Inactivating and activating mutations of the Gs alpha gene

A. Spada, G. Mantovani, A. Lania
Institute of Endocrine Sciences, University of Milan, Ospedale Maggiore IRCCS, Via F. Sforza 35-20122 Milano Italy.

Reprint request: A. Spada, address above.
e-mail: anna.spada@unimi.it

INTRODUCTION

Most polypeptide hormones, all monoamine neurotransmitters, prostaglandins and even ions signal their target cells through membrane receptors belonging to a superfamily that shares a common structural and functional motif, i.e. a single polypeptide with seven membrane-spanning domains, and a common transduction mechanism, i.e. coupling to G proteins. Therefore, G proteins play a key role in relaying signals from plasma membrane to intracellular effectors. In the past few years, defects in G protein coupled signal transduction have been identified as cause of endocrine disorders. However, while a number of gain or loss of function mutations of G protein coupled receptors have been identified, to date only one G protein gene, i.e. the gene coding for the alpha subunit of G stimulatory protein (GNAS1), has been identified to harbor naturally occurring mutations causing endocrine disorders [5, 10, 23, 24].

GS PROTEIN STRUCTURE AND FUNCTION

Gs protein is a heterotrimer composed of the three distinct subunits α, β and γ, the α subunit conferring functional specificity and the β and γ being at least partially interchangeable. The Gs α subunit contains high affinity binding sites for the guanine nucleotides GDP and GTP and have intrinsic GTPase activity. The GDP-bound form binds tightly to βγ and is inactive, whereas the GTP-bound α dissociates from βγ and serves as a regulator of the effector, i.e. the adenylyl cyclase enzyme. Hormones that activate Gs coupled receptors cause the exchange of GTP for GDP on the α subunit while the signal deactivation is induced by the intrinsic GTPase activity. Proteins of the Gs class have been defined as ubiquitous activators of all adenylyl cyclase isoforms, whereas their effects on ion channel activity are restricted to selected cell types [21, 29].

ABNORMALITIES OF GS PROTEIN SIGNALING PATHWAYS

As previously demonstrated for nuclear hormone receptors and growth factor receptors, it has been proposed that components of G protein signaling pathways may potentially be involved in the development of human diseases [5, 10, 23, 24]. The abnormal transduction may be due to mutations in the genes encoding either G protein coupled receptors or G proteins or effectors.

The first indication that alterations in the structure of Gs protein could lead to development of disease was suggested by the observation that the Vibrio Cholerae toxin possesses an ADP-ribosyl transferase activity, the target amino acid for this reaction being Arg 201 in the Gsα subunit. The ADP ribosylation of this residue and the subsequent blockade of the intrinsic GTP-ase activity induce the constitutive activation of Gsα, leading to activation of adenylyl cyclase, particularly in intestinal epithelial cells, increased secretion of electrolytes into the bowel lumen and watery diarrhea.

In the past few years, molecular biological approaches have provided important insights into the pathogenetic role of naturally occurring mutations of GNAS1 gene with consequent altered signal transduction. The phenotypic expression of these mutations depend on several determinants; in particular, mutations may occur as germ-line mutations, affecting every cell in which the gene is expressed vs. somatic mutations that lead to focal manifestations of the disease. Moreover, GNAS1 mutations may cause either loss or gain of function, by inactivating or activating signal transduction, leading to the clinical phenotype...
of hormone defect or excess, respectively [5, 10, 23, 24].

**INACTIVATING MUTATIONS OF GNAS1**

**Albright hereditary osteodystrophy and pseudohypoparathyroidism**

In 1942 Fuller Albright described the first hormone resistance syndrome [1] in which hypocalcemia and hyperphosphatemia were due to resistance to PTH action, termed pseudohypoparathyroidism (PHP). Moreover, these patients displayed a constellation of physical features including short stature, centripetal obesity, rounded face, short neck, brachydactyly, subcutaneous ossifications and mental retardation which is now referred to as Albright hereditary osteodystrophy (AHO). Few years later, Albright described a new syndrome, termed pseudopseudohypoparathyroidism (PPHP), that may be present either in kindreds with PHP either as an isolated defect, characterized by the presence of physical features of AHO without any evidence of PTH resistance [2]. The majority of familial PHP are inherited in an autosomal dominant manner [6].

Pseudohypoparathyroidism now refers to a heterogeneous group of disorders differing in several clinical, biochemical and genetic characteristics. Moreover, it is worth noting that many features of AHO are quite unspecific or are present in other disorders, some of which ascribed to specific chromosomal defects, as for the small terminal deletions on chromosome 2 in AHO-like syndrome.

**Pseudohypoparathyroidism type Ia and Pseudopseudohypoparathyroidism**

In 1990 Pattern et al. [18] detected and described the first heterozygous inactivating mutation in the GNAS1 gene, responsible for PHP type Ia in one family. The genetic defect in the majority of patients with PHP Ia and in their relatives with PPHP has been then confirmed by the identification of multiple heterozygous loss of function mutations within this gene [3].

The human GNAS1 gene maps to 20q13 and contains 13 exons (fig. 1). Mutations have been localized in the entire coding region of the gene, with the exception of exon 3 where an alternative splicing site is present. On the other hand, mutations in exon 1 are probably under-estimated, as the extremely GC-rich nature of the flanking sequences has precluded its analysis by many authors. Considering the type of mutations, small insertions/deletions and amino acid substitutions predominate, but nonsense mutations and point mutations that lead to altered translation initiation or aberrant mRNA splicing have also been documented.

An intriguing missense mutation has been identified in two unrelated males who presented with AHO, resistance to PTH and testotoxicosis. This substitution (A366S) leads to constitutive activation of adenylyl cyclase by causing accelerated release of GDP, thus increasing the fraction of active GTP-bound Gsα. However, while this mutant protein is stable at the reduced temperature of the testis, it is thermolabile at 37 °C, resulting in reduced Gsα activity in almost all tissues and AHO phenotype [16].

Although each mutation is usually associated to a single kindred, a mutational hot-spot involving 20% of all mutations so far described in literature (about 60) has

---

*Figure 1: Schematic representation of Gsα gene with the different functional domains. The human GNAS1 gene maps to 20q13 and contains 13 exons. In patients with PHP 1a and PPHP inactivating mutations are localized in the entire coding region of the gene, with the exception of exon 3 where an alternative splicing site is present (dark circles). Somatic activating mutations detected in sporadic endocrine tumors and in the affected tissues from patient with the McCune-Albright syndrome are located in exons 8 and 9, replacing either Arg 201 or Gln 227 (open circles).*
been identified within exon 7. It is a 4-bp deletion which coincides with a defined consensus sequence for arrest of DNA polymerase α, a region known to be prone to sporadic deletion mutations. In most cases it has been found as a de novo mutation, thus representing a recurring new mutation rather than a founder effect. Moreover, four families have been found to carry mutations within exon 5, affecting prolines 115 and 116, while three different insertion/deletions have been found to be clustered at nucleotides 1106-1108 in exon 13, probably representing two new potential mutational hot-spots [15].

In families in which PHP la and PPHP coexist, mutations in GNAS1 can be detected in all the affected members, i.e. members affected with either PHP la or PPHP. Several questions arise when studying these families. Firstly, why does the same GNAS1 mutation possibly lead to presence or absence of hormone resistance in PHP la and PPHP, respectively. Secondly, why do PHP la patients display a resistance to some (PTH, TSH and gonadotropins) but not all hormones that activate the Gs-coupled pathway. The observation that only maternal transmission of GNAS1 mutations leads to PHP la while paternal transmission of the same mutations is associated to PPHP in the offsprings suggested that GNAS1 gene may be subject to genomic imprinting, an epigenetic phenomenon by which one allele undergoes a partial or total loss of expression [28]. Indeed, GNAS1 in the mouse (Gnas1) maps within a region on distal chromosome 2 presumed to have more than one imprinted genes.

Recent studies on the GNAS1 locus in the human confirm that this region is extremely complex, with multiple alternatively spliced transcripts encoding multiple protein products (the Gsα protein, the extra large XLas, the neuroendocrine secretory protein NESP55, a nontranslated transcript deriving from Exon 1A and a poly-adenylated antisense transcript) that are oppositely imprinted. In particular, XLas is expressed from the paternal allele, while NESP55 is expressed from the maternal allele. In contrast with previous negative results, recent studies demonstrate that in specific endocrine tissues also Gsα is imprinted, its transcription mainly deriving from the maternal allele. In particular, a predominant maternal origin of Gsα has been observed in human thyroid, gonad and pituitary [8, 13]. These data support the hypothesis that GNAS1 imprinting is the potential mechanism responsible for resistance to the action of specific hormones in patients with GNAS1 mutation. In accordance with the predominant maternal origin of GNAS1 transcript in the pituitary, it has been recently reported that patients with PHP type 1a also have resistance to GHRH action, resulting in GH deficiency [14].

### Pseudohypoparathyroidism Ib

PHP Ib refers to a condition characterized by resistance to PTH in the absence of AHO phenotype. The defect is usually sporadic but occasionally is familial, with a pattern of transmission consistent with an autosomal dominant one. Linkage analysis has recently permitted to map the genetic locus for PHP Ib to a small region of chromosome 20q13.3, where GNAS1 is located, in four unrelated families [9]. Analysis of a region upstream of the Gsα promoter point to the biallelic expression of exon 1A, normally expressed only from the paternal allele, as pathogenetic mechanism of PHP Ib. Therefore, PHP Ib would be associated with an abnormal expression of exon 1A, leading to decreased Gsα expression in renal proximal tubules. The involvement of other target tissues in which Gsα only derives from the maternal allele in patients with PHP 1b is still unclear. However, recent studies report a slight increase in TSH levels, consistent with a certain degree of hormone resistance.

### Pseudohypoparathyroidism Ic

This term refers to a small subset of patients with all the clinical and biochemical features of PHP la (generalized hormone resistance and AHO), without evidence of reduced Gsα activity and GNAS1 mutations. The molecular defect responsible for this disease, that may involve any component of the proximal cAMP pathway (adenyl cyclase, Gi, phosphodiesterases), has not been established yet.

### Pseudohypoparathyroidism II

Patients affected with PHP II show clinical evidence of PTH resistance with a normal urinary cAMP response to TSH treatment. This condition is usually sporadic but occasionally familial, with a pattern of transmission consistent with an autosomal dominant one. Linkage analysis has recently permitted to map the genetic locus for PHP II to a small region of chromosome 20q13, where GNAS1 is located, in four unrelated families [9]. Analysis of a region upstream of the Gsα promoter point to the biallelic expression of exon 1A, normally expressed only from the paternal allele, as pathogenetic mechanism of PHP Ib. Therefore, PHP Ib would be associated with an abnormal expression of exon 1A, leading to decreased Gsα expression in renal proximal tubules. The involvement of other target tissues in which Gsα only derives from the maternal allele in patients with PHP 1b is still unclear. However, recent studies report a slight increase in TSH levels, consistent with a certain degree of hormone resistance.

**Figure 2:** Genomic organization of human GNAS1 locus. The figure shows 4 alternative first exons that splice into exon 2 generating 4 different transcripts, the Gsα from exon 1 and the extra large XLas, the neuroendocrine secretory protein NESP and a nontranslated gene product from Exon 1A. XLas and untranslated Exon 1A are expressed from the paternal allele, NESP is expressed from the maternal allele while Gsα is biallelically expressed in most tissues and prevalently maternally expressed in specific endocrine organs (pituitary, thyroid, gonads).
the injection of exogenous PTH but a blunted phosphaturic response to the same hormone, thus indicating a defect distal to cAMP production in the PTH-mediated transduction pathway. Until now, there is no evidence of the specific alterations responsible for this disorder.

It has also been hypothesized that in most cases PHP II may be an acquired defect secondary to vitamin D deficiency, as suggested by the observation that calcium and vitamin D replacement is able to normalize the phosphaturic response to PTH in these patients.

**ACTIVATING MUTATIONS OF GNAS1**

The first clue of the possible existence of activating mutations of G protein genes as cause of human neoplasia arose from the identification of a subset of GH-secreting pituitary adenomas characterized by high levels of in vitro GH release, intracellular cAMP accumulation and membrane adenylyl cyclase activity [25]. The subsequent analysis of DNA from these tumors revealed amino acid substitutions in exons 8 and 9, replacing either Arg 201 with Cys or His or Ser, or Gln 227 with Arg or Leu that are the only location for mutations so far identified. When transfected into S49 cyc cells, mutant Gsα showed a 30-fold decrease in intrinsic GTPase activity, consistent with the notion that both residues are known to be important in GTP hydrolysis [10]. Since somatotrophs belong to a set of cells that recognizes cAMP as a mitogenic signal, Gsα may be considered the product of a proto-oncogene that is converted into an oncogene, designated gsp (for Gs protein) in selected cell types.

Studies on cell lines transfected with mutant Gsα yielded important insights into the series of events resulting from the activation of cAMP cascade. Indeed, the transcription of a variety of common cAMP-responsive genes, including the immediate early genes such as c-fos, c-jun and jun B, are enhanced by the expression of mutant Gsα. Moreover, mutant Gsα stimulates GH and PRL promoter activity in GH3 cells expressing this protein. As far as the mitogenic effect of gsp mutations is concerned, the introduction of mutant Gsα results in enhanced function and growth of selected cell types in which cAMP cascade activates proliferation processes.

Although these results suggest that the expression of mutationally activated Gsα is sufficient to bypass the requirement for the specific growth factor and promotes autonomous cell growth of specific cell types, most of these effects were observed only when cAMP hydrolysis was blocked by phosphodiesterase (PDE) inhibitors, raising the question whether this mutation may be oncogenic in vivo [11, 19].

**gsp oncogene in pituitary adenomas**

Several screening studies confirmed that approximately 30-40% of GH-secreting adenomas is associated with gsp mutations, that most frequently replace Arg 201 with Cys. These mutations are somatic in origin as indicated by the presence of wild type Gsα in the peripheral blood leukocytes from affected patients and dominant, as indicated by the presence of both mutant and wild type Gsα in genomic DNA from the tumor.

Several in vivo studies indicate no difference in clinical features, duration of the disease or cure rate in patients with or without gsp mutations. However, tumors expressing gsp mutations are most frequently very small in size, consistently with an hypersecretory activity of tumoral somatrophs. Secretory patterns generally observed in patients with gsp positive tumors are the lack of serum GH increase by GHRH administration and a high sensitivity to the inhibitory action of somatostatin analogues [22].

**gsp oncogene in thyroid neoplasia**

Following the identification of gsp mutations in GH-secreting adenomas, mutations involving the same two hot-spots of GNAS1 gene have been identified in hyperfunctioning thyroid adenomas [17]. This finding is consistent with the key role of the cAMP pathway in mediating TSH action on both thyroid hormone secretion and thyrocyte proliferation. The frequency of gsp mutations in thyroid hot nodules is variable from one series to another, ranging from 5 to 30%, and is definitely lower than that of TSH receptor gene mutations. As it occurs in GH-secreting adenomas with gsp mutations, the phenotype of thyroid adenomas carrying mutant Gsα or TSH receptor is similar to that observed in tumors with wild-type proteins.

Mutant Gsα may also be present with low frequency (<10%) in hypofunctioning thyroid adenomas (cold nodules) as well as in differentiated thyroid adencarcinomas. In particular, gsp mutations were detected in a subset of papillary and follicular carcinomas selected on the basis of high adenylyl cyclase activity in basal conditions not further stimulated by TSH. No gsp mutations have been detected in anaplastic carcinoma. The data collected from the different studies indicate that whereas gsp oncogene may be considered as an initiator for a minority of hyperfunctioning thyroid adenomas, its role in thyroid tumorigenesis is much less certain.

**gsp oncogene in the McCune-Albright syndrome**

The McCune-Albright syndrome (MAS) is a sporadic disorder characterized by polyostotic fibrous dysplasia,
càfe-au-lait skin hyperpigmentation and autonomous hyperfunction of several endocrine glands, such as gonads, pituitary, thyroid and adrenal cortex, i.e. glands sensitive to trophic agents acting through cAMP dependent pathway. Mutations of the GNAS1 gene have been detected in all affected subjects and Arg 201 is the only location so far reported. Mutant Gsα is expressed in the affected endocrine and non endocrine organs, the highest proportion of mutant alleles being found in regions of abnormal proliferation [20, 27]. This mosaic distribution is consistent with the hypothesis that this syndrome is due to a somatic mutation in Gsα gene occurring as an early postzygotic event. Therefore, the time of occurrence of GNAS1 mutations seems to be an important factor in determining the nature of the disease. Due to the ubiquitous expression of Gsα, late occurring mutations cause focal disease such as acromegaly and toxic thyroid adenomas, while when the same mutations occur very early in embryogenesis they cause disorders with widespread manifestations, such as the McCune Albright syndrome. It is tempting to speculate that activating germ-line mutations of Gsα would be incompatible with life.

Recent studies have provided insights into the pathological role of mutant Gsα in non-endocrine organs involved in MAS. On this line, it has been shown that melanocytes from the café-au-lait spots of MAS patients have high mRNA levels of tyrosinase gene, probably responsible for alteration in skin pigmentation. As far as fibrous dysplasia is concerned, high levels of c-fos expression, presumably a consequence of increased adenylyl cyclase activity, have been detected in the bone lesions from all MAS patients studied, consistent with the bone disorders present in transgenic mice overexpressing c-fos proto-oncogene. Moreover, transplantation of skeletal progenitor cells obtained from fibrous dysplastic marrow of patients with MAS into immunocompromized mice caused abnormal ossicle formation, resembling human fibrous dysplasia. Interestingly, the lesion development required the coexistence of normal cells and cells with a mutant allele, thus reproducing the mosaic distribution of Gsα mutations that characterizes the syndrome. Finally, substitutions at Arg 201 of GNAS1 gene have also found in isolated fibrous dysplasia occurring outside of the context of typical MAS [4].

**gsp oncogene in other endocrine disorders**

Other endocrine organs have been screened for gsp mutations since they contain cell types in which cAMP is a positive growth stimulus, such as the endocrine pancreas, the parathyroid, the gonads and the adrenal gland. No Gsα mutations has been so far identified in hyperfunctioning neoplasia from the pancreas and the parathyroid [26]. By contrast, Arg 201 to Cys substitution have been detected in a significant proportion (4 out of 6) of ovarian and testicular stromal Leydig cell tumors, that had caused hormonal hypersecretion resulting in virilization and gynecomastia in female and male patients, respectively [7]. Similarly, gsp mutations are present in a subset of hyperfunctioning adrenal adenomas. As reported for other endocrine neoplasia with gsp mutations, there was no evidence of clinical or hormonal differences between patients with gsp positive and negative tumors.

**CONCLUSIONS**

It is well established that proteins involved in signal transduction may be target for naturally occurring mutations resulting in human diseases. To date, GNAS1 is the only gene encoding a G protein that has been identified as target for mutations that unequivocally cause endocrine diseases. Indeed, inactivating germ line mutations of this gene cause AHO and pseudohypoparathyroidism while activating somatic mutations lead to the proliferation of endocrine cells in which cAMP is a mitogenic signal. Although in the last years several screening studies have detected the presence of new inactivating or activating mutations of GNAS1 gene and established their prevalence in the different diseases, several questions arise when studying the genotype/phenotype relationships. In particular, why do apparently identical Gsα deficiency associated to the same GNAS1 mutation lead to the presence or absence of generalized hormone resistance, why is the resistance limited to some hormones, i.e. PTH, TSH, gonadotropins and GHRH and why are the resulting hormone defects variable in entity and age of appearance? Activating mutations of GNAS1 gene would in principle confer growth advantage in the selected cell types in which cAMP acts as a mitogenic signal, and on this basis these mutations were referred to as gsp oncogene. However, studies of neoplasia carrying this oncogene failed to detect differences in the clinical and hormonal phenotypes, probably reflecting the existence of mechanisms able to counteract the activation of the cAMP pathway, that are still insufficiently understood. Finally, the identification of naturally occurring mutations of G proteins has already had major implications for understanding the structure and function of these signaling proteins. Unfortunately, the implications of identifying G protein mutations for diagnosis and treatment of endocrine disorders are, as yet, rather limited.
Inactivating and activating mutations of the Gs alpha gene

REFERENCES


