**DIFFICULT CASE**

**A Novel, Complex Heterozygous Mutation Within Gsα Gene in Patient with McCune-Albright Syndrome**

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McCune-Albright syndrome (MAS) is caused by embryonic somatic mutations leading to the substitution of His or Cys for Arg at amino acid 201 of the α-subunit of the signal transduction protein Gs (Gsα). The mutations have been found in many affected tissues of patients with MAS. Recently, a new missense mutation was detected in a patient with MAS, leading to the substitution of glycine for arginine at amino acid 201 of the Gsα gene, whereas no mutations have been reported at other sites in this gene. In the present study, we identified the activating mutations in the gene encoding Gsα protein in the osseous lesions of fibrous dysplasia and peripheral blood leukocyte in a 17-yr-old male patient with MAS. In addition, a heterozygous mutation encoding substitution of Arg201 of Gsα with His was found. Interestingly, we also found the other two types of mutations within the Gsα gene in the patient’s affected osseous tissue. One is a combination mutation in the same allele at codons 209 and 210 of the Gsα gene, and the other the missense mutation at codon 235.

**Key Words:** McCune Albright syndrome; Gsα gene; gene mutation.

**Introduction**

First reported by McCune (1) in 1935 and shortly thereafter by Albright et al. (2), McCune-Albright syndrome (MAS) is an uncommon clinical disorder characterized by the tetrad of polyostotic fibrous dysplasia, cutaneous hyperpigmentation, and some hyperfunctional endocrinopathies, such as sexual precocity, GH excess, hyperthyroidism, and hypercortisolism (3–5).

The pathogenesis of this peculiar syndrome affecting multiple systems has remained unknown for many years. By examining a 4-yr-old female patient and reviewing the photographs published in the literature, Happle found that the cutaneous pigmentation displayed a typical segmental distribution frequently following the lines of Blaschko, reflecting an underlying mosaicism. He proposed that MAS resulted from a postzygotic somatic cell mutation (6,7).

Recent evidence has shown that the clinical manifestations of MAS are caused by embryonic somatic mutations leading to the substitution of His or Cys for Arg at amino acid 201 of the α-subunit of the signal transduction protein Gs (Gsα) (8,9). The mutations in the gene coding for the Gsα protein have been found in many affected tissues of patients with MAS, including the skin, bone, pituitary, thyroid, adrenal gland, testis, ovary, and other nonendocrine tissues (8–14). The mutations inhibit the guanosine-5’-triphosphatase (GTPase) activity of Gsα, and adenylate cyclase is constitutively activated. The clinical characteristics are postulated to be caused by autonomous signaling in tissues, such as those of endocrine organs, which express a Gsα-linked signaling mechanism. Identification of Gsα mutations may help to define a more complete clinical spectrum of MAS.

In the present study, we identified the activating mutations in the gene encoding Gsα gene in the osseous lesions of fibrous dysplasia and peripheral blood leukocyte in a 17-yr-old male patient with MAS, and we found an unusual complex of heterozygous mutations in the Gsα gene in this patient.

**Results**

**Case Report**

The patient, a 17-yr-old Chinese male high school student, had been suffering from recurrent pathologic fractures following minor traumas. At the age of 12, he sought medical attention for chronic nasosinusitis and a diagnosis of polyostotic fibrous dysplasia was made by radiograph. Pubertal development was normal. His parents are nonconsanguineous and of average height, and there is no family history of similar bone diseases or any endocrinopathy. Before age 14 he was of average height for his age group. During the past 3 yr, his height has increased at a rate of 10 cm/yr, and he has become much taller than his peers. On...
physical examination, the patient’s height was 182 cm and weight was 70 kg. Multiple, large café-au-lait lesions with irregular borders were present over the skin and mucosa of the labium maxillae and labium inferius oris (Fig. 1A–B). His face was slightly asymmetric as a result of prominent right ossa malare.

Radiologic examination showed multiple bone lesions characteristic of polyostotic fibrous dysplasia in the humera, femurs, ilia, ribs, and skull (Fig. 1C). 99mTc-MDP scanning of the skeleton revealed high radioactive uptake at multiple areas of bone lesions (Fig. 1E). Computed tomography (CT) and magnetic resonance imaging (MRI) of the brain demonstrated bony frontotemporal overgrowth affecting ethmoidal and sphenoidal bones. The pituitary was normal (Fig. 1D). Visual fields appeared normal. Histologic examination of bone showed fibrous dysplasia (Fig. 2).

Serum and urinary concentrations of phosphate and calcium were normal. Serum alkaline phosphatase was elevated to 1942 IU/L (reference value: 42–121 IU/L), and urinary pyridol was elevated to 19.9 nmol/mmol of urinary creatinine. Plasma concentrations of levorotatory thyroxine (T4), triiodothyronine (T3), triiodothyronine (T3), free T4, free T3, thyroid-stimulating hormone (TSH), cortisol, adrenocorticotropic hormone (ACTH), testosterone, E2, follicle-stimulating hormone, luteinizing hormone, and leptin were normal. Serum levels of growth hormone (GH) (15.3 and 14.3 ng/mL; normal range: 0–10 ng/mL), parathyroid hormone (PTH) (75.3 and 238 pg/mL; normal range: 13–53 pg/mL), and prolactin (PRL) (19 ng/mL; normal range: 2.9–17.1 ng/mL) were slightly elevated.

**Polymerase Chain Reaction**

**Amplification and DNA Sequencing**

Amplification of genomic DNA with primers produced a 375-bp fragment that included exons 8 and 9 of the Gsα gene. The bands were purified using the Qiaquick Gel Extraction Kit (Qiagen) according to the manufacturer’s recommendation and subcloned to PGM-T easy vector (Promega, Madison, WI). Ten and four clones were selected from osseous tissue and leukocytes, respectively, and sequenced by BigDye Terminator Cycle Sequencing Ready Reaction Kits (Perkin-Elmer) on an ABI 377 DNA sequencer.

The results revealed that three clones from the patient’s affected bone and one clone from leukocytes presented a single C-to-T transition within the codon for Arg201. This mutation changes the normal CGT to TGT and results in the substitution of cysteine for arginine (Arg201 → Cys201). The DNA sequence analysis of the other clones revealed the wild-type sequence at this position (Fig. 3A). Interestingly, two types of mutations were newly found within the Gsα gene amplified from the patient’s affected osseous tissue. One type consisted of the combination mutation in the same allele at positions 209 and 210 of the Gsα gene. Sequencing showed the presence of a single A-to-G and C-to-T transversion in the same clone at positions 209 and 210, respectively (Fig. 3B), leading to Glu209Gly and Thr210Ile substitution. The other was a mutation at position 235 with a single A-to-G transition (Fig. 3C), leading to Ile235Val substitution. These two type of mutations were not found in exons 8 and 9 of DNA amplified from the patient’s leukocytes. The presence of both normal and mutant sequences of the Gsα gene indicates the occurrence of a somatic mutation and thus a mosaicism of normal and abnormal cells as observed in the case of MAS.

**Confirmation of Two Types of Novel Mutations**

To exclude the possibility of polymerase chain reaction (PCR) amplification errors or polymorphism for the newly found mutations at these positions, the fragments containing codons 209 and 210 and those including codon 235 were amplified by pair primers 1 and 2, respectively, as described in the Materials and Methods, from the genomic DNA extracted from the leukocytes of 10 normal subjects and the patient’s leukocytes and osseous tissue and were digested by AlwI. As shown in Fig. 4, the fragments containing codons 209 and 210 amplified from peripheral leukocytes of control subjects and the patient produced one fragment of 201 bp, but the combination mutation made the fragments amplified from the genomic DNA extracted from the patient’s affected bone with one AlwI site and thus generated two fragments of 142 and 59 bp. The fragments containing codon 235 from the genomic DNA extracted from peripheral leukocytes of control subjects and the patient were cut at two AlwI sites, producing three fragments of 159, 73, and 9 bp, whereas the missense mutation at position 235 made the fragments amplified from genomic DNA extracted from the patient’s affected bone with one AlwI site, thus generating only two fragments of 232 and 9 bp. The fact that these mutations were found in the patient’s affected bone but not in leukocytes of the patient and normal subject indicates that these mutations are not polymorphic.

The 36 clones selected from the patient’s affected osseous tissue were amplified by the two pair primers as already mentioned, then digested by AlwI. As a result, a combination mutation in the same allele at positions 209 and 210 of the Gsα gene was found in one clone, and a missense mutation at position 235 was detected in two clones (data not shown).

**Discussion**

MAS is a sporadic disease characterized by polyostotic fibrous dysplasia, café-au-lait spots, and various endocrine disorders, including precocious puberty, hyperthyroidism, hypercortisolism, GH excess, and hyperprolactinemia. The diverse metabolic abnormalities seen in MAS are related to the involvement of cells that respond to extracellular signals through activation of the hormone-sensitive adenylyl cyclase system (9). In 1991, Weinstein et al. (8) first reported a missense mutation at codon 201 of the gene encoding the