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GNAS Mutations in Fibrous Dysplasia: A Comparative Study of Standard Sequencing and Locked Nucleic Acid PCR Sequencing on Decalcified and Non-Decalcified Formalin Fixed Paraffin Embedded Tissues

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Abstract

It is well-known that fibrous dysplasia is characterized by the presence of activating mutations involving G-nucleotide binding protein alpha sub unit (*GNAS*) involving codon R201 and rarely codon 227 with a mutation frequency between 45–93%. Herein, we investigate the sensitivity of detection of *GNAS* mutations in exons 8 and 9 using standard and a high sensitive locked nucleic acid PCR (LNA-PCR) sequencing in 52 cases of fibrous dysplasia. In view of the recent report of *GNAS* mutations in a small number of low grade osteosarcomas, we also tested in addition 12 cases of low grade osteosarcomas. *GNAS* exon 8 mutations p.R201H (31%), p.R201C (15%) and p.R201S (2%) were identified in 48% of fibrous dysplasia cases. LNA-PCR/sequencing identified only one codon 201 positive case within the mutation negative cases tested by standard PCR and Sanger sequencing. No mutations were identified in any of the low grade osteosarcomas by standard and LNA-PCR/sequencing. There was no association between age, site, size, specimen type and mutational status. No exon 9 or codon 227 mutations were identified in any of tested cases. There was a significant difference in the sensitivity of the assay between decalcified and non decalcified FDs (31 vs. 70%, p=0.002). LNA-PCR has no added value in enhancing detection sensitivity for *GNAS* mutations in fibrous dysplasia. In addition to decalcification, innate somatic mosaicism contributes to the decreased sensitivity in mutation detection.

Keywords

Fibrous dysplasia; *GNAS* mutations; Sanger sequencing; Locked nucleic acid PCR sequencing (LNA-PCR/sequencing)

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Disclosure/conflict of interest

The authors declare no conflict of interest.

INTRODUCTION

Fibrous dysplasia (FD) is a benign medullary osteofibrous lesion that may involve one (monostotic FD) or more (polyostotic FD) bones. Fibrous dysplasia occurs in children and adults and affects all populations with an equal sex distribution [1]. Fibrous dysplasia may occur in isolation or as a part of McCune-Albright syndrome, which includes endocrine abnormalities, café-au-lait pigmented skin lesions, and FD [2]. Microscopically fibrous dysplasia has biphasic morphology with both fibrous and osseous components. The fibrous component consists of cytologically bland spindle cells with low mitotic rate blending with and surrounding the osseous component. The latter shows irregular curvilinear trabeculae of woven bone. Typically, osteoblastic rimming of the bony trabeculae is absent. *GNAS* gene encodes the stimulatory G-protein α -subunit ($G\alpha$), which serves as a ubiquitously expressed signal transducer that transmits hormonal and growth factor signals to effector proteins. The most well characterized function of *GNAS* is activation of the membrane-associated enzyme adenylate cyclase, which stimulates the production of cAMP and can activate protein kinase A (PKA). Mutations that occur at *GNAS* codons 201 (exon 8) or 227 (exon 9) have been shown to constitutively activate $G\alpha$ and therefore lead to constitutive cAMP signaling. [3]. Studies showed that this constitutive signaling leads to increased cAMP in osteoblastic cells and impedes their normal differentiation. This confers the spindle cell morphology seen in fibrous dysplasia and the immature partially formed bone trabeculae [4,5,6]. Over the last decade, *GNAS* mutational analysis has been used an ancillary test in challenging cases of fibrous dysplasia. Studies have shown that *GNAS* α mutation occur in FD with a frequency ranging from 45% to 100% [7,8]. The technologies used to detect these mutations from paraffin-embedded tissues and frozen samples, range from traditional Sanger sequencing (requiring 25% mutant allele frequency) to more sensitive Pyrosequencing (detects 1–2% mutant allele frequency) [8,9].

Herein, we report our institutional experience with *GNAS* mutational analysis in Fibrous dysplasia taking into consideration the effect of decalcification treatment. Additionally, we investigate the added value of Locked nucleic acid PCR sequencing (LNA-PCR/sequencing) method in enhancing the assay's sensitivity. In addition, we have investigated a series of low grade osteosarcomas for the presence of *GNAS* mutations with LNA-PCR/sequencing.

MATERIALS AND METHODS

Patient Selection

Institutional pathology database was reviewed to identify patients with fibrous dysplasia treated at our center over a 20 year period. Sixty cases were selected. Only cases with the final diagnosis of “fibrous dysplasia” in Pathology database were included. Seven cases were excluded after pathological examination due to atypical histological and/or imaging features. Twelve cases of Low grade paraosteal osteosarcoma were also included.

DNA extraction

Genomic DNA was extracted from formalin-fixed paraffin-embedded tissue blocks using DNeasy Tissue kit (Qiagen, Valencia, CA), following the manufacturer's protocol.

GNAS mutational Analysis by standard-PCR Sanger sequencing and LNA-PCR/sequencing

GNAS exons 8 and exons 9 were amplified using the following forward and reverse primers: *GNAS*-Ex8F, 5'-GTAAAACGACGGCCAGT-3'; *GNAS*-Ex8R, 5'-CAGGAAACAGCTATGACC-3'; *GNAS*-Ex9F, 5'-GTAAAACGACGGCCAGT-3', and *GNAS*-Ex9R, 5'-CAGGAAACAGCTATGACC-3' (figure 1). Forward and reverse primers were M13 tailed to facilitate Sanger sequencing. All samples were tested in duplicate. The PCR amplification was performed in a final volume of 50 μ L containing 100ng to 200ng of genomic DNA, 1X JumpStart ReadyMix REDTaq DNA (Sigma P-0982), and forward and reverse primers (20 pmol each) for standard PCR. For the LNA-PCR reaction, 20 pmol of LNA probe (5'-G+G+A+C+A+C+G+GC+AGCG/3InvdT/) complementary to the wild type (WT) *GNAS* exon 8 sequence (figure 1A) was added to the reaction (figure 1). Briefly, LNAs are nucleic acid analogs that contain a 2' to 4' C-methylene bridge in the ribose moiety. LNA bases get incorporated into wild type DNA oligonucleotide, which increases the thermal stability of DNA/probe heteroduplexes and as a result suppresses the wild-type allele amplification leading to preferential amplification of the mutant allele if present. The sensitivity achieved by this method is in the range of 0.5 to 1% detection of the mutant allele in any given sample ([10,11](see below)).

The PCR cycling parameters included initial denaturation at 94°C for 2 minutes, 42 cycles of denaturation at 94°C (0 seconds), annealing at 60°C (30 seconds), and extension at 72°C (2 minutes), followed by a final extension at 72°C (10 minutes). The PCR products of *GNAS* exons 8 (272 bp) and 9 (237bp) were separated on a 3% agarose gel and visualized by ethidium bromide staining. All PCR products were purified using Spin Columns (Qiagen) and sequenced in both directions with forward M13F2: 5'-GTAAAACGACGGCCAGT-3' and reverse M13R2: 5'-CAGGAAACAGCTATGACC-3' primers using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's protocol on an ABI3730XL DNA sequencer running ABI Prism DNA Sequence Analysis Software. The *GNAS* reference sequence and the positions of the primers used in this assay are shown in Figure 1.

Technical Sensitivity of standard and LNA PCR sequencing methods

The sensitivity study for *GNAS* sequencing analysis was performed by using six serial dilutions (50, 25, 12.5, 6.25, 3, and 1.5%) from a known positive case harboring the common p.R201C mutation in exon 8 and mixed into normal DNA (figure 2).

Statistical Analysis

Pearson correlation test X^2 and Fisher exact test were used to study correlation between different co-variables. Z test was used to compare proportions. P Student test was used to compare different numerical co-variables. A p value <0.05 was considered significant. Graph pad prism software package 6.0 was used for data analysis.

RESULTS

Clinical and Pathological Findings

Clinical and pathological data related to the 52 patients and tumors in the study group is shown in table 1. Two of the Fibrous dysplasia group cases showed extensive adipocytic differentiation with myxoid and fibrous background consistent with the diagnosis of “Liposclerosing fibromyxoid tumor”, a variant of classic Fibrous dysplasia. Additionally, two cases showed an associated desmoplastic fibroma (figure 3). The low grade osteosarcoma group included 11 surface low grade parosteal osteosarcoma and one central low grade osteosarcoma. Three of the parosteal osteosarcomas showed evidence of high grade dedifferentiation

GNAS exons 8 and 9 mutations in the Fibrous dysplasia and osteosarcoma groups

GNAS mutations in the R201 residue were detected by Sanger sequencing in 25 tested cases (48%) including 17 non decalcified and 8 decalcified specimens, respectively. The CGT>CAT p.R201H mutation was the most frequently detected mutation (n=16) followed by the CGT>TGT p. R201C mutation (n=8). One additional mutation leading to substitution of the arginine residue with serine (p.R201S) was detected in one case (Table 1). Cases which were negative by standard-PCR/Sanger sequencing (n=27, 52%) were tested for exon 8 mutation with LNA-PCR/Sanger sequencing method and exon 9 mutations standard-PCR/sequencing. Except for one case which showed positive CGT>TGT, p.R201C mutation rest of the tested cases were negative by LNA-PCR for *GNAS* exon 8 and no mutations were found in exon 9 by standard-PCR/sequencing in these cases. Twelve additional positive cases by traditional Sanger were confirmed by LNA-PCR method. All positive mutations were present at codon 201. None of the tested osteosarcoma tumors showed any mutations in *GNAS* exon 8 using standard (n=12) and LNA-PCR/sequencing methods (n=8). No *GNAS* exon 9 sequencing was performed on these cases.

Technical sensitivity of standard Sanger sequencing and the LNA PCR sequencing

The results of the serial dilutions are shown in figure 2 and summarized in table2, B. Based on these results; the technical sensitivity for standard-PCR/sequencing and the LNA-PCR/sequencing is 6.25% and 1.5%, respectively.

Correlation of different clinicopathological variables with mutational status

No significant correlation was identified between the mutational status and any of the tested variable including, type of specimen, average size, site (long versus flat bone) and histological subtype (table 3). Correlation between decalcified vs non-decalcified cases:

There was a significant difference in the sensitivity of the assay between decalcified and non decalcified FDs (31 vs. 70%, p=0.002).

DISCUSSION

GNAS gene is located on chromosome 20q13.3. The gene products derive from the use of multiple promoters and first exons that splice onto a common set of downstream exons

(exons 2–13) [12]. Numerous splicing products are generated during *GNAS* transcription. The major product is alpha-subunit of the heterotrimeric G protein complex (G α). It is generated by splicing occurring at the most downstream promoter at exon 1 [13]. G α is ubiquitously expressed and interacts with numerous hormonal as well as other transmembrane receptors to adenylyl cyclase and is required for the receptor-stimulated intracellular cAMP production [12, 13]. G α expression is biallelic in most tissues including the bone; however, a small subset of tissues such as renal proximal tubules, pituitary, gonads, and thyroid expresses G α predominantly from the maternal allele [12, 13]. Fibrous dysplasia is characterized by activating missense mutations of the *GNAS* gene, encoding the subunit of the stimulatory G. These mutations occur post zygotically leading to a focal somatic mosaic state within the lesional tissue itself [14,15]. This mechanism allows understanding the non hereditary pattern of presentation in fibrous dysplasia and the coexistence of a normal and “abnormal” genotype within the same lesional cell population [16]. Recent studies showed that activating *GNAS* mutations disrupt a pathway that is required for skeletal stem cell self renewal and development of fibrous disease. As such, mutant cells fail to regenerate and die while residual normal stem cells selectively survive. This leads to progressive remodeling that can replace dysplastic bone into normal bone over time [16]. Fibrous dysplasia can be a challenging diagnosis even for experienced bone pathologists as it can present with overlapping features with low grade central osteosarcoma. Distinguishing between these two entities is of critical as it carries major implications for patient management. While diagnosis can be achieved by correlation of radiological and histopathological findings, overlapping features can be seen in some cases necessitating molecular testing. Hence, over the last decade, *GNAS* mutations have emerged as a useful ancillary molecular test in diagnosing fibrous dysplasia. Two main nucleotide substitutions in codon 201 have been identified within exon 8 of the *GNAS* gene. These mutations lead to the replacement of an arginine by a histidine (R201H) or a cysteine (R201C) [7, 8]. In rare cases, serine (R201S), leucine (R201L) and glycine (R201G) substitutions have been reported [17, 18, and 19]. Exceptional cases of fibrous dysplasia have been linked to mutation in exon 9, leading to the substitution of the glutamine at position 227 by a leucine (Q227L), an arginine (Q227R), a lysine (Q227K) or a histidine (Q227H) [20]. All mutations lead to constitutive G α adenylylase activity with overproduction of cyclic adenosine monophosphate (cAMP) in dysplastic cells [3].

Several studies have reported variable sensitivity with different molecular detection methods for *GNAS* exon 8 mutations. In a seminal meta-analysis study by Lee et al, the authors reviewed 9 series from different countries over a 14 year period. They reported a *GNAS* exon 8 activating mutation incidence rate of 71.9% of across studies. The authors’ institutional experience with 48 fibrous dysplasia cases yielded a positive rate of 58.3% [8]. In a recent study by Tabareau et al, the authors reported their experience with a series of 51 cases of fibrous dysplasia using parallel High Resolution Melting (HRM), allele-specific PCR, followed by standard Sanger sequencing. Their series yielded a *GNAS* exon 8 activating mutations positive rate of 45%. This is similar to the range of what was reported by Lee et al. and lower than that obtained from the meta-analysis of different studies [3, 7]. Our series showed similar results to the two previously cited studies with a positive rate of 48% with inclusion of decalcified and non-decalcified cases. The sample size is an important

factor that can introduce a significant sample size bias; indeed the highest sensitivities reported in the literature (positive rate of 100%) are from four different studies which included less than 10 patients each [6, 21, 22 and 23]. Of note, each of these studies used a different detection method including restriction fragment analysis with standard sequencing or Peptide Nucleic acid/PNR. The number of samples studied, the specimen pretreatment (decalcification versus no decalcification), the decalcification methods and detection methods used are all important issues to consider when analyzing results from various studies. These factors may explain the differences seen between the meta-analysis results and our results. One of the most sensitive methods described for *GNAS* mutation detection is Pyrosequencing. In a recent series of 24 cases of Fibrous dysplasia by Liang et al., the authors reported a clinical sensitivity of 96% and an analytical sensitivity of 95% for their assay [9]. This high sensitivity and specificity has not been reported in other series and was not seen in our group using a highly sensitive technique such as LNA-PCR/sequencing. When choosing a mutation detection method for clinical DNA testing, sensitivity is one of the major points to consider. Direct sequencing still remains the gold standard method for mutation analysis. Yet, it requires a relatively high amount of mutant DNA of more than 20% in the total DNA population. LNA-PCR/sequencing is a useful method to lower this threshold by amplifying selectively low copy number of mutant alleles increasing the sensitivity of the sequencing assay. In a study by Lietman et al, the authors reported a very high sensitivity for this method. They report a threshold of detection of one mutant *GNAS* allele out of 1000–5000 cells [23]. However in their study, DNA was extracted from peripheral blood of patients Mc-Cune Albright syndrome and not fibrous dysplasia tissue samples. In our study we aimed to investigate whether LNA-PCR/sequencing could provide additional value by increasing the sensitivity of the assay compared to standard-PCR/Sanger sequencing. Clearly, it did not show an added value and our analytical sensitivity studies (figure 2) reflects this. One possible explanation for lack of detection by LNA-PCR is the described genotypic mosaicism of mutant and non-mutant cells within the lesion. As demonstrated by Kuznetsov et al [16], there is a tight inverse correlation between the percentage mutant colony forming unit fibroblast CFU-F versus age of fibrous dysplasia patients, suggesting progressive demise of mutant stem cells caused by exuberant apoptosis. This “normalization” phenomenon replaces the mutant cells that are unable to regenerate with non mutant cells leading to formation of normal bone. Interestingly, we found identical median and average ages for both groups of positive and negative *GNAS* exon 8 mutation in our patients. Also, no significant association was seen between age and mutational status in both decalcified and non-decalcified groups (table 3).

In our study, decalcification was the major limiting factor. There was a clear difference in the clinical sensitivity between the non-decalcified specimen group and specimens pretreated with decalcification (74% versus 31%, $p=0.002$) (table 4). Also, neither specimen size nor type (biopsy versus resection) showed significant correlation with the mutational status in the tested samples ($p=0.356$, $p=0.456$, respectively). This reinforces the fact that DNA quality is more important than quantity for sequencing method. All of our decalcified samples underwent acid treatment using formic acid. Ethylene diamino tetraacetic acid (EDTA) sequesters metallic ions, including calcium in aqueous solution and acts as a chelating factor that can be used for decalcification. EDTA decalcification has been shown

to provide better DNA quality for molecular studies [24]. Yet, the duration of this decalcification protocol remains an impediment to implement in routine practice.

Overall our results are in agreement with the previously reported literature in terms of type of substitutions identified. Indeed, the most commonly identified p.R201H and p.R201C mutations constituted 62 % and 30% of the positive cases, respectively.

Overall *GNAS* exon 8 mutations are accepted as very specific to fibrous dysplasia among all fibro-osseous lesions. Until recently, *GNAS* exon 8 mutations have been reported in only one case outside of fibrous dysplasia and its morphological variants [7, 8, 25]. Pollandt et al. reported an R201C mutation in one Fibrous dysplasia-like low grade central osteosarcoma [25]. A very recent study of 9 parosteal osteosarcomas by Carter et al revealed activating *GNAS* mutations in 3 osteosarcomas with dedifferentiation and 2 low grade osteosarcomas (n=5, 55%) using direct sequencing. The mutations included 4 R201C mutated tumors and 1 tumor with an R201H mutation. Based on these results the authors concluded that *GNAS* R201 mutation is not specific for fibrous dysplasia and may not be as valuable as it was thought to be [26]. None of the low grade central or parosteal osteosarcomas in our study showed *GNAS* exon 8 mutations.

In conclusion *GNAS* mutational analysis remains a valuable adjunct method for the diagnosis of fibrous dysplasia. Decalcification is one of the major limiting factors in molecular testing in fibrous dysplasia. Highly sensitive methods such as locked nucleic acid do not add additional advantage for mutation detection. Finally, as proposed by some authors, tumorigenesis in fibrous dysplasia cases negative for activating *GNAS* mutations may be due to alternative pathways.

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Figure 1. *GNAS* exon 8 and 9 reference sequences

A, *GNAS* exon 8 reference sequence (*GNAS*-015 ENST00000371085). The forward and reverse primers are shown boxed in black in lower case lettering at the beginning and the end of the reference sequence. *GNAS*R201 (c.601_603CGT) mutated codon is shown. B, *GNAS* exon 9 reference sequence (*GNAS*-015 ENST00000371085). The forward and reverse primers are shown boxed in black in lower case lettering at the beginning and the end of the reference sequence. *GNAS*Q227 (c.679_681CAG) mutated codon is shown.

Figure 2. Comparison electropherograms: standard vs. LNA-PCR assay sensitivity for *GNAS* exon 8 mutations

Serial dilutions of mutant DNA from a known positive case admixed with normal DNA from a non mutated case to evaluate sensitivity of *GNAS* direct sequencing. %M denotes percent of mutant DNA. The tested sample showed about 50% of tumor cells based on peak height on the forward and reverse sequences, so the 50% dilution represent 25% of tumor DNA and so forth. **LNA-PCR:** The addition of 0.4uM of locked oligonucleotide (LNA) blocks the amplification of the wild-type allele in the PCR reaction allowing the mutant allele to amplify.

Figure 3. Morphological spectrum of fibrous dysplasia, A-D

A, 10X magnification, showing characteristic irregular woven bone trabecula associated with moderately cellular fibroblastic tissue with bland spindle nuclei lacking significant atypia. B, 10x magnification, liposclerosing fibromyxoid tumor showing admixed areas with spindle cell stroma associated with woven bone spicules devoid of osteoblasts. Near these areas, mature adipocytic tissue is seen along with fibrous stroma. C, 20X magnification, aneurysmal bone cyst like changes within fibrous dysplasia characterized by large cystic hemorrhagic areas surrounded by osteoclasts like giant cells (circle) D. 20X magnification, desmoplastic fibroma associated with fibrous dysplasia showing stellate and spindle cells without significant atypia surrounding thick stromal collagen fibers (arrows).

Figure 4. Summary of the different mutations seen in the FD group (A-D)

A, negative case of parosteal osteosarcoma showing wild-type (WT) sequence. B, a case of classic FD showing mutations affecting the c.601 nucleotide; in the CGT codon the C position is replaced with T resulting in the Arg (CGT)→Cys (TGT) substitution. C, a case of FD with cystic changes showing mutations affecting the c.601 nucleotide; in the CGT codon the C position is replaced with A resulting in Arg (CGT)→Ser (AGT) substitution. D, a case of liposclerosing fibromyxoid tumor showing the most frequent mutation involving nucleotide c.602; in the CGT codon the G position is replaced with A resulting in Arg (CGT)→His (CAT).

Table 1

Summary of clinical, pathological and molecular features of 52 cases of FD in our series

Feature	Number n=52 (%)
Median age (range)	33 (5–72)
Gender	
Male	30 (58)
Female	22 (42)
Anatomic location	
Long bone (%)	41 (80)
Flat bone (%)	11 (20)
Sample type	
Resection (%)	4 (8)
Curettage (%)	34 (63)
Biopsy (%)	14 (29)
Sample size, cm (range)	2.23 (0.3–10)
Decalcification treatment	
Yes (%)	29 (56)
No (%)	23 (44)
Histological features	
Classic Fibrous dysplasia (%)	41 (78)
Liposclerosing fibromyxoid (%) tumor (LSFMT)	2 (4)
Secondary ABC like changes (%)	5 (10)
Secondary chondroid metaplasia (fibrocartilaginous dysplasia) (%)	2 (4)
Associated Desmoplastic fibroma (%)	2 (4)
Mutational Analysis (<i>GNAS</i> codon 201)	
CGT>CAT p. R201H (%)	17 (31)
CGT>TGT p. R201C (%)	8 (15)
CGT>AGT p. R201S (%)	1 (2)

Table 2

GNAS primer sequences for exon 8 and 9.

A)			
Gene / Exon F/R	Primer (5'→3')	Size (bp)	
GNAS-E8F-M13F2	<i>GTAAAAACGACGGCCAGT</i> ¹ gagccctcttccaaacta	272 (237+35)	
GNAS-E8R-M13R2	<i>CAGGAAACAGCTATGACC</i> ² actgggggaatgtcaagaa		
GNAS-R201-LNA-8R	5'-G+G+A+C+G+GC+AGCG/3InvdT/		
GNAS-E9-M13F2	<i>GTAAAAACGACGGCCAGT</i> accgccagtcctcttggaata	237 (202+35)	
GNAS-E9-M13R2	<i>CAGGAAACAGCTATGACC</i> ccaagagagcaagccaag		
M13F2 * (17mers)	<i>GTAAAAACGACGGCCAGT</i>	-	
M13R2 * (18mers)	<i>CAGGAAACAGCTATGACC</i>	-	

B)							
Ratio of Wild-Type/Mutant (WT/MUT) peak heights in DNA serial dilution							
PCR		100%	50%	25%	12.50%	6.25%	1.50%
	F	2/1	3/2	2/1	2/1	3/1	4/1
Standard	R	2/1	3/2	2/1	2/1	3/1	4/1
	F	All mutant	All mutant	All mutant	All mutant	1/4	1/1
LNA	R	All mutant	All mutant	All mutant	All mutant	1/3	2/1

* M13 primers for sequencing (35 nucleotides are added to the PCR product) (c.679_681CAG)
F= forward, R= Reverse, LNA= Locked nucleic acid.

Table 3

Correlation of clinicopathological features with mutational status.

Feature	GNAS mutation positive (n=25)	GNAS mutation negative (n=27)	P values
Age (Median)	33.2	33	p=0.661
Gender			
Male (n=)	14	16	p=0.456
Female (n=)	11	11	
Sample type			
Resection (n=)	1	3	p=0.561
Curettage (n=)	16	17	
Biopsy (n=)	8	7	
Anatomic location			
Long bone (n=)	21	22	p=0.461
Flat bone (n=)	3	5	
Sample size, cm (mean)	3.73	2.23	p=0.356
Histological features			
Classic Fibrous dysplasia (n=)	20	17	p=0.632
Liposclerosing fibromyxoid tumor (LSFMT) (n=)	1	1	
Secondary ABC like changes (n=)	1	4	
Secondary chondroid metaplasia (n=)	2	2	
Associated lesion [*] (n=)	1	3	

* Other lesions include associated desmoplastic fibroma and low grade fibrosarcomatous transformation in FD. (can we look at this case?) don't recall

Table 4

Analysis of the sensitivity of standard-PCR vs LNA-PCR Sanger sequencing assay across decalcified and non decalcified Fibrous dysplasia FD specimens.

	Positive for GNAS exon 8 Codon 201 mutation	Negative for GNAS exon 8 Codon 201 mutation	Clinical Sensitivity
Decalcified n=29	9	20	31%
Non decalcified n=23	16	7	70%
	<i>Z score= 3.081; p= 0.002</i>		
Total n=52			

Z score was used to compare between the sensitivities in both decalcified and calcified groups.