McCune-Albright Syndrome in a Discordant Monozygotic Twin

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ABSTRACT: **Background:** McCune-Albright syndrome is a sporadic disorder characterized by polystotic fibrous dysplasia, pigmented patches of skin, and endocrinological abnormalities.

Objectives: To compare the genetic characteristics of the *GNAS1* gene in a monozygotic pair of twins, one of whom was diagnosed with MAS while the other had no indication of the syndrome.

Methods: We performed a molecular analysis of the *GNAS1* gene in DNA extracted from peripheral blood cells and quantification of mRNA extracted from lymphoblastoid cells from both twins by quantitative real-time polymerase chain reaction.

Results: Monozygosity of the twins was confirmed by typing them to four highly polymorphic microsatellites. Molecular analysis of the *GNAS1* gene extracted from both twins did not reveal the cause of this discordance.

Conclusions: It is possible that the exact molecular mechanism for the MAS discordance can only be determined by sampling affected tissues.

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KEY WORDS: McCune-Albright syndrome, fibrous dysplasia, discordant monozygotic twin, *GNAS1* mutation analysis, real-time RT-PCR

The McCune-Albright syndrome was first described by McCune and Bruch [1] and Albright et al. [2,3]. It is usually caused by postzygotic activating mutations of the guanine nucleotide-binding protein, alpha-stimulating activity polypeptide 1 (*GNAS1*) [4]. This syndrome is a sporadic disorder characterized by polystotic fibrous dysplasia, pigmented patches of skin, and endocrinological abnormalities. In all three systems, the extent of the abnormality and, in the case of the endocrine system, the nature of the abnormality are highly variable from case to case depending on the specific tissues involved in the mosaicism and the extent of involvement. The frequency of this disorder is similar in males and females [5]. In addition, *GNAS1* is subject to a complex pattern of genomic imprinting. Its various promoters direct the production of maternally, paternally, and biallelically derived gene products [6], and identical inactivating mutations in *GNAS1* can give rise to either pseudohypoparathyroidism type 1a or Albright hereditary osteodystrophy in the same kindred depending on the transmitting parent [7].

We describe a pair of monozygotic twins, one of whom was diagnosed with MAS while the other had no indication of the syndrome. Molecular analysis of the *GNAS1* gene in DNA extracted from peripheral blood cells and mRNA extracted from lymphoblastoid cells attained from both twins did not reveal the cause of the discordance.

MAS = McCune-Albright Syndrome

SUBJECTS AND METHODS

TWIN 1

The first twin is a 22 year old woman, the first of the single-placenta monozygotic twins, and a smoker. A computed tomography scan was performed following a traffic accident that resulted in relatively mild head trauma. The CT revealed as an incidental finding that the skull structure was consistent with fibrous dysplasia. In light of this finding an evaluation was conducted including endocrinological blood tests, which revealed acromegaly and hyperprolactinemia. She had regular menses beginning at age 13, a year after that of her healthy twin sister.

- **Physical examination:** The pulse rate was 60 beats/minute, blood pressure 110/70, respiratory rate 16/min, height 168 cm and weight 61 kg (body mass index 22). The zygoma was very prominent on the left side and the facial structure was typical of acromegaly (broad nose, elongated jaw, large mandibular, and sharp facial features). There was an oval-shaped hyperpigmented lesion, 1 x 2 cm, with irregular margins on the lower chest, left of the median line. Hirsutism was noted. The heart sounds were normal without murmurs. The lung sounds were clear. The abdo-

Table 1. Results of blood tests

Test	Result	Normal range
Luteinizing hormone	3.2 mIU/ml	
Follicle-stimulating hormone	3.5 mIU/ml	
DHEA sulphate	3.67 µg/ml	0.35–4.3
Thyroid-stimulating hormone	1.99 µl/U	0.39–4
Blood cortisol	11.7 µg/dl	
Growth hormone	56.6 ng/ml	0–5
Prolactin	100 ng/ml	1–29
Insulin growth factor	91.2 nmol/L	7–47
Parathyroid hormone	73.8 pg/ml	12–65
Alkaline phosphatase	211 U/L	35–104
Vitamin D25-OH	16 ng/ml	20–45
Glucose	98 mg/dl	60–115
Urea	23 mg/dl	0–50
Creatinine	0.51 mg/dl	0.5–0.9
Calcium	8.7 mg/dl	8.6–10.2
Phosphorus	4.2 mg/dl	2.7–4.5
Sodium	142 mEq/L	135–145
Potassium	4.2 mEq/L	3.5–5.1

men was soft to palpation without evidence of an enlarged liver or spleen. The hands and legs were large compared to those of the second twin. The cranial nerves were normal with the exception of impaired vision of the left eye with scotomas. A neuro-ophthalmologic examination revealed retinal atrophy and myopia. The muscle tone and reflexes were normal.

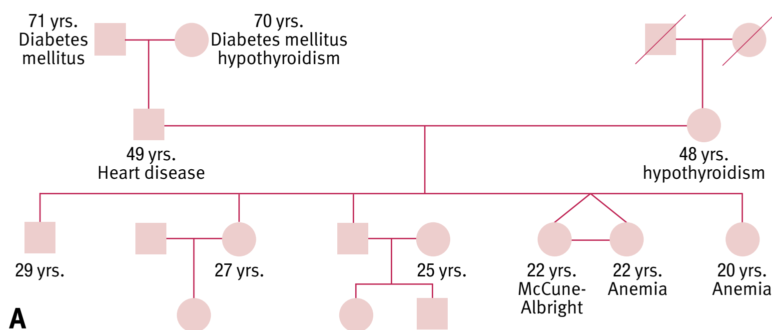
- **Laboratory test results:** Laboratory tests showed high levels of serum growth hormone, prolactin, and insulin growth factor-1 [Table 1].
- **Ancillary test results:** A bone scan showed strong uptake in the left side including the skull, the maxillary bone, the frontal and ethmoidal regions, and the jaw. A CT scan showed typical osseous changes of fibrous dysplasia. The pituitary gland was slightly enlarged. Magnetic resonance imaging showed diffuse fibrous dysplasia causing significant distortion of the base of the skull in the front. It was difficult to evaluate the patient for a hypophyseal adenoma because of the osseous changes that bordered on and involved the sella turcica. The osseous part of the left orbit was involved. It was pushed forward and to the left, causing narrowing and pressure on the optic nerve.
- **Therapy:** Treatment was initiated with a somatostatin analog, lanreotide acetate (Somatuline®, 20 mg/week), a dopamine agonist, cabergoline (Dostinex®, 0.5 mg/week), and bisphosphonate (Fosalan®, 70 mg/week). Under medical therapy the laboratory test values stabilized at: prolactin 13 ng/ml, growth hormone 26.9 ng/ml, IGF-1 75.5 nmol/L, and parathyroid hormone 54.9 pg/ml.
- **Family history:** The twins' mother suffered from hypothyroidism that was treated with thyroid replacement therapy, thyroxine sodium (Eltroxin®). The paternal grandmother had diabetes mellitus and hypothyroidism and the grandfather had diabetes mellitus. The family tree is shown in Figure 1A.

TWIN 2

The second twin had no characteristic indicators of MAS. Her height was 160 cm and her weight 65 kg.

- **DNA analyses:** DNA was prepared by the salting-out method from fresh peripheral blood lymphocytes. Typing for polymorphic microsatellite markers to test for monozygosity was done by polymerase chain reaction amplification and separation of the PCR products on polyacrylamide sequencing gels. The PCR products

IGF = insulin growth factor
PCR = polymerase chain reaction



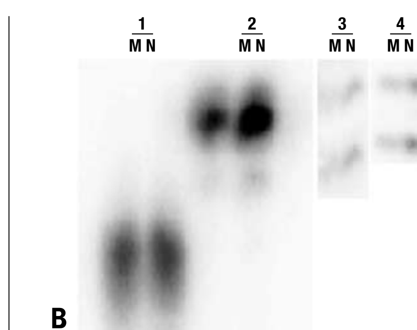


Figure 1. [A] Family pedigree. **[B]** The identical genotypes of the monozygotic twins (M = McCune-Albright patient, N = healthy twin) are presented for four polymorphic markers: 1: D22S425, 2: D10S677, 3: An (AC) dinucleotide polymorphic repeat on BAC RP11-704J17 on chromosome 6 (acc. No. AL450270) and 4: D2S1722.

were visualized by silver staining. Primers to enable PCR amplification of each exon and its borders were designed using the primer3 server (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Following the PCR reaction the remaining free PCR primers were digested by a combination of shrimp alkaline phosphatase 0.6 U and 6 U Exonuclease I (both from Fermentas, Lithuania). The PCR products were directly sequenced on an AB373 apparatus. To analyze the *GNAS1* mRNA expression from a homogeneous population of cells, lymphoblastoid cell lines were established from the blood of both twins. RNA was extracted from the lymphoblastoid cell using the EZ-RNA II kit of Biological Industries (Israel) according to the manufacturer's instructions and cDNA was synthesized by the Reverse–iT 1st strand synthesis kit of ABgene (UK) with an oligo d(T) primer. The *GNAS1* cDNA was PCR amplified in overlapping fragments that were directly sequenced. Subcloning of the PCR product into the pGEM-T vector (Promega, USA) was done by ligating the PCR product at a molar ratio of 3:1 to the plasmid and electroporating the ligated product into DH5α101 bacteria. Plasmids DNA were extracted using the HiYield plasmid mini kit (RBC Bioscience, Taiwan).

- **Quantification of mRNA by quantitative real-time PCR:** Transcript levels of the *GNAS* gene were determined by QPCR using the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA) according to the instructions of the manufacturer. Gene-specific primers were designed on exon junctions using the Primer Express Software (Applied Biosystems). The forward primer: AACCTGAAAGAGGCGATTGAAAC, the reverse primer: CACACTCAGGATGTAGTCCACTCTG. Primer and cDNA concentrations were optimized following the guidelines of the supplier. For each primer pair used, melting curve analysis (slowly ramping the temperature from 60ºC to 95ºC with continuous measurement of fluorescence) showed a single melting peak after amplification, indicating a specific product. The internal reference was ROX.

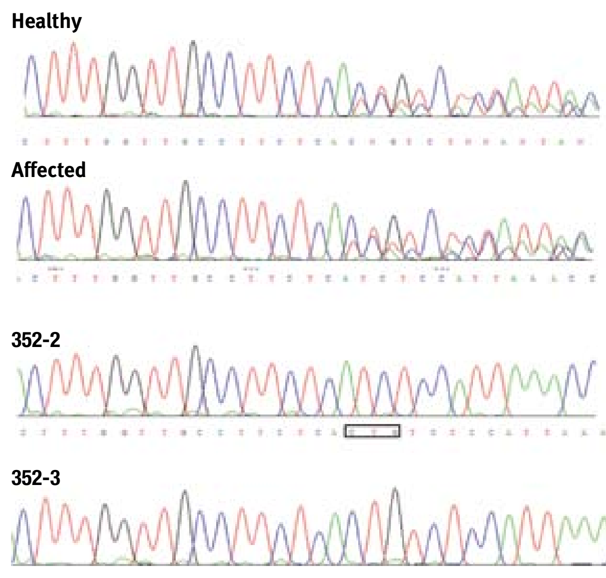
Each 20 μl reaction contained 2 μl (1–2 μg first strand cDNA), 10 μl PCR master mix (Applied Biosystems), and 300–700 nM of each forward and reverse primer (according to the optimization of the primers). All reactions were performed under the following conditions: pre-incubation at 50ºC for 2 minutes followed by denaturation at 95ºC for 10 min and 40 cycles at 95ºC for 15 sec, annealing and elongation at 60ºC for 1 minute. Reactions were characterized by comparing the threshold cycle (Ct) values. For each sample, results were normalized by the transcript level of internal control gene beta-actin.

QPCR = real-time PCR

RESULTS

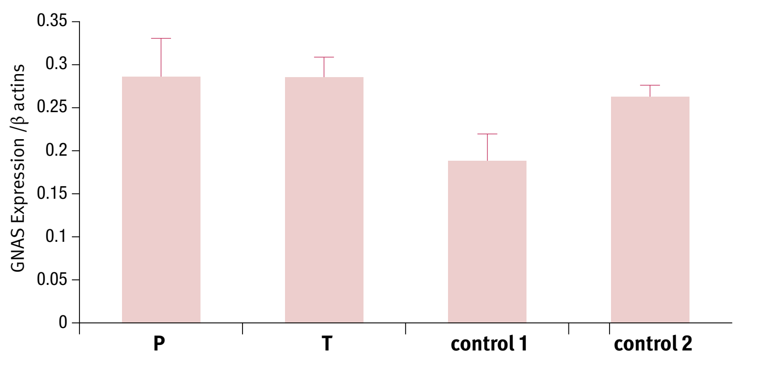
Monozygosity of the twins was confirmed by typing them to four highly polymorphic microsatellite markers. In addition, the archive medical records confirmed that the twin sisters were identical and born of one placenta. To verify whether the MAS in one of the twins was the result of an activating mutation, we used DNA extracted from her peripheral blood, the sole available source of DNA from the patient, to PCR-amplify all 13 coding exons and their borders and sequenced the PCR product. No mutation was identified. We further verified the possibility of a mutation in the MAS twin in lymphoblastoid cells by reverse transcriptase-PCR amplification of the *GNAS1* cDNA in overlapping fragments and directly sequenced the PCR products that amplified the coding sequence. This analysis excluded any mutation causing either a mutation in the coding sequence or a splicing variant, since only the expected-size products were amplified by the RT-PCR. The cDNA sequencing revealed that both twins were heterozygous for a polymorphism of an addition of a Ser codon at position 72 (the coding sequence with the Ser Acc.

Figure 2. Biallelic expression of the *GNAS1* gene in lymphoblastoid cells of the twin patient, upper lane (affected) and her sister, second lane (healthy). The presence of the additional three nucleotides in one of the *GNAS1* alleles causes appearance of the two superimposed sequences starting at the point of the insertion. Two plasmid sequences showing each of the polymorphic sequences are shown in the third and fourth lanes (352-2 and 352-3). The three nucleotides differing between the alleles leading to the addition of the Ser codon are boxed in the fourth lane. The sequence was performed with the reverse primer.



RT = reverse transcriptase

Figure 3. *GNAS1* mRNA levels of lymphoblastoid cells. Total RNA was isolated and subjected to QPCR. For each sample, transcript levels of the GNAS gene were determined by QPCR and were normalized to beta-actin (housekeeping gene). Results are expressed as mean ± SE. P = patient, T = healthy twin, controls 1, 2 = two unrelated controls.



No. NM_080426, without the Ser Acc. No. NM_001077489, named transcript variant 7). Both alleles were expressed at approximately equal levels as judged by the equal signals of both sequences of the PCR-amplified cDNA [Figure 2]. The presence of the polymorphism was validated by subcloning the PCR product into the pGEM-T plasmids and sequencing individual plasmids.

High activity of the *GNAS1* gene could result from increased production of the enzyme. To address this possibility we compared the quantity of *GNAS1* mRNA from lymphoblastoid cells of the patient, her twin and two unrelated healthy controls by real-time RT-PCR. No significant difference was observed [Figure 3].

DISCUSSION

McCune-Albright syndrome has been described previously in twins, but the underlying cause was not identified. Lemli [8] reported female monozygotic twins with fibrous bone dysplasia. One of the twins presented with the classic signs of MAS, while the other twin had only radiological evidence of bone disease and an increased serum concentration of alkaline phosphatase. In another report Endo et al. [9] described monozygotic twins who had advanced bone age but were discordant for the other major signs of MAS. We searched for a mutation that could cause constitutive activation of the *GNAS1* gene, which was demonstrated previously to be the cause of MAS [10,11]. It was suggested that such mutations occur postzygotically, leading to mosaic distribution of mutant-bearing cells and to a constellation of abnormal tissues specific to each patient that can explain the large variability of phenotypic expression in spite of identical genotype [12]. We hypothesized that an activating mutation could have occurred in the affected twin after separation from her healthy twin. We assume that we failed to identify the mutation in the DNA extracted from her peripheral blood lymphocytes because the quantity of mutated DNA in these cells was very low. Previously, Lumbroso and co-authors [12] made a similar observation. Similarly, we can assume that since the quantity of mutated DNA in the lymphoblastoid cells is very low the mutation might have occurred at a later stage of development without it being distributed to blood cells.

Another possible explanation for the discordance could be epigenetic differences arising during the lifetime of the monozygotic twins. Fraga et al. [13] examined the global and locus-specific differences in DNA methylation and histone acetylation of a large cohort of monozygotic twins and found that they exhibited remarkable differences in their overall content and genomic distribution of 5-methylcytosine DNA and histone acetylation, especially at older ages. Moreover, increased DNA methylation at the *AXIN1* gene in a monozygotic twin was demonstrated to cause discordance for a caudal duplication anomaly, probably by repression of transcription of the gene [14]. The *GNAS1* gene has a highly complex imprinted expression pattern. It encodes maternally, paternally and biallelically expressed proteins that are derived from alternatively spliced transcripts with alternate 5' exons. Each of the upstream exons is within a differentially methylated region commonly found in imprinted genes [6]. The presence of the addition of the Ser codon at position 72 polymorphism enabled us to conclude that *GNAS1* is biallelically expressed in lymphoblastoid cells of both the patient and her twin sister. This finding is in agreement with other studies showing that transcripts of *GNAS1* are biallelically expressed in most human tissues [6]. Since DNA methylation affects the levels of RNA transcription we verified directly whether our MAS twin expresses a higher level of *GNAS1* mRNA relative to her healthy twin and two independent controls. Such an enhanced level could not be detected in lymphoblastoid cells. However, our inability to identify a difference in the lymphoblastoid cells' mRNA level or the expression of a mono-allele of a specific parent does not preclude the possibility that a higher level of *GNAS1* mRNA or a specifically mutated allele exists in the affected tissues since epigenetic modification is tissue specific. Thus, it is possible that the exact molecular mechanism causing the MAS discordance could be determined only by sampling the affected tissues

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