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Original contribution

The diagnostic utility of the *GNAS* mutation in patients with fibrous dysplasia: meta-analysis of 168 sporadic cases ☆

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Summary *GNAS* mutations have been implicated in the development of fibrous dysplasia and multiple endocrinopathies of the Albright-McCune syndrome. To investigate the diagnostic utility of *GNAS* mutations in patients with fibrous dysplasia, we performed mutational analyses of histologically confirmed fibrous dysplasia and conducted a meta-analysis of the literature. We collected 48 cases of fibrous dysplasia from 3 institutions from 2002 to 2011 and performed polymerase chain reaction and direct bidirectional sequencing of exons 8 and 9 of *GNAS* using paraffin-embedded tissues. We searched MEDLINE, PubMed, and the KoreaMed databases from 1997 to 2011 and included an additional 155 cases of fibrous dysplasia from 8 representative studies to conduct a meta-analysis. In our sample, 28 (58.3%) of 48 cases showed point mutations of codon 201 at exon 8. Twenty-five cases had a substitution of arginine at codon 201 for histidine (p.R201H), and 3 cases had a substitution for cysteine (p.R201C). One case had a new mutation at codon 224 (p.V224A). The incidence of *GNAS*

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mutations was significantly greater in cases that involved long bones than in cases that involved flat bones (P = .017) and was higher in polyostotic cases than in monostotic cases (P = .067). In meta-analysis, 9 studies and 203 patients were included. The overall positive rate of *GNAS* mutation in fibrous dysplasia was 71.9% (146/203). The major types of mutations were missense mutations such as R201H (66.4%) and R201C (30.8%). As a result, the detection of *GNAS* mutation could be a valuable adjunct to conventional histopathologic diagnosis of fibrous dysplasia.

1. Introduction

Fibrous dysplasia (FD) is a benign medullary osteofibrous lesion that may involve 1 (monostotic FD) or more (polyostotic FD) bones. FD occurs in children and adults and affects all populations with an equal sex distribution [1]. FD may occur in isolation or as a part of McCune-Albright syndrome (MAS), which includes endocrine abnormalities, café-au-lait pigmented skin lesions, and FD [2]. The histopathology of FD shows replacement of the marrow space by fibrous and osseous components. The fibrous component is composed of cytologically bland spindle cells with low mitotic rates. The osseous component comprises irregular curvilinear trabeculae of woven (or rarely, lamellar) bone. The bony trabeculae are typically not rimmed by osteoblasts but merge with the surrounding fibrous tissue and unmineralized osteoids. Monostotic and polyostotic variants of FD and soft tissue myxomas coexisting with FD are known to be developed as a result of activating missense mutations of codon 201 in exon 8 of the *GNAS* encoding the stimulatory G-protein α-subunit (Gsα) of the heterotrimeric G-protein complex [2-4]. Substitution of arginine at codon 201 for cysteine (R201C) or histidine (R201H) results in the constitutive activation of the GSα-cyclic adenosine monophosphate (cAMP) signaling pathway and overproduction of cAMP in the affected cells. In bones, increased concentration of cAMP in cells of osteoblastic lineage leads to increased proliferation and abnormal differentiation, which explains their spindle cell morphology and failure to form a mature bone [5,6]. Bianco et al [7] found that stromal cells isolated from FD failed to recapitulate normal ossicles; instead, they generated a miniature replica of FD in an in vivo transplantation assay. These findings further emphasize the link between stromal cell dysfunction and *GNAS* mutation in FD tissues [7].

Detection of *GNAS* mutations is a valuable adjunct to the histopathologic diagnosis of FD [8]. One of the most important differential diagnoses of FD is FD-like low-grade central osteosarcoma, which shares substantial histologic and radiologic attributes with FD. Initial correct diagnosis of this tumor is extremely difficult, even for specialists. Sometimes, only the clinical course can reveal the true nature of the lesion. The aims of this study were to evaluate the clinicopathologic features of *GNAS* mutation and to investigate the diagnostic utility of *GNAS* mutation in patients with FD. We performed mutational analysis on histologically confirmed FD and conducted a meta-analysis of the literature to assess the incidence and types of *GNAS* mutations.

2. Materials and methods

2.1. Patients and histology

This study included 48 patients who were histologically diagnosed as having FD and underwent surgery at the Samsung Medical Center (26), Samsung Changwon Hospital (11), and National Policy Hospital (1), South Korea, from 2002 to 2011. Patients with FD who exhibited endocrine hyperfunction or café-au-lait pigmented skin lesions were excluded from the study. Five cases of histologically confirmed osteofibrous dysplasia were included. We reviewed hematoxylin-and-eosin slides, clinical charts, and pathologic reports. In all cases, archival paraffin-embedded specimens suitable for mutational analysis (>50% of tumor area in section) were selected after review of the histologic slides. *Polyostotic FD* was defined as involvement of multiple lesions, and *monostotic FD* referred to single-bone involvement.

2.2. *GNAS* DNA sequence analysis

Genomic DNA was extracted from paraffin-embedded tissues using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). We subjected 100 ng of genomic DNA to polymerase chain reaction (PCR) amplification for exons 8 and 9 of the *GNAS*. Primer pairs were used to amplify the complete coding sequences of *GNAS* exons 8 and 9. PCR was performed in 20-μL reaction mixtures containing 100 ng of template DNA, 2 μL 10× PCR buffer, 0.25 mmol/L deoxynucleoside triphosphate (dNTP), 10 pmol primers, and 1.25 U Taq DNA polymerase (iNtRON, Seoul, Korea). PCR products were subjected to electrophoresis on 2% agarose gels and were purified using a QIAquick PCR purification kit (QIAGEN). Bidirectional sequencing was performed using the BigDye Terminator v1.1 kit (Applied Biosystems, Foster City, CA) on the ABI 3130XL genetic analyzer (Applied Biosystems).

Sequencher version 4.10.1 (Gene Codes Corporation, Ann Arbor, MI) was used along with a manual review of chromatograms for sequence analysis. Confirmatory resequencing from replicate PCR amplification reactions was performed for any sequence that was ambiguous or that deviated from the wild type so that all abnormal sequences were verified in at least quadruplicate for replicate amplification reactions.