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Research Paper

Diagnostic value of *H3F3A* mutation and clinicopathological features of giant cell tumours in non-long bones

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HIGHLIGHTS

- *H3F3A* mutation is a useful diagnostic marker for GCTB in non-long bones.
- GCTB of non-long bones have a female preference.
- G34L/V/R mutations are more frequent in younger patients.

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ABSTRACT

Aims: A histone *H3F3A* (H3.3) mutation involving a substitution in H3.3 G34 recently has been reported in GCTB within the frequency range (from 69 % to 96 %) and is a helpful diagnostic indicator of GCTB. However, the relationship between *H3F3A* mutations and the clinicopathological feature of GCTB involving non-long bones (irregular bones and small bones) is unclear.

Methods and results: H3F3A mutations were observed in a cohort of specimens (230 samples of GCTB) using immunohistochemistry and Sanger sequencing. The relationship between *H3F3A* mutations and the clinicopathological characteristics of patients with GCTB occurring in the non-long bones of the appendicular skeleton was investigated. No significant difference between *H3F3A* mutations in GCTB arising in non-long bones and the classic sites was found (P = 0.483). GCTB in non-long bones occurred more common in female (31/49, 63.3 %) than in male patients (P = 0.016). GCTB with *H3.3* G34L/V/R mutation occurred more often in younger patients compared with those with *H3.3* G34W mutation (P = 0.009). The majority of GCTB with soft tissue extension developed in irregular bones but not in small bones (P = 0.061). The *H3.3* G34L/V/R mutations rate (7/45) in the non-long bones was significantly higher than that in long bones. The recurrence rate of the GCTB in long bones and non-long bones was 23.3 % (45/193) including 43 cases with local recurrene and 2 cases with lung metastasis. No recurrence occurred in cases with G34V/L/R mutations.

Conclusions: H3F3A was an effective diagnostic marker for GCTB of the non-long bones. The younger patients with GCTB of the non-long bones harboured *H3.3* G34L/V/R mutations and may had a female preference and rarely recurrent.

1. Introduction

Giant cell tumour of bone (GCTB) is a locally aggressive primary bone tumour that rarely metastasizes, accounted for 5 % of all primary bone neoplasms [1]. It is composed of three main cellular components: mononuclear spindle-like stromal cells (the main neoplastic components), mononuclear cells of the macrophage lineage and multinucleated osteoclast-like giant cells. GCTB is typically located in the epiphysis of long bones, such as the distal femur, proximal tibia, distal radius, and proximal humerus [2]. However, it is rare in non-long bones, such as the

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pelvis, sacrum, ischium, ilium, especially in the small bones of the hands and feet [2–6].

Recently, a histone *H3F3A* (H3.3) gene mutation (G34W) in 69–96 % of GCTB was reported using Sanger sequencing and immunohistochemistry (IHC) [2,7–9]. *H3F3A* mutations are less in other giant cell-rich lesions (such as osteosarcomas and osteoblastomas) than in GCTB [10,11], which is a key marker for the differential diagnosis of histological mimics [2,7,12]. It is not challenging to diagnose GCTB with typical radiological and morphological features. However, the radiographic features and morphological phenotype of GCTB usually overlap with the features of solid aneurysmal bone cysts (ABCs), chondroblastoma, giant cell reparative granulomas and other giant cell lesions in non-long bones. It may be difficult to reach a definite diagnosis of GCTB in the non-long bones, especially those with the atypical morphology and imaging.

H3F3A p.G34R or *H3F3A* p.G34V mutations in glioblastomas are involved in key regulatory posttranslational modifications [13]. Using a fission yeast system, *H3.3* G34V showed no defects in H3K36 acetylation, chromosome loss, replicative stress, or homologous recombination (HR), but the sensitivity to irradiation (IR) was enhanced. *H3.3* G34R, but not *H3.3* G34V, causes malignant transformation including dominant hydroxyurea sensitivity, homologous recombination defects, and dominant subtelomeric silencing [14]. Rare *H3.3* G34 mutations have recently been reported in GCTB. Amary *et al.* found 6 of 235 GCTB with different *H3.3* G34 substitutions (G34L/M/V), and Yamamoto *et al.* reported four cases of GCTB with the *H3.3*G34V or *H3.3* G34R mutations [2,3]. The *H3.3* G34L (3/180, 1.67 %), *H3.3* G34V (3/180, 1.67 %), and *H3.3* G34R (2/180, 1.11 %) mutations were also found in the literature [4]. However, there have been no reports on *H3.3* G34R/G34V mutations involved in malignant biological behaviours of GCTB.

The recurrence rate in the patients with GCTB varies greatly ranging from 14.2 % to 52.9 %. Recurrence typically presents within three years after surgery [15–17]. Pulmonary metastasis of GCTB was observed in 3–7 % patients with conventional GCTB [18,19]· Multiple factors were associated with the recurrence in patients with GCTB, including the type of surgery performed, adjuvants used, use of cement, and tumour location [15,17,20]. However, the limited published researches on the recurrence of GCTB in non-long bones, especially on the recurrence of GCTB in small bone, were reported mostly in the form of small series and case reports [21]. There have been no studies on the correlation between *H3F3A* mutation and recurrence of GCTB, especially in non-long bones.

In the present study, we investigated *H3F3A* mutation in GCTB of non-long bones in a single institution and examined the relationship between *H3F3A* mutation and the clinicopathological characteristics of these patients.

2. Materials and methods

2.1. Patients

The present study was approved by the Ethics Committee of -Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (2013–87), in accordance with the Declaration of Helsinki. The participants were fully informed about the procedures, and written informed consent for participation was obtained. Forty-nine cases of GCTB of non-long bones were collected from January 2013 to May 2022, including 35 cases of irregular bones, such as the vertebrae, clivus pelvis, ischium, and ribs, and 14 cases of short bones of the hands and feet and the patella (Table 1). The other cases of GCTB (n = 181) in long bones (femur, tibia, radius, ulna, fibula, and humerus) were also randomly collected including 110 cases of primary GCTB, 23 patients with recurrent GCTB, 15 cases of GCTB with secondary aneurysmal bone cyst (sABC), 6 cases GCTB received denosumab treatment, 2 cases GCTB with lung metastasis, 16 cases of primary malignant GCTB (MGCTB) and 9 cases of secondary malignant GCTB (MGCTB) (Table 1). According to morphology, 107 cases of their histological mimics (osteosarcoma, n = 30, chondroblastoma, n = 16, sABC, n = 30, giant cell reparative granuloma, n = 4, chondrosarcomas, n = 25 and non-ossifying fibroma, n = 2) as control tissues were also selected.

Table 1
Location and *H3.3* G34 mutations of giant cell tumor of bone.

Location	Number of tumors	G34W mutation (Sequencing)	G34W (IHC)	Other G34 substitutions detected
Long bone	181	162	158	10
Femur	76	66	65	G34V*2, G34L*1, G34R*1
Tibia	58	53	51	G34V*1, G34L*1, G34R*1
Fibula	6	6	6	0
Humerus	18	16	15	G34V*2
Radius	19	19	19	0
Ulna	4	2	2	G34V*1
Non-long bone	49	38	25	7
Patella	2	1	0	G34L*1
Phalanx hand	1	1	1	0
Metacarpal	1	1	0	0
Metatarsal	2	2	0	0
Navicular	2	1	1	G34V*1
Talus	5	3	2	G34R*1
Vertebra	4	4	3	0
Rib	3	3	1	0
Sacrum	2	1	0	0
Clivus	2	2	2	0
Pelvis	4	4	3	0
Acetabulum	6	2	2	G34V*2, G34L*1
Scapula	2	1	0	G34L*1
Pubis	2	2	2	0
Ilium	7	6	4	0
Ischium	3	3	3	0
Cuboid	1	1	0	0
Total	230	200	183	17

The age of the patients in our study ranged from 9 to 72 years. The mean age of patients with GCTBs was 37.35 ± 13.56 in a classic location, and 36.39 ± 12.99 in the non-long bones of the appendicular skeleton. The follow-up period was December 2021 to May 2022. Follow-up information was available for 193 patients totally (n = 193; from 1 month to 255 months; median, 37.14 ± 13.42 months), the recurrence rate was 23.3% including 43 patients with local recurrence and 2 patients with lung metastasis. The overall recurrence time for these patients was calculated from the date of surgery to the date of recurrence. Eighty-one patients with GCTB had a soft tissue extension, and relevant information was not available for 34 patients. Patients were followed up with radiographic examinations every 3 months for the first 1 year after surgery. They were followed up with radiographic examination every 6 months until the 3rd year and then annually until the 5th year. Radiographs of the involved area and CT images of the chest were obtained to evaluate GCTB recurrence and metastasis.

3. Immunohistochemistry

We used formalin-fixed specimens cut into 4-μm-thick sections for immunohistochemistry (IHC). Antigen retrieval was performed and the sections were incubated at room temperature for 10 min with 100 μl peroxidase. The sections were further incubated with rabbit monoclonal antibody against H3F3A p.G34W (clone RM263; RevMAb Biosciences, USA) (diluted 1:500), H3F3A p.G34R (rabbit monoclonal, clone RM240, dilution 1:1000; RevMAb Biosciences), and H3F3A p.G34V mutant protein (rabbit monoclonal, clone RM307, dilution 1:4000; RevMAb Biosciences) for 1 h at 37 °C, followed by incubation for 30 min at 37 °C with a horseradish peroxidase-conjugated secondary antibody (Dako EnVision + System HRP, DAB). The slides were stained with

haematoxylin (Harris Formula, Surgipath Medical Industries, Inc., Richmond, IL, USA) at 25 °C for 1 min. Positive H3H3A reaction was characterised by unequivocal strong, crisp nuclear staining.

4. Sanger sequencing

DNA was extracted from FFPE tissue specimens. A QIAamp DNA extraction kit (Qiagen GmbH, Hilden, Germany) was used, and according to the manufacturer's protocol *H3F3A* mutations were identified using direct sequencing. Polymerase chain reaction (PCR) amplification and direct sequencing of *H3F3A* (exon2) were performed on 230 samples with GCTB and 107 control tissues. Primers (forward AAATCGACCGGTGGTAAAGC; reverse: ATACAAGAGAGACTTTGTCCCA) were designed to amplify exon 2 of *H3F3A* gene. PCR was performed using a Master cycler gradient PCR machine (Eppendorf, Hamburg, Germany) under the following amplification conditions:37 °C for 2 min and 94 °C for 10 min, 45 cycles of 94 °C for 15 s and 60 °C for 45 s, and a final extension at 25 °C for 1 min. The resulting PCR product was subjected to cycle sequencing using standard procedures.

5. Imaging and statistical analysis

Imaging was reviewed when there was a discrepancy. We investigated the relationship between molecular features and clinicopathologic characterizations using the $\chi 2$ test or two independent samples *t*-test. The data were analysed using IBM SPSS Statistics version 13.

6. Results

6.1. H3.3 G34W expression in GCTBs of long bones and non-long bones

The common imaging features of patients with GCTB were osteolytic lesions on the long bone ends (Fig. 1A). Based on anatomic sites, 230 cases of GCTB were classified into two groups. The classic group including the 181 tumours in the long appendicular bones (femur, tibia, radius, ulna, humerus, and fibula, Fig. 1A); The others were classified into the non-long appendicular bone group including tumours in irregular bones (35 cases) and small bones (14 cases) (Fig. 1B-D, Table 1). The morphology of traditional GCTB shows mononuclear cells with interspersed osteoclastic giant cells (Fig. 1E-G), while only spindle cells were observed in three cases of GCTB in the small bones (Fig. 1H). Patients with GCTB mainly harboured H3.3 G34W mutation by IHC

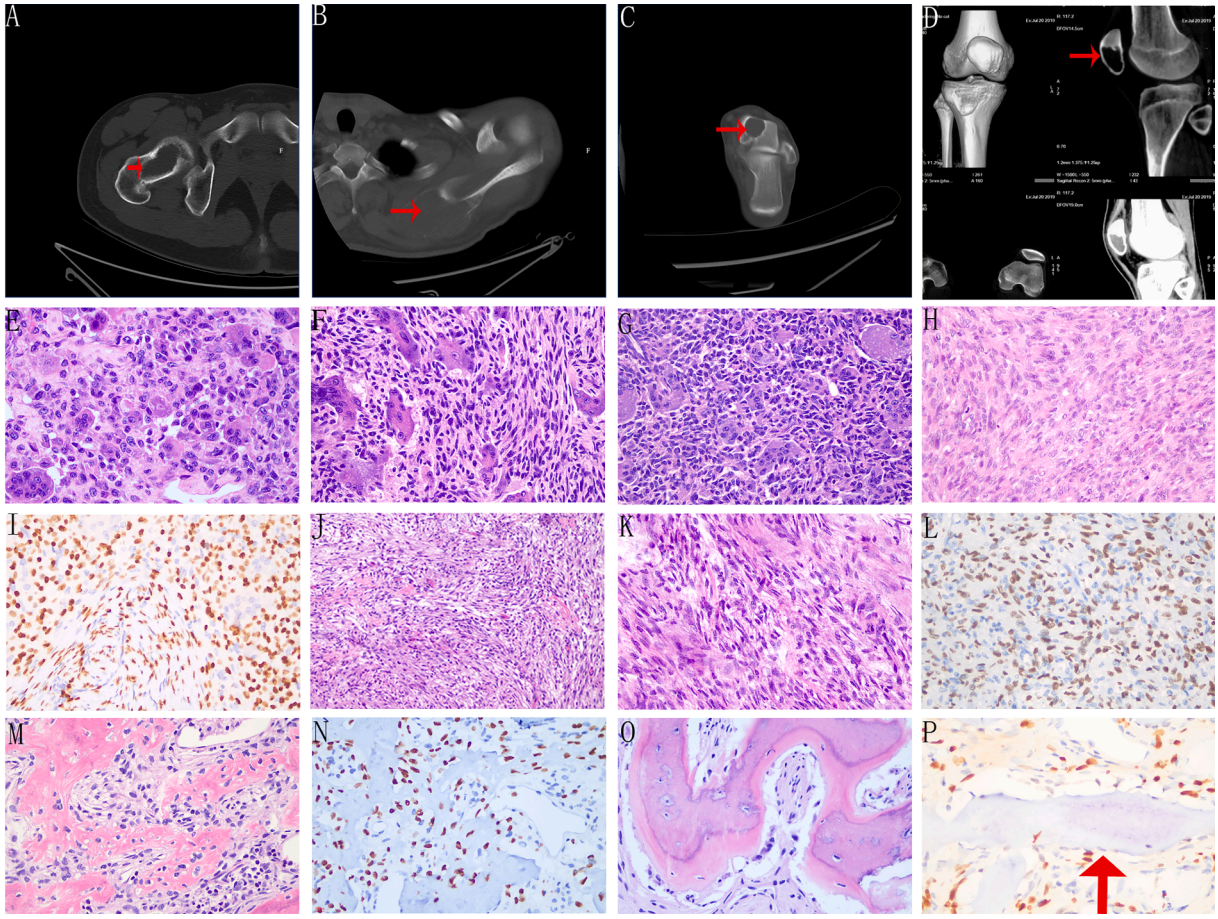

Fig. 1. Imaging, histological appearance, immunohistochemical features of H3.3 G34W in GCTB. Osteolytic lesions are radiographic features (A-D) of GCTB (red arrows) in proximal femur(A), scapula(B), calcaneus(C) and patella(D). Mononuclear stromal cells and osteoclast-like multinucleated giant cells are typical histological features of GCTB (E-G), whereas H show spindle cells in GCTB of small bone (patella). Diffuse positive immunoreaction with H3.3 G34W was found in the nucleus of mononuclear stromal cells but not giant cells in GCTB (I). Bland spindle cells without giant cells in sheet and storiform pattern were seen in GCTB after neoadjuvant therapy by denosumab (J, K), which show positive immunoreaction with H3.3 G34W (L). Various degrees of new bone formation and positive H3.3 G34W immunoreaction were found in GCTB after neoadjuvant therapy by denosumab (M−P). Immature bone formation was found, and newly formed bone show long cord, curvilinear, branching or anastomosing woven bone in GCTB after denosumab treatment (M). Nuclear positive expression for H3.3 G34W mutation protein is observed in inactive osteoblast-like cells in the bone lacunae(N). Reactive proliferation of mature bone (O) is covered with mature osteoblasts (arrow) without H3.3 G34W mutant protein expression in GCTB after denosumab treatment (P) (original magnification, J ×200 , E-I ×400 , K-P ×400). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(79.6%, 183/230, Fig. 1). Diffuse strong positive nuclear immunoreaction with H3.3 G34W antibody was found in mononuclear stromal cells but not giant cells in GCTB without neoadjuvant therapy (Fig. 1I). The same immunoreaction was detected in all spindle cells in all 3 cases of GCTB with neoadjuvant therapy by denosumab (Fig. 1J-L). Various degrees of new bone formation and positive H3.3 G34W immunoreaction were found in GCTBs after neoadjuvant therapy by denosumab (Fig. 1M-P).

6.2. The correlation between the H3F3A genotype and expression of H3.3 G34W/L/V/R in GCTB

In our experiments, the main type of *H3F3A* mutation in GCTB is *H3.3* G34W, which show positive immunoreaction with H3.3 G34W but not H3.3 G34V or G34R (Fig. 2A). Only a few H3F3A wild-type patients with GCTB were detected by IHC and sequencing (Fig. 2B). Seventeen cases of GCTB harboured *H3.3* G34L/V/R mutation (17/230, 7.39 %). *H3.3* G34L mutation was found in 5 cases of GCTB, which showed negative immunoreaction with H3.3 G34W/V mutation protein (Fig. 2C). Expression of H3.3 G34W/V/R in GCTB were observed in a mutually exclusive manner. The H3.3 G34R–positive cases (n = 3) harbored *H3.3* G34R mutation, and H3.3 G34V–positive cases (n = 9) harbored *H3.3* G34V mutation (Fig. 2D, E).

Ten patients had *H3.3* G34L/V/R mutations (10/181, 5.52 %) in the long bones (x6 p. G34V, x2 p. G34L, and x2 p. G34R), and seven patients with the *H3.3* G34 substitutions (x3 p. G34V, x3 p. G34L, and x1 p. G34R) in the non-long bones group (14.3 %, 7/49).

6.3. No significant correlation between H3F3A mutations and clinicopathological factors

The mutation frequency of *H3F3A* in GCTB samples was 217/230 (94.3 %) by Sanger sequencing. In contrast, all the 107 control tumours were *H3F3A* wild type using Sanger detection and IHC. *H3F3A* mutation was not associated with the clinicopathological parameters, including patient age, sex, tumour site, tumour size and local recurrence (P = 0.991, 1.000, 0.483, 0.062, and 0.736, respectively) (Table 2).

6.4. Correlation between H3F3A mutations and clinicopathological parameters of GCTB in different locations

The *H3F3A* mutation rate in non-long appendicular bones (91.8 %, 45/49) was similar to that in long bones (95.0 %, 172/181) (Table.2). Unexpectedly, GCTB in females more frequently occurred in non-long bones than in conventional sites (31/49, 63.3 % and 79/181, 43.6 %, respectively, $P = 0.016$) (Table.3). Soft tissue involvement was significantly more frequent in GCTB of the non-long appendicular bones (57.5 %, 23/40) than in GCTB of long bones (37.2 %, 58/156) except 34 cases

Table 2
Relationship between *H3.3* mutations and the clinicopathological features of 230 cases of giant cell tumour of bone.

Variants	H3F3A mutation			P
	G34	WT		
Location				0.483
LGCTB(n = 181)	172	9	95.0 %	
NGCTB (n = 49)	45	4	91.8 %	
Gender				1.00
Male (n = 120)	113	7	94.2 %	
Female (n = 110)	104	6	94.5 %	
Soft tissue involvement				1.00
No (n = 115)	108	7	93.9 %	
Yes (n = 81)	77	4	95.1 %	
Missing (n = 34)				
Recurrence				0.736
No (n = 148)	138	10	93.2 %	
Yes (n = 45)	43	2	95.6 %	
Missing(n = 37)				
Age	37.31 ± 13.43	34.31 ± 13.43		0.991
Size	5.55 ± 2.27	5.21 ± 2.03		0.062
Missing(n = 29)				

LGCTB is the abbreviation of giant cell tumor of long bone, and NGCTB is the abbreviation of giant cell tumor of non-long bone.

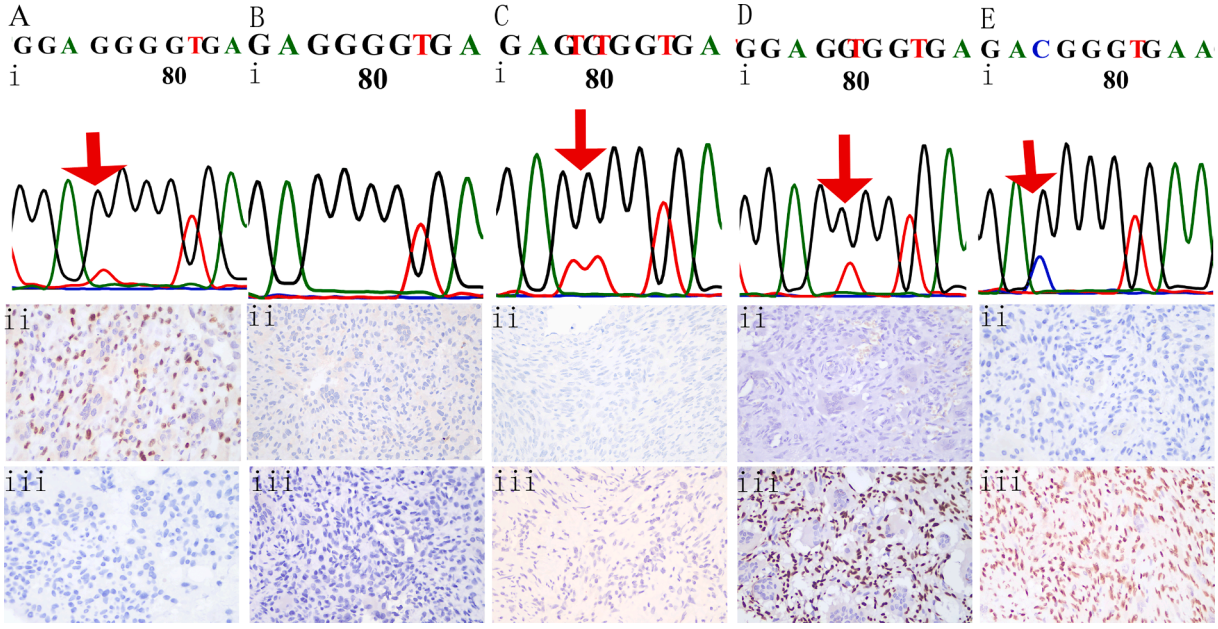

Fig. 2. The mutations and immunohistochemical features of H3.3 G34 in GCTB.A showed the *H3.3* G34W mutation (arrow) of GCTB (Ai).The mononuclear stromal cells are immunohistochemically positive for H3.3 G34W mutation protein (Aii) but negative for H3.3 G34V (Aiii). B. *H3.3* G34 mutation was not found in GCTB of distal tibia (Bi) with H3.3 G34W/V mutation protein negative expression (Bii-iii). C. Patient with GCTB of the acetabulum harboured *H3.3* G34L mutation (arrow) without H3.3 G34W/V expression (Ci-iii). D showed *H3.3*p.G34V mutation (arrow) in GCTB of the radius (Di), which was positive for H3.3 G34V but negative for H3.3 G34W (Dii-iii). E. *H3.3* G34R mutation (arrow) was observed in GCTB by Sanger sequncing (Ei), which showed positive immunoreaction with H3.3 G34R but not H3.3 G34W (Eii-iii) (original magnification × 400).

Table 3
Correlations between location and clinicopathological parameters of GCTB.

Variables	LGCTB	NGCTB	P-value
Gender			**0.016**
Male	102	18	
Female	79	31	
Soft tissue involvement			**0.030**
No	98	17	
Yes	58	23	
Missing (n = 34)			
Recurrence			0.681
No	117	31	
Yes	34	11	
Missing (n = 37)			
Age	37.35 ± 13.56	36.39 ± 12.99	0.064
Size	5.50 ± 2.14	5.73 ± 2.68	0.328
Missing(n = 29)			

LGCTB is the abbreviation of giant cell tumor of long bone. Giant cell tumor of bone in non-long bone is abbreviated to NGCTB.

of GCTB without the data of soft tissue involvement ($P = 0.030$). Further analysis showed that soft tissue involvement of GCTB of non-long bones group occurred more frequently in irregular bones than in small bones (20/29, 69 %; 3/10, 30 %, respectively) except 10 cases without information of the surrounding soft tissue. However, no significant difference in the recurrence rates was observed between the long bones (20.7 %, 31/150) and non-long bones (25.6 %, 11/43) (P = 0.681, 37 cases with missing related data) (Table 3). There was no difference in patient age or tumour volume of GCTB in the long bones and non-long bones (P = 0.064, 0.328).

6.5. The recurrence rate of GCTB with H3F3A p.G34L/V/R mutation was lower than those with H3.3 G34W mutation

The rate of the *H3.3* G34L/V/R mutations in the small bones was significantly higher than that in the classic group ($P = 0.012$, Table.4). Follow-up data were available for 193 of 230 (83.9 %) GCTB cases, and a total of 45 cases had recurrence, including 43 cases with local recurrence and 2 cases with lung metastasis. None of GCTBs with *H3.3* G34V/L/R mutations recurred, which was different from those cases harboured *H3.3* G34W mutation. *H3.3* G34L/V/R mutations occurred in the younger patients (P = 0.009). However, there was no significant difference in the tumours size and soft tissue involvement of GCTB between the *H3.3* G34W and G34L/V/R mutations ($P = 0.19$, and 0.205, respectively, Table 4). No statistical significance was examined by multiple factor analysis.

Table 4
Correlations between *H3.3* G4V/L/R mutation and clinicopathological parameters of 217 giant cell tumours of the bone with *H3F3A* mutation.

Variable	G34W	G34L/V/R	P-value
Location			0.055
LGCTB	162	10	
NGCTB	38	7	
Small bones			0.012
No	191	13	
Yes	9	4	
Irregular bone			
No	171	14	0.722
Yes	29	3	
Soft tissue involvement			
No	104	4	0.205
Yes	70	7	
Undetected	32		
Recurrence			
No	123	15	0.024
Yes	43	0	
Missing(n = 36)	34	2	
Size	5.58 ± 2.29	5.21 ± 2.09	0.190
Missing(n = 27)			
Age	37.88 ± 13.52	30.71 ± 10.52	0.009
Gender			
Male	104	9	1.00
Female	96	8	

LGCTB is the abbreviation of GCTB of long bone, and NGCTB is the abbreviation of GCTB of non-long bone.

6.6. H3F3A mutation and its immunohistochemical features in malignant GCTB and other giant cell-rich tumors in non-long bones

Nineteen of the 25 cases of MGCTB (76 %) harboured the *H3.3* G34 mutation, and the rate of *H3.3* G34 mutation was significantly higher in the GCTB groups of long bones than in the malignant GCTB (P = 0.001). In the primary MGCTB with *H3.3* G34W mutation, two components were observed histologically including GCTB component with mild atypia and corresponding osteosarcoma component with severe atypia in the above case of the distal femur (Fig. 3A, B). Positive immunoreaction with *H3.3* G34W was detected in the GCTB component but not the osteosarcoma component (Fig. 3C). No immunoreaction with H3.3 G34W was found in chondroblastoma with osteoclast-like giant cells (Fig. 3D-F) and giant cell reparative granuloma of small bones (Fig. 3G-I). Fibrous stromal cells of giant cell repair granuloma are usually spindle shaped (Fig. 3H). Osteosarcoma, chondroblastoma, sABC and non-ossifying fibroma were also negative expression for H3.3 G34.

7. Discussion

Recent studies have reported *H3F3A* mutation in GCTB within the range of 69–96 % [2,7–9]. GCTB often occurs in long bones, while extremely rare in non-long bones [2–6]. Importantly, GCTB of non-long bones were more common in women than in men, and in young patients in our study. Secondly, GCTB with soft tissue extension in irregular bones were more than in small bones. Then, *H3.3* G34L/V/R mutation rate was higher in small bones than in long bones. Lastly, patients with *H3.3* G34V/L mutations had no recurrences.

GCTB are sporadic and rare in non-long bones [2–4]. In our analysis, patients with GCTB harboured *H3F3A* mutation, which is a useful diagnostic tool for GCTB in long bones and non-long appendicular bones similar with previous reports [10,22]. We confirm that *H3F3A* mutation may contribute to the differential diagnosis of GCTB of non-long appendicular bones. Positive nuclear immunoreactivity was observed in 213/235 GCTB patients in the previous study [2]. However, the *H3.3* G34W mutation rate of GCTB in our research was lower than the previous reports, which was probably due to the different tissue processing and decalcification strategy [12,23].

Only a slight female predominance in conventional GCTB has been reported in the literature [6] and in paediatric lesions [24–27]. However, regional differences in the sex distribution of GCTB patients are observed in our study. A slight male predominance in GCTB of the long bones was found in our study similarly with a large series of studies of GCTB patients from China [28,29]. Interestingly, a female preponderance among GCTB in the non-long bones was more likely to occur (P = 0.023) in our series, while the reason was still unclear. The predominance of women was also confirmed in paediatric lesions [30]. The younger patients with GCTB may harboured *H3.3* G34L/V/R mutations in our study. Some studies have found *H3.3* G 34L/V/R mutations in small bones, and 4/235 cases of *H3.3* G34L/V/R mutations in GCTB have been reported [2]. Four of 51 primary GCTB with G34V/R mutations and eight of 180 cases with *H3.3* G34L/V/R mutations have also been reported in the literatures [3,4]. In this study, the mutation rate of *H3.3* G34L/V/R in non-long bones, especially in small bones, was higher than that in the classic locations, which was consistent with previous studies. One GCTB with *H3.3* G34M mutation was reported in the literatures [2,12]. However, no *H3.3* G34M mutation was determined in our study, which is in accordance with a previous Chinese report [4]. These findings indicate that there are regional differences in the

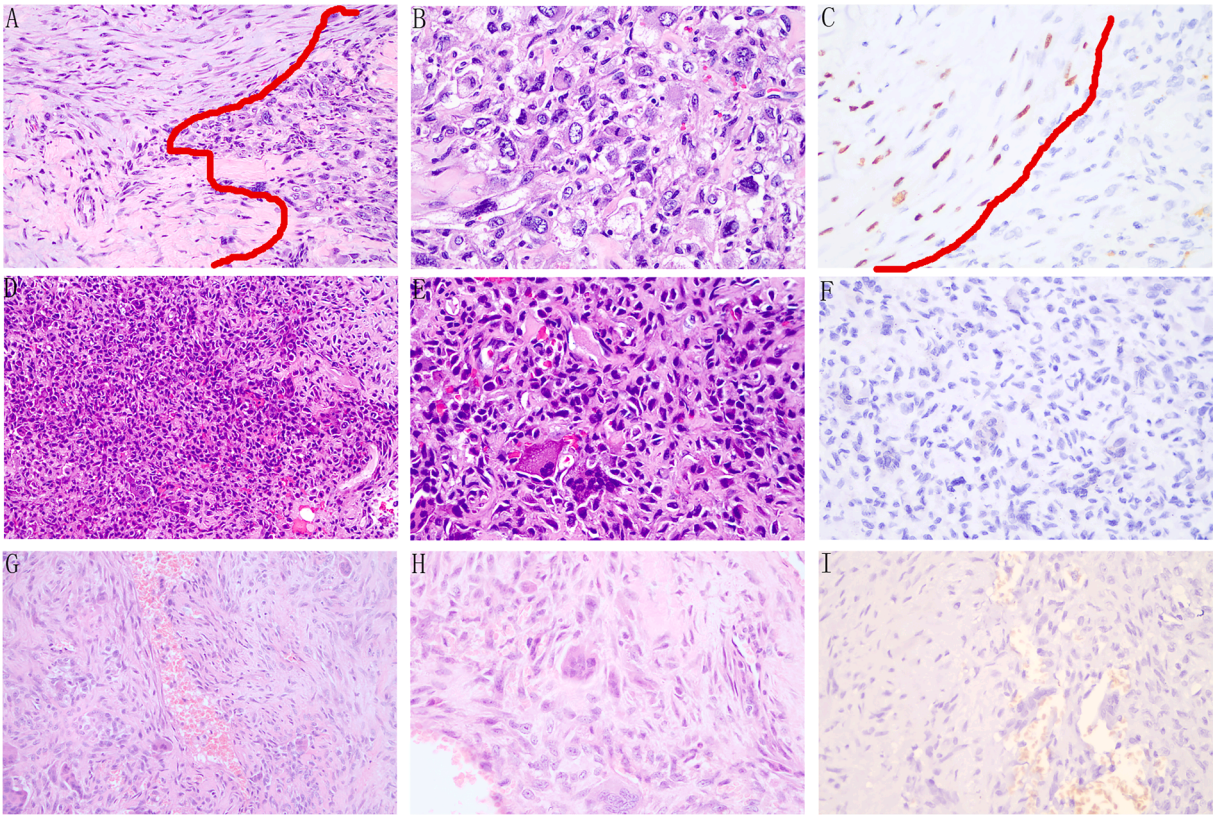

Fig. 3. Histological and immunohistochemical features of H3.3 G34W in primary malignant giant cell tumours of bone and other giant cell-rich lesions of small bone. A-C show the histological and immunohistochemical features of H3.3 G34W in primary MGCTB of bone. Giant cell tumour of bone (left part of A and C) with osteosarcoma transformation (right part of A-C, B) were observed in the primary MGCTB in the distal femur (A-C). Positive immunoreaction with H3.3 G34W was examined in the GCTB component (left part of C), but not the osteosarcoma transformation component (right part of C). Giant cell reparative granuloma of small bones with a lot of giant cells around the hemorrhagic focus (G, H) showed negative expression for H3.3G34W (I) (original magnification A, D G × 200; B, C, E, F, H, I × 400).

mutation variants of *H3.3* G34 in GCTB.

The local recurrence rate for GCTB of the pelvis and sacrum is higher than that for GCTB in other location in the present study [31]. However, Balke *et al.* reported that the recurrence rate of GCTB in the pelvis was very low (1/20) [15]. No significant differences were observed in the recurrence rates of GCTB in different locations. In our study, tumors with soft tissue extensions were mostly found in irregular bones but not in small bones. However, we did not find a significant relationship between soft tissue involvement and GCTB recurrence in non-long bones, which is different from those reports from western countries [25–28]. There are two possible reasons for this finding. First, the surgical procedure is an important factor that affects local GCTB recurrence [8,15,29]. Second, our study focused on GCTB in non-long bones but not the conventional location of GCTB. We propose that the *H3.3* G34V/L/R mutation may be negatively correlated with GCTB recurrence. However, *H3.3* G34L/V/R mutations are very rare, and no statistical significance was found in our study by multivariate analysis due to case number limitation.

H3.3 G34V and G34R mutations differentially affect H3K36 modifications, subtelomeric silencing, genomic stability, sensitivity to irradiation, and hydroxyurea and influence DNA repair [13]. *H3.3* G34R, but not *H3.3* G34V, induces dominant hydroxyurea sensitivity, homologous recombination defects, and dominant subtelomeric silencing in their analysis [14]. In this study, eleven cases of GCTB harboured *H3.3* G34V mutation, and only three cases had *H3.3* G34R mutation. One of three cases was secondary MGCTB. Amary *et al.* reported that 2 of 11 cases of MGCTB harboured *H3.3* G34R mutation. Therefore, we speculate that *H3.3* G34R may be associated with the malignant transformation of GCTB. Moreover, we found that *H3.3* G34W mutation is less in MGCTB than in conventional GCTB. Our results are consistent with these data in the literature [32]. Yoshida *et al.* found that two of seven MGCTB cases harboured the *H3.3* G34W mutation, but this mutation was absent in the sarcomatous components of the remaining five cases. The decrease of the H3F3A gene mutation rate due to the deletion of *H.3.3* gene was confirmed by FISH analysis [32].

GCTB in non-long bones display a female preponderance. *H3F3A* mutation is a useful diagnostic marker for GCTB in unusual locations, such as small and irregular bones. The younger patients with GCTB may harbour H3.3 G34L/V/R mutations. The small number of GCTB patients in non-long bones is one of the limitations of this study because of the rarity, a multi-institutional study should be performed in the future.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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