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ARTICLE

GNAS mutation analysis assists in differentiating chronic diffuse sclerosing osteomyelitis from fibrous dysplasia in the jaw

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Chronic diffuse sclerosing osteomyelitis of the mandible (DSOM) and fibrous dysplasia (FD) are distinct lesions with overlapping clinicopathological features that complicate their diagnosis. This study aimed to evaluate the efficacy of *GNAS* mutation analysis in differentiating between these two conditions. DNA samples from patients with DSOM ($n = 35$) and FD ($n = 29$) were collected to analyze the presence of *GNAS* mutations in exons 8 and 9, the two previously reported hotspot regions, using polymerase chain reaction and direct sequencing. Twenty-four of 29 patients (83%) with FD showed missense mutations in codon 201 in exon 8, whereas no mutation was detected in exon 9. No mutations were found in any of the 35 cases with DSOM. We also identified one case with an uncertain diagnosis due to overlapping clinicopathological features of DSOM and FD. A Q227H mutation was detected in this case, that confirmed the diagnosis of FD. Taken together, the findings indicate that mutational analysis of the *GNAS* is a reliable approach to differentiate between DSOM and FD of the jaw.

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INTRODUCTION

Chronic diffuse sclerosing osteomyelitis of the mandible (DSOM) is a rare auto-inflammatory disease of the mandible, and is also known as chronic non-bacterial osteomyelitis (CNO) of the mandible. DSOM can occur solely or present as a manifestation of syndromes, such as chronic recurrent multifocal osteomyelitis (CRMO), and synovitis, acne, pustulosis, hyperostosis and osteitis syndrome (SAPHO)[1–4]. DSOM is characterized with cyclic episodes of pain and swelling in the mandible, and the radiographic features of patients with DSOM include intermingled osteosclerosis and osteolysis in the mandibular bone and subperiosteal bone formation.

Fibrous dysplasia (FD) is a benign dysplastic disease[5]. DSOM and FD are distinct conditions with overlapping clinicopathological features[6–8]. Clinically, DSOM is often misdiagnosed as monostotic FD of mandible.

In addition, the management of these two diseases is different[2,5]. Surgery is the primary treatment for FD[9]. At present, the indications for surgical treatment in FD patients are functional impairments, such as compressive neuropathies, otic canal obstruction, severe malocclusion, and symptomatic cranial base deformities[10,11]. The surgical interventions for cosmetic purposes must be individualized, and are preferably after childhood, during which period FD tends to be more metabolically active[9]. However, there is no standard management of DSOM. Surgical procedures have been attempted to treat DSOM, but with a poor outcome[12]. Drug therapy with agents, such as antibiotics, non-steroidal anti-inflammatory drugs and glucocorticoids have also been used to treat DSOM, although these can only alleviate the symptoms temporarily. Some advocate NSAIDs as first line therapy, but patients need to take the drug for a long time and the lesions are more likely to relapse when patients stop taking it[13–16]. Bisphosphonates[17] and denosumab[18,19] have recently shown good results in the treatment of DSOM. Therefore, it is imperative to distinguish between the two lesions for their treatment and prognosis.

Although our previous research demonstrated that clinical and radiographic features is significant in differentiating between the two diseases, there are still cases which are difficult to diagnosis solely based on clinicopathological and radiographic features[20]. Molecular techniques may be helpful in differentiating these two conditions. The FD is known to be associated with postzygotic activating mutations in *GNAS* that encodes the α-subunit of the stimulatory G-protein Gs ($G_s\alpha$)[5]. Mutations occur at either Arg201 (>95% of the reported cases)[21,22] or Gln227 (<5%)[22]. However, the etiology of DSOM remains unknown. Additionally, *GNAS* mutation profiling has been reported in a few case reports of DSOM as well as series of benign fibro-osseous lesions[23–25], which warrants assessment of the possibility to perform mutational analysis in differentiating the two conditions.

This study aimed to evaluate the role of *GNAS* mutation analysis in differentiating between the two conditions. We examined both Arg201 and Gln227 positions in 35 patients with DSOM and 29 patients with FD in the jaw using polymerase chain reaction (PCR) and direct sequencing.

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MATERIALS AND METHODS

Patients and samples

Samples from 35 cases with DSOM/SAPHO syndrome and 29 cases with FD/ McCune-Albright syndrome (MAS) in the jaw, collected between 2011 and 2021, were obtained from the tissue bank of the Peking University Hospital of Stomatology. As per an institutionally approved protocol, fresh tissues from craniomaxillofacial bone lesions including hard tissue component of the bone and endosseous medullary tissue were obtained during surgery. After collection, all specimens were stored at −80 °C. All cases were re-evaluated and confirmed by three experts according to the current clinical, radiographic, and histological criteria for FD and DSOM. Detailed information on these cases is listed in Tables 1 and 2. Additionally, data of a case pathologically diagnosed with "fibro-osseous" lesion with overlapping clinical features of FD and DSOM was also retrieved from the files. All fresh frozen and craniomaxillofacial bone lesion tissues from 29 cases of FD, 35 cases of DSOM, and one case with a confusing diagnosis were used for mutational analysis.

Mutation analysis of GNAS at Arg201 and Gln227 codons

Genomic DNA was isolated from tissue samples using the QIAamp DNA Blood & Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. In all samples, mutation analysis was performed by direct DNA sequencing of the PCR-amplified target sequence of *GNAS*. DNA (100 ng) was amplified in a standard 30 µL PCR mixture using GoTaq Green Master Mix (Promega, Madison, WI, USA) according to the manufacturer's instructions. A 270-bp fragment of *GNAS*, including the Arg201 codon, was amplified using the following primers: forward, 5′-TGACTATGTGCCGAGCGA-3′ and reverse, 5′-AACCATGATCTCTGTTATATAA-3′; another 316-bp sequence of *GNAS*, including the Gln227 codon, was amplified using the following primers: forward, 5′-GACCTGCTTCGCTGCCGTGT-3′ and reverse, 5′-AGCCAAGAGCGTGAGCAGCG-3′. The optimized PCR procedure was as follows: denaturation at 94 °C for 15 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C (for 270 bp sequence) or 65 °C (for 316 bp sequence) for 30 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min. The PCR products were purified using a DNA purification system (Promega) and sequenced using an automated DNA sequencer model 373 (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 8.0.2) statistical software package. Descriptive statistics were used as appropriate.

RESULTS

Clinicopathological features

The clinical data of the patients enrolled in the study are summarized in Tables 1 and 2. Of the 35 patients with DSOM, 13 were men and 22 were women, with a men-to-women ratio of 1:1.7. The age of onset ranged between 5 years and 67 years, with a mean of 20.94 ± 16.53 years. The duration ranged from 2 months to 12 years, with a mean of 1.50 ± 2.26 years. The mandible was affected in all the patients, while 15 cases (43%) were diagnosed with SAPHO syndrome.

Of the 29 patients with FD, there were 12 men and 17 women, with a men-to-women ratio of 1:1.4. The age of onset ranged between 0 years and 56 years, with a mean of 13.14 ± 10.36 years. The duration ranged from 4 months to 45 years, with a mean of 12.08 ± 9.24 years. Twelve cases showed defects in only one bone, including in the maxilla ($n = 8$), mandible ($n = 3$), and zygoma ($n = 1$); while 14 cases showed multiple gnathic bone involvement, including one case affecting both the gnathic and extragnathic bones, and three cases with MAS.

Pain (97.1%), soft-tissue swelling (97.1%), and trismus (60.0%) were more common in patients with DSOM. However, in patients with FD, pain was rare (13.8%), and none of them showed soft-tissue swelling or trismus. All patients with FD showed bone swelling, which was not observed in patients with DSOM.

Mutations in GNAS

The results of the *GNAS* mutation analysis are shown in Tables 1 and 2. A mutation in the Arg201 codon of Gsα protein was found in 24 of the 29 (83%) cases with FD, with a preference for Arg-to-His (p.R201H) substitution (Fig. 1B; 14 cases, 58%), as opposed to Arg-to-Cys (p.R201 C) substitution (Fig. 1C; 10 cases, 42%). The rarely reported mutation at Gln227 was not detected, and no mutation was detected in any of the 35 cases with DSOM.

A case report: differential diagnosis assisted by GNAS mutation analysis

A 19-year-old girl was referred to our hospital with complaint of recurrent swelling and pain in the left mandible for 12 years, 2–3 times a month. The symptoms were relieved after the administration of antibiotics or non-steroidal anti-inflammatory drugs. Physical examination showed significant bone swelling and soft-tissue swelling with tenderness in the left mandible (Fig. 2A, B). The skin showed mild facial acne without increased skin temperature (Fig. 2A, B), and there was no palmoplantar pustulosis or other skin lesion. Moreover, there were no signs of trismus or numbness in the lower lip. There were no dental caries or periodontitis. Additionally, panoramic radiography and CT revealed an obvious expansion of the left mandible with diffuse sclerosis and partial cystic change, and the cortex was continuous, although thin, with a mild subperiosteal bone formation (Fig. 2C–F). Technetium-99 (99Tc) pyrophosphate bone scanning showed a local radionuclide accumulation in the mandible, with no obvious abnormality in the other parts. The histological features of the biopsy lesion showed a fibrous stroma containing small, irregular, disconnected woven bones (somewhat resembling cementicles) (Fig. 2G), and some of these bones were rimmed with osteoblasts (Fig. 2H). Sixteen months before, the patient was treated with an intravenous drip of pamidronate disodium at our hospital, after which, the pain disappeared.

Due to the overlapping clinicopathological features of DSOM and FD, diagnosis was difficult to achieve. The clinical signs of recurrent swelling and pain were consistent with those of DSOM. However, the imaging features were more consistent with those of FD, while the signs of subperiosteal bone formation tended to be consistent with those of DSOM, and thus, the diagnosis remained ambiguous. Additionally, the histopathological features were suggestive of FD. Therefore, we examined mutations in both Arg201 and Gln227 codons of *GNAS* in this patient using PCR and direct sequencing. The analysis indicated a Q227H mutation in the bone lesion in the mandible (Fig. 2I), and thus, helped confirm the diagnosis of FD.

DISCUSSION

This study aimed to assess the role of *GNAS* mutation analysis in the differential diagnosis of DSOM and FD. To the best of our knowledge, this is the first study to provide evidence of a significant difference between DSOM and FD in the jaw based on PCR and direct sequencing analyses. Molecular diagnosis overcomes limitations of the traditional clinicopathological diagnosis; and the findings emphasized the importance of molecular features in differentiating between the two diseases.

We reconfirmed that pain, soft-tissue swelling or bone enlargement, and trismus are essential for differential diagnosis of DSOM and FD[20]. Usually, the diagnosis of FD or DSOM can solely be made on the basis of combination of clinical, radiological, and histological estimation; but in some situations, pathologists have a dilemma, especially in the absence of typical features and specific histology. Meanwhile, our results demonstrated that the patients younger than 18 years accounted for 57% (20/35) and 90% (26/29) in each condition. The overlap in age of onset also increased the difficulty of differential diagnosis. Moreover, DSOM is frequently misdiagnosed as FD or FD with

Table 1. The clinical features and *GNAS* mutations in patients with DSOM.

No. of patient	Sex	Onset age (years)	Operation age (years)	Duration	Location	Pain	Bone swelling	Soft-tissue swelling	Trismus	SAPHO syndrome	Type of GNAS mutation
1	M	26	27	12 y	Mandible	N	N	Y	Y	N	N
2	M	12	14	2 y	Mandible	Y	N	Y	N	N	N
3	M	34	35	6 m	Mandible	Y	N	N	Y	N	N
4	M	20	21	18 m	Mandible	Y	N	Y	Y	Acne, Sternoclavicular joint, Sacroiliac joint	N
5	F	28	31	3 y	Mandible	Y	N	Y	N	N	N
6	M	11	11	2 m	Mandible	Y	N	Y	Y	PPP	N
7	M	7	7	3 m	Mandible	Y	N	Y	Y	Left femur	N
8	F	58	59	8 m	Mandible	Y	N	Y	Y	Left clavicle, Lumbar vertebra	N
9	F	10	11	1 y	Mandible	Y	N	Y	N	Psoriasis vulgaris	N
10	F	64	65	16 m	Mandible	Y	N	Y	N	Lumbar vertebra, Right ankle	N
11	F	67	67	9 m	Mandible	Y	N	Y	N	Sternum	N
12	F	11	11	7 m	Mandible	Y	N	Y	N	PPP	N
13	F	11	11	9 m	Mandible	Y	N	Y	Y	Sternoclavicular joint	N
14	F	6	6	7 m	Mandible	Y	N	Y	Y	N	N
15	M	22	25	3 y	Mandible	Y	N	Y	N	Sternoclavicular joint, First rib	N
16	F	11	11	7 m	Mandible	Y	N	Y	Y	N	N
17	F	5	5	7 m	Mandible	Y	N	Y	Y	N	N
18	M	11	11	2 m	Mandible	Y	N	Y	Y	N	N
19	F	16	16	6 m	Mandible	Y	N	Y	Y	Acne	N
20	F	50	53	3 y	Mandible	Y	N	Y	Y	Sternoclavicular joint	N
21	F	36	36	5 m	Mandible	Y	N	Y	N	N	N
22	F	12	12	6 m	Mandible	Y	N	Y	Y	N	N
23	M	7	7	4 m	Mandible	Y	N	Y	N	N	N
24	M	23	23	5 m	Mandible	Y	N	Y	N	N	N
25	M	17	17	6 m	Mandible	Y	N	Y	Y	N	N
26	M	13	13	6 m	Mandible	Y	N	Y	Y	N	N
27	F	11	12	15 m	Mandible	Y	N	Y	Y	N	N
28	F	9	9	2 m	Mandible	Y	N	Y	N	PPP, Appendicular skeleton	N
29	F	34	37	3 y	Mandible	Y	N	Y	Y	N	N
30	F	22	23	6 m	Mandible	Y	N	Y	N	N	N
31	M	19	20	1 y	Mandible	Y	N	Y	Y	Sternoclavicular joint	N
32	F	13	14	1 y	Mandible	Y	N	Y	N	N	N
33	F	6	67	2 y	Mandible	Y	N	Y	N	Sacroiliac joint	N
34	F	23	24	1 y	Mandible	Y	N	Y	Y	N	N
35	F	8	15	7 y	Mandible	Y	N	Y	Y	N	N

F female, *M* male, *Y* yes, *N* no, *y* year, *m* month, *PPP* palmoplantar pustulosis.

Table 2. The clinical features and *GNAS* mutations in patients with FD.

No. of patients	Sex	Onset age (years)	Operation age (years)	Duration	Location	Pain	Soft-tissue swelling	Bone swelling	Trismus	Type	Type of *GNAS* mutation
1	F	14	23	9 y	Multiple gnathic bones	N	N	Y	N	PFD	R201H
2	F	9	39	30 y	Multiple gnathic and extragnathic bones	N	N	Y	N	MAS	R201C
3	F	6	16	10 y	Multiple gnathic bones	N	N	Y	N	PFD	R201H
4	F	6	24	18 y	zygoma	Y	N	Y	N	MFD	R201H
5	F	15	23	8 y	maxilla	N	N	Y	N	MFD	R201H
6	F	13	23	10 y	maxilla	N	N	Y	N	MFD	R201C
7	F	23	26	3 y	Multiple gnathic bones	N	N	Y	N	PFD	R201C
8	M	8	18	10 y	Multiple gnathic bones	N	N	Y	N	PFD	R201C
9	M	8	27	19 y	Multiple gnathic and extragnathic bones	Y	N	Y	N	MAS	R201C
10	F	12	22	10 y	maxilla	N	N	Y	N	MFD	R201H
11	F	10	24	14 y	Multiple gnathic bones	N	N	Y	N	PFD	R201H
12	M	11	17	6 y	Multiple gnathic bones	N	N	Y	N	PFD	R201C
13	F	10	17	7 y	Multiple gnathic bones	N	N	Y	N	PFD	R201H
14	F	11	18	7 y	Multiple gnathic bones	N	N	Y	N	PFD	R201H
15	M	14	20	6 y	maxilla	N	N	Y	N	MFD	R201H
16	M	11	21	10 y	Multiple gnathic bones	N	N	Y	N	PFD	R201H
17	F	7	17	10 y	Multiple gnathic bones	N	N	Y	N	PFD	R201C
18	M	9	19	10 y	Multiple gnathic bones	N	N	Y	N	PFD	R201C
19	M	6	23	17 y	Multiple gnathic bones	N	N	Y	N	PFD	R201H
20	F	10	40	30 y	Multiple gnathic and extragnathic bones	N	N	Y	N	PFD	R201C
21	F	18	22	4 y	maxilla	Y	N	Y	N	MFD	R201H
22	M	15	23	8 y	maxilla	Y	N	Y	N	MFD	R201C
23	F	0	45	45 y	Multiple gnathic bones	N	N	Y	N	PFD	R201H
24	M	3	17	14 y	Multiple gnathic bones	N	N	Y	N	MAS	R201H
25	M	56	56	4 m	mandible	N	N	Y	N	MFD	N
26	F	34	44	10 y	maxilla	N	N	Y	N	MFD	N
27	F	14	24	10 y	mandible	N	N	Y	N	MFD	N
28	M	15	20	5 y	maxilla	N	N	Y	N	MFD	N
29	M	13	23	10 y	mandible	N	N	Y	N	MFD	N

F female, *M* male, *Y* yes, *N* no, *y* year, *m* month, *MFD* monostotic fibrous dysplasia, *PFD* polyostotic fibrous dysplasia, *MAS* McCune-Albright syndrome.

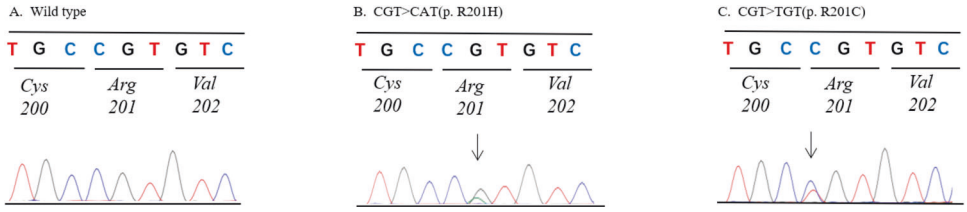

Fig. 1 Mutational analysis of *GNAS* at the Arg201 codon. *GNAS* Mutational Analysis of FD Lesions. **A** The wild-type sequence of *GNAS* at the Arg201 codon. **B** The missense mutation at the Arg201 codon (arrow). **C** The missense mutation at the Arg201 codon (arrow).

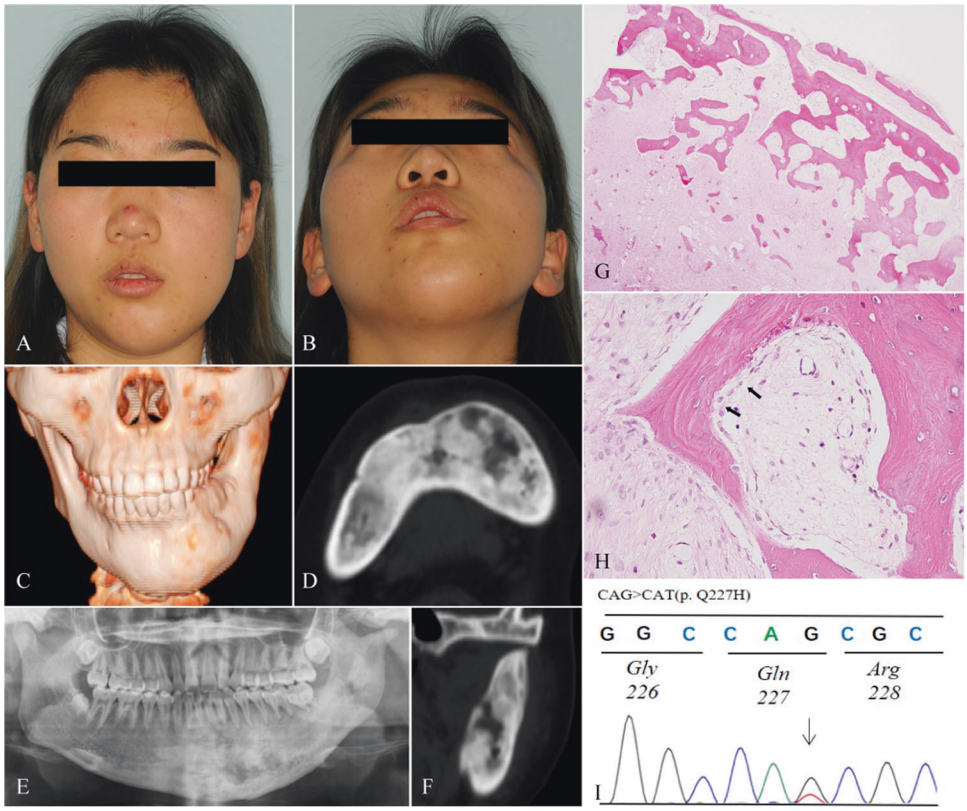

Fig. 2 *GNAS* mutational analysis in one case with overlapping clinical characteristics of FD and DSOM. A, **B** Clinical image of the patient. **C** Three-dimensional reconstruction of the CT images showed swelling of the left mandible. **D**, **E** CT and panoramic radiograph revealed an expansion of ramus and corpus of the left mandible with a "ground-glass" appearance. Cystic change without a sclerotic margin could be seen in the body of the mandible. **F** The sign of subperiosteal bone formation on the left mandible. **G** Histologic features of the lesion: the low-power view (HE, 40×) showing small, round and disconnected bones lying within a fibrous stroma. **H** Part of these bones were rimmed with osteoblasts in the high-power view (arrow; HE, 400×). **I** The sequence of polymerase chain reaction (PCR)-amplified product showed a mutation at the Arg201 codon (arrow), CAG > CAT (p.Q227H).

infection. FD is easily diagnosed when lesions occur in the maxillofacial bone rather than in the mandible. Patients with FD secondary to an infection may present with suppurative lesions, such as fistula, abscess, and cutaneous temperature elevation[26]; and the situation can be more challenging, especially when there is a typical clinical pitfall with ambiguous radiographic characteristics, such as the case described in the Results. The patient showed recurrent pain and swelling as observed for DSOM, while the radiographic and pathological features were unclear. Therefore, we performed molecular analysis to clarify the diagnosis. As identification of somatic mutations in *GNAS* has been used to improve the diagnostic accuracy of FD, mutational analysis may facilitate differential diagnosis.

The results indicate that mutational analysis of the *GNAS* at codon 201 (exon 8) and codon 227 (exon 9) by direct sequencing is a rapid and effective method to differentiate between DSOM and FD. Mutations in *GNAS* were specific to FD and were detected in a majority of the cases with FD (83%), whereas no mutations were detected in cases with DSOM in the study. Of the *GNAS* mutations reported in FD, R201H and R201C mutations were observed in 58% and 42% of the cases, respectively. Taken together, molecular analysis may be helpful when the diagnosis is difficult due to overlapping clinical or pathological characteristics.

Furthermore, Jour et al.[27] found that there was a significant difference in the sensitivity of the *GNAS* mutational status assay between decalcified and non-decalcified FDs (31% vs. 70%, $p = 0.002$) and this was also the case in another study[28] (9.6% vs. 65.7%, $p = 0.001$). Although it is uncertain whether there is a difference between fresh and fixed tissues, testing for *GNAS* mutations is preferably on cryopreserved material if possible[24]. The specimens used in this study were all fresh tissues, which avoids the effect of decalcification on the results. Thus, fresh tissues or part of non-decalcified tissues can be reserved for gene

mutation detection when the diagnosis is difficult due to ambiguous clinical or radiographic characteristics.

Notably, five of 29 FD cases revealed no mutation, which indicated that the possibility of FD cannot be ruled out in the absence of mutations. The negative results mainly due to the technical concerns regarding conventional PCR and direct sequencing, which requires high quality and quantity of DNA, and also a mutant threshold of about 20% in the total population[29]. However, the somatic nature of the mutations in FD may not meet this level in some cases, especially for the older patients, as reported by Isobe et al.[30] that the frequency of mutated cells had decreased in the older lesion. In our study, two of the five FD cases with no detectable *GNAS* mutations were older than 40 years of age. In addition, Shin et al.[28] and Lee et al.[31] had also reported that *GNAS* mutation was more likely to occur in polyostotic FD than in monostotic form. In our study, mutation was higher in polyostotic cases (17/17, 100%) than in monostotic cases (7/12, 58%) and all the negative cases are the monostotic form, which indicated that the positive detection rate may be related to the type of lesion. Thus, the FD cases without detectable *GNAS* mutation may be explained by the mosaicism of FD, with low rates of mutated cells compared with non-mutated cells, or by the existence of new mutations, which were not sought in our study.

Due to the limitations of molecular detection methods (e.g., no mutations detected), it is particularly important to clarify the pathogenesis of DSOM. The etiology of DSOM remains unknown and poorly understood due to its rarity and lack of clear diagnostic criteria. The recent studies on nonsynonymous homozygous mutations in the proline serine threonine phosphatase interacting protein 2 (*PSTPIP2*) in two mouse models[32,33], that share some manifestations with the human SAPHO syndrome, have provided new insights into the molecular basis of SAPHO syndrome. However, analysis of the *PSTPIP2* coding sequence revealed no specific variants in 38 samples from patients with SAPHO[34]. Determining the etiology of DSOM is of great significance for diagnosis and treatment, so we expect further progress in etiology research.

Taken together, the present study confirmed the efficacy of *GNAS* mutation analysis (including both exons 8 and 9) in differentiating DSOM and FD using PCR and direct sequencing. However, the diagnosis of FD, that has mosaic features, cannot not be ruled out in the absence of mutations.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

J.A. and J.Z. performed study concept and design; J.X. and K.J. performed development of methodology and writing, review and revision of the paper; J.X. and K.J. provided acquisition, analysis and interpretation of data; J.A., T.L. and J.Z. provided technical and material support.

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COMPETING INTERESTS

The regional Ethical Review Board of Peking University School and Hospital of Stomatology approved this study (PKUSSIRB-202272025), and the study was performed in accordance with the Declaration of Helsinki. The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The regional Ethical Review Board of Peking University School and Hospital of Stomatology approved this study (PKUSSIRB-202272025), and the study was performed in accordance with the Declaration of Helsinki.

ADDITIONAL INFORMATION

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