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Genetic and Molecular Aspects of McCune-Albright Syndrome

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Abstract

McCune-Albright syndrome (MAS) is characterized by the clinical triad of polyostotic fibrous dysplasia, café-au-lait pigmented skin lesions and endocrinopathy (1,2). The molecular lesion in MAS is a postzygotic mutation in the GNAS gene that leads to activation of G5α, the alpha chain of the heterotrimeric G protein, G5α. Cells that carry the activating mutation are distributed in a mosaic pattern. A clinical diagnosis of MAS can be made when a patient is found to have at least two features of the classical triad (3). Because of the restricted pattern of distribution of the GNAS mutation, termed gsp, initial molecular analyses were limited to lesional tissue, but recent techniques such as peptide nucleic acid clamping have improved the sensitivity of current assays and now enable the detection of gsp mutations in circulating cells from many patients with MAS.


Key words: McCune-Albright; G Protein; Adenyllyl Cyclase; Peptide Nucleic Acid; Cyclic AMP

Introduction

The McCune-Albright syndrome (MAS) is characterized by the clinical triad of polyostotic fibrous dysplasia, café-au-lait pigmented skin lesions and endocrinopathy (1,2). MAS is a sporadic genetic disorder, but it is not inherited. A mutation in the GNAS gene that encodes the α-subunit of the stimulatory G protein of adenyl cyclase, G5α, is present in affected tissues of patients with MAS. The mosaic distribution of cells that contain the GNAS mutation is consistent with the hypothesis that MAS results from a somatic mutation that occurs during early embryogenesis. The GNAS mutation, termed gsp, results in constitutive (i.e., ligand-independent) activation of G5α and in turn stimulation of adenyl cyclase and is the molecular basis for autonomous function and hyperplasia of the affected cells. The restricted pattern of distribution of these mutations has presented a challenge to molecular analyses, as few cells are affected and even lesional tissue (endocrine tissues, pigmented skin, fibrous dysplasia) contains an admixture of normal and abnormal cell populations. In this chapter we shall discuss the molecular pathophysiology of MAS as well as recent advances in the polymerase chain reaction (PCR) that have enhanced the sensitivity of the technique to make it possible to detect gsp mutations in small samples of lesional tissue or even in circulating cells (4).

Molecular Pathogenesis

Identification of GNAS Mutations: Insight into the pathogenesis of MAS came from early laboratory studies that indicated that replacement of either arginine201 or glutamine227 of G5α inhibits the intrinsic GTPase activity and results in constitutive activation of adenyl cyclase and increased production of cAMP (5-9) initially described in a subset of human growth hormone secreting pituitary tumors that exhibited increased adenyl cyclase activity in vitro in the absence of added growth hormone releasing hormone. The molecular basis for constitutive activation of adenyl cyclase in these somatotropic tumors was subsequently identified as an oncogenic form of G5α, termed gsp, that lacked GTPase activity due to somatic mutations in GNAS that led to the replacement of either arginine201 or glutamine227 (10,11). The gsp protein lacks GTPase activity. Arg201 is the site for covalent modification by cholera toxin, while Gln227 is analogous to
the Gln that is commonly replaced in the oncogene p21. Replacement of either Arg207 or Gln227 inhibits the intrinsic GTPase activity of p21 and leads to constitutive (i.e., ligand-independent) activation of adenyl cyclase. Increased intracellular cAMP may account for both autonomous function and proliferation of cells expressing the mutation (Figure 1). Missense mutations in codon 201 of the GNAS gene (gsp) encoding the α chain of the stimulatory heterotrimeric Gα, G-protein, Gs lead to constitutive activation of GSs by inactivating the intrinsic GTPase. Hormone binding to a heptadecil receptor leads to a conformation change in the GS heterotrimer that facilitates exchange of bound GDP for GTP on the β chain. The binding of GTP to Gs leads to the dissociation into free Gsα and βγ dimers, with Gsα (and in some cases βγ dimers) activating adenyl cyclase, leading to synthesis of cAMP. Under normal conditions, the intrinsic GTPase of the α chain would hydrolyze GTP to GDP, with subsequent dissociation of GSs from adenyl cyclase and re-association of the heterotrimeric GSs. The gsp mutation is unable to hydrolyze GTP and thus remains in a state of persistent activation. In susceptible cells, the continuous and unregulated, production of cAMP leads to increased cellular proliferation and/or hormone secretion, thus producing the features of McCune-Albright syndrome.

These gsp mutations occur in approximately 40% of somatotrophic tumors and may distinguish a subset of tumors that are more sensitive to inhibition of growth hormone secretion by somatostatin analogs (12,13). In addition to growth hormone secreting pituitary tumors, gsp mutations are also present in a small number of ACTH-secreting pituitary tumors (12,14) a subset of thyroid neoplasms and testicular and ovarian stromal leydig tumors (15), but are rare in other endocrine tumors. Moreover, gsp mutations have been described in ovarian cysts that cause sexual gonadotropin-independent precocious puberty (16,17) and in isolated fibrous dysplasia of the bone (18).

Similar mutations in Arg201 of GNAS in patients with MAS have been identified in DNA from surgical and autopsy specimens of endocrine tumors (19), café-au-lait pigmented skin lesions (20), fibrous dysplasia of bone (21) and in peripheral blood cells (4). Each patient had a single mutation in GNAS, either Arg201-Cys, His, or rarely Leu (4). To date no mutations in Gln227 have been reported in patients with MAS. The gsp mutation was present in some but not all of tissues examined and in many cases, the gsp mutation was present in abnormal portions of a tissue but not in histologically normal portions of the same tissue. Even more remarkably, gsp alleles may be present in only cell types within tissues that are derived from different embryological precursors. For example, a 3 year old male MAS patient with macro-orchidism but no precocious puberty was recently reported to have a R201H gsp allele present only in Sertoli cells, resulting in isolated Sertoli cell hyperplasia, evidenced by increased AMH expression and cell hyperplasia leading to prepubertal macro-orchidism. There were no signs of Leydig cell activation and no evidence of excess androgen action (22,23)

The different early embryologic origin of precursors contributing to Sertoli and Leydig cell lineages may underlie the differential existence of the mutated GNAS gene. These observations are all consistent with somatic mutation of the GNAS gene as a postzygotic event that occurs during early embryogenesis, with mosaic distribution of mutation-bearing cells following a developmental pattern.

Genetic Modulation - Imprinting of GNAS

GNAS is a highly complex locus that achieves tremendous transcriptional plasticity through the use of alternative exons, generation of antisense transcripts and reciprocal imprinting (24,25) GNAS encodes at least six transcripts, four of which utilize alternative first exons that are spliced to exons 2-13; the complexity of each of these transcripts is increased by variable inclusion of exon 3 and by use of an alternative 3'-UTR (26). Transcripts starting with exon 1 encode Gsa, the stimulatory G-protein of adenyl cyclase and are expressed from both the maternal and paternal alleles in most tissues. However, in some cells (e.g. renal proximal tubule cells, thyroid follicular cells and pituitary somatotrophs) there is preferential expression of the maternal allele (6,27) Approximately 2.5 kb upstream of exon 1, an alternative exon 1A is expressed exclusively from the paternal allele. Transcripts beginning with exon 1A are probably untranslated (28). Transcripts beginning approximately 38kb upstream from
Genetics of McCune-Albright Syndrome

exon 1 are expressed exclusively from the paternal allele and encode two distinct proteins with extensively overlapping reading frames, XLαs and ALEX. XLαs shares carboxyl terminal sequences with Gαs but has a larger amino terminal end encoded by the alternate first exon. XLαs is enriched in the golgi of neuroendocrine tissues and functions in G-protein coupled signal transduction (29). A recent study has shown that gsp mutations in XLαs can affect signal transduction in vitro (30), however a role for gsp mutations in XLαs in human disease has yet to be defined. ALEX is encoded by a shifted reading frame in the same transcripts that encode XLαs and thus shares no protein homology with XLαs or Gαs (31). Finally, a transcript with an alternative first exon approximately 52 kb upstream of exon 1, is expressed exclusively from the maternal allele and encodes the neurosecretory protein NESP55 (32). NESP55 is a chromogranin-like protein found in secretory granules and shares no protein homology with Gαs. Activating mutations in Arg201 of Gαs would also be present in XLαs, but not in NESP55 or ALEX.

One current model that has been proposed to explain the tissue specific imprinting of GNAS implicates the differential methylation of the maternal allele of exon 1A as a mechanism to inhibit binding of a tissue specific repressor protein. Thus, the repressor protein can bind only to the paternal allele and silences its expression, but only in those cells that express the repressor protein (25).

Imprinting may play a role in the pathogenesis of MAS. Theoretically, in those cells in which Gαs is expressed predominately if not exclusively from the maternal allele, only somatic mutations of the maternal allele will have pathophysiological consequences. This is the case for sporadic GH-secreting pituitary adenomas as well as patients with MAS who have GH-secreting pituitary adenomas, where activating mutations of Gαs are almost always found to be on the maternal allele (33,34). By contrast, the involvement of other endocrine organs in MAS patients is not associated with a particular parent specificity, as precocious puberty and hyperthyroidism are present in patients with mutations on either the maternal or the paternal allele (34). This would be consistent with full (or even some) expression of the paternal GNAS alleles in these cells.

Molecular Pathogenesis

Taken in context, it seems reasonable to conclude that the clinical and endocrinological expression of MAS will be determined by several key variables:

1. Timing of the gsp mutation. The number of tissues in which the gsp is present and the proportion and distribution of affected cells in a tissue, will be determined by the precise stage in development in which the mutation occurred. Thus, mutational events that occur later in embryogenesis are likely to give rise to fewer mutant cells and a milder phenotype, than mutational events that occur very early. By contrast, acquisition of a gsp mutation months or even years after birth could explain the development of a solitary endocrine tumor or a single fibrous dysplasia lesion in some patients.

2. The variable ability of cAMP to induce proliferation in different cells. Constitutive activation of Gαs, with increased adenyl cyclase activity, will produce the most clinically obvious consequences in those tissues in which cAMP stimulates cellular proliferation and/or hormone secretion rather than differentiation. Cyclic AMP is not mitogenic in all cell types and in some cell types cAMP can actually inhibit growth. Moreover, even in cells in which cAMP is a strong growth stimulator, changes in the expression of other genes (13) or induction of counter-regulatory responses (such as increased expression of tissue-specific forms of cAMP phosphodiesterase (18,35-39)) could mitigate or even reverse the effects of the gsp oncogene.

3. Parental origin of the GNAS allele that carries the gsp mutation. Mutations that occur on the maternal allele will be expressed in all tissues, including those tissues (e.g. somatotrophs and thyroid follicular cells) in which the imprinting mechanism suppresses expression of the paternal GNAS allele. Thus, MAS patients who have maternal allele gsp mutations might have a more severe or widespread phenotype than patients who have paternal gsp mutations, as a gsp mutation on a paternal allele will not be expressed in all cells (see above).

Molecular Diagnosis

Detection of gsp in Lesional Tissue

The mosaic pattern of distribution of cells bearing the GNAS mutation and the variable number of affected cells in a tissue, makes it technically difficult to detect mutant GNAS alleles, which may represent only a small proportion of the GNAS alleles present in a DNA sample. The gsp mutation can be most readily identified using DNA from affected tissues from patients with MAS (19,20,35,40). These mutations are present in abnormal portions of a tissue but not in histologically normal portions of the same tissue, consistent with the notion that somatic mutations in the GNAS gene arise during early embryogenesis with mosaic distribution of mutation-bearing cells. Detection of a gsp mutant in DNA samples can be greatly enhanced by protocols that employ multiple rounds of nested PCR in conjunction with restriction endonuclease treatment to digest wild type products, thus enriching the relative abundance of mutant alleles as PCR targets for selective amplification (41,42). However, restriction endonuclease digestion does not enrich for all mutant alleles and overall the technique has variable sensitivity (Table). As an alternative to restriction endonuclease digestion to reduce the abundance of PCR targets from wild type GNAS alleles, Bianco et al. included a peptide nucleic acid (PNA) in the PCR to block amplification of wild type GNAS targets (43). PNA is a nucleic acid analog in which the sugar phosphate backbone of natural nucleic acid has
been replaced by a synthetic peptide backbone usually formed from N-(2-amino-ethyl)-glycine units, resulting in an achiral and uncharged mimic. It is chemically stable and resistant to hydrolytic cleavage. PNA is capable of sequence-specific recognition of DNA (and RNA) and the hybrid complexes exhibit extraordinary thermal stability and unique ionic strength effects. To enhance the ability of PCR to detect gsp, the PNA is designed to bind specifically to the wild type sequence at Arg201 and to overlap with the annealing site for one of the two oligonucleotide primers used to amplify the DNA (Figure 2). Thus, inclusion of the PNA during PCR selectively inhibits amplification of the wild type allele and enhances sensitivity. Using the PNA-clamping technique enabled Blanco et al. to detect gsp mutations in DNA samples from lesional tissue from patients with MAS that were not detectable by standard PCR (43). Similarly, Kaila et al. (44) directly compared the sensitivity and ease of use of nested PCR and PNA-clamping to examine 148 DNA samples from 88 patients with symptoms compatible with MAS. DNA samples were obtained from peripheral blood, ovarian tissue, or cyst liquid and bone lesions. The nested PCR method required 4 days and PNA clamping required 1.5 days. The sensitivity of mutation detection was 54% (n=80) for nested PCR and 46.6% (n=69) for PNA (P>0.05). Importantly, the 11 cases where PNA failed to detect the mutation were mainly incomplete and atypical clinical forms of MAS (n=10/11). PNA clamping was more rapid, reliable and economical than nested PCR.

More recently, Karadag et al. (45) used labeled PNA hybridization probes and fluorescence resonance energy transfer (FRET) to develop a PCR method that allows for the direct and rapid quantification of gsp alleles with a sensitivity that allows detection in tissues that contain as few as 5% mutant cells.

In the scenario depicted in the upper panel of figure 2, the perfectly complementary PNA will preferentially bind to the target wild-type genomic DNA, thus preventing the polymerase chain reaction primer from annealing. This effectively inhibits amplification. Conversely, in the scenario depicted in the lower panel, the PNA probe will not bind to the target DNA because of an internal mismatch in the Arg201 codon, thus permitting the polymerase chain reaction primer to anneal. The result is amplification of the mutant GNAS amplicon [reproduced with permission from Li et al. (4)]

Detection of gsp in Peripheral Blood

While analysis of DNA from lesional tissue affords great sensitivity, it is neither practical nor expedient to biopsy affected tissue(s) in all patients. Candelieri et al. found mutations in the peripheral blood in 7 of 8 patients with fibrous dysplasia or clinical MAS using nested PCR and restriction endonuclease digestion (Table) (41,46). The ability of PNA-clamping to enhance the sensitivity of PCR analysis of gsp mutations suggested to us that this technique might be suitable for analysis of peripheral blood samples (4,47). We detected mutations in DNA prepared from peripheral blood in 11 of 13 patients with fibrous dysplasia or MAS using PNA clamping, but in only 3 of 13 patient samples without PNA clamping (Table) (4). PNA-clamping provides significant enhancement over standard PCR and is an alternative to nested PCR schemes in the detection of gsp mutations. Compared to nested PCR, the PNA-clamping technique takes less time to perform and requires fewer technical steps and thus is likely to be a less expensive procedure with similar sensitivity. All of these techniques will require additional refinement and further development, however, before they can be considered as standard diagnostic tests and at the present time no molecular technique is offered as a test for MAS in a commercial reference laboratory.

Limitations of Molecular Diagnosis

In our study examining mutations detected with PNA clamping we were still unable to find mutations in 2 of 13 (15%) of MAS patients, indicating a sensitivity of only 85% (4). Others have reported even lower sensitivity for molecular testing of

<table>
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<tr>
<td>PNA Clamping</td>
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<td>+</td>
<td>35-85%</td>
<td>+</td>
<td>0.05% Mutant Alleles (4)</td>
<td>4, 44, 47</td>
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<td>Nested PCR with Restriction Endonuclease</td>
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<td>+</td>
<td>37-88%</td>
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<td></td>
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<td>FRET with PCR</td>
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<td>-</td>
<td>23%</td>
<td>+</td>
<td>2.5% Mutant Alleles (49)</td>
<td>45</td>
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Table. Comparison of Molecular Techniques to Detect gsp Mutations
DNA isolated from circulating cells (Table). Patients with apparently isolated FD have a high likelihood of having clinically unsuspected MAS (47), but the detection of a gsp mutation in circulating cells from a patient with FD or an isolated endocrinopathy [e.g., gonadotropin-independent precocious puberty secondary to ovarian cysts (15-17)] does not necessarily imply that the patient has the complete form MAS. Additional studies and clinical interpretation will be needed to distinguish between these alternatives. The presence of café au lait skin lesions and endocrine abnormalities in MAS or the presence of extensive FD enhances the likelihood that their cells with gsp mutations will be present in the circulation, but these cells could be leukocytes with a mutant GNAS allele and/or cells shed from affected tissues.

By contrast, identification of a gsp mutation can distinguish between FD lesions and lesions that resemble FD, such as osteofibrous dysplasia (42).

Conclusion

The diagnosis of MAS remains a clinical one and requires a careful integration of physical findings, biochemical evaluation and radiological examination. Specific GNAS gene mutations are detectable in DNA from affected tissues and in many cases peripheral blood cells and can confirm a clinical diagnosis of MAS. However, similar gsp mutations are present in many patients with sporadic lesions that are otherwise found in MAS (e.g., isolated FD and solitary endocrine adenomas) and the molecular techniques for detection of the gsp mutation are not currently offered by clinical reference laboratories. The growing understanding of the diverse presentation of MAS now compels a more thorough evaluation of patients who present with only FD or café-au-lait pigmented skin lesions, as some of these patients will be found to have endocrinopathies that are associated with MAS. Recognition of the molecular basis of MAS and careful analysis of the phenotype has led to the appreciation that MAS involves many other tissues, including liver, heart, pancreas and thymus. This has enabled recognition of a severe form of the disease that is fatal in infancy or early childhood. Finally, the genetic basis for MAS, mosaicism of a somatic gsp mutation, provides new insights into the role of imprinting as a modulator of human disease.

Disclosure

The authors have no conflict of interest to declare related to this manuscript.

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