Clinicopathological and genetic study of a rare occurrence: Malignant transformation of fibrous dysplasia of the jaws

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Abstract

Background: Malignant transformation of fibrous dysplasia (FD) is very rare and little is known about this occurrence.

Methods: We present the detailed clinical course of three cases of osteosarcoma arising from FD of the jaws and explore the genetic aberrations by Sanger sequencing, whole-exome sequencing (WES) and immunohistochemistry (IHC). A literature review of important topics related to this occurrence was also performed.

Results: It was observed that patients with secondary sarcoma from FD showed a wide range of ages, with most during the third decade. Female and males were equally affected. Craniofacial bones and femurs were the most affected sites. High-risk factors for this occurrence included polyostotic FD, McCune-Albright syndrome and excess growth hormone. Notably, a potential relationship between thyroid hormones and sarcoma development was suggested in one patient, who began to show malignant features after hypothyroidism correction. Sanger sequencing revealed GNAS mutations of FD retained in all malignant tissues. Additionally, abnormal TP53 was demonstrated in all three cases by WES and IHC. WES also revealed two other driver mutations, ROS1 and CHD8, and large amounts of somatic copy number alterations (CNAs) where various oncogenes and tumour suppressors are located.
1 | INTRODUCTION

Fibrous dysplasia (FD) is a skeletal disorder arising from somatic mutations in GNAS (OMIM *139320) and is associated with bone marrow stromal cells (BMSCs; Saggio, 2019). GNAS mutations in FD are gain-of-function alterations, which constitutively activate adenylyl cyclase (AC) to generate excess cyclic adenosine monophosphate (cAMP) through loss of GTPase activity of GTP-bound Gαs (Landis et al., 1989) and abnormal activation of GDP-bound Gαs (Hu & Shokat, 2018), resulting in abnormal proliferation and differentiation of mutation-bearing BMSCs (Marie, 2001).

In FD, normal bone and bone marrow are replaced by abnormal trabeculae and fibrous tissue, with enhanced osteoclastogenesis, devoid of haematopoiesis and adipogenesis (Lichtenstein, 1938; Riminucci et al., 1999, 2003). The fibrotic area of FD consists of cells with phenotypic features of pre-osteogenic cells, whereas the lesional bone formed de novo within fibrous areas represents the biosynthetic output of mature but abnormal osteoblasts (Riminucci et al., 1997). The bone trabeculae within the fibrous tissues are heterogeneous in overall amount, cellularity, structure and architecture (Riminucci et al., 1997, 1999).

The clinical presentation of FD demonstrates an extensive spectrum. Based on the amount of affected bone and whether it is accompanied by extraskeletal manifestations, FD is generally categorized into three forms: monostotic or polyostotic FD and McCune-Albright syndrome (MAS, polyostotic FD with café-au-lait macules and/or hyperfunctioning endocrinopathies; Boyce & Collins, 2020; Lichtenstein, 1942).

FD itself is a benign disease, however, with a variable clinical course (Han et al., 2014; Sweeney & Kaban, 2020). Malignant transformation is one of the most damaging courses. Ruggieri and coworkers reported a mortality rate of 53.6% in FD patients with malignant transformation (Ruggieri et al., 1994). According to a recent report focused on the prognosis of malignant transformation of FD in craniofacial bones, it was confirmed that the prognosis was disappointing, with a median survival ranging from 4 to 62 months (Li et al., 2020). All forms of FD, namely, monostotic and polyostotic FD and MAS, can transform into sarcomas (Li et al., 2020; Ruggieri et al., 1994). The malignant transformations reported in the literature include osteosarcoma, fibrosarcoma, chondrosarcoma, malignant fibrous cell tumours, and angiosarcoma (Fukuroku et al., 1999; Li et al., 2020; Ruggieri et al., 1994). Among them, osteosarcoma is the most frequently occurring histologic type, with a frequency of 48%–70% in the literature (Li et al., 2020; Riddle & Bui, 2013; Ruggieri et al., 1994; Schwartz & Alpert, 1964; Yabut et al., 1988). Malignancy is suggested by rapid growth, pain, or a significant rapid change in radiographic appearance, especially in mineralization (Qu et al., 2015; Ruggieri et al., 1994). Computed tomography scans can be helpful in recognizing malignancy as well as in determining their extent (Riddle & Bui, 2013). Microscopically, the features of FD-derived sarcoma showed no difference from those in normal bone (Schwartz & Alpert, 1964).

It has been reported that the malignancy of FD ranges from 0.4% to 4% (Schwartz & Alpert, 1964). Due to its rarity, its clinical characteristics and molecular pathogenesis remain largely unclear. To obtain a better understanding of this rare but damaging disorder, we reported three new cases and performed whole-exome sequencing (WES) using both benign FD tissues and malignant tissues in the present study. A literature review regarding the limited known knowledge about the genetic aberrations associated with sarcoma change of FD, a rare entity, and several important topics related to its clinical features was also conducted.

2 | MATERIALS AND METHODS

2.1 | Patients and specimens

Three patients at Peking University School and Hospital of Stomatology with malignant tumours arising from FD were enrolled. Their clinical information, including age, sex, symptoms, serum alkaline phosphatase (ALP) level, clinical history and radiographic examinations, were retrieved from their medical records and reviewed retrospectively in detail. Haematoxylin–eosin (H&E) stained

Conclusion: This study demonstrated and reviewed the clinical features and risk factors for a rare occurrence, secondary sarcoma from FD, and provided important new knowledge about its genetics.

KEYWORDS

copy number alterations, fibrous dysplasia, GNAS, malignant transformation, TP53
sections of formalin-fixed paraffin-embedded tissue were examined for pathological evaluation by three specialized pathologists.

2.2 | DNA extraction

Genomic DNA was extracted from both the FD tissues and the matched malignant tissues of each patient using QIAamp DNA Mini Kit (Qiagen) according to the manufacturer’s instructions. For patient #1, formalin-fixed tissue prior to decalcification was used, whereas formalin-fixed paraffin-embedded (FFPE) sections from decalcified tissues were used for the other two patients. NanoDrop8000 (ThermoFisher Scientific) and Qubit2.0 fluorometer (ThermoFisher Scientific) were used to assess the quality and quantity of DNA.

2.3 | GNAS mutation analysis

Sanger sequencing of regular PCR-amplified exon 8 and exon 9 of the GNAS (GenBank and NCBI reference sequence: AH002748.2/NG_016194.2/NM_000516.7) gene was performed as described previously (Kuznetsov et al., 2008).

2.4 | Whole-exome sequencing

Qualified DNA for WES was obtained from patient #1. For WES, DNA was sheared into 180–200 bp segments and submitted to library preparation with an Agilent SureSelect Human All Exon V5/V6 kit (Agilent Technology) according to the manufacturer’s protocol. Paired-end sequencing (150 bp) was conducted on an Illumina HiSeq platform (Novogene). At least 14 giga bases of raw data were produced for each sample.

After quality control of raw data, clean sequencing reads were mapped to the human reference genome (human_B37) using BWA (Li & Durbin, 2009) and Samblaster (Faust & Hall, 2014). Through comparison with matched FD tissue data, somatic single nucleotide variations (SNVs) and insertions/deletions (INDELs) specific to malignant specimens were identified from malignant tumours using MuTect (Cibulskis et al., 2013) and Strelka (Saunders et al., 2012), respectively. Functional annotation of somatic mutations on their encoded amino acids was performed using ANNOVAR. By comparison with known driver genes using in-house software, driver genes of malignancy were identified. Somatic copy number alterations (CNAs) were identified using control-FREEC software (Boeva et al., 2012).

2.5 | Immunohistochemistry

For immunohistochemistry (IHC), FFPE tissues of both benign FD and sarcoma specimens were sectioned at a thickness of 4 µm. They were deparaffinized using xylene and hydrated through graded alcohols. For antigen unmasking, sections were treated with citrate buffer (pH 6.0). Sections were incubated with mouse monoclonal p53 antibody (ZM-0408, ZSGB-BIO) at 4°C overnight, followed by treatment with PV9001 (ZM-0408, ZSGB-BIO) and visualization with DAB (ZM-0408, ZSGB-BIO). Negative controls were obtained by omitting the primary antibody.

3 | RESULTS

3.1 | The clinicopathological data of three patients with malignant transformation of FD

Three patients with osteosarcoma arising from FD of the jaws were identified from a total of 253 patients with FD in the maxillofacial bones over a duration of 20 years (2000–2020). The clinicopathological information of these patients was summarized in Table 1.

The first patient was a 39-year-old female patient, who presented with complaints of facial swelling for more than 30 years. She underwent three operations at the ages of 9, 19 and 25 years old in external hospitals due to a gradual increase in swelling and was consistently diagnosed with FD. Three months before her referral to our hospital, she was found to have hypothyroidism and Euthyrox was prescribed. It was noted that since being treated with medicine for hypothyroidism, her facial swelling began to grow rapidly, with significant tooth displacement and proptosis of her left eye. Routine blood tests revealed an increased serum ALP level of 247 U/L. A CT scan showed an ill-defined mass with a ground-glass appearance involving her left zygomatic, ethmoid, sphenoid, temple and maxillary bones and mandible extending from the right body to the left condyle. Thinning of cortical bone and narrowing of the left maxillary sinus and nasal cavity were also observed (Figure 1b,c). X-ray chest film showed radiopaque widening in the right fourth rib and disappearance of the medullary cavity (Figure 1a). There was no significant disorder suggestive of MAS. Contour correction of the left maxilla and segmental osteotomy of the mandible were carried out, and histopathological analysis was performed, which showed FD (Figure 1d–f) in the maxilla and an osteosarcoma lesion in the mandible (Figure 1h–j).
Patient #2 was a 56-year-old male, who was referred to our hospital due to progressively worsening pain in the front area of the left ear (Figure 2a), with obvious swelling and limitation of mouth opening for 10 months. With the uncertainty of the nature of the disease, an incisional biopsy was carried out, and histopathological analysis revealed a diagnosis of FD, with a very low risk of malignant transformation, composed of bone and fibrous tissue (Figure 2d–f). The mass was found to be significantly increased three months postoperatively, as demonstrated on CT examination, measuring 5 cm in the buccal lingual direction and 4.6 cm in the axial direction, with numbness (Figure 2b,c). Blood tests revealed an elevated level of ALP of 265 U/L. Segmental osteotomy of the left mandible involving the lesion of the condyle was then conducted, and pathological analysis revealed a more proliferating area with many osteoclasts and atypical nuclei, which is the manifestation of osteosarcoma of the osteoclast-rich type (Figure 2h–j).

Patient #3 was a 31-year-old female, who complained of a progressive increase in mandible swelling for more than 18 years. Except for the mandible lesion, other bone abnormalities were also present, including abnormal ribs (Figure 3a) and both lower limbs, with a history of broken legs. Furthermore, she had precocious puberty and pigmentation, which were signs of MAS. Six months before her referral to our hospital, the mass grew rapidly, with severe pain, breathing problems and numbness of the lower lip. A blood test revealed an elevated ALP level of 1323 U/L. Chest X-ray revealed curved spines and radiopaque segmental bulging of multiple ribs (Figure 3a). CT examination revealed a huge bone mass of bilateral mandible bodies, with mixed radiopaque-radiolucent density and resorption of the tooth root. An inferior border of the mandibles was observed (Figure 3b). Resection of the bilateral mandible mass was then carried out, and pathological examination revealed both benign FD lesions (Figure 3c–e) and osteosarcoma (Figure 3g–i).

### 3.2 | GNAS mutation was detected in both FD and malignant tissues

GNAS mutation analysis was performed in both the benign and malignant tissues of each patient. For patient #1 and patient #2, GNAS mutation was detected at exon 8, demonstrating c.601 C>T, resulting in the substitution of Arg of 201 to Cys (R201C), a previously reported hotpot activating missense mutation of GNAS, in both FD tissue (Figures 1g and 2g) and malignant tissue (Figures 1k and 2k). For patient #3, no mutation was revealed in the benign tissue (Figure 3f), whereas
an R201C mutation was found in the malignant tissue (Figure 3j).

3.3 | WES revealed multiple SNVs and CNAs

By comparison with benign FD tissues, we obtained genomic abnormalities specific to malignant tissues, including SNVs and CNAs, using WES. In total, 117 somatic SNVs (Figure 4a, left) and 4 INDELS (Figure 4a, middle) were identified, including 46 SNVs and 2 INDELS in the coding regions (CDSs; Figure 4a, right). By further analysis of genetic aberrations in the CDSs, 30 missense mutations, 2 frameshift deletions and 1 stop-gain SNV were revealed (Table 2), among which three driver genes were found, including TP53 (frameshift deletion, NM_000546.6, c.582del), ROS1 (missense, NM_002944.3, c. T4025>C) and CDH8 (missense, NM_001170629.2, c.C2586>G). Analysis of somatic CNAs (Figure 4b) revealed multiple chromosomal abnormalities, except for chromosomes 5, 9 and 15. In total, there were 134 gain counts with a size of 413 Mb and 11 loss counts with a size of 20 Mb. In these CNVs, 29 enes were found to have a relationship with tumours in the OMIM database: RSPO1, PTCH1, MUTYH and RAD54L on chromosome 1; GALNT3 and HOXD4 on chromosome 2; ATR, TFG and PIK3CA on chromosome 3; PDGFR, KIT and CHIC2 on chromosome 4; RNF139,
RAD54B, EXT1, MYC, RB1CC1 and PLAG1 on chromosome 8; RRAS2, TSG101 and CD82 on chromosome 11; FGF23 on chromosome 12; DICER1 on chromosome 14; WWOX and ZFHX3 on chromosome 16; SMARCA4 on chromosome 19; GNAS on chromosome 20; and BACH1 on chromosome 22. Except for WWOX, which showed loss, all other 28 genes above were amplified. The details of the somatic CNAs were summarized in Table 3.

3.4 | IHC analysis showed positive staining of p53 in malignant tissues arising from FD

Alterations in the TP53 gene in sarcomas often lead to stabilization of the p53 protein and make it visible on IHC, whereas the wild-type protein has a short half-life (Yamamoto & Iwakuma, 2018). To test whether there were also TP53 aberrations in the other two patients without qualified DNA for WES, IHC was performed, which revealed a positive expression of p53 in the malignant tissues of all the patients (Figure 5).

4 | DISCUSSION

Three well-documented cases of osteosarcoma arising in FD of the jaws were reported, extending the knowledge of this rare occurrence. More importantly, WES analysis was performed, which revealed significant SNA and CNA involvement on the basis of GNAS activating mutations in sarcomas, adding novel and valuable insight into genome alterations underlying FD malignancy.
Although no precise estimation of the frequency of malignant transformation in FD is currently available, it is considered extremely rare. Several groups have made independent estimations based on large populations with FD. In 1964, Schwartz reported a ratio of 0.4%, with 6 cases of sarcoma in a total of 1517 cases of FD (including 1480 cases of monostotic FD and 37 cases of polyostotic FD) and a higher ratio (4%) in a population of 100 patients with MAS (Schwartz & Alpert, 1964). In 1994, a review of Mayo Clinic data revealed 28 cases of malignancy out of 1122 total cases of FD, with a prevalence of ~2% (Ruggieri et al., 1994). More recently, in 2012, three cases of malignancy
| CytoBand | Position | Ref | Alt | GeneName | Exonic Func | AAChange | dbSNP/COSMIC ID | SIFT | Polyphen 2_HVAR | Polyphen 2_HDIV | Mutation Taster |
|----------|----------|-----|-----|----------|-------------|----------|----------------|------|----------------|----------------|----------------|
| 1p36.12  | 21016692 | C   | T   | KIF17   | Missense    | NM_001122819.3:c.G1370>A p.(Arg457Gln) | rs186246358 | 0.019,D | 0.032,B | 0.144,B | 1.000,N |
| 2q31.1   | 170493312| G   | C   | PPIG    | Missense    | NM_004792.3:c.G1544>C p.(Arg515Thr) | COSM334392 | 0.001,D | 0.533,P | 0.948,P | 0.993,D |
| 2q23.1   | 149539242| C   | T   | EPC2    | Stopgain    | NM_015630.4:c.C1750>T p.(Gln584Ter) | —            | —     | —     | —     | 1,A         |
| 3p21.31  | 47043946 | A   | T   | NBEAL2  | Missense    | NM_015715.3:c.A5237>T p.(Asp1746Val) | —            | 0.002,D | 0.999,D | 1.0,D  | 1,D         |
| 5q31.2   | 139060331| C   | T   | CXXC5   | Missense    | NM_016463.9: c.C223>T p.(Asp75Cys) | COSM292855  | 0.001,D | 0.642,P | 0.999,D | 1.000,D |
| 5q31.3   | 140735915| C   | T   | PCDHGA4 | Missense    | NM_018917.4:c.C1244>T p.(Ile414Ile) | —            | .      | 0.081,B | 0.019,B | 1,N         |
| 5q35.5   | 176813546| G   | A   | SLC34A1 | Missense    | NM_001167579.2:c.G511>A p.(Val171Ile) | rs570463028  | 0.085,T | 0.881,P | 0.995,D | 1.000,D |
| 6p12.1   | 54095537 | T   | A   | MLIP    | Missense    | NM_001281747.2:c.T2744>A p.(Val915Asp) | —            | 0.003,D | 0.653,P | 0.911,P | 1,D         |
| 6q22.1   | 117677908| A   | G   | ROS1    | Missense    | NM_002944.3:c.T4025>C p.(Ile1342Thr) | —            | 0.004,D | 0.11,B  | 0.319,B | 0.540,N |
| 7p21.3   | 8790825  | C   | T   | NXPH1   | Missense    | NM_015745.3:c.C242>T p.(Pro81Leu) | —            | 0.125,T | 0.037,B | 0.029,B | 1,D         |
| 7p15.3   | 23293048 | G   | A   | GPNMB   | Missense    | NM_001005340.2:c.G193>A p.(Gly65Arg) | —            | 0.011,D | 0.956,D | 0.998,D | 1,D         |
| 7q36.1   | 150325784| C   | T   | GIMAP6  | Missense    | NM_00124072.2:c.G112>A p.(Val38ffe) | rs561321166  | —     | —     | —     | 1,N         |
| 8q24.3   | 145698002| G   | A   | KIFC2   | Missense    | NM_014575.4:c.G1774>A p.(Ala592Thr) | —            | 0.405,T | 0.017,B | 0.11,B  | 1,N         |
| 8q12.3   | 63902756 | CT  | C   | NKA1N3  | Frameshift deletion | NM_0173688.2:c.563del p.(Leu188fs) | —            | —     | —     | —     | —           |
| 9q21.11  | 70176851 | A   | G   | FOXD4L5 | Missense    | NM_001126334.1:c.T1133>C p.(Leu378Pro) | rs3000494; COSM4592895, COSM4592896 | 1.0,T   | 0.0,B  | 0.0,B  | 0.940,D |
| 10q11.23 | 49984948 | C   | A   | WDFY4   | Missense    | NM_020945.2:c.C3017>A p.(Thr1006Asn) | —            | 0.0,D  | 0.997,D | 1.0,D  | 1.000,D |
| CytoBand | Position   | Ref | Alt | GeneName | Exonic Func | AAClange      | dbSNP/COSMIC ID       | Polyphen 2_HVAR | Polyphen 2_HDIV | Mutation Taster |
|----------|------------|-----|-----|----------|-------------|----------------|-----------------------|-----------------|-----------------|-----------------|
| 11q13.3  | 70333254   | G   | T   | SHANK2   | Missense    | NM_13266.5:c.C1380>A p.(Ser460Arg) | —                     | 0.007,D        | 0.999,D        | 1.0,D           | 1.000,D         |
| 12p13.33 | 1017657    | G   | A   | WNK1     | Missense    | NM_001184985.2:c.G7628>A p.(Arg2543Lys) | —                     | 0.044,D        | 0.99,D         | 0.998,D        | 1.000,D         |
| 14q11.2  | 21876615   | G   | C   | CHD8     | Missense    | NM_001170629.2:c.G2586>G p.(Phe862Leu) | —                     | 0.001,D        | 0.883,P        | 0.924,P        | 1,D             |
| 16p13.3  | 2570530    | G   | T   | AMDHD2   | Missense    | NM_001145815.2:c.G71>T p.(Gly24Val) | —                     | 0.213,T        | 0.773,P        | 0.965,D        | 0.997,N         |
| 16q22.1  | 67861244   | C   | T   | TSNAXIP1 | Missense    | NM_001288990.3:c.C1757>T p.(Ala586Val) | —                     | 0.42,T         | 0.124,B        | 0.279,B        | 1.000,N         |
| 16p11.2  | 30709563   | C   | A   | LOC730183| Missense    | NM_001256932.2:c.G67>T p.(Ala23Ser) | —                     | —              | —              | —              | —               |
| 16q12.1  | 48177945   | G   | T   | ABCC12   | Missense    | NM_033226.3:c.C151>A p.(Leu51Ile) | —                     | 0.009,D        | 0.945,D        | 0.988,D        | 0.723,D         |
| 17p11.2  | 20370767   | G   | C   | LGALS9B  | Missense    | NM_001042685.3:c.C17>G p.(Ser6Cys) | rs4985834, COSM308341 | 0.086,T        | 0.001,B        | 0.0,B          | 1,P             |
| 17p13.1  | 7578266    | TA  | T   | TP53     | Missense    | NM_000546.6:c.S82del p.(Leu194fs) | COSM308341, COSM308343, COSM308344, COSM308342, COSM308345 | —              | —              | —              | —               |
| 18q12.2  | 33828939   | G   | A   | MOCOS    | Missense    | NM_017947.4:c.G2015>A p.(Arg672His) | rs75369462  | 0.367,T        | 0.106,B        | 0.219,B        | 1.000,N         |
| 18q21.1  | 46447770   | C   | G   | SMAD7    | Missense    | NM_001190821.2:c.G1250>C p.(Trp417Ser) | —                     | 0.0,D          | 0.998,D        | 0.999,D        | 1,D             |
| 19p13.3  | 3961100    | G   | A   | DAPK3    | Missense    | NM_001348.3:c.G689>T p.(Ser230Leu) | rs866486559 | 0.044,D        | 0.246,B        | 0.758,P        | 1.000,D         |
| 19p13.2  | 10114755   | G   | C   | COL5A3   | Missense    | NM_015719.4:c.C661>G p.(Leu221Val) | —                     | 0.401,T        | 0.236,B        | 0.767,P        | 0.925,N         |
| 19q13.11 | 35434615   | C   | T   | ZNF30    | Missense    | NM_001099437.2:c.C748>T p.(Arg250Trp) | rs373065289 | 0.08,T         | 0.015,B        | 0.069,B        | 1,N             |
| 19q13.43 | 58863024   | G   | A   | A1BG     | Missense    | NM_130786.4:c.C643>T p.(His215Tyr) | —                     | 0.437,T        | 0.015,B        | 0.001,B        | 1,N             |

(Continues)
out of 266 cases in a large Chinese population with cranio-maxillofacial FD were identified, revealing a prevalence of 1.1% (Cheng et al., 2012). Similarly, in the present study, we found 3 cases of sarcoma out of 253 cases of FD of the jaws. Therefore, the development of sarcoma from FD is rare. The first well-documented case was reported in 1945 by Coley and Stewart (1945). A recent review regarding malignant transformation in FD specifically in the craniofacial bones identified only 48 cases in the literature (Li et al., 2020), calling for a larger sample to obtain a better and comprehensive understanding of this entity. In this context, reporting these three new cases in the present study is helpful.

We carefully reviewed the detailed clinicopathological information of these three patients. Moreover, some of the most concerning topics related to this occurrence were reviewed from the literature, including risk factors for malignant transformation, malignant tendency in different sexes and types of FD (monostotic FD, polyostotic FD, MAS) and onset age of sarcoma transformation.

Generally, secondary sarcomas affect male and female patients with FD equally (Li et al., 2020; Ruggieri et al., 1994; Yabut et al., 1988). Similar to the skeletal distribution of FD, craniofacial bones and femur were the most frequently affected sites when sarcomas occurred (Ruggieri et al., 1994; Schwartz & Alpert, 1964). Regarding the onset age of sarcoma occurrence in FD, a wide range of ages were identified, mostly beyond the third decade. The onset ages of all three patients in the present study were all over 30 years old. Interestingly, de novo osteosarcoma from normal bones occurred in jaw bones two decades later than in long bones (Bertin et al., 2020). Whether this rule of primary osteosarcoma is applied to sarcomas secondary to FD is still unknown. We combined and analysed data from two large populations of this specific entity in the literature (Ruggieri et al., 1994; Schwartz & Alpert, 1964) and found that the onset ages for sarcoma were 34.96 ± 16.80 years and 40.22 ± 14.75 years for craniofacial bones and other bones, respectively. There was a site-related variation in this entity as well; however, statistical analysis (Student’s t test) showed no significant difference (p value: 0.2188).

Regarding potential risk factors leading to malignant degeneration, radiotherapy was the first one suggested in the literature. It was claimed for two reasons. First, radiation itself had carcinogenic effects (Yannopoulos et al., 1964), which can induce primary bone sarcomas (Schwartz & Alpert, 1964). Second, it was originally observed that sarcoma developed in FD exclusively with previous radiation (Schwartz & Alpert, 1964) and specifically in the field of prior irradiation (Ruggieri et al., 1994), which raised the possibility that radiation might play a significant role in sarcoma transformation.
| Chr | Start   | End      | CNV type | GeneName related with OMIM | OMIM                                                                 |
|-----|---------|----------|----------|----------------------------|----------------------------------------------------------------------|
| 1   | 36480000| 41090000 | Gain     | RSPO1                      | Palmoplantar hyperkeratosis and true urachal cysts; Palmoplantar hyperkeratosis with squamous cell carcinoma of skin and sex reversal |
| 1   | 44090000| 46370000 | Gain     | PTCH2                      | Basal cell carcinoma, somatic; Medulloblastoma                         |
| 1   | 44090000| 46370000 | Gain     | MUTYH                      | Adenomas, multiple colorectal; Colorectal adenomatous polyposis, autosomal recessive, with pilomatrixomas; Gastric cancer, somatic |
| 1   | 46370000| 47520000 | Gain     | RAD54L                     | Adenocarcinoma, colonic, somatic (3); Lymphoma, non-Hodgkin, somatic; [Breast cancer, invasive ductal] |
| 2   | 16370000| 17004000 | Gain     | GALNT3                     | Tumoral calcinosis, hyperphosphatemic, familial                        |
| 2   | 17769000| 17831000 | Gain     | HOXD4                      | [Leukemia, acute lymphoblastic, susceptibility to] (3)                   |
| 3   | 14078000| 15802000 | Gain     | ATR                        | Cutaneous telangiectasia and cancer syndrome, familial; GAPO syndrome; Seckel syndrome 1; [Hemangioma, capillary infantile, susceptibility to] |
| 3   | 93590000| 11200000 | Gain     | TFG                        | Chondrosarcoma, extraskeletal myxoid; Hereditary motor and sensory neuropathy, proximal type |
| 4   | 52930000| 75860000 | Gain     | PDGFRA                     | Gastrointestinal stromal tumor, somatic; Hypereosinophilic syndrome, idiopathic, resistant to imatinib                 |
| 4   | 52930000| 75860000 | Gain     | KIT                        | Gastrointestinal stromal tumor, familial; Germ cell tumors; Leukemia, acute myeloid; Mast cell disease; Piebaldism |
| 4   | 52930000| 75860000 | Gain     | CHIC2                      | [Leukemia, acute myeloid]                                              |
| 8   | 107740000| 134250000| Gain    | RNF139                     | Renal cell carcinoma                                                   |
| 8   | 89090000| 99230000 | Gain     | RAD54B                     | Colon adenocarcinoma (3) | Lymphoma, non-Hodgkin (3)                                             |
| 8   | 107740000| 134250000| Gain    | EXT1                       | Chondrosarcoma; Exostoses, multiple, type 1                            |
| 8   | 107740000| 134250000| Gain    | MYC                        | Burkitt lymphoma; Myelodysplasia, familial cortical                    |
| 8   | 49100000| 80540000 | Gain     | RB1CC1                     | Breast cancer, somatic                                                 |
| 8   | 49100000| 80540000 | Gain     | PLAG1                      | Adenomas, salivary gland pleomorphic                                   |
| 11  | 292000000| 187900000| Gain    | RRAS2                      | Ovarian carcinoma (3)                                                  |
| 11  | 292000000 | 187900000 | Gain   | TSG101                     | Breast cancer, somatic                                                 |
| 11  | 356400000| 460100000| Gain    | CD82                       | [Prostate cancer, susceptibility to]                                   |
| 12  | 364000000| 486000000| Gain    | FGF23                      | Hypophosphatemic rickets, autosomal dominant; Osteomalacia, tumor-induced (1); Tumoral calcinosis, hyperphosphatemic, familial |

(Continues)
| Chr | Start   | End       | CNV type | GeneName related with OMIM | OMIM                                      |
|-----|---------|-----------|----------|-----------------------------|-------------------------------------------|
| 14  | 94780000| 99960000  | Gain     | Dicer1                      | Goiter, multinodular 1, with or without Sertoli-Leydig cell tumors; Pleuropulmonary blastoma |
| 16  | 77750000| 80720000  | Loss     | Wwox                        | Esophageal squamous cell carcinoma        |
| 16  | 72110000| 73170000  | Gain     | Zfhx3                       | [Prostate cancer, susceptibility to]      |
| 17  | 16390000| 18200000  | Gain     | Flcn                        | Birt-Hogg-Dube syndrome; Colorectal cancer, somatic; Pneumothorax, primary spontaneous; Renal carcinoma, chromophobe, somatic |
| 19  | 5610000 | 11730000  | Gain     | Smarca4                     | Mental retardation, autosomal dominant 16; Rhabdoid tumor predisposition syndrome 2 |
| 20  | 55740000| 60080000  | Gain     | Gnas                        | ACTH-independent macronodular adrenal hyperplasia; Acromegaly; McCune-Albright syndrome; Osseous heteroplasia, progressive; Prolonged bleeding time, brachydactyly and mental retardation (3) | |
| 21  | 28200000| 31730000  | Gain     | Bach1                       | Breast cancer, early-onset; Fanconi anemia, complementation group J |

Note: GenBank and NCBI reference sequence: Rspo1, AK098225.1/NG_012239.2; Ptcch2, AF091501.1/NG_013369.1; Mutyh, U63329.1/NG_008189.1; Rad54l, X97795.1/NG_012144.1; Galnt3, NG_012069.1; Hoxd4, NG_012080.1; Attr, U76308.1/NG_008951.1; Tfg, BC009241.2/NG_027821.2; Pik3c4, NG_012113.2; Pdgfra, D50017.1/NG_009250.1; Kit, S79639.1/NG_007456.1; Chic2, AF159423.1/NG_028924.1; Rnf139, AF064801.1/NG_012158.1; Rad54b, AF112481.1/NG_012878.2; Ext1, S79639.1/NG_007455.2; Myc, NG_007161.2; Rb1cc1, AB059622.1/NG_015833.2; Plag1, U65002.1/NG_023310.1; Rras2, M31468.1/NG_017058.1; Tsg101, U82130.1/NG_012138.2; Cdh2, U20770.1/NG_023234.1; Fgf23, AF265537.1/NG_007087.1; Dicer1, AB028449.1/NG_016311.1; Wwox, AF187015.1/NG_011698.1; Zfhx3, D10250.1/NG_013211.2; Flcn, AF517523.1/NG_008001.2; Smarca4, D26156.1/NG_011556.3; Gnas, AH002748.2/NG_016194.2; Bach1, AF026200.1/NG_029658.2.

**FIGURE 5** IHC analysis revealed positive expression of p53 in the malignant tissues of all three patients. (a,b) Representative views of patient #1. (a) 10X view, (b) 40X view. (c,d) Representative views of patient #2. (c) 10X view, (d) 40X view. (e,f) Representative views of patient #3. (e) 10X view, (f) 40X view. P53 staining was observed in the nuclei.
However, more cases without prior radiation were reported later (Cheng et al., 2012; Li et al., 2020; Ruggieri et al., 1994; Schwartz & Alpert, 1964) and finding that no major differences in onset age were found between patients with and without radiotherapy (Ruggieri et al., 1994; Schwartz & Alpert, 1964), its role in malignant transformation became weak and controversial. Here, none of the three patients received radiation, again suggesting that there are additional unknown factors contributing to sarcoma development. It has been observed that polyostotic FD and MAS have more malignant potential than monostotic FD (Li et al., 2020; Schwartz & Alpert, 1964). In addition, in a study by Schwartz, the rate of metastasis was reported to be greater in patients with polyostotic FD than in those with monostotic FD (Schwartz & Alpert, 1964). Taken together, polyostotic FD seems to be a high-risk factor for sarcoma progression. Several case reports have also demonstrated that excess growth hormone (GH; Collins et al., 2012) is associated with malignant transformation and may be a potential driver. Interestingly, patient #1 in the present study showed rapid growth after correction of her hypothyroidism, providing evidence that thyroid-related hormones may also contribute to malignant transformation.

Based on the above discussion, it is clear that every FD has potential for malignant transformation, regardless of the sex and age of the patient or the location and type of FD. Therefore, all FD patients should be carefully followed up periodically. Specifically, some kinds of FD (polyostotic FD and MAS, especially those with abnormal hormones) have more potential for malignant transformation and need more attention. The role of radiation in malignant transformation in FD is still controversial, and radiotherapy treatment for FD should be avoided.

Very little has been reported in the literature about the genomic and molecular mechanisms underlying sarcoma transformation in FD, with only 7 papers published so far (Hagelstein-Rotman et al., 2020; Hatano et al., 2014; Jhala et al., 2003; Kanazawa et al., 2009; Sugiura et al., 2018; Yap et al., 2020; Zreik et al., 2017), which are summarized in Table 4. It has been well demonstrated that FD was caused by GNAS activating mutations, which can constitutively activate cAMP signalling and result in the abnormal proliferation and osteoblast differentiation of bone marrow stromal cells, the two major features of this disorder. Whether these mutations in FD are retained in malignant tissues has drawn much attention. In the literature, GNAS mutation analysis of malignant tissues has been performed in only 7 patients, and 5 of them showed mutations, with 3 R201C (p.Arg201Cys; Kanazawa et al., 2009; Yap et al., 2020; Zreik et al., 2017) and 2 R201H (p.Arg201His; Hatano et al., 2014; Sugiura et al., 2018). In the present study, we detected R201C GNAS mutations in all three patients. Collectively, GNAS mutations were retained in most of the sarcomas (8/10), with more R201C (75%) than R201H (25%) mutations. Whether a sarcoma in patients with FD comes from pre-existing FD or de novo has been controversial. Since the evaluation by Schwartz, which revealed that sarcomas always developed in bones affected by FD (Schwartz & Alpert, 1964), the view of sarcomas originating from pre-existing FD has become less controversial and has become more widely accepted. However, direct evidence of sarcoma development in FD has been lacking for a long time. The finding that GNAS mutations exist only in FD other than primary osteosarcoma (Salinas-Souza et al., 2015; Tabareau-Delalande et al., 2013) provides an opportunity to study the origin of sarcomas in FD patients. The combined data from the literature and our study now allow us to make more confident conclusions that FD has malignant potential.

In addition to the GNAS mutation analysis above, other molecular mechanisms underlying sarcoma development in FD, including aberrant TP53 (Sugiura et al., 2018; Yap et al., 2020), multiple chromosomal abnormalities (Hatano et al., 2014; Jhala et al., 2003), increased cell proliferation (Sugiura et al., 2018; Yap et al., 2020), MDM2 (Sugiura et al., 2018), c-fos (Kanazawa et al., 2009), PTH/PTHrP (Kanazawa et al., 2009) and keratins (Zreik et al., 2017), have been reported in case reports using traditional chromosome abnormality methods and IHC, which can reveal only limited information. Next-generation sequencing has illuminated rich and unappreciated genomic alterations for various diseases; however, only one study using WES analysis for malignant transformation in FD was found in the literature (Yap et al., 2020) when we prepared for the present study. Through WES analysis, we found several valuable genomic aberrations for FD malignancy. First, three-driver genes were found in the malignant tissues associated with FD: ROS1, CHD8 and TP53. Among them, TP53 is the only one that has been reported in the literature for this occurrence. However, different from the reported TP53 point mutation (Asp281Asn; Yap et al., 2020), it was an INDEL in the present study. Functionally, TP53 is a tumour suppressor gene that is essential for regulating cell division and preventing tumour formation. According to a recent study (Sayles et al., 2019), TP53 gain-of-function alterations can be detected in 74% of osteosarcoma cases. We also confirmed the abnormality of TP53 by IHC in the other two patients here. Taking the data from the literature and our present study, it is obvious that similar to primary osteosarcoma, TP53 abnormalities may be a significant event in osteosarcoma secondary to FD.

Regarding TP53 aberration in the malignant transformation of FD, it is notable that its coexistence with GNAS activating mutations has been reported in other malignant
| Country                        | Sample size | Onset age of malignancy (years) | Sex | Site       | Type of FD | Malignant pathology | Genetic mechanisms for sarcoma transformation | Methods for other molecular analysis |
|-------------------------------|-------------|---------------------------------|-----|------------|-------------|---------------------|-----------------------------------------------|----------------------------------|
| US (Jhala et al., 2003)       | 1           | 44                              | F   | Right elbow | Concommitant MAS and Mazabraud’s syndrome | OS                                | Not included Chr5 and Chr7 trisomies, multiple chromosomal abnormalities | G-banded karyotype on short-term primary cells, FISH on paraffin-embedded tissues, CGH of DNA from paraffin-embedded tissues |
| Japan (Kanazawa et al., 2009) | 1           | 38                              | F   | Mandible   | MAS         | OS                  | R201C mutation | Expression of c-fos and PTH/PTHrP; excess serum GH and IGF-1 | Immunohistochemistry on paraffin-embedded tissues; laboratory examinations |
| Japan (Hatano et al., 2014)   | 1           | 72                              | M   | Femur      | PFD         | OS                  | R201H mutation | 44X,-Y, add(4)(p11), add(5)(p15), add(11)(p15)t(1:11) (q21;q23), add(12)(q11), -13, der(22)(12:22) (q11:p12) | G-banded karyotype |
| US (Zreik et al., 2017)       | 1           | 45                              | M   | Femur      | PFD         | NS                  | R201C mutation | Aberrant expression of multiple keratins | Immunohistochemistry |
| Japan (Sugiura et al., 2018)  | 1           | 33                              | M   | Hip joint  | MFD         | OS                  | R201H mutation | Positive staining for p53 and MDM2; MIB-1 index: 15%; negative for CDK4; no MDM2 amplification | Immunohistochemistry and FISH on paraffin-embedded tissues |
| Netherlands (Hagelstein-Rotman et al., 2020) | 7 | NS                              | NS  | NS         | 4 MFD, 3 NS | OS                  | 2 out of 7 underwent GNAS mutation detection, with one negative and one inconclusive | Not included | not included |
| Australia (Yap et al., 2020)  | 1           | 21                              | M   | Maxilla    | MFD         | OS                  | R201C mutation | TP53 mutation (Asp281Asn); positive staining for p53; Ki-67 proliferation index: 25%; no MDM2/CDK4 amplification | Next-generation sequencing; immunohistochemistry; FISH |

Abbreviations: F, female; M, male; MAS, MuCune-Albright syndrome; MFD, monostotic fibrous dysplasia; NS, not specified; OS, osteosarcoma; PFD, polyostotic fibrous dysplasia; R201C, p.Arg201Cys; R201H, p.Arg201His.
tumours, including colorectal adenocarcinoma, oesophageal squamous cell carcinoma, pancreatic adenocarcinoma and non-small-cell lung cancer (Table 5, data from TCGA database). Furthermore, in a recent study (Patra et al., 2018), malignant transformation (pancreatic ductal adenocarcinomas, PDAs) of a benign pancreatic disorder, intraductal papillary mucinous neoplasm (IPMN), induced by GNAS mutation, with the addition of TP53 loss was reported, supporting the significant role of TP53 in the progression of benign disease with pre-existing GNAS mutation to a malignant state. Whether this coexistence of TP53 and GNAS variations makes the osteosarcoma different from the counterpart without GNAS mutation is unclear. Pathologically, no significant difference could be found, with all three of our cases being the conventional OS. However, based on the surprising finding by Patra et al that mutant GNAS was still required for the maintenance and growth of tumours after malignant transformation of IPMN, it is probably true that GNAS mutation also retained an important role after FD malignancy. Therefore, it might be feasible to treat secondary malignancies from FD and other diseases bearing this mutation by gene targeting therapy in the future. Indeed, molecular therapy targeting GNAS activating mutations has been demonstrated to be effective for FD in vitro (Piersanti et al., 2010).

Somatic CNAs are another important aspect underlying the genetic mechanism of primary osteosarcoma. It has even been hypothesized that CNAs rather than point mutations may be the dominant oncogenic mechanism for osteosarcoma progression and maintenance (Sayles et al., 2019). Furthermore, Sayles et al. (2019) identified some somatic CNAs most likely to be of direct clinical relevance, targeting these somatic CNAs could lead to a significant decrease in tumour burden. Somatic CNAs across cases of osteosarcoma are highly complex and heterogeneous; thus, their analysis in each patient is of great importance. In the literature, only one paper has demonstrated the gain of chromosomal regions in FD malignancy using comparative genomic hybridization (CGH) analysis (Jhala et al., 2003). In the present study, through WES, more detailed information about somatic CNVs in a secondary osteosarcoma of FD was revealed. Various oncogenes and tumour suppressors, including KIT, PDGFR, MYC, EXT1, PIK3A and WWOX, are located in these somatic CNAs. Based on Sayles's study, targeting oncogenes within these CNAs may provide hope for the treatment of patients with these specific genomic abnormalities.

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**CONFLICT OF INTERESTS**

The authors declare that they have no conflict of interest.

**AUTHOR CONTRIBUTIONS**

Study design: RRS, TJL. Study procedures and data collection: RRS, XFL, MM, YRF. Data analysis and interpretation: RRS, XFL, JYZ, FC, TJL. Manuscript preparation: RRS, FC, TJL.
ETHICAL COMPLIANCE
This study was approved by the Institutional Review Board of Peking University and conducted in accordance with the Declaration of Helsinki.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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