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CASE

Genetic diagnosis of multiple affected tissues in a patient with McCune–Albright syndrome

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Abstract McCune–Albright syndrome (MAS) is a sporadic disorder characterized by the classic triad of polyostotic fibrous dysplasia, 'café-au-lait' skin pigmentation, and hyperfunctional endocrinopathy. It is caused by embryonic somatic mutations leading to the substitution of His or Cys for Arg at amino acid 201 of the alpha-subunit of the signal transduction protein Gs (Gsα). A 32-year-old man was diagnosed as McCune–Albright syndrome with the following findings: polyostotic fibrous dysplasia, 'café-au-lait' spots and acromegaly. An ultrasonic examination showed that he had left-pleural effusion, which disappeared after almost a year without special treatment. Genomic DNA was isolated from the peripheral blood, bone tissue, skin lesion and pleura samples of the patient. Then PCR and direct sequencing were performed. An activating mutation of the Gsα gene (Arg201Cys) was found in the genomic DNA isolated from the peripheral blood and the bone tissue, but not in genomic DNA isolated from the skin and pleura samples.

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Introduction

McCune–Albright syndrome (MAS) is a sporadic disorder characterized by the classic triad of polyostotic fibrous dysplasia, 'café-au-lait' skin pigmentation and hyperfunctional endocrinopathies, such as sexual precocity, GH excess, hyperthyroidism, and hypercortisolism . It was first reported by McCune in 1936 [1] and shortly after by Albright et al. in 1937 [2]. This syndrome is rare and less than 100 cases associated with acromegaly have been described in literature [3, 4]. The clinical manifestations of MAS are caused by embryonic somatic mutations. This leads to the substitution of His or Cys for Arg at amino acid 201 of the alpha-subunit of the signal transduction protein Gs (Gsα) [5, 6]. The activating Gsα mutation could explain many features of MAS [7].

Here, we presented a case of MAS with acromegaly and self-resolving pleural effusion, and we studied gene mutation of Gsα in the peripheral blood leukocytes, osseous lesion, skin lesion, and pleura samples. We identified the activating mutation of Gsα gene in the peripheral blood leukocytes and osseous lesion of the patient.

Results

Case report

The patient, a 32-year-old Chinese man, had been noted with an increase in size and distortion of the face since the age of 12. The enlargement of the hands and feet had also begun at

the same age. Four months before coming to our hospital, he had upper abdominal pain and was unexpectedly found left-pleural effusion in the ultrasonic examination. He didnot have chest pain, dyspnea, and cough. Polyostotic fibrous dysplasia was diagnosed by radiograph at the same time. Anti-tuberculosis and anti-inflammatory treatments were started. But since there was no change of pleural effusion, both therapies were discontinued two months later. When he came to our hospital, he was given cytological examination of pleural fluid and histopathologic examination of pleura sample. Both examinations indicated mesothelial hyperplasia. As the amount of pleural effusion tended to decrease, we didnot give him any special treatment. Then after six months, the pleural effusion disappeared. The development of his secondary sex features was normal. On physical examination at admission, the patient's height was 183 cm and weight was 70 kg. Multiple 'café-au-lait' lesions with irregular borders were present over the skin of left neck (Fig. 1A) and mucosa of the labium maxillae and labium inferius oris (Fig. 1B). He had an asymmetric face with coarse features and acral enlargement.

Serum and urinary concentrations of calcium and phosphates were normal. Serum alkaline phosphatase was elevated to 1130 IU/L (reference value: 38–121 IU/L). Plasma concentrations of thyroxine (T4), triiodothyronine (T3), free T4, free T3, thyroid-stimulating hormone (TSH), cortisol, adrenocorticotropic hormone (ACTH), testosterone, luteinizing hormone (LH), and prolactin (PRL) were normal. Serum levels of parathyroid hormone (PTH) (151.5 pg/ml; normal range: 13.0–53.0 pg/ml), growth hormone (GH) (38.1 ng/ml; normal range: 0.1–10.0 ng/ml), and follicle-stimulating hormone (FSH) (21.59 mIU/mL; normal range: 1.3–10.1 mIU/mL) were elevated. The lowest-level of GH was 26.7 ng/ml in OGTT (normal range: <1 ng/ml), and his glucose tolerance was normal. Plasma concentration of E2 (15.00 pg/ml; normal range: 25–107 pg/ml) was slightly lowered.

Radiologic examination showed multiple bone lesions characterized with polyostotic fibrous dysplasia in the skull, ribs, scapula, vertebrae, femurs, and left ilium. Computed tomography (CT) and magnetic resonance imaging (MRI) of the brain demonstrated thickening of cranial bones. The signal of the pituitary was not uniform, without indication of pituitary adenoma (Fig. 2). Histopathology of left ilium lesion confirmed the diagnosis of fibrous dysplasia (Fig. 3). Histopathology of pleura sample indicated mesothelial hyperplasia (Fig. 4).

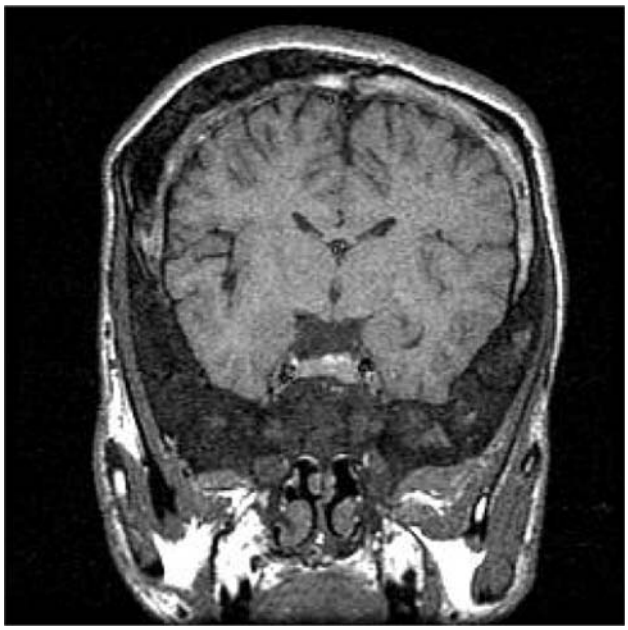

Fig. 2 Frontal MRI showing normal size of pituitary with nonuniform signal, and irregular thickening of cranial bones

Confirmation of gene mutation

An Arg201Cys (R201C) mutation was found in genomic DNA which was isolated from peripheral blood and the bone tissue (from the left ilium). This is a missense point mutation (CGT → TGT) leading to the substitution of Cys for Arg at amino acid 201. However, no mutation was detected in the patient's genomic DNA isolated from the skin and pleura samples (Fig. 5).

Discussion

McCune–Albright syndrome (MAS) is a sporadic syndrome characterized by the clinical triad of polyostotic

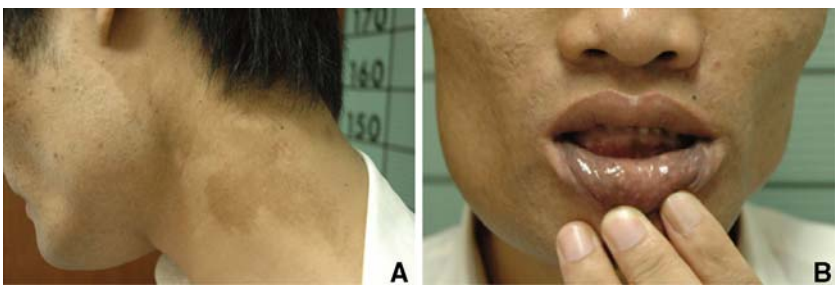

Fig. 1 Skin lesions in the patient with MAS. Multiple 'café-au-lait' lesions with irregular borders over the skin of left neck (**A**) and the mucosa of labium inferius oris (**B**)

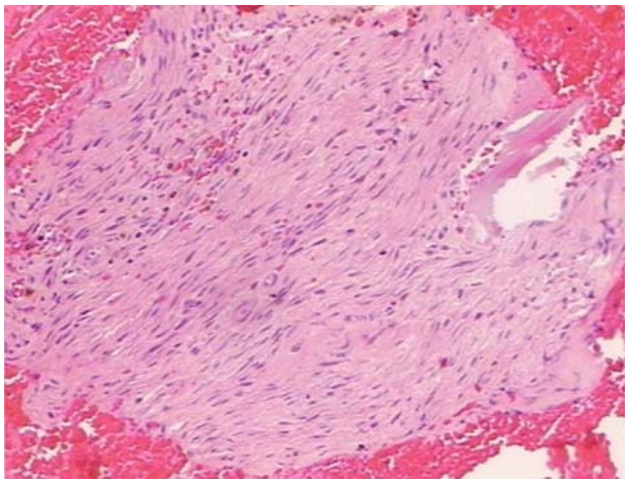

Fig. 3 Hematoxylin and eosin-stained bone sections from the left ilium showing irregular bone trabeculae infiltrated with benign spindle cells (magnification 100×)

fibrous dysplasia, 'café-au-lait' skin lesions, and endocrine hyperfunction. Endocrine disorders include precocious puberty, hyperthyroidism, hypercortisolism, hyperparathyroidism, hyperprolactinemia, GH excess, hypophosphatemic rickets, and hypothalamic hypogonadotropic hypogonadism [3, 4, 8]. The diagnosis of the MAS requires at least the presence of two components of the triad [9]. The patient had polyostotic fibrous dysplasia, 'café-au-lait' skin pigmentations, manifestations of acromegaly such as coarse facial features, and acral enlargement and an increase in serum GH, which was not suppressed in oral glucose tolerance test (OGTT). The diagnosis of MAS was therefore made according to the clinical data.

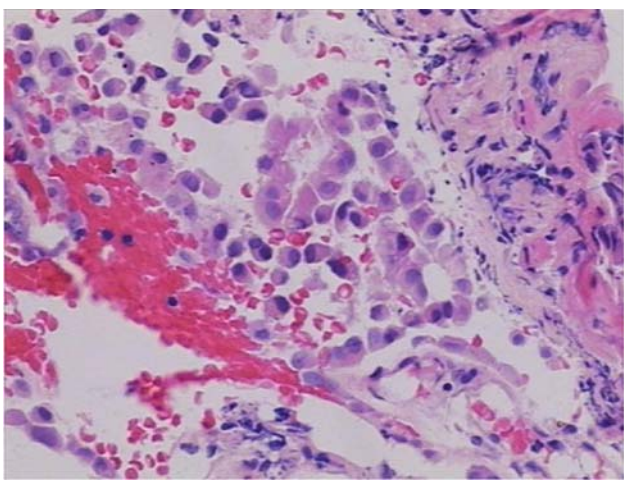

Fig. 4 Hematoxylin and eosin-stained pleura sections showing mesothelial hyperplasia with no cellular heterotype. (magnification 200×)

Nearly all patients (98%) with MAS have solitary or multiple expansile fibrous dysplasia lesions. Most of the lesions are located in the craniofacial bones or long bones. The lesions can cause local pain, progressive deformity, fractures and nerve entrapment. Radiographs of affected bones reveal expansile, lytic lesions with a ''ground glass'' pattern. In this case, polyostotic fibrous dysplasia with involvement of the craniofacial bones, ribs, scapular bones, vertebrae, ilium, and femur were noted.

The most common extra skeletal involvement is skin lesions, known as 'café-au-lait' macules. Lesions rarely extend beyond the midline, and the majority tends to be on the same side of the body as the skeletal lesions. These lesions consist of one or more light to dark brown macules with irregular borders [10]. In our case, 'café-au-lait' lesions were present over the mucosa of cavitas oris and the skin of left neck, which located similarly to a reported case of MAS associated with pituitary tumor [11].

Endocrine disorders are common in MAS and are characterized by autonomous and excessive function of hormone-producing tissues [10]. Serum concentrations of tropic or stimulating hormones are typically normal or reduced. The most common endocrine disorder is precocious puberty. Although seldom observed in males [3], precocious puberty is a common initial manifestation of MAS in girls, and characteristically presents as the larche and/or vaginal bleeding in a girl prior to age 9 years. Sexual precocity was also not observed in our case.

GH excess is also common in patients with MAS. Many patients will have features of acromegaly. The biochemical behavior of growth hormone-producing pituitary tumors in patients with MAS is indistinguishable from that of sporadic tumors either with or without Gs protein mutations. However, only 65% of MAS patients with excess growth hormone have radiographic evidence of a pituitary tumor; a much lower incidence than in sporadic cases of acromegaly (99%) [12]. Patients with MAS are generally younger than sporadic cases of acromegaly [13]. Regarding the patient's history, the complaints of our patient also started at the age of 12. The latest MRI scan couldnot show the existence of a pituitary adenoma. There may be two possible reasons for the result. One possibility is that the pituitary adenoma is at its early stage and therefore too small to be detected by MRI scan; another being that there is just proliferation of GH-secreting cells without the form of tumor. Thus, the patient should be followed up regularly.

MAS is a disease caused by somatic gene mutation. Its classical mutation is a missense point mutation at amino acid 201 in exon 8 of Gsα gene located on chromosome 20 q [5]. The mutation is nearly always a substitution of the residue arginine at position 201 by histidine or cysteine. It is very infrequent that arginine is replaced by serine, glycine, or leucine [14–16]. The mutation inhibits GTPase

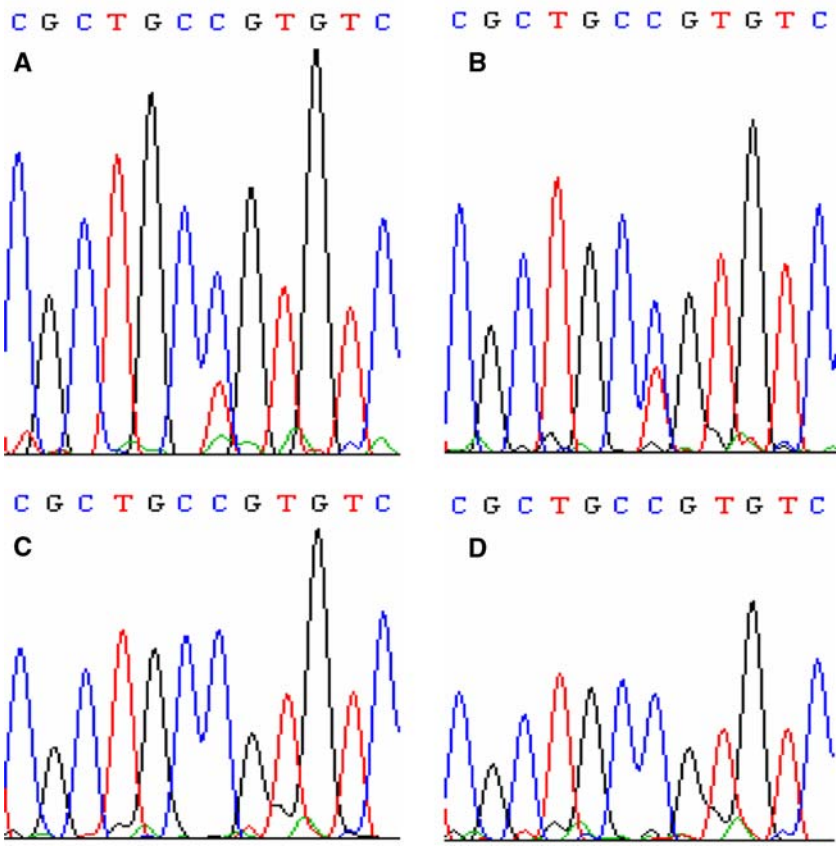

Fig. 5 Automatic sequencing result of Gsα gene from the peripheral blood (**A**), bone tissue (**B**), skin lesion (**C**) and pleura sample (**D**): the mutation associated with substitution of Cys for Arg at amino acid 201 was demonstrated in the genomic DNA isolated from the blood (**A**) and tissue taken from the left ilium (**B**) of the patient, but not in genomic DNA isolated from the skin (**C**) and pleura (**D**) samples

activity of the Gsα subunit and leads to constitutive activation of adenylate cyclase [17]. MAS is caused by post-zygotic somatic mutation and has a mosaic distribution of mutant-bearing cells, which are not present in all cells of affected patients, even within affected organs [18, 19].

Here we studied the gene mutation of Gsα gene in multiple affected tissues of this MAS patient. The genetic diagnosis of multiple affected tissues confirms the patient has an R201C mutation in Gsα gene isolated from the peripheral blood and bone tissue. The fact that the mutation is present in peripheral blood suggests that the mutation occurred early in embryogenesis, and the clinical manifestations can thus be variable, whereas later mutations would have a more limited distribution, and the frequency of detecting the mutation in peripheral blood is lower. This coincides with the results of the largest study related to MAS [4], in which the mutation was detected in 46% of the peripheral blood samples in patients presenting the classic triad. This figure fell to 8% and 21% in patients with one and two signs, respectively. Furthermore, in the largest study, analysis of bone tissues revealed a very high-proportion of positive samples (9/11; 82%) and the two cases in which mutation was not detected corresponded to isolated fibrous dysplasia [4]. The mutation was also detected in the osseous lesion in our case. Thus, we consider that bone tissues are of great value for genetic diagnosis of MAS.

Besides the peripheral blood leukocytes and bone tissues, activating Gsα mutations have also been identified in liver, heart, thymus, and the gastrointestinal tract, associated with clinical consequences such as hepatitis, cardiac arrhythmias, or intestinal polyps [6, 20]. Our patient was unexpected to demonstrate left-pleural effusion and it seems to be self-limited. We are not sure whether such kind a pleural effusion is a new feature of MAS or not. Interestingly, the pleural effusion of this patient was localized on the left, same side as his skin lesions. Both the cytological analysis of pleural effusion and the histopathologic examination of pleura sample of the patient indicated mesothelial hyperplasia. We do not know what is the pathogenesis of this lesion. Since the gene analysis of correlated cells in pleural fluid was interfered by the large amount of leucocytes in the exuded pleural fluid, and no GNAS mutation was found in the pleura sample of this patient, there is no direct evidence to confirm the correlation between the pleural effusion and Gsα gene mutation. Nevertheless, it is still necessary to notice and further study this clinical manifestation in patients with McCune–Albright syndrome.

No mutation was found in this patient's skin lesion either. This result confirms previous reports showing the

difficulty of detecting the mutation in skin [6], likely due to the low proportion of melanocytes, which are the cells potentially mutated in skin tissue.

Because of the mosaic distribution of mutant-bearing cells in MAS, the number of abnormal cells in a given tissue and thus the quantity of mutated DNA is quite variable and may be extremely low. Thus, the molecular diagnosis of MAS is difficult and somewhat depends on the detection technique. However, as some authors used the most sensitive methods, some samples still remain negative [4]. Mutated cells may be confined to specific loci in the affected tissue, and some samples will be negative if the biopsy has missed these loci. Normal-functioning Gsα protein in a patient with MAS has also been reported, which suggest a defect beyond the Gsα protein [21]. Therefore, several factors are responsible for the negative results.

In conclusion, MAS is caused by postzygotic somatic mutation and has a mosaic distribution of mutant-bearing cells, which leads to various manifestations of the syndrome. Diagnostic efforts should also be oriented toward those numerous atypical or partial forms of MAS.

Materials and methods

Subject

The patient gave informed consent for this study. The samples from his left ilium, skin pigmentation, pleura, and peripheral blood were taken for the analysis of Gsα mutations.

Amplification of DNA by PCR

Genomic DNA was isolated from leukocytes by protease-K digestion and phenol-chloroform extraction [22]. The osseous tissue in the lesion of the left ilium was obtained by harpoon biopsy under CT and immediately frozen in liquid nitrogen. The tissue was rapidly homogenized by milling and then digested with 0.5 mg/ml of proteinase K (Sigma, Louis, MO), in the digestion buffer (50 mmol/l of Tris, pH 8.5; 1 mmol/l of EDTA; 0.5% Tween-20) for 5 h at 50°C. The sample was extracted with a mixture of phenol, chloroform, and isoamyl alcohol (25:24:1). The DNA was precipitated and washed with ethanol. The process of extracting the other tissues was the same as that of osseous tissue.

One pair of PCR primers was designed by Primer 5.0 software for amplifying exons 8 and 9 of the gene encoding the Gsα protein. The oligonucleotide primers with sequence identical to the introns flanking exons 8 and 9 of the Gsα gene were synthesized as follows: upstream, 5′-TGGCTTT GGTGAGATCCATT-3′; downstream, 5′-CAAACCTGTT GTTCCAGATGC-3′. PCR reaction mixtures (20 μl) contained 2 μl 10× Buffer, 0.5 μl dNTPs, 0.5 μl TaqDNA polymerase, 1 μl genomic DNA (50 ng/μl), 1 μl of each primer, and 14 μl ddH_2O. A Peltier Thermal cycler 225 was used to perform primary denaturation for 3 min at 95°C, and then 35 cycles of denaturation for 45 s at 94°C, annealing for 45s at 60°C, and extension for 1 min at 72°C. The total final extension was 10 min at 72°C.

DNA sequencing analysis

PCR fragments amplified from genomic DNA were analyzed by electrophoresis using 1% agarose gel containing EB and visualized with UV light. PCR products were purified by SAP. The purified PCR products were sequenced from two directions. The sequencing reactions were performed on a Peltier Thermal cycler 225 using a BigDye Deoxy Terminator Cycle Sequencing Kit, and then analyzed using an ABI PRISM 3700 Sequencer.

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