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GNAS mutational analysis in differentiating fibrous dysplasia and ossifying fibroma of the jaw

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Differential diagnosis of fibrous dysplasia and ossifying fibroma may often pose problems for pathologists. The purpose of this study was to evaluate the value of mutational analysis of the *GNAS* gene in differentiating these two conditions. DNA samples from patients with fibrous dysplasia ($n=30$) and ossifying fibroma ($n=21$) were collected to analyze the presence of *GNAS* mutations at exons 8 and 9, the two previously reported hotspot regions, using polymerase chain reaction and direct sequencing. In all, 90% (27/30) of cases with fibrous dysplasia showed missense mutations of codon 201 at exon 8, with a predilection of arginine-to-histidine substitution (p.R201H, 70%) as opposed to arginine-to-cysteine substitution (p.R201C, 30%), whereas no mutation was detected at exon 9. No mutation was found in all 21 cases with ossifying fibroma. In addition, a meta-analysis of previously published reports on *GNAS* mutations in fibrous dysplasia and ossifying fibroma was performed to substantiate our findings. A total of 24 reports including 307 cases of fibrous dysplasia and 23 cases of ossifying fibroma were reviewed. The overall incidence of *GNAS* mutations in fibrous dysplasia was 86% (264/307), and the major types of mutations were also R201H (53%) and R201C (45%). No *GNAS* mutation was detected in all patients with ossifying fibroma. We also reported one case with uncertain diagnosis due to overlapping clinicopathological features of fibrous dysplasia and ossifying fibroma. An R201H mutation was detected in this case, thus confirming a diagnosis of fibrous dysplasia. Taken together, our findings indicate that mutational analysis of *GNAS* gene is a reliable adjunct to differentiate ossifying fibroma and fibrous dysplasia of the jaws.
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Fibrous dysplasia and ossifying fibroma of the jaws are fibro-osseous lesions with different clinical course and treatment strategies.[1] Ossifying fibroma is a benign tumor thought to arise from the periodontal ligament,[2] which can occur in almost any bone in the craniofacial region, predominantly in the jaws. The clinical course of the tumor varies from indolent to aggressive progression.[3] Generally, it is slow-growing and incidentally diagnosed by routine dental examinations, but in some instances, it can be destructive, causing facial deformity, sinus obstruction, proptosis, infection and intracranial complications, as a result of which complete surgical removal is needed.[4] Fibrous dysplasia is a benign dysplastic disease of the bone, which occurs in three forms: monostotic fibrous dysplasia, which involves one bone; polyostotic fibrous dysplasia, which affects multiple bones; and McCune–Albright syndrome, in which polyostotic fibrous dysplasia is accompanied by cafe-au-lait spots or hyperfunctioning endocrinopathies.[5] No bone can be spared.[6] According to Hart *et al*'s report,[7] 90% of the total body disease skeletal burden is established by age 15 years, and most of the monostotic fibrous dysplasia tend to stop growing when skeletal maturity has been attained;[1] thus, it is best to perform bone contouring subsequent to growth arrest of the lesion.[8] Differential diagnosis of fibrous dysplasia and ossifying fibroma is of great importance for their treatment and prognosis.

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According to the latest WHO classification, there are two important features that can be used to differentiate ossifying fibroma and fibrous dysplasia. First, ossifying fibroma is a radiologically and histologically well-demarcated lesion, whereas fibrous dysplasia lesional bones merge with its surroundings. Furthermore, fibrous dysplasia can be diagnosed by its typical histological characteristics: isolated trabeculae of woven bone generally without rimming of osteoblasts.[9,10] In addition to the WHO criteria, it was reported that bundles of collagen fibers oriented perpendicular to the bone surface, compatible with Sharpey's fibers, were characteristic of the fibrous dysplasia lesion.[11,12] However, not all fibrous dysplasia or ossifying fibroma cases exhibit these classic features; instead, they can present overlapping clinical, radiographic and histological features posing diagnostic challenge to the clinicians and pathologists.[4] Under such circumstances, investigation at the molecular levels may be useful.

It has been well established that fibrous dysplasia is associated with postzygotic activating mutations of the *GNAS* gene, encoding the α-subunit of the stimulatory G-protein Gs (Gsα).[13] Somatic mutations at Arg^{201} and Gln^{227} codon of Gsα have been identified in many fibrous dysplasia lesions, but absent in ossifying fibroma lesions,[14,15] which points to a possible role of the mutational analysis in differentiating these two conditions. Furthermore, Toyosawa's study also suggest that polymerase chain reaction (PCR) analysis with peptide nucleic acid (PNA) for *GNAS* mutations at the Arg^{201} codon is a useful method to differentiate between fibrous dysplasia and ossifying fibroma.[1]

To further explore the role of *GNAS* gene mutational analysis in differential diagnosis of fibrous dysplasia and ossifying fibroma, we examined both Arg^{201} and Gln^{227} codon in 30 patients with fibrous dysplasia and 21 with ossifying fibroma in the jaws using PCR and direct sequencing. A meta-analysis was also conducted from the published literature evaluating the *GNAS* mutations in fibrous dysplasia and ossifying fibroma in both the jaws and extragnathic bones. In addition, a mutation of *GNAS* gene was detected in one case with fibro-osseous lesions with overlapping clinical and pathological features of fibrous dysplasia and ossifying fibroma, thus confirming a diagnosis of fibrous dysplasia. Our results demonstrate that the mutational analysis of *GNAS* gene could be a clinically feasible method in differentiating fibrous dysplasia and ossifying fibroma of the jaws.

Materials and methods

Patients and Samples

A total of 30 cases of fibrous dysplasia and 21 cases of ossifying fibroma arising from the jaws were retrieved from the repository of the Department of Oral Pathology, Peking University School and Hospital of Stomatology from 2005 to 2011. Under an institutionally approved protocol, fresh tissues from the bone lesions were obtained during the surgical removal procedure. Once collected, all the specimens were kept at $-80\,^{\circ}C$. In addition, one extra case diagnosed as 'fibro-osseous' lesions with overlapping pathologic features of fibrous dysplasia and ossifying fibroma was also retrieved from our files. The formalin-fixed, paraffin-embedded tissues of the patient were obtained for mutational analysis. All of the cases were re-evaluated and confirmed by three experts according to the current histological, radiographic and clinical criteria for fibrous dysplasia and ossifying fibroma.[9] The detailed information of these cases was listed in Tables 1 and 2.

Mutational Analysis of *GNAS* Gene at Arg^{201} and Gln^{227} Codon

Genomic DNA was isolated from tissue samples as described above using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. For all patients, mutational analysis was undertaken by direct DNA sequencing of PCR-amplified target sequence of the *GNAS* gene. DNA (200 ng) was amplified in a standard 100-μl PCR reaction mixture using GoTaq

Table 1 The clinical features and *GNAS* mutations in patients with fibrous dysplasia

No. of patients	*Gender*	*Onset age (years)*	*Operation age (years)*	*Duration (years)*	*Location*	*Type of* GNAS *mutation*
1	F	10	18	8	Both	R201H
2	F	10	21	11	Both	R201H
3	F	9	36	27	Both	R201H
4	M	5	18	13	Both	R201H
5	M	8	19	11	Both	R201H
6	M	13	23	10	Both	R201H
7	F	14	19	5	Mandible	R201H
8	M	10	20	10	Mandible	R201H
9	M	17	21	4	Mandible	R201H
10	M	18	28	10	Mandible	R201H
11	F	8	18	10	Maxilla	R201H
12	M	7	9	2	Maxilla	R201H
13	M	6	13	7	Maxilla	R201H
14	M	11	18	7	Maxilla	R201H
15	M	10	28	18	Maxilla	R201H
16	M	12	19	7	Maxilla	R201H
17	M	11	19	8	Maxilla	R201H
18	F	7	19	12	Multiple	R201H
19	M	11	19	8	Multiple	R201H
20	F	8	15	7	Both	R201C
21	M	8	21	13	Both	R201C
22	F	8	13	5	Maxilla	R201C
23	M	14	19	5	Maxilla	R201C
24	M	15	24	9	Maxilla	R201C
25	F	7	21	14	Multiple	R201C
26	F	12	30	18	Multiple	R201C
27	F	15	32	17	Multiple	R201C
28	F	12	27	15	Maxilla	No mutation
29	F	40	45	5	Maxilla	No mutation
30	M	27	47	20	Maxilla	No mutation

Abbreviations: F, female; M, male.
'Both' indicates that the lesion occurred in both mandible and maxilla.

Green Master Mix (Promega, Madison, WI, USA) according to the manufacturer's instructions. A 270-bp fragment of the *GNAS* gene including the Arg^{201} codon was amplified using the following primers: forward, 5′-TGACTATGTGCCGAGCGA-3′ and reverse, 5′-AACCATGATCTCTGTTATATA A-3′,[13] while another 316-bp sequence of the *GNAS* gene including the Gln^{227} 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 by DNA purification system (Promega) and sequenced using an automated DNA sequencer model 373 (Applied Biosystems, Foster City, CA, USA).

Table 2 The clinical features and *GNAS* mutations in patients with ossifying fibroma

No. of patient	*Gender*	*Onset age (years)*	*Operation age (years)*	*Duration (years)*	*Location*	*Type of* GNAS *mutation*
1	F	45.9	46	0.1	Mandible	No mutation
2	F	19	22	3	Mandible	No mutation
3	F	9.8	10	0.2	Maxilla	No mutation
4	M	1	1	0	Mandible	No mutation
5	M	13	14	1	Mandible	No mutation
6	F	16	16	0	Mandible	No mutation
7	F	8	20	12	Maxilla	No mutation
8	F	17.8	18	0.2	Mandible	No mutation
9	F	38.7	39	0.3	Mandible	No mutation
10	F	43	45	2	Mandible	No mutation
11	F	37	40	3	Mandible	No mutation
12	F	34	36	2	Maxilla	No mutation
13	M	11.9	12	0.1	Mandible	No mutation
14	F	40.5	41	0.5	Maxilla	No mutation
15	M	46.7	47	0.3	Maxilla	No mutation
16	F	39	41	2	Maxilla	No mutation
17	M	5.8	6	0.2	Mandible	No mutation
18	F	27.9	28	0.1	Maxilla	No mutation
19	F	21.7	22	0.3	Maxilla	No mutation
20	M	14	15	1	Mandible	No mutation
21	M	8	13	5	Maxilla	No mutation

Abbreviations: F, female; M, male.

Meta-Analysis

We searched the PubMed (National Library of Medicine) database from 1966 to July 2012 using the following terms: '*GNAS*' OR '*GNAS1*' and 'fibrous dysplasia', '*GNAS*' OR '*GNAS1*' and 'ossifying fibroma'. Reference lists of retrieved articles were hand-searched for further publications. Two reviewers independently performed the literature search and evaluation. Papers were rejected at the initial screening if the articles were published in a language other than English or titles/abstracts showed that they were clearly irrelevant or the mutation analysis were not examined in the lesional bones. Full-text versions of potentially relevant articles were obtained and reviewed to assess their suitability for inclusion in this study. Study selection process was described in Figure 1.

Statistical Analysis

Statistical analysis was performed using the SPSS for windows (version 11.0) statistical software package. Descriptive statistics were used as appropriate.

Results

Clinicopathological Features

The clinical characteristics of patients enrolled in this study were summarized in Tables 1 and 2. Of the 30 patients with fibrous dysplasia (13 females and 17 males), 13 occurred in the maxilla, 4 in the mandible, 8 patients presented both mandibular and maxillary lesions, 5 cases showed multiple bone

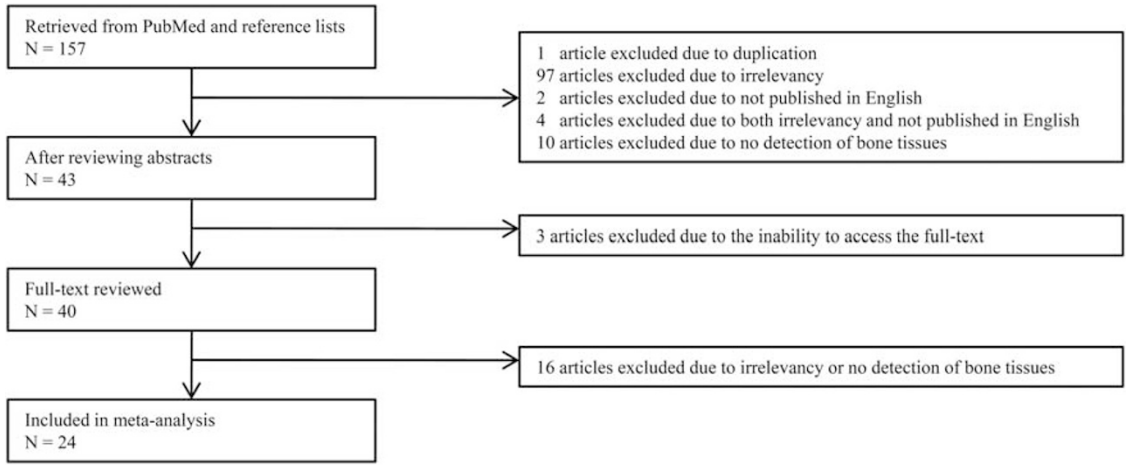

Figure 1 Flow chart of the selection process for meta-analysis.

lesions affecting both gnathic and extragnathic bones and 4 of whom were diagnosed as McCune–Albright syndrome. The onset age ranged from 5 to 40 years with a mean of 12.1 ± 6.9 years, and the age at operation ranged from 9 to 47 years with a mean of 22.6 ± 8.6 years. The duration ranged from 2 to 27 years with a mean of 10.5 ± 5.5 years. The 21 cases with ossifying fibroma (7 males and 14 females) included 9 occurring in the maxilla and 12 in the mandible. The onset age ranged from 1 to 46.7 years old with a mean of 23.7 ± 14.9 years, and the age at operation ranged from 1 to 47 years with a mean of 25.3 ± 14.6 years. The duration ranged from 0 to 12 years with a mean of 1.6 ± 2.7 years. Histologically, fibrous dysplasia was composed of fibrous stroma containing irregular-shaped woven bone generally without obvious osteoblastic rimming (Figure 2a). Ossifying fibroma featured as spherical and small bone spicules resembling normal cementicles, which were present in the periodontal ligament (Figure 2b).

GNAS Mutations

The results of *GNAS* mutational analysis were shown in Tables 1 and 2. A mutation of Arg^{201} codon of Gsα protein was found in 27 of the 30 (90%) cases of fibrous dysplasia, with a predilection of Arg-to-His (p.R201H) substitutions (Figure 3b, 19 cases, 70%) as opposed to Arg-to-Cys (p.R201C) substitutions (Figure 3c, 8 cases, 30%). The rarely reported mutation of Gln^{227} was not detected, and no mutation was detected in all 21 cases of ossifying fibroma.

A case report. A 23-year-old woman was referred to our hospital complaining of an asymptomatic expansion of the left mandible for more than 10 years with a chronic progression. The patient took no medical treatment during the past 10 years. Physical examination revealed a painless, hard and immobile mass of size approximately 4.0 cm × 4.0 cm in the left mandible. The skin overlying the expansion appeared intact. Intraorally, a firm mass was present in the left mandible with buccal and lingual expansion, there was no sign of tenderness and the oral mucosa was normal. On the basis of the above clinical findings, a diagnosis of benign tumor was suggested.

Panoramic radiograph (Figure 4a) showed an enlargement of ramus and corpus of the left mandible with a 'ground-glass' appearance, which was consistent with fibrous dysplasia. However, the cystic change with a sclerotic margin in the corpus made the diagnosis of fibrous dysplasia difficult.

The patient underwent conservative surgery with trimming of the affected bone. The histological features of the removed lesion showed (Figure 4b) a cellular fibrous stroma within which were small, irregular and disconnected bone spicules (somewhat resembling cementicles), and part of these spicules were rimmed with osteoblasts, which was the characteristic of ossifying fibroma.

Owing to the overlapping features of fibrous dysplasia and ossifying fibroma described above (the radiologic appearance was suggestive of fibrous dysplasia, but the histopathology revealed features consistent with an ossifying fibroma), a diagnosis of 'fibro-osseous' lesion was then made. By *GNAS* mutational analysis of exons 8 and 9, we identified an R201H mutation in the lesional bone (Figure 4c), which confirmed a diagnosis of fibrous dysplasia. Because of the conservative surgery (trimming) of the lesion, the patient was followed up for one and half years postoperatively. The lesion was stable with no apparent enlargement.

Meta-analysis. The flow chart of the meta-analysis was present in Figure 1. Initially, 157 publications

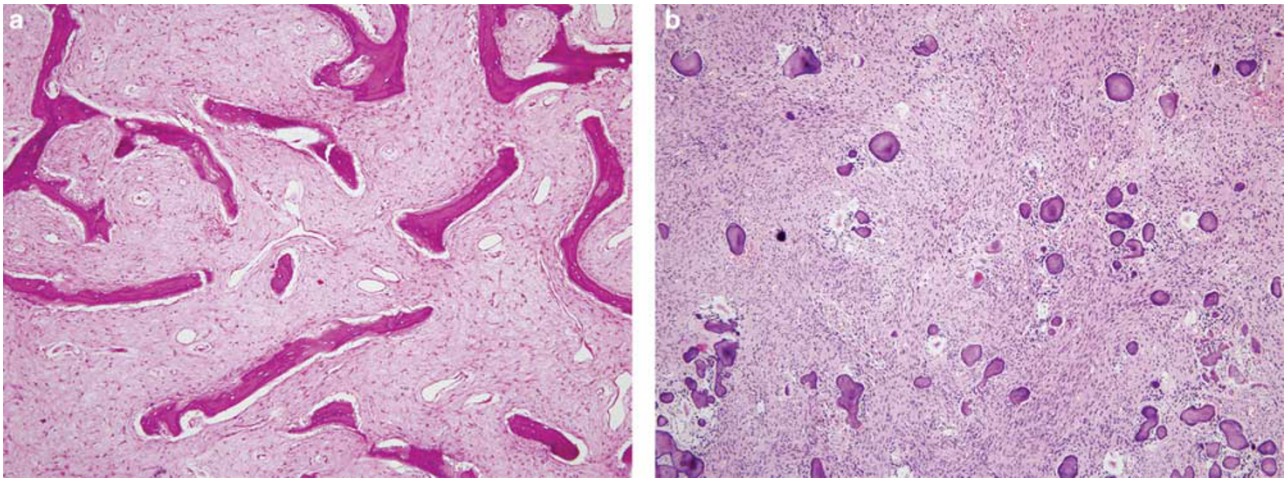

Figure 2 Histologic features of fibrous dysplasia and ossifying fibroma. (**a**) Fibrous dysplasia is featured as irregular trabeculaes of woven bone within fibrous stroma, no osteoblasts could be seen around the bone. (**b**) Ossifying fibroma showed calcified spherules similar to cementicles, which lie in a moderately cellular, dense fibrous stroma. Magnifications: × 40.

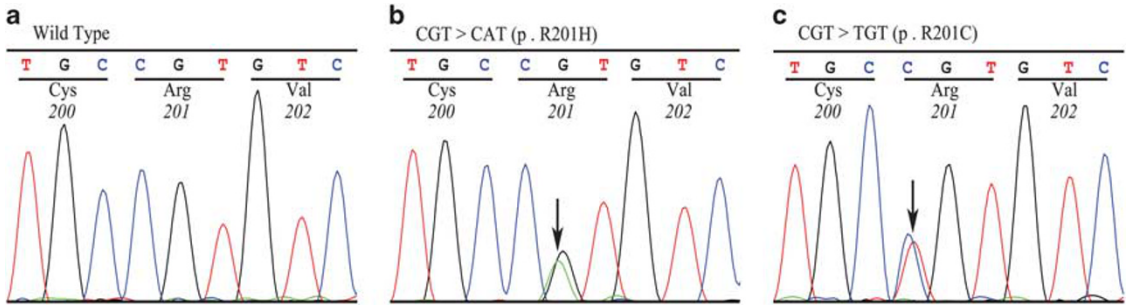

Figure 3 Mutational analysis of *GNAS* at the Arg^{201} codon. (**a**) The wild-type sequence of *GNAS* at the Arg^{201} codon. (**b**) The missense mutation at the Arg^{201} codon (arrow), CGT>CAT (p.R201H). (**c**) The missense mutation at the Arg^{201} codon (arrow), CGT>TGT (p.R201C).

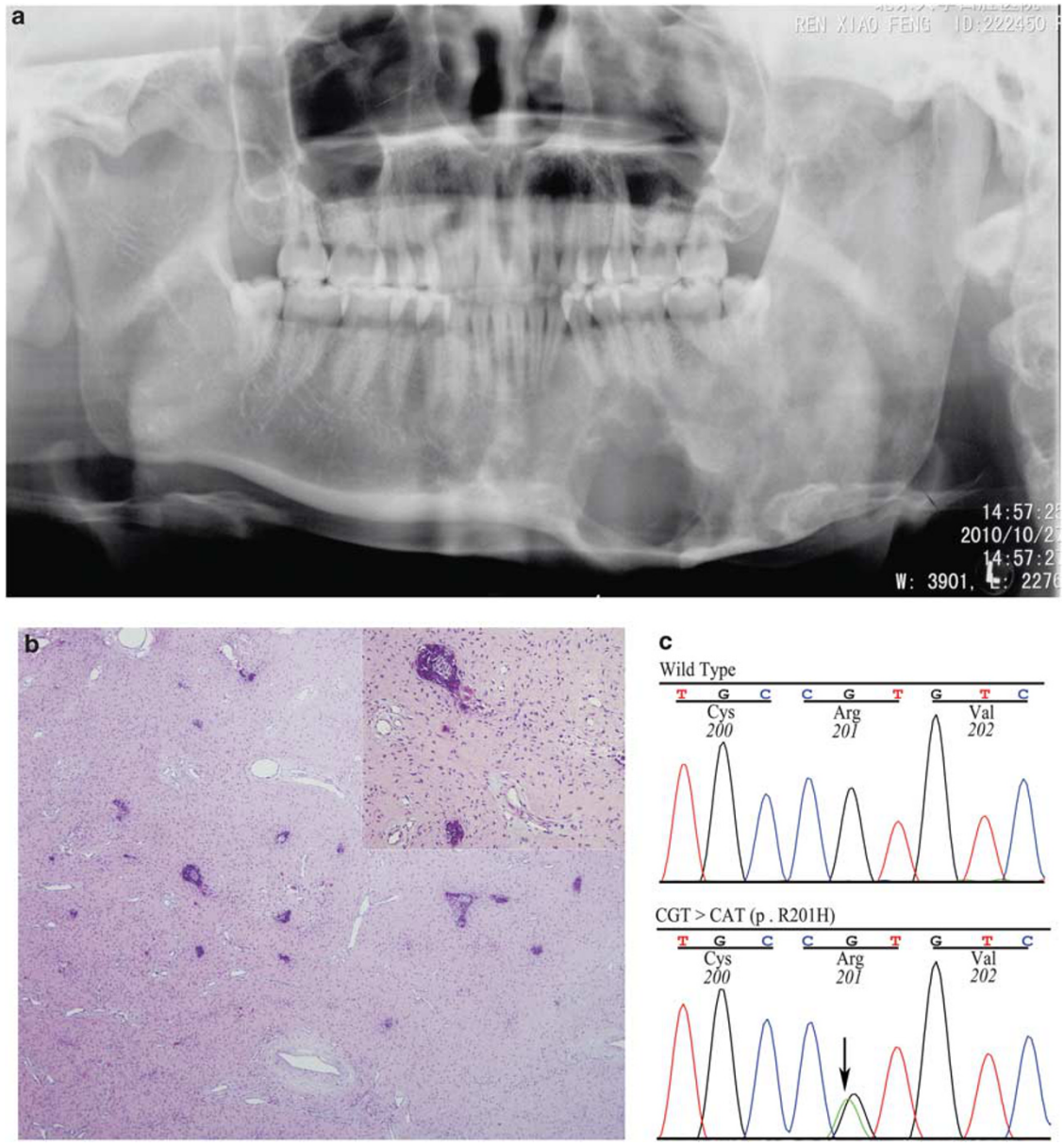

Figure 4 *GNAS* mutational analysis in one case with overlapping clinicopathological characteristics of fibrous dysplasia and ossifying fibroma. (**a**) The panoramic radiograph revealed an expansion of ramus and corpus of the left mandible with a 'ground-glass' appearance. Cystic change with a sclerotic margin could be seen in the corpus. (**b**) Histologic features of the lesion: the low-power view (×40) showing small, round and disconnected bone lying within a cellular fibrous stroma, which was abundant compared with the area of the bone; osteoblasts could be seen around the bone surface as revealed in the high-power view (inset, ×200). (**c**) The sequence of polymerase chain reaction (PCR)-amplified product showed a mutation at the Arg^{201} codon (arrow), CGT>CAT (p.R201H).

were retrieved, including 155 reports from PubMed and 2 additional articles from the reference lists. After a screening of the title and abstract available, 114 articles were excluded, of which 1 was excluded for the duplication, 101 due to irrelevancy, 2 published in languages other than English, 3 for unavailable full texts and another 10 were excluded because the mutations were not examined in the lesion tissues. After reviewing the remaining 43 full texts available, 24 articles including a total of 330 patients (307 cases of fibrous dysplasia and 23 cases of ossifying fibroma) were analyzed. The details of the 24 studies are summarized in Table 3.[1,13–35]

Among the 24 studies, the numbers of cases ranged from 1 to 64, various techniques have been used for the detection of *GNAS* mutations, such as reverse transcription PCR and clone sequencing, conventional PCR and direct sequencing, PCR-restriction fragment length polymorphism, pyrosequencing, PCR with mutation-specific restriction enzyme digestion and so on. A variety of materials were used for the extraction of genomic DNA, including fresh bone biopsy, fresh bone tissue, lesional stromal cell cultures, formalin-fixed, paraffin-embedded tissues and so on. All of these studies examined the exon 8 of *GNAS*, six of which also studied the exon 9.[17–19,21,23,34] One study examined the exon 7, as well as exons 10–13.[19]

Totally, the overall positive rate of *GNAS* mutation in fibrous dysplasia was 86%. The major types of *GNAS* mutation were R201H and R201C, except for one report[22] that did not mention the type of mutation. The incidences of R201H and R201C were 53% and 45%, respectively. The other types of missense mutations rarely encountered were Q227L in three cases, R201S in one case and R201G in one case.

In the 13 studies that mentioned the types of the bone involved,[1,13,16,17,19,21,23,27,28,30,31,33,34] the positive rate of *GNAS* mutation was 84% in the extragnathic bones and 78% in the craniofacial bones.

GNAS mutation analysis in ossifying fibroma was performed in three reports[1,14,15] including a total of 23 cases, and no mutations were detected.

Discussion

According to the latest WHO classification,[9] the fibro-osseous lesions in the oral and maxillofacial region referred to a group of entities including fibrous dysplasia, ossifying fibroma and osseous dysplasia. Usually, the diagnosis of these fibro-osseous lesions could be made solely based on a combination of clinical, radiological and histological estimation, but under some situations, it still can be challenging, especially for fibrous dysplasia and ossifying fibroma.

Our results demonstrated that mutational analysis of *GNAS* gene at codon 201 (exon 8) and codon 227 (exon 9) by direct sequencing is a rapid and effective method to differentiate ossifying fibroma and fibrous dysplasia of the jaws. *GNAS* mutations were specific to fibrous dysplasia and could be detected in most of the fibrous dysplasia cases (90%), whereas no mutations were detected in ossifying fibroma. Of the *GNAS* mutations in fibrous dysplasia, R201H was the primary mutation type with a ratio of 70%, whereas R201C mutation accounted for 30%. The results of meta-analysis further substantiated our findings, which revealed that an overall of 86% of the fibrous dysplasia cases from both the extragnathic and the craniofacial bones had *GNAS* mutations, including R201H, R201C, R201S and Q227L, with R201H and R201C being the two most frequent types. On the basis of the above findings, the molecular analysis might be a helpful method when the fibro-osseous lesions in the jaws were difficult to diagnose. By using this method, we successfully identified one case with overlapping clinical and histological features of fibrous dysplasia and ossifying fibroma, and the diagnosis of fibrous dysplasia was established based on the presence of *GNAS* mutation.

Notably, three cases with fibrous dysplasia in this study had no detectable mutation, which indicated that the diagnosis of fibrous dysplasia could not be ruled out when no mutation could be detected, mainly because of the technical concerns regarding regular PCR and direct sequencing, which requires high quality and quantity of DNA, and also a mutant threshold of about 20% in the total population;[15] however, the somatic nature of the mutations in fibrous dysplasia may not meet this level of sensitivity in some cases, especially for the older ones, as reported by Kuznetsov *et al*[13] that the percentage of mutated cells within a given lesion may decrease with age. Two of the three fibrous dysplasia cases with no detectable *GNAS* mutations in this study were older than 40 years of age, probably caused by the decreasing percentage of the mutant cells. To raise the detection rate of mutant *GNAS* in samples, a few methods had been employed, such as pyrosequencing, PNA-clamping PCR and multiple rounds of nested PCR in conjunction with restriction endonuclease treatment. However, because of the complexity, none of these mentioned methods could be a good candidate for routine clinical examination. Compared with the above methods, regular PCR and direct sequencing employed in this study was a relatively easier and more practical method for the detection of multiple mutations in routine practice. In some cases, this approach was proven to be sensitive enough even when DNA samples extracted from formalin-fixed, paraffin-embedded tissues were used.

Taken together, the present large series as well as a systematic review of the literature confirmed the role of *GNAS* mutational analysis (including both exons 8 and 9) in differentiating fibrous dysplasia and ossifying fibroma by using regular PCR and

Table 3 The characteristics of studies included in meta-analysis

Country	*Year*	*Disease included*	*Method*	*Exon*	*Types of samples*	*No. of FD cases*	*Positive FD cases (%)*	*Positive extragnathic*	*Genotype (no. of cases)*	*Positive gnathic*	*Genotype (no. of cases)*	*Total genotype (no. of cases)*	*No. of OF cases*	*Positive OF cases (%)*
China[16]	2012	FD	RT-PCR and CS	8	Cell	3	3 (100)	3/3	R201H (3)			R201H (3)	0	
South Korea[17]	2012	FD	Conventional PCR and DS	8,9	Paraffin-embedded tissue	48	28 (58)	19/30	R201H (17) R201C (2)	9/18	R201H (8) R201C (1)	R201H (25) R201C (3)	0	
USA[15]	2011	FD and OF	Pyrosequencing	8	FFPE tissue	24	23 (96)					R201H (19) R201C (4)	10	0 (0)
USA[18]	2011	FD	RT-PCR and DS	8,9	Biopsy sample	6	5 (83)					R201H (3) R201C (2)	0	
Japan[19]	2011	FD	Conventional PCR and DS	7–13	Bone tissue	1	0 (0)	0/1		0/0			0	
USA[14]	2010	OF	Nested PCR-RFLP and CS	8	FFPE tissue	0	0 (0)						8	0 (0)
USA[13]	2008	FD	PNA-based PCR or conventional PCR and DS	8	Cell	25	22 (88)	16/18	R201H (1) R201C (15)	6/7	R201H (2) R201C (4)	R201H (3) R201C (19)	0	
USA[20]	2007	FD	PNA-based PCR or conventional PCR and DS	8	Fresh bone tissue, cell	17	14 (82)					R201H (2) R201C (12)	0	
UK[21]	2007	FD	MSRED	8,9	Paraffin-embedded tissue	64	56 (88)	51/59	R201H (31) R201C (18) Q227L (2)	5/5	R201H (1) R201C (3) Q227L (1)	R201H (32) R201C (21) Q227L (3)	0	
Japan[1]	2007	FD and OF	PNA-based nested PCR-RFLP and CS	8	FFPE tissue	9	9 (100)	4/4	R201H (2) R201C (2)	5/5	R201H (2) R201C (3)	R201H (4) R201C (5)	5	0 (0)
France[22]	2006	FD	Nested PCR-RFLP and PNA-based PCR and DS	8	Paraffin-embedded tissue, frozen bone tissue, fresh bone tissue, cell	15	12 (80)					NM	0	
Italy[23]	2006	FD	PNA-based PCR-RFLP and DS	8,9	Paraffin-embedded tissue, fresh bone tissue	2	1 (50)	1/2	R201H (1)			R201H (1)	0	
Japan[24]	2006	FD	PNA-based PCR and CS	8	Paraffin-embedded tissue	16	16 (100)					R201H (8) R201C (8)	0	
USA[25]	2006	FD	NM	8	NM	5	5 (100)					R201H (1) R201C (4)	0	
USA[26]	2004	FD	PNA hybridization probe and FRET principle	8	Paraffin-embedded tissue, biopsy sample, cell	9	9 (100)					R201H (2) R201C (7)	0	
USA[27]	2003	FD	Conventional PCR and DS	8	Cell	4	4 (100)	2/2	R201H (1) R201C (1)	2/2	R201H (1) R201C (1)	R201H (2) R201C (2)	0	
Italy[28]	2003	FD	PNA-based PCR and DS	8	Fresh bone tissue, cell	13	13 (100)	13/13	R201H (3) R201C (10)			R201H (3) R201C (10)	0	
Italy[29]	2003	FD	Conventional PCR and DS	8	Paraffin-embedded tissue, formaldehyde-fixed tissue, fresh bone biopsy, fresh bone tissue, cell	7	7 (100)					R201H (5) R201 (2)	0	
Germany[30]	2001	FD	PCR-RFLP and SSCP and DS	8	Paraffin-embedded tissue, frozen bone tissue	9	9 (100)	9/9	R201H (6) R201C (3)			R201H (6) R201C (3)	0	
USA[31]	2000	FD	PNA-based PCR or conventional PCR and DS	8	Paraffin-embedded tissue, frozen bone tissue, fresh bone tissue, cell	8	8 (100)	2/2	R201H (2)	6/6	R201H (2) R201C (4)	R201H (4) R201C (4)	0	
USA[32]	1999	FD	PCR-RFLP and DS	8	Bone tissue, cell	11	10 (91)					R201H (5) R201C (5)	0	
Italy[33]	1999	FD	PNA-based PCR and DS	8	Fresh bone tissue	1	1 (100)			1/1	R201G (1)	R201G (1)	0	
Germany[34]	1999	FD	Conventional PCR and DS	8,9	Formalin-fixed tissue, frozen bone tissue, cell	1	1 (100)			1/1	R201H (1)	R201H (1)		
Canada[35]	1997	FD	Nested PCR and DS	8	Methylmethacrylate-embedded tissue	9	8 (89)					R201H (4) R201C (3) R201S (1)	0	
Total						307	264 (86)	120/143 (84%)	R201H (66) R201C (52) Q227L (2)	35/45 (78%)	R201H (17) R201C (16) Q227L (1) R201G (1)	R201H (133, 53%) R201C (114, 45%) Q227L (3, 1%) R201G (1, 4‰) R201S (1, 4‰)	23	0 (0)

Abbreviations: FD, fibrous dysplasia; OF, ossifying fibroma; RT-PCR, reverse transcription-polymerase chain reaction; CS, clone sequencing; DS, direct sequencing; RFLP, restriction fragment length polymorphism; PNA, protein nucleic acid; MSRED, PCR with mutation-specific restriction enzyme digestion; NM, not mentioned; SSCP, single-strand conformational polymorphism; FFPE, formalin-fixed paraffin-embedded.

direct sequencing. However, considering the mosaic features of fibrous dysplasia and the limitations of the method, diagnosis of fibrous dysplasia could not be ruled out when no mutations are detected.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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