the other paternally expressed GNAS products (see below).

The A/B Transcript

About 2.5 kb upstream of the Gsa promoter lies another promoter that drives the nearly ubiquitous expression of another transcript termed A/B (also termed 1A and 1′) [84-86]. The A/B promoter, despite being in close proximity to the Gsa promoter, reside within a DMR, where the maternal allele is methylated and repressed, while the paternal allele is non-methylated and active (Fig. 2). The first exon of the A/B transcript, as in Nesp55 and XLas, splices onto Gsa exons 2-13. However, exon A/B does not contain an in-frame translation initiation codon, and as supported by evidence from in vitro translation assays [86], translation can be initiated by an in-frame AUG located in exon 2, leading, presumably, to a variant of Gsa that has a truncated N-terminus. Transfection of COS cells with A/B cDNA results in the expression of a protein that localizes to the plasma membrane [86]. Based on these features, it is conceivable that the A/B protein interacts with adenylyl cyclase and may, therefore, exert a dominant negative effect on Gsa actions. This possibility remains to be investigated.

On the other hand, it appears more likely that the A/B transcript is non-coding, as the existence of an endogenous A/B protein is not supported by experimental evidence. Furthermore, similar to other non-coding RNA molecules in the genome, the A/B transcript and/or the exon A/B DMR has an important role in regulating gene expression from GNAS. Unlike the DMRs comprising the promoters of Nesp55 and XLas, the exon A/B DMR has been shown to be a germ-line imprint mark [85]. It has also been shown that this DMR is associated with allele-specific differences in histone modifications consistent with an active paternal (histone acetylation and histone-3 Lys^4^ methylation) and an inactive maternal (histone-3 Lys^4^ methylation) promoter [42, 87]. In addition, paternal ablation of the A/B DMR results in derepression of the Gsa transcript in those tissues where paternal Gsa expression is normally silenced, such as the renal cortex [81, 88]. Thus, it appears that the non-methylated A/B DMR and/or active A/B transcription is necessary for the tissue-specific paternal silencing of Gsa. Consistent with this finding, paternal exon A/B is unmethylated and the maternal A/B promoter is derepressed in patients with pseudohypoparathyroidism type-Ib [63, 89, 90], who are thought to have a lack of Gsa expression in the proximal tubule due to silencing of the maternal Gsa promoter (in addition to the silencing of the paternal Gsa promoter that occurs normally in the same tissue; see below for further discussion).

The mechanisms underlying the role of A/B in the tissue-specific silencing of paternal Gsa expression are not well understood. Considering that the A/B transcription takes place more broadly than does paternal Gsa silencing, a mechanism that involves simple competition between the promoters of these two transcripts is unlikely. On the other hand, a plausible hypothesis involves a mechanism whereby the paternal A/B DMR binds either a repressor that directly silences the Gsa promoter or an insulator that prevents the effects of an upstream enhancer on the Gsa promoter. A mechanism similar to the latter has been demonstrated for the Igf2-H19 locus, in which methylation-sensitive binding of CTCF hinders Igf2 enhancer activity [91, 92]. To address these possibilities, it may be necessary to generate and study additional mouse models in which A/B transcript is disrupted without the deletion of its promoter region.

The GNAS Antisense Transcript

A promoter located immediately upstream of the XLas promoter drives expression of an antisense transcript, which extends past the exon(s) encoding NESP55 [93, 94] (Fig. 2). In mice, the mature antisense transcript (termed Nespas or Gnasas) encompasses both Nesp55 exons 1 and 2 and the intervening intron [94], while in humans, it has no overlap with exon NESP55 [93]. There are at least five distinct exons that form the human GNAS antisense transcript, but alternative splicing results in at least six variants. The largest open reading frame is predicted to encode a polypeptide of 97 amino acids that share no homology to known proteins [93]. Taken together these features suggest that the antisense transcript is non-coding. Indeed, similar to the promoter of A/B, the promoter of the GNAS antisense promoter resides in a DMR and is active exclusively on the paternal allele [65, 93, 94]; however, the antisense transcript shows biallelic expression in the adrenal and testes [65]. Consistent with allele-specific expression, the paternal antisense promoter is associated with acetylated histone and histone-3 Lys^4^ methylation, while the maternal promoter lacks histone acetylation and carries histone-3 Lys^9^ methylation [87]. Furthermore, studies in mice have shown that the maternal methylation at the antisense promoter is present even in the oocytes and, therefore, represents a germ-line imprint mark [95].

Paternal deletion of the antisense promoter in mice results in derepression of Nesp55 in cis, thereby leading to biallelic expression of the latter [96]. This finding indicates that at least one of the roles of the antisense transcript is to silence the paternal Nesp55 promoter. However, the effect of the deletion of the antisense promoter is not limited to Nesp55 expression. First, the deleted region evidently comprises an enhancer of the XLas promoter, resulting in a dramatic reduction in XLas expression and, thus, a phenotype similar to that observed in Gnasxl knockout mice. Second, mice with the paternal deletion exhibit a modest decrease in A/B expression combined with an increase in the methylation of the A/B promoter. Third, in those tissues where Gsa
is normally silenced from the paternal allele, the diminished A/B expression is associated with an increase in Gsa expression in cis, i.e. the tissue specific Gsa imprinting is relaxed. These findings clearly demonstrate the importance of the antisense transcript in the regulation of imprinted gene expression from the GNAS locus. Nonetheless, what causes the reduction of paternal A/B expression remains unclear. Is it the derepression of Nesp55, the reduction of XLas expression, or the deletion of the genomic region containing the antisense promoter?

**HUMAN DISEASES ASSOCIATED WITH GNAS MUTATIONS**

Consistent with the pivotal role of Gsa in multiple biological responses, mutations that affect the activity or expression of Gsa lead to human disease. However, there are no disorders caused by inactivation of both Gsa alleles, i.e. homozygous inactivating Gsa mutations, and this is consistent with the early embryonic lethality observed in mice with homozygous disruption of either Gnas exon 2 or Gnas exon 1 [40, 41, 82]. Thus, complete loss of Gsa activity is not compatible with life. Moreover, there are mutations that cause constitutive Gsa activity, but these are virtually never inherited and are of somatic origin, indicating that universal Gsa overactivity is embryonic lethal, as well.

**Endocrine Adenomas and other Tumors**

Many hormones bind Gsa-coupled receptors in order to activate their endocrine glands for proliferation, differentiation, and hormone secretion. Accordingly, mutations that cause constitutive Gsa activity are found in various functionally active endocrine adenomas, including those that originate from pituitary somatotrophs. In about 40% of patients with growth hormone secreting pituitary adenomas, constitutively activating Gsa missense mutations at either Arg201 or Gln227 have been identified in DNA from the tumor tissue, but not in DNA from peripheral blood [20, 97]. Since these mutant Gsa forms are identified in tumors and are present in one of the Gsa alleles only, they are referred to as the gsp oncogene [20, 97]. Other endocrine tumors also bare the gsp oncogene, including corticotroph, thyroid, parathyroid, and adrenocortical tumors, but their frequency in the patient population appears to be low based on many studies (reviewed in [98]). Some studies have recently identified the gsp oncogene in ovarian granulosa cell tumors and testicular stromal Leydig cell tumors as a possible cause of tumorigenesis and as a possible prognostic marker [99, 100]. In a more recent study, 5 of 30 patients with clear cell renal carcinoma have been shown to carry constitutively activating Gsa mutations in the tumor tissue [101]. Hence, the gsp oncogene can be present in a wide variety of tumors that mostly, but not exclusively, involve classic endocrine tissues. Data from transgenic mouse models and cell culture assays have shown that constitutive Gsa activity can lead to hyperplasia and increased hormone secretion in endocrine cells [102, 103]. However, although a recent study implicates sustained activation of extracellular signal-regulated kinase in increased hormone secretion [104], the signaling pathways downstream of the gsp oncogene currently remain incompletely understood.

**McCune-Albright Syndrome**

This syndrome, independently described by McCune [105] and Albright et al. [106], is characterized by a triad of sexual precocity, fibrous dysplasia of bone, and hyperpigmented skin lesions termed café-au-lait spots. Patients with the McCune-Albright syndrome (MAS) are mosaic for constitutively activating Gsa mutations that occur during early embryonic development [107-109]. This is consistent with the observation that MAS occurs sporadically and is never transmitted to the next generation. All identified mutations are at residue Arg201 (Cys or His), suggesting that changes in residue Gln227 may result in higher constitutive activity and are, therefore, less viable. Because of the mosaicism, patients with MAS show significant variation in their clinical presentation. In general, the abnormalities involve bone, skin, and endocrine organs. Fibrous dysplastic bone lesions are usually found in multiple bones. It is important to note that some patients with the constitutively activating Gsa mutations present with fibrous dysplasia alone, affecting either a single bone or multiple bones. In patients with isolated fibrous dysplasia, histological changes appear to be indistinguishable from those seen in the context of MAS [110, 111]. As in patients with MAS, nearly all isolated cases of fibrous dysplasia are associated with GNAS mutations at Arg201; however, a study using a mutation-specific restriction enzyme digest assay has recently identified three Gln227 (to Leu) mutations among a total of 56 samples [112]. The skin lesions, which typically have irregular borders, can be single or multiple light brown hyperpigmented areas arranged in segmental patterns that follow the developmental lines of Blaschko [106, 109]. Endocrine abnormalities, in addition to precocious puberty, can be summarized as hyperplasia and increased function of many different glands, including thyroid, adrenal, and pituitary [113-115]. Some patients with MAS also exhibit urinary phosphate wasting, hypophosphatemia, and bone mineralization defects observed as rickets or osteomalacia [113, 114]. While the latter findings are consistent with the role of Gsa in mediating the phosphaturic actions of parathyroid hormone in the renal proximal tubule, recent data shows that serum phosphate in MAS patients is negatively correlated with the level of fibroblast growth factor-23 [116-118], suggesting that the elevation of this phosphaturic factor is responsible, at least partly, for the hypophosphatemia observed in these patients. In addition to the various endocrine defects discussed above, there are rare reports of non-endocrine abnormalities associated with MAS, including liver and cardiac abnormalities, and neurological defects. The endocrine and non-endocrine findings in MAS have been reviewed elsewhere in greater detail [98].

**Pseudohypoparathyroidism Type-Ia, Pseudopseudohypoparathyroidism, and Progressive Osseous Heteroplasia**

Fist described by Albright and colleagues [119], Pseudohypoparathyroidism (PHP) refers to end-organ resistance to multiple hormones that primarily involves the actions of parathyroid hormone. PTH exerts its actions in bone and kidney through the PTH/parathyroid hormone-related peptide receptor (PTH-R1), which couples to Gs and, less effectively, to Gq [120, 121]. PTH increases bone turnover, leading to mobilization of calcium and phosphate from bone [122, 123]. In the proximal renal tubule, it induces the syn-
thesis of 1,25-dihydroxyvitamin D and inhibits phosphate reabsorption from the glomerular filtrate, while in the distal renal tubule, it enhances the absorption of calcium mediated via transcellular mechanisms. Exogenous administration of biologically active PTH, used previously as a diagnostic test [124], results in a blunted excretion of urinary phosphate in both PHP type-I and PHP type-II, but this defect is accompanied by blunted nephrogenous cAMP production in PHP type-I only; the PTH-induced generation of nephrogenous cAMP is normal in PHP type-II [119, 125]. Clinically, PHP-I is far more frequent than PHP-II, for which underlying molecular defects are not well understood. On the other hand, significant advances have been recently made regarding the molecular pathology underlying PHP-I.

Some patients with PHP-I display distinctive physical features collectively termed Albright’s hereditary osteodystrophy [119]. These features include obesity, short stature, ectopic ossification, brachydactyly, and mild mental retardation, although there is significant patient-to-patient variation in the range and severity of these features. The presence of both PTH-resistance and AHO defines patients with PHP type-Ia. Gsa mRNA and protein levels are reduced to half in easily accessible tissues from these patients [126-128]. This defect results from heterozygous inactivating mutations within one of the thirteen Gsa coding GNAS exons [129, 130]. Scattered throughout the gene, various different types of mutations, such as insertions, deletions, and missense and nonsense changes, have been identified, which is consistent with their inactivating nature. An extensive list of the mutations associated with this disorder can be found under OMIM entry #139320 at http://www.ncbi.nlm.nih.gov.

Because Gsa is not exclusive for PTH signaling, PHP-Ia patients also show resistance to some other hormones whose actions depend on Gsa signaling, including thyroid stimulating hormone (TSH), gonadotropins, and growth hormone releasing hormone (GHRH) [131-135]. It is worth noting that not all hormone actions that rely on Gsa are impaired in PHP-Ia. For example, there is no resistance to vasopressin [136, 137] or to hormones in the hypothalamo-pituitary-adrenal axis [134, 136, 138].

Inactivating Gsa mutations found in PHP-Ia patients are also present in patients who lack hormone resistance but present with AHO features, a condition referred to as pseudopseudohypoparathyroidism (PPHP) [139]. Mutations in patients with PPHP are often identical to those in PHP-Ia patients. In fact, both disorders typically co-exist in the same kindred [129, 140], with the gender of the affected parent determining whether the offspring will have PHP-Ia or PPHP: maternal inheritance leads to PHP-Ia, whereas paternal inheritance leads to PPHP [141, 142]. Thus, hormone resistance (PHP-Ia) is inherited only from female obligate carriers, a mode of inheritance that is consistent with the predominantly maternal expression of Gsa in certain tissues. It is important to note that the repertoire of hormone resistance in PHP-Ia correlates well with the tissues in which maternal, monoallelic Gsa expression takes place, underscoring the significance of Gsa imprinting in the pathogenesis of PHP-Ia.

AHO features are present in both PHP-Ia and PPHP patients, and therefore, the molecular mechanisms underlying AHO presumably entail Gsa haploinsufficiency rather than imprinting. Gsa haploinsufficiency has been demonstrated in the growth plate of mice chimeric for wild-type cells and cells heterozygous for disruption of either the maternal or the paternal Gnas exon 2 [143]. In the chimeric setting, the mutant chondrocytes undergo hypertrophic differentiation sooner than wild-type chondrocytes, and because this finding is qualitatively similar to (albeit far less severe than) that observed in chondrocytes with homozygous Gsa ablation under the same conditions, it indicates Gsa haploinsufficiency. While this study strongly suggests that the short stature and the brachydactyly seen in patients with AHO reflect, at least in part, Gsa haploinsufficiency in the growth plate, some features of AHO may still involve Gsa imprinting in the pathogenesis. For example, a recent study has clearly shown that obesity is more prominent in PHP-Ia patients than PPHP patients [144]. Thus, it appears that Gsa may be imprinted in more tissues than currently recognized, such as in parts of the brain that controls satiety and body weight. It is also possible that disruption of other imprinted GNAS gene products contribute to the pathogenesis of AHO. Supporting this hypothesis, chondrocytes with paternal Gnas exon 2 disruption exhibit a slightly, but significantly, higher degree of Gsa haploinsufficiency than chondrocytes with maternal Gnas exon 2 disruption [143]. More detailed characterization of the different AHO features between patients with paternally and maternally inherited Gsa mutations are likely to provide further insights into the understanding of the mechanisms underlying AHO.

Progressive osseous heteroplasia (POH) describes a severe, debilitating disease characterized by ectopic intramembranous bone formation that affects not only the subcutis, but also the skeletal muscle and the deep connective tissue [145]. Heterozygous inactivation mutations within the Gsa coding GNAS exons have also been identified in patients with POH. In fact, some of those mutations are identical to those found in patients with PHP-Ia or PPHP [145-147]. It is therefore possible that POH is an extreme manifestation of the ectopic bone formation of AHO that normally involves the subcutaneous tissue. However, POH is rarely accompanied with any AHO features or hormone resistance [146, 148]. Furthermore, in many kindreds, it has been shown that the disease develops only after paternal inheritance [147], suggesting that genomic imprinting also plays a role in the pathogenesis of POH. Because this disorder is paternally inherited, deficiency of GNAS products that show paternal specific expression and share exons with Gsa, such as XLas, could contribute to ectopic bone formation. Consistent with this hypothesis, no mutations in exon 1 has thus far been reported in cases with isolated POH [147, 149, 150]. However, the roles of XLas and other imprinted GNAS products in POH remain currently undefined.

**PHP Type-Ib**

Some patients with PTH-resistance lack AHO and any additional hormone resistance, defining the typical features of PHP type-Ib (PHP-Ib). Recent studies have demonstrated that some PHP-Ib patients also have mild TSH resistance in addition to PTH resistance [89, 90, 151]. Furthermore, a single study described several patients who showed both the epigenetic defects characteristic of PHP-Ib (see below) and
mild features of AHO [152], suggesting more variation in the phenotype. Unlike in PHP-Ia, Gsa activity is typically normal in easily accessible cells from these patients, thus excluding mutations within Gsa coding GNAS exons [131, 153]. Nonetheless, in three related patients with an apparent diagnosis of PHP-Ib an in-frame, tri-nucleotide deletion has been identified within exon 13 [154]. Expressed in HEK293 cells, an embryonic kidney derived cell line, this Gsa mutant was shown to affect the signaling of PTH, but not of TSH, LH, or isoproterenol, thus explaining the isolated PTH resistance. The selective effect of this mutation on PTH signaling, however, could not be verified in a subsequent study [70], and it is possible that the discrepancy between the two studies stems from the use of different cell types and/or assays; the second study used mouse embryonic fibroblasts null for endogenous Gsa [70, 71]. Alternatively, the three patients may have PHP-Ia, consistent with the observation that two of them exhibited advanced bone age [154], which is a typical sign of AHO. Since the urinary cAMP response to exogenously administered PTH is blunted in PHP-Ib patients [131], defects in the gene encoding PTHR1 seemed like a good candidate at the time. However, several studies have ruled out this possibility [153, 155-157].

PHP-Ib is often sporadic, but a number of familial cases have also been described. A careful analysis of these pedigrees has revealed that the PTH resistance in PHP-Ib develops only if the genetic defect is inherited from a female carrier [158], i.e. the mode of inheritance is identical to that observed for hormone resistance in PHP-Ia. Genetic linkage studies using some of these kindreds have mapped the genetic defect to a region of chromosome 20q that comprises the GNAS locus [158, 159]. Moreover, most PHP-Ib patients exhibit GNAS imprinting defects [63, 89]. Although these defects involve multiple GNAS DMRs in some cases, the most consistent defect is a loss of imprinting at the A/B DMR, i.e. loss of methylation at the A/B promoter and exon combined with biallelic A/B expression, which appears to be an isolated defect in most familial PHP-Ib cases [89, 160]. Based on these findings, the genetic mutation responsible for PHP-Ib is thought to disrupt an imprinting regulatory element of GNAS. The most frequent mutation, identified thus far in more than 30 unrelated kindreds, is a unique 3-kb microdeletion located about 220 kb upstream of exon A/B [160-163] (Fig. 4). Flanked by two 391-bp repeats, the 3-kb microdeletion removes exons 4-6 of STX16, the gene encoding syntaxin-16. The second mutation is a 4.4-kb microdeletion that overlaps with the former and removes STX16 exons 2-4; this mutation, unlike the 3-kb microdeletion, has been found in only one kindred thus far [164]. These mutations cause disease only after maternal inheritance, and each affected individual carrying either of these mutations displays an isolated loss of exon A/B imprinting, thereby indicating that the mutations disrupt a cis-acting element that controls imprinting at the exon A/B DMR [160, 164]. This element may lie within the 1.3-kb region where the two deletions overlap. The overlapping region comprises exon 4, which is evolutionarily conserved and lies within a small CpG-rich region that lacks differential methylation [160]. It is also possible that the identified deletions independently disrupt a large control element that spans STX16. On the other hand, disruption of STX16 is not considered to be involved in the pathogenesis of PHP-Ib, because there is no evidence that this gene is imprinted [160, 164].

All sporadic and some familial PHP-Ib cases show epigenetic defects at one or more GNAS DMRs in addition to the exon A/B DMR [63, 89]. These defects often consist of a loss of imprinting at exon A/B, exon XL, and the promoter of the antisense transcript and a gain of imprinting at exon NESP55. Two unrelated familial cases with such GNAS imprinting abnormalities have been shown to carry maternally inherited deletions of the entire NESP55 DMR including exons 3 and 4 of the antisense transcript [165], revealing the putative location of another control element required for the imprinting of the entire maternal GNAS allele (Fig. 4). The presence of similarly large deletions at the NESP55 DMR has been excluded in a number of sporadic PHP-Ib cases [165].

The broad GNAS epigenetic defects seen in the sporadic cases are often such that the maternal allele has attained a paternal epigenotype. Accordingly, a sporadic PHP-Ib case has been shown to have paternal uniparental isodisomy of the entire chromosome 20q (patUPD20q) [90]. Leading to the diagnosis was PTH-resistance and mild TSH resistance in the absence of typical AHO findings, although the patient had additional abnormalities, including developmental delay and craniosynostosis, which may have resulted from either disrupted expression of other imprinted genes or from unmasking of recessive defects present on paternal chromosome 20q. Hence, patUPD20q is a cause of sporadic PHP-Ib, and it is possible that interstitial paternal UPDs in this region could lead to PHP-Ib as a more common cause of this disorder in the sporadic cases. It has also been suggested that some cases of sporadic PHP-Ib is caused by stochastic defects in the imprinting process [162]. In either case, the offspring of affected females would be predicted to have normal GNAS imprinting and normal proximal tubular PTH responsiveness even if they inherit the disease-associate allele.

Sporadic PHP-Ib cases typically differ from familial PHP-Ib cases in the nature of the GNAS epigenetic defects they exhibit, yet evaluation of many cases with these PHP forms indicates that the divergence in the epigenetic features do not directly translate into differences in clinical presentation. The age of onset and the severity of hypocalcemia and hyperphosphatemia that result from PTH resistance appear to be similar in sporadic and familial cases [62]. As explained above, Gsa expression is also predominantly maternal in the pituitary somatotrophs [43]; however, growth hormone deficiency and short stature are not typical clinical features of PHP-Ib. It is possible that the epigenetic defects do not affect Gsa expression in this tissue, thereby allowing unimpaired GHRH signaling. This could suggest that the mechanisms underlying Gsa imprinting are different between the pituitary and the proximal tubule. Alternatively, GHRH resistance may be present in patients with PHP-Ib, but it may be too mild to become clinically manifest. This is similar to the TSH resistance in PHP-Ib, which can be absent in many PHP-Ib patients, is milder than in PHP-Ia, and can be accounted for by the partial imprinting of Gsa in the thyroid [151]. Mild short stature has been recently reported in some patients who show PTH-resistance and GNAS imprinting...
defects [152], consistent with GHRH resistance and resultant growth hormone deficiency. Nevertheless, it remains unknown whether patients with PHP-Ib have reduced Gs expression in the pituitary and whether they display GHRH resistance.

The common defect in nearly all PHP-Ib cases is the loss of GNAS exon A/B imprinting. In fact, this epigenetic defect is necessary for the development of PTH resistance, as documented in a PHP-Ib kindred in whom some individuals lacked both loss of A/B imprinting and PTH resistance despite maternally inheriting the disease-associated haplotype on 20q [166]. The loss of A/B imprinting on the maternal allele is predicted to silence, in cis, Gs transcription in the proximal tubule. Therefore, methylation of exon A/B DMR and/or the repression of A/B transcription appear to be required for maternal Gsa expression in this and, probably, other tissues, such as the thyroid gland. Supporting this notion, ablation of the paternal exon A/B region derepresses Gsa in cis in tissues where this signaling protein is paternally silenced [81, 88] and, furthermore, rescues the PTH resistance phenotype observed in mice with a point mutation in maternal Gnas exon 6 [80, 81].

ANIMAL MODELS OF THE GNAS-RELATED DISEASES

PHP-Ia/PPHP

Before the evidence that several different GNAS transcripts, in addition to Gsa, utilize exon 2, a mouse model of PHP-Ia was generated by targeted disruption of this exon [40]. Homozygous disruption of exon 2 results in early embryonic lethality. Furthermore, paternal heterozygous disruption leads to lethality within the first 24 h after birth, and maternal heterozygous disruption results in death within the first three weeks. Surviving animals are fertile and appear to have normal life spans. As explained above, mice with paternal disruption of Gnas exon 2 have severe defects that are similar to those seen in mice with paternal disruption of Gnasxl [67], including reduced adiposity, which is not seen in patients with paternally inherited inactivating Gs mutations (PPHP); these patients are typically overweight. On the other hand, both the maternal and the paternal Gnas exon 2 knockout mice appear smaller than their littermates, reminiscent of the short stature observed in patients with AHO. Furthermore, the exon 2 knockout mice prove to be a good model of PTH resistance. Reflecting the tissue-specific silencing of paternal Gs in the proximal tubule, PTH-resistance, characterized by hypocalcemia, hyperphosphatemia, and elevated serum PTH, is present only after maternal inheritance of the disrupted allele [40]. In addition, PTH stimulation of proximal tubule extracts from mice with the maternal disruption, but not with the paternal disruption, fail to increase cAMP levels. Similar to the observations in PHP-Ia patients, the urine concentrating ability of the kidney in response to vasopressin is unimpaired in both the maternal and the paternal knockouts. Hence, although the findings resulted from the disruption of exon 2 are rather complicated due to the use of this exon by not only Gsa but also several other GNAS products (particularly XLs), this animal model is able to phenocopy PHP-Ia to a significant extent.

Chen et al. [82] and Germain-Lee et al. [41] have independently generated another model of PHP-Ia through tar-
geted disruption of Gnas exon 1. In addition to early embryonic lethality observed upon homozygous inheritance of exon 1 disruption, even the heterozygous disruption is associated with some pre-weaning mortality regardless of the parental origin. While this finding is not observed in families with PHP-Ia/PPHP, the overall phenotype of mice heterozygous for maternal disruption of Gnas exon 1 resembles that of PHP-1a remarkably. Mice with maternal disruption of exon 1 develop biochemical features consistent with PTH resistance [41]. In humans with inactivating Gsα mutations it is accepted that obesity develops regardless of the parent-of-origin of the introduced mutation, but recent evidence indicates that patients with PHP-1a exhibit more prominent obesity than patients with PPHP [144]. Consistent with this recent observation, mice with the maternal Gnas exon 1 disruption is more obese than the paternal disruption [82]. In contrast to these similarities between the mouse models of exon 1 ablation and PHP-Ia/PPHP, the serum PTH level seems to be moderately elevated in mice with paternal Gnas exon 1 ablation, suggesting the presence of PTH-resistance [41]. This finding is not observed in mice with paternal ablation of exon 2 and may therefore reflect the preservation of Gnas transcripts that use exon 2, such as XLαs. Although this conclusion is contrary to the evidence that XLαs can mimic Gsa [70, 71, 143], it correlates well with the notion that Gsα and XLαs, which are oppositely imprinted in certain tissues, mediate opposing actions [67].

**PHP-Ib**

Since a 3-kb deletion removing exons 4-6 of the STX16 locus has been identified in numerous familial PHP-Ib cases, and since this locus, with its exon-intron structure and its proximity to the GNAS locus, is similar in mice and humans, an attempt at generating a PHP-Ib mouse model has been made through targeted deletion of Stx16 exons 4-6 [167]. Both heterozygous and homozygous mice with this deletion lack gross abnormalities, are fertile, and appear to have a normal life span. More important, regardless of the parental origin of the deletion, the mutant mice fail to phenocopy the epigenetic abnormalities found in patients with familial PHP-Ib. In fact, neither any other Gnas methylation defects nor any biochemical features that would suggest PTH resistance could be observed in these animals. Thus, the nearly exact change in the mouse Stx16 gene is not sufficient to disrupt Gnas imprinting and to result in PTH resistance, ruling out a disrupted STX16 mRNA and/or protein as the molecular cause of familial PHP-Ib. This conclusion, which is consistent with the evidence that the STX16 locus is not imprinted, supports the hypothesis that the deletions in this gene disrupt a cis-acting long-range regulatory element required for the proper imprinting of GNAS exon A/B. It appears that this putative control element is not located within Stx16 exons 4-6 in mice.

**SUMMARY AND CONCLUSION**

GNAS is a complex locus located on the telomeric end of chromosome 20q. At least five distinct promoters reside in this locus, leading to biallelically, paternally, and/or maternally expressed transcripts from both the sense and antisense strands. Expression from this locus is tightly regulated by epigenetic mechanisms and cis-acting regulatory elements, including non-coding RNA molecules. GNAS encodes Gsα, one of the subunits of the heterotrimeric stimulatory G protein, which is essential for the actions of numerous agonists. Several human disorders are caused by heterozygous mutations that affect Gsα expression and/or activity, and consistent with the tissue-specific paternal silencing of Gsα expression, these disorders are inherited in a parent-of-origin specific manner. In contrast to Gsα, the roles of most of the other GNAS products remain unknown at the cellular and molecular level, even though the biological significance of these products are clear from gene knockout studies. Depending on their parental origin, mutations that affect the Gsα transcript also disrupt some of the additional GNAS transcripts, such as XLαs. It is likely that the disruption of these additional gene products or alterations in the balance between the expression of Gsα and these proteins contributes to disease pathogenesis. It will therefore be important to investigate the factors regulating the expression of each GNAS product and to determine their cellular actions. Consequently, we will know more about the biological role of this gene locus and improve our understanding of the molecular mechanisms underlying the GNAS-related disorders. In addition, we will likely gain further insights into the functional significance of complex genes in the genome.

**REFERENCES**

[1] Lander, E.S., Linton, L.M., Birren, B., Nilsback, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczky, J., LeVine, R., McEwan, P., McKernan, K., Meldrim, J., Mesirov, J.P., Miranda, C., Morris, W., Naylor, J., Raymond, C., Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Ghahram, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J.C., Mungall, A., Pumph, R., Ross, M., Shownkeen, R., Sims, S., Waterston, R.H., Wilson, R.K., Hillier, L.W., McPherson, J.D., Marra, M.A., Mardis, E.R., Fulton, L.A., Chinwalla, A.T., Pepin, K.H., Gish, W.R., Chissoe, S.L., Wendl, M.C., Delehaunty, K.D., Miner, T.L., Delehaunty, A., Kramer, J.B., Cook, L.L., Fulton, R.S., Johnson, D.L., Minx, P.J., Clifton, S.W., Hawkins, T., Branscombe, P., Redki, P., Richardson, S., Wenning, S., Sleazak, T., Doggett, N., Cheng, J.F., Olsen, A., Sulkas, S., Elkin, C., Uberbacher, E., Frazier, M., Gibbs, R.A., Muzny, D.M., Scherer, S.E., Bouck, J.B., Sodergren, E.J., Worley, K.C., Rives, C.M., Gorrell, J.H., Metzker, M.L., Naylor, S.L., Kucherlapati, R.S., Nelson, D.L., Weinstock, G.M., Sakaki, Y., Fujiymama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Weiessenbach, J., Heilig, R., Saurin, W., Artigue, E., Botttger, P., Bruls, T., Pelletier, E., Robert, C., Wincker, P., Smith, D.R., Doucette-Stamm, L., Rubenfeld, M., Weinstock, K., Lee, H.M., Dubois, J., Rosenthal, A., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Hood, L., Rowen, L., Madan, A., Qin, S., Davis, R.W., Federspiel, N.A., Absa, A.P., Proctor, M.J., Myers, R.M., Schmutz, J., Dickson, M., Grimwood, J., Cox, D.R., Olson, M.V., Kaul, R., Raymond, C., Shimizu, N., Kawasaki, K., Minoshima, S., Evans, G.A., Athanasiou, M., Schulz, R., Roe, B.A., Chen, F., Pan, H., Ramser, J., Lehrach, H., Reinhardt, R., McCombie, W.R., de la Bastide, M., Dedhia, N., Blocker, H., Hornischer, K., Nordsiel, G., Agarwala, R., Aravind, L., Bailey, J.A., Bateman, A., Batzoglou, S., Birney, E., Bork, P., Brown, D.G., Burge, C.B., Cerutti, L., Chen, H.C., Church, D., Clark, M., Copley, R.R., Doerks, T., Eddy, S.R., Eichler, E.E., Furey, T.S., Galagan, J., Gilbert, J.B., Harmon, C., Hayashizaki, Y., Haussler, D., Hermjakob, H., Hofkamp, K., Jang, W., Johnson, L.S., Jones, T.A., Kasif, S.
Robishaw, J.D., Smigel, M.D., Gilman, A.G. Molecular basis for two forms of the G protein that stimulates adenylate cyclase. J. Biol. Chem. 1986, 261(21): 9587-90.

Sterneweis, P.C., Northup, J.K., Smigel, M.D., Gilman, A.G. The regulatory component of adenylate cyclase. Purification and properties. J. Biol. Chem. 1981, 256(22): 1517-26.

Walseth, T.F., Zhang, H.J., Olson, L.K., Schroeder, W.A., Roberton, R.P. Increase in Gs and cyclic AMP generation in HIT cells. Evidence that the 45-kDa alpha-subunit of Gs has greater functional activity than the 52-kDa alpha-subunit. J. Biol. Chem. 1989, 264(35): 21106-11.

Graziano, M.P., Freimuth, M., Gilman, A.G. Expression of G alpha in Escherichia coli. Purification and properties of two forms of the G protein. J. Biol. Chem. 1990, 265(11): 409-18.

Seifert, R., Wenzel-Seifert, K., Lee, T.W., Gether, U., Sanders-Bush, E., Kobilka, B.K. Different effects of Galpha splice variants on beta2-adrenergoreceptor-mediated signaling. The beta2-adrenoceptor coupled to the long splice variant of Gsalpha has properties of a constitutively active receptor. J. Biol. Chem. 1998, 273(9): 5109-16.

Kvapil, P., Novotny, J., Svoboda, P., Ransnas, L.A. The short and long forms of the alpha subunit of the stimulatory guanine-nucleotide-binding protein are unequally redistributed during ( -) isoprotenerol-mediated desensitization of intact 3T4 lymphoma cells. Eur. J. Biochem. 1994, 226(1): 193-9.

el Jamali, A., Rachdouani, N., Jacquemin, C., Correze, C. Long-term effect of forskolin on the activation of adenyl cyclase in astrocytes. J. Neurochem. 1996, 67(6): 2532-9.

Bourgeois, C., Duc-Gogoian, P., Queille-Roussel, B., Mondon, F., Ferre, F. G protein expression in human fetoplacental vascularization. Functional evidence for Gs alpha and Gi alpha subunits. J. Mol. Cell Cardiol. 1996, 28(5): 1009-21.

Thiele, S., Werner, R., Ahrens, W., Hoppe, U., Marschke, C., Staedt, P., Hiort, O. A disruptive mutation in exon 3 of the GNAS gene with albright hereditary osteodystrophy, normocalcemic pseudohypoparathyroidism, and selective long transcript variant Gs alpha associated with Albright hereditary osteodystrophy, normocalcemic pseudohypoparathyroidism type Ib. J. Clin. Invest. 2005, 115(11): 3217-27.

Sakamoto, A., Chen, M., Nakamura, T., Xie, T., Karsenty, G., Stein, L.S. Deficiency of the G protein alpha -subunit Gs alpha in osteoblasts leads to differential effects on trabecular and cortical bone. J. Biol. Chem. 2005.

Peters, J., Wroe, S.F., Wells, C.A., Miller, H.J., Bode, D., Beechey, C.V., Williamson, C.M., Kelsey, G. A cluster of oppositely imprinted transcripts at the Gnas locus in the distal imprinting region of mouse chromosome 2. Proc. Natl. Acad. Sci. U.S.A. 1999, 96: 3830-5.

Ichisita, R., Lovisetti-Scinamihorn, P., Hogue-Angeletti, R., Wolkersdorfer, M., Winkler, H., Fischer-Colbrie, R. Molecular cloning and characterization of NESP55, a novel chromogranin-like protein containing a G alpha-signal sequence. FEBS Lett. 1994, 347(1): 17-21.

Bauer, R., Weiss, C., Marksteiner, J., Doblinger, A., Fischer-Colbrie, R., Laslop, A., Fischer-Colbrie, R. Secretion and molecular forms of NESP55, a novel genomically imprinted neuroendocrine secretory protein 55 (NESP55): alternative splicing onto transcripts of the GNAS gene and posttranscriptional processing of a maternally expressed protein. Neuroendocrinology 2000, 71(3): 177-86.

Bauer, R., Weiss, C., Marksteiner, J., Doblinger, A., Fischer-Colbrie, R., Laslop, A. The novel chromogranin-like protein NESP55 is preferentially localized in adrenaline-synthesizing cells of the bovine and rat adrenal medulla. Neurosci. Lett. 1999, 263(1): 13-6.

Fischer-Colbrie, R., Laslop, A., Fischer-Colbrie, R. Neuroendocrine secretory protein 55 (NESP55): alternative splicing onto transcripts of the GNAS gene and posttranscriptional processing of a maternally expressed protein. Neuroendocrinology 2000, 71(3): 177-86.

Fischer-Colbrie, R., Laslop, A., Fischer-Colbrie, R. Neuroendocrine secretory protein 55 (NESP55): alternative splicing onto transcripts of the GNAS gene and posttranscriptional processing of a maternally expressed protein. Neuroendocrinology 2000, 71(3): 177-86.

Bauer, R., Weiss, C., Marksteiner, J., Doblinger, A., Fischer-Colbrie, R., Laslop, A. The novel chromogranin-like protein NESP55 is preferentially localized in adrenaline-synthesizing cells of the bovine and rat adrenal medulla. Neurosci. Lett. 1999, 263(1): 13-6.

Fischer-Colbrie, R., Laslop, A., Fischer-Colbrie, R. Neuroendocrine secretory protein 55 (NESP55): alternative splicing onto transcripts of the GNAS gene and posttranscriptional processing of a maternally expressed protein. Neuroendocrinology 2000, 71(3): 177-86.
The GNAS Locus

Current Genomics, 2007, Vol. 8, No. 6 411

[60] Plagge, A., Isles, A.R., Gordon, E., Humby, T., Dean, W., Gritsch, S., Fischer-Colbrie, R., Wilkinson, L.S., Kelsey, G. Imprinted Nesp55 influences behavioral reactivity to novel environments. *Mol. Cell Biol.* 2005, 25(8): 3019-26.

[61] Bauer, R., Ischia, R., Marksteiner, J., Kapeller, I., Fischer-Colbrie, R. Localization of neuroendocrine secretory protein 55 messenger RNA in the rat brain. *Neuroscience* 1999(7): 685-94.

[62] Pinglart, A., Bastepo, M., Jüppner, H. Similar clinical and laboratory findings in patients with symptomatic autosomal dominant and sporadic pseudohypoparathyroidism type Ib despite different epigenetic changes at the GNAS locus. *Clin. Endocrinol.* (Oxf) 2007.

[63] Ljub, L., Litman, D., Rosenberg, M., Yu, S., Biesecker, L., Weinstein, L.S. Increased insulin sensitivity in paternal Gnas knockout mice is associated with increased lipid clearance. *Endocrinology* 2004, 145(9): 4094-102.

[64] Skinner, J., Cattanach, B., Peters, J. The imprinted oedematous small mutation on mouse chromosome 2 identifies new roles for gnas and gnasxl in development. *Genomics* 2002, 80(4): 373.

[65] Williamson, C.M., Ball, S.T., Nottingham, W.T., Skinner, J.A., Pincus, J.E., Sugimoto, T., Tojo, K., Kanatani, M., Baumer, L. XLalphas, the extra-long form of the alpha-subunit of the stimulatory G protein alpha-subunit leads to Gs hyperfunction in adult mice. *J. Clin. Invest.* 2000, 105: 615-23.

[66] Chen, M., Haluzik, M., Wolf, N.J., Lorenzo, J., Dietz, K.R., Reitman, M.L., Weinstein, L.S. Increased insulin sensitivity in paternal Gnas knockout mice is associated with increased lipid clearance. *Endocrinology* 2004, 145(9): 4094-102.

[67] Yu, S., Castle, A., Chen, M., Lee, R., Takeda, K., Weinstein, L.S. Increased insulin sensitivity in Gsalpha knockout mice. *J. Biol. Chem.* 2001, 276(23): 19994-8.

[68] Yu, S., Gavrilova, O., Chen, H., Lee, R., Liu, J., Pacak, K., Parlow, A., Quon, M., Reitman, L., Weinstein, L. Paternal versus maternal transmission of a stimulatory G-protein alpha subunit knockout produces opposite effects on energy metabolism. *J. Clin. Invest.* 2000, 105: 615-23.

[69] Yu, S., Castle, A., Chen, M., Lee, R., Takeda, K., Weinstein, L.S. Increased insulin sensitivity in paternal Gnas knockout mice is associated with increased lipid clearance. *Endocrinology* 2004, 145(9): 4094-102.

[70] Skinner, J., Cattanach, B., Peters, J. The imprinted oedematous small mutation on mouse chromosome 2 identifies new roles for gnas and gnasxl in development. *Genomics* 2002, 80(4): 373.

[71] Williamson, C.M., Ball, S.T., Nottingham, W.T., Skinner, J.A., Pincus, J.E., Sugimoto, T., Tojo, K., Kanatani, M., Baumer, L. XLalphas, the extra-long form of the alpha-subunit of the stimulatory G protein alpha-subunit leads to Gs hyperfunction in adult mice. *J. Clin. Invest.* 2000, 105: 615-23.

[72] Chen, M., Haluzik, M., Wolf, N.J., Lorenzo, J., Dietz, K.R., Reitman, M.L., Weinstein, L.S. Increased insulin sensitivity in paternal Gnas knockout mice is associated with increased lipid clearance. *Endocrinology* 2004, 145(9): 4094-102.

[73] Yu, S., Gavrilova, O., Chen, H., Lee, R., Liu, J., Pacak, K., Parlow, A., Quon, M., Reitman, L., Weinstein, L. Paternal versus maternal transmission of a stimulatory G-protein alpha subunit knockout produces opposite effects on energy metabolism. *J. Clin. Invest.* 2000, 105: 615-23.

[74] Yu, S., Castle, A., Chen, M., Lee, R., Takeda, K., Weinstein, L.S. Increased insulin sensitivity in Gsalpha knockout mice. *J. Biol. Chem.* 2001, 276(23): 19994-8.
the mouse Gnas imprinted locus. Mol. Cell Biol. 2003, 23(16): 5475-88.

[96] Williamson, C.M., Turner, M.D., Ball, S.T., Nottingham, W.T., Glenister, P., Fray, M., Tymowska-Lalanne, Z., Pliage, A., Powlis-Glover, N., Kelsey, G., Maconochie, M., Peters, J. Identification of an imprinting control region affecting the expression of all transcripts in the Gnas cluster. Nat. Genet. 2006, 38: 550-55.

[97] Lyons, J., Landis, C.A., Hash, G., Varral, L., Grunewald, K., Feichtinger, H., Duh, Q.Y., Clark, O.H., Kawasaki, E., Bourne, H.R., et al. Two G protein oncogenes in human endocrine tumors. Science 1990, 249(4969): 665-9.

[98] Weinstein, L.S., Yu, S., Warner, D.R., Liu, J. Osteitis fibrosa cystica; the case of a nine-year old girl. Am. J. Dis. Child. 1991, 145(12): 1208-12.

[99] Shenker, A., Weinstein, L.S., Sweet, D.E., Spiegel, A.M. Activating mutation in the stimulatory G protein (gs) as a putative cause of ovarian and testicular human stromal Leydig cell tumors. J. Clin. Endocrinol. Metab. 1998, 83(6): 2074-8.

[100] Kafka, N., Echard, A., Patte, C., Duvald, P., Audran, F., Pienkowski, C., Thibaud, E., Brauner, R., Leconte, C., Plantaz, D., Guedj, A.M., Paris, F., Baldet, P., Lumbruso, S., Sultan, C. Activating mutations of the stimulatory G protein in juvenile ovarian granulosa cell tumors: a new prognostic factor? J. Clin. Endocrinol. Metab. 2006, 91(5): 1842-7.

[101] Kafka, N., Lumbruso, S., Boulle, N., Guiter, J., Jousete, L., Costa, P., Chapuis, H., Baldet, P., Sultan, C. Activating mutations of Gnas in kidney tumors. J. Urol. 2006, 176(5): 801-8.

[102] Zeiger, M.A., Saji, M., Gusev, Y., Westra, W.H., Tendonca, B.B., Villares, S.M. activating mutation of the stimulatory G protein (gs) in testicular seminoma. Nat. Genet. 2003, 33(2): 133-40.

[103] Muca, C., Varral, L. Expression of mutationally activated G alpha s stimulates growth and differentiation of thyroid FRTL5 cells. On cocogene 1994, 9(2): 3647-53.

[104] Romano, D., Magalon, K., Pertuit, M., Rasolonjanahary, R., Barlier, A., Enjalbert, A., Gerard, C. Overexpression of the wild-type Gs alpha as the gsp oncogene initiates chronic extracellularly regulated kinase 1/2 activation and hormone hypersecretion in pituitary cell lines. Endocrinology 2007, 148(6): 2973-83.

[105] McCune, D. Osteitis fibrosa cystica; the case of a nine-year old girl who also exhibits precocious puberty, multiple pigmentation of the skin and hyperthyroidism. Am. J. Dis. Child. 1936, 52: 743-4. Albright, F., Butler, A., Hampton, A., Smith, P. Syndrome characterized by osteitis fibrosa disseminata, areas of pigmentation and endocrine dysfunction, with precocious puberty in females. New Engl. J. Med. 1937, 216: 727-46.

[106] Weinstein, L.S., Shenker, A., Gejman, P.V., Merino, M.J., Friedman, E., Spiegel, A.M. Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. New Engl. J. Med. 1991, 323: 1688-95.

[107] Schwindinger, W.F., Franchomano, C.A., Levine, M.A. Identification of a mutation in the gene encoding the alpha subunit of the stimulatory G protein of adenyl cyclase in McCune-Albright syndrome. Proc. Natl. Acad. Sci. U.S.A. 1992, 89(11): 5152-6.

[108] Happle, R. The McCune-Albright syndrome: a lethal gene surviving by mosaicism. Clin. Genet. 1986, 29(4): 321-4.

[109] Bianco, P., Riminucci, M., Majolagbe, A., Kuznetsov, S.A., Collins, M.T., Mankani, M.H., Corsi, A., Bone, H.G., Wientroub, S., Spiegel, A.M., Fisher, L.W., Roby, P.G. Mutations of the GNAS1 gene, stromal cell dysfunction, and osteomalacic changes in non-McCune-Albright fibrous dysplasia of bone. J. Bone Miner. Res. 2000, 15(1): 120-8.

[110] Shenker, A., Weinstein, L.S., Sweet, D.E., Spiegel, A.M. An activating Gs alpha mutation is present in fibrous dysplasia of bone in the McCune-Albright syndrome. J. Clin. Endocrinol. Metab. 1994, 79(3): 750-5.

[111] Idowu, B.D., Al-Adnani, M., O'Donnell, P., Lu, Y., Odell, E., Diss, T., Gale, R.E., Planagan, A.M. A sensitive mutation-specific screening technique for GNAS1 mutations in cases of fibrous dysplasia: the first report of a codon 227 mutation in bone. Histopathology 2007, 50(6): 691-704.

[112] Danon, M., Crawford, J.D. The McCune-Albright syndrome. Ergeb. Inn. Med. Kinderheilkd. 1987, 55: 81-115.

[113] Maira, M., Luzzatto, G., Frezzetti, G., Sertoli, G., Di Stefano, G., Neri, G., Assis, C. An activating mutation in the alpha subunit of the stimulatory G protein in McCune-Albright syndrome type IA. Proc. Natl. Acad. Sci. U.S.A. 1987, 84(12): 7266-9.

[114] Weinstein, L.S., Gejman, P.V., Friedman, E., Kadowaki, T., Collins, R.M., Gershon, E.S., Spiegel, A.M. Mutations of the Gs alpha-subunit gene in Albright hereditary osteodystrophy detected by denaturing gradient gel electrophoresis. Proc. Natl. Acad. Sci. U.S.A. 1990, 87(3): 8267-90.

[115] Patten, J.L., Johns, D.R., Velle, D., El, C., Gruppuso, P.A., Steele, G., Smallwood, P.M., Levine, M.A. Mutation in the gene encoding
the stimulatory G protein of adenylyl cyclase in Albright's hereditary osteodeystrophy. *New Engl. J. Med.* 1990, 322: 1412-9.

[131] Levine, M.A., Downs, R.W., Jr., Moses, A.M., Breslau, N.A., Marx, S.J., Lasker, R.D., Rizzoli, R.E., Aubach, G.D., Spiegel, A.M. Resistance to multiple hormones in patients with pseudohy-poparathyroidism. Association with deficient activity of guanine nucleotide regulatory protein. *Am. J. Med.* 1993, 74: 545-56.

[132] Meguro, G., Marghie, M., Weber, G., De Menis, E., Brunelli, V., Cappia, M., Lolfi, P., Beck-Pecco, P., Spada, A. Growth hormone-releasing hormone resistance in pseudohypoparathyroidism type 1a: new evidence for imprinting of the Gs alpha gene. *J. Clin. Endocrinol. Metab.* 2003, 88(9): 4070-4.

[133] Germain-Lee, E.L., Groman, J., Crane, J.L., Jan de Beur, S.M., Levine, M.A. Growth hormone deficiency in pseudohypoparathyroidism type 1a: another manifestation of multihormone resistance. *J. Clin. Endocrinol. Metab.* 2003, 88(9): 4059-69.

[134] Faust, R.A., Shore, E.M., Stevens, C.E., Xu, M., Shah, S., Phillips, C.D., Kaplan, F.S. Progressive osseous heteroplasia in the face of a child. *Am. J. Med.* 2003, 118(1): 71-5.

[135] Liu, J., Erlichman, B., Weinstein, L.S. The stimulatory G protein-coupled receptor. *Clin. Endocrinol. Metab.* 2003, 88(9): 4336-41.

[136] Silve, C., Santora, A., Breslau, N., Moses, A., Spiegel, A. Selective resistance to parathyroid hormone in cultured skin fibroblasts from patients with pseudohypoparathyroidism type Ib. *J. Clin. Endocrinol. Metab.* 2007, 92(6): 2370-3.

[137] Marra, S., Breslau, N., Moses, A., Spiegel, A. Selective resistance to parathyroid hormone caused by a novel uncoupling mutation in the carboxyl terminus of Gs: A cause of pseudohypoparathyroidism type Ib. *Am. J. Med.* 2003, 105: 640-4.
arathyroidism Type Ib Redefines the Boundaries of a cis-Acting Imprinting Control Element of GNAS. *Am. J. Hum. Genet.* 2005, 76(5): 804-14.

[165] Bastepe, M., Fröhlich, L.F., Linglart, A., Abu-zahra, H.S., Tojo, K., Ward, L.M., Jüppner, H. Deletion of the NESP55 differentially methylated region causes loss of maternal GNAS imprints and pseudohypoparathyroidism type-Ib. *Nat. Genet.* 2005, 37(1): 25-37.

[166] Jan de Beur, S., Ding, C., Germain-Lee, E., Cho, J., Maret, A., Levine, M.A. Discordance between genetic and epigenetic defects in pseudohypoparathyroidism type 1b revealed by inconsistent loss of maternal imprinting at GNAS1. *Am. J. Hum. Genet.* 2003, 73(2): 314-22.

[167] Fröhlich, L.F., Bastepe, M., Ozturk, D., Abu-Zahra, H., Jüppner, H. Lack of Gnas epigenetic changes and pseudohypoparathyroidism type Ib in mice with targeted disruption of syntaxin-16. *Endocrinology* 2007, 148(6): 2925-35.