



Original contribution

# Is immunohistochemical staining for $\beta$ -catenin the definitive pathological diagnostic tool for desmoid-type fibromatosis? A multi-institutional study<sup>☆,☆☆</sup>



Hiroshi Koike MD<sup>a</sup>, Yoshihiro Nishida MD, PhD<sup>b,\*</sup>, Kei Kohno MD, PhD<sup>c</sup>,  
Yoshie Shimoyama MD, PhD<sup>c</sup>, Toru Motoi MD, PhD<sup>d</sup>, Shunsuke Hamada MD, PhD<sup>e</sup>,  
Akira Kawai MD, PhD<sup>f</sup>, Akira Ogose MD, PhD<sup>g</sup>, Toshifumi Ozaki MD, PhD<sup>h</sup>,  
Toshiyuki Kunisada MD, PhD<sup>i</sup>, Yoshihiro Matsumoto MD, PhD<sup>j</sup>,  
Tomoya Matsunobu MD, PhD<sup>k</sup>, Keisuke Ae MD, PhD<sup>l</sup>, Tabu Gokita MD, PhD<sup>m</sup>,  
Tomohisa Sakai MD, PhD<sup>a</sup>, Koki Shimizu MD<sup>a</sup>, Naoki Ishiguro MD, PhD<sup>a</sup>

<sup>a</sup>Department of Orthopedic Surgery, Nagoya University Graduate School of Medicine, Nagoya, Aichi 466-8550, Japan

<sup>b</sup>Department of Rehabilitation, Nagoya University Hospital, Nagoya, Aichi 466-8550, Japan

<sup>c</sup>Department of Pathology and Laboratory Medicine, Nagoya University Hospital, Nagoya, Aichi 466-8550, Japan

<sup>d</sup>Department of Pathology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo 113-8677, Japan

<sup>e</sup>Department of Orthopedic Surgery, Aichi Cancer Center Hospital, Nagoya 464-8681, Japan

<sup>f</sup>Department of Musculoskeletal Oncology, National Cancer Center Hospital, Tokyo 104-0045, Japan

<sup>g</sup>Department of Orthopedic Surgery, Uonuma Institute of Community Medicine, Niigata University Medical and Dental Hospital, Niigata 949-7302, Japan

<sup>h</sup>Department of Orthopedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama 700-8558, Japan

<sup>i</sup>Department of Medical Materials for Musculoskeletal Reconstruction, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama 700-8558, Japan

<sup>j</sup>Department of Orthopedic Surgery, Kyushu University, Fukuoka 812-8582, Japan

<sup>k</sup>Department of Orthopedic Surgery, Kyushu Rosai Hospital, Fukuoka 800-0296, Japan

<sup>l</sup>Department of Orthopedic Surgery, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo 135-8550, Japan

<sup>m</sup>Department of Orthopedic Surgery, Saitama Cancer Center, Saitama 362-0806, Japan

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\* Corresponding author.

E-mail address: ynishida@med.nagoya-u.ac.jp (Y. Nishida).

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**Summary** Immunohistochemical staining with anti- $\beta$ -catenin antibody has been applied as a diagnostic tool for desmoid-type fibromatoses (DFs). In recent years, specific gene mutation (*CTNNB1*) analysis has also been reported to be useful for diagnosis of DF; however, the association between *CTNNB1* mutation status and immunohistochemical staining pattern of  $\beta$ -catenin is rarely reported. The purposes of this study are to clarify the relationship of the staining pattern of  $\beta$ -catenin with the *CTNNB1* mutation status and various clinical variables, and to investigate the significance of immunohistochemical staining of  $\beta$ -catenin in cases diagnosed as DF. Between 1997 and 2017, 104 cases diagnosed as DF from 6 institutions in Japan were enrolled in this study: Nagoya University, National Cancer Center Hospital, Niigata University, Okayama University, Kyushu University, and Cancer Institute Hospital. For all cases, immunohistochemical staining of  $\beta$ -catenin and gene mutation analysis of *CTNNB1* were performed. Of 104 cases, 87 (84%) showed nuclear staining of  $\beta$ -catenin, and 95 (91%) showed positive staining in the cytoplasm. The proportion of cases showing strong nuclear staining of  $\beta$ -catenin was significantly higher in the cases with S45F than in those with T41A or wild type. The proportion of cases stained strongly in the cytoplasm rather than in the nucleus was significantly higher in the group of T41A than that of S45F or wild type. Among 17 cases in which nuclear immunostaining was absent, *CTNNB1* mutation was observed in 5 cases (29.4%). There were unignorable cases of DF with negative  $\beta$ -catenin immunostaining despite a definitive clinical and pathological diagnosis of DF and/or positive *CTNNB1* mutation.

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## 1. Introduction

Desmoid-type fibromatoses (DFs) are soft tissue tumors with clonal origin and mesenchymal fibroblastic/myofibroblastic proliferation, with an incidence of 2 to 4 cases per million persons per year [1]. Most cases develop sporadically in adults, whereas a few occur against a family background of familial adenomatous polyposis [2]. DFs are generally highly invasive and locally aggressive, but do not metastasize [3,4], and are categorized as intermediate tumors in the World Health Organization classification [5].

Somatic mutations at exon 3 of *CTNNB1* gene have been reported as a molecular pathogenesis of sporadic DF. The mutation generally occurs at codon 41 or 45. These mutations inhibit phosphorylation of  $\beta$ -catenin, which protect  $\beta$ -catenin from degradation by adenomatous polyposis coli complex, resulting in nuclear accumulation of  $\beta$ -catenin and activation of the T-cell factor/lymphoid enhancer factor pathway [6].

A recent study showed that *CTNNB1* mutations were highly prevalent and detected in 88% of sporadic DFs in contrast to no hotspot *CTNNB1* mutations in any of the spindle cell nondesmoid lesions [7]. The hotspot mutation of *CTNNB1* is thought to be very specific for DF. Recent studies exhibited that tumors with *CTNNB1* S45F mutation were reported to exhibit resistance to surgical and conservative treatment [8,9], suggesting that *CTNNB1* mutation analysis might be important not only for the diagnosis of DF but also for determining the subsequent treatment options. However, *CTNNB1* mutation analysis is performed only in limited facilities in Japan and has not yet been generally applied for clinical use as a diagnostic tool, whereas immunohistochemical staining of  $\beta$ -catenin has been considered useful for the diagnosis of DF [6,10-16]. These studies investigated the significance of immunohistochemical staining of  $\beta$ -catenin or *CTNNB1* mutation status independently, and

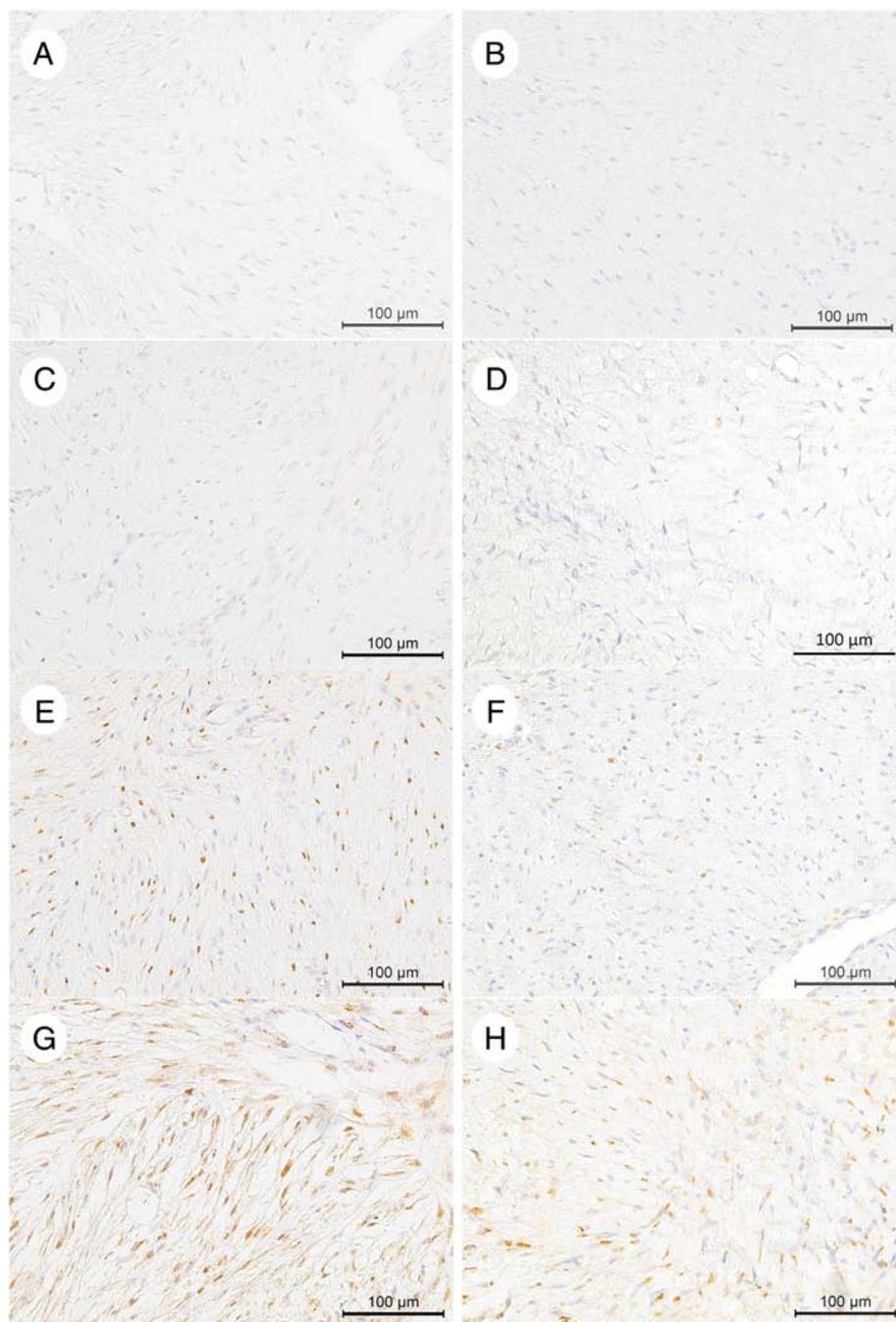
few reports demonstrated the correlation between immunohistochemical staining pattern of  $\beta$ -catenin and *CTNNB1* mutation status.

In the present study, we aimed to clarify the association between staining pattern of  $\beta$ -catenin, patient characteristics, and *CTNNB1* mutation status and assess the significance of immunohistochemical staining for nuclear or cytoplasmic  $\beta$ -catenin in 104 sporadic DF cases.

## 2. Materials and methods

### 2.1. Tissue samples

This study has been performed according to the Declaration of Helsinki, and ethical approval from the appropriate committees of Nagoya University was obtained for this study. Formalin-fixed, paraffin-embedded specimens accumulated between 1994 and 2017 were retrieved from 6 institutions: Nagoya University, National Cancer Center Hospital, Niigata University, Okayama University, Kyushu University, and Cancer Institute Hospital. There were 113 cases diagnosed as sporadic DF. To confirm the histologic diagnosis of DF, sections of all cases were transmitted to the Pathology Laboratory at Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital for central pathology review (T. M.). Based on the evaluation of central review and careful discussion between the pathologist and physicians, 9 cases were excluded. No cases with an obvious history of familial adenomatous polyposis were included. Finally, this study was composed of 104 cases of DF. Specimens obtained by biopsy or surgical excision were formalin fixed and paraffin embedded, and subjected to examination of



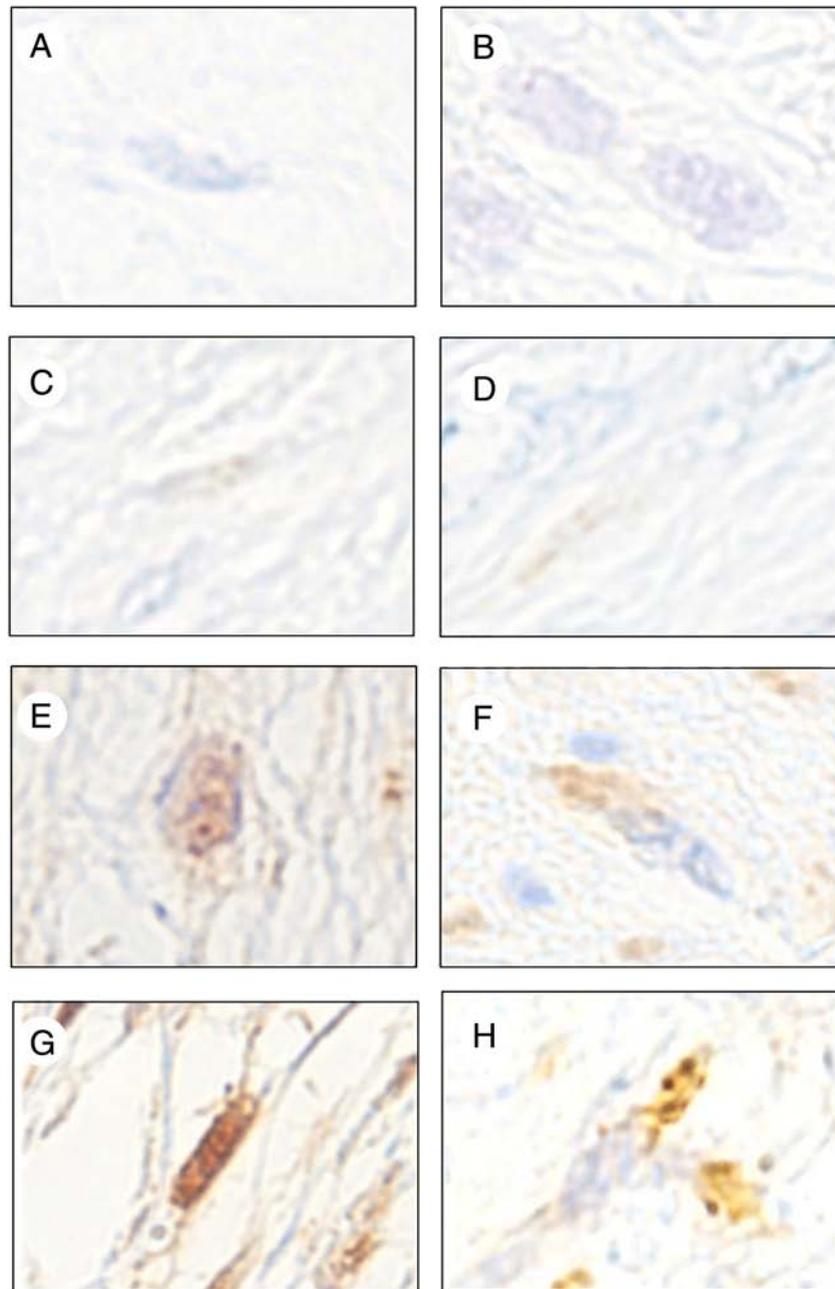
**Fig. 1** Representative images of immunohistochemical staining for  $\beta$ -catenin (original magnification  $\times 200$ ). Differential staining positivity of the nucleus and cytoplasm. Staining positivity: absent in the nucleus (A) and cytoplasm (B), weak in the nucleus (C) and cytoplasm (D), moderate in the nucleus (E) and cytoplasm (F), and strong in the nucleus (G) and cytoplasm (H).

immunohistochemistry for  $\beta$ -catenin and *CTNNB1* mutation analysis. The following clinical data were collected: age at the diagnosis, gender, tumor location, and tumor size.

## 2.2. Mutation analysis

DNA was extracted from frozen tissue or formalin-fixed, paraffin-embedded tissue with the High Pure PCR Template

Preparation Kit (Roche Molecular Diagnostics, Mannheim, Germany) according to the manufacturer's protocol. Extracted DNA was amplified by polymerase chain reaction using LightCycler 480 System (Roche). To analyze the point mutations in codon 41 or 45 of *CTNNB1* exon 3, we designed 2 pairs of primers: forward 59-GATTTGATGGAGTTGGA CATGG-39, reverse 59-TCTTCCTCAGGATTGCCTT-39, and forward 59-TGGAACCAGACAGAAAAGCG-39, reverse



**Fig. 2** Representative images of  $\times 400$  magnification. Staining intensity: absent in the nucleus (A) and cytoplasm (B), weak in the nucleus (C) and cytoplasm (D), moderate in the nucleus (E) and cytoplasm (F), and strong in the nucleus (G) and cytoplasm (H).

59-TCAGGATTGCCTTTACCACTC-39. Polymerase chain reaction products were isolated by gel electrophoresis, and amplified bands were extracted and purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA). Purified products were subjected to direct sequencing using the above primers (forward), with Applied Biosystems Big Dye Terminator V3.1 and Applied Biosystems 3730x DNA analyzer (Applied Biosystems, Foster City, CA) at FASMAC (Kanagawa, Japan). All sequencing results were compared with those in the databases of National Center for Biotechnology Information BLAST to confirm the mutation sites.

### 2.3. Immunohistochemistry

All tumor samples were obtained by needle or incisional biopsy. Biopsy specimens were fixed in 10% formaldehyde and embedded in paraffin. Immunohistochemistry was performed using a Ventana BenchMark XT automated slide staining system (Roche). For antigen retrieval, slides were heated in an oven for 60 minutes. Endogenous peroxidase was blocked with the appropriate kit. Primary antibody against  $\beta$ -catenin (dilution 1:50; Dako, Carpinteria, CA, US mouse monoclonal antibody, M3539) was then applied for 32 minutes at 37°C in

**Table 1** Patient characteristics

| Variables             |             |
|-----------------------|-------------|
| Age, mean (y)         | 42.3 (8–80) |
| Sex (%)               |             |
| Male                  | 32 (30%)    |
| Female                | 72 (70%)    |
| Tumor size (cm), mean | 7.9 (2–20)  |
| Tumor location (%)    |             |
| Abdominal wall        | 18 (17%)    |
| Extremity             | 28 (27%)    |
| Head and neck         | 12 (12%)    |
| Trunk                 | 46 (44%)    |

BenchMark XT stainer and revealed with the 3,3'-diaminobenzidine detection kit, yielding a brown reaction product. Nuclear or cytoplasmic positivity of  $\beta$ -catenin was evaluated by 2 independent observers (S. H., H. K.) without any knowledge of the clinicopathological information under the guidance of the experienced pathologists (Y. S. and K. K.), and the stainability was also validated by them. Staining positivity was divided into 4 groups; absent (0%), weak (1%-9%), moderate (10%-50%), or strong (51%-100%) by the numbers of positively stained cells (Fig. 1). Images of higher magnification are shown in Fig. 2.

## 2.4. Statistical analysis

Data are summarized by frequency and percentages for categorical variables and by mean and range for continuous variables. Differences between groups were assessed using  $\chi^2$  test for categorical variables. Statistical analyses were

**Table 2** Patient characteristics vs *CTNNB1* mutation status

| Variables       | <i>CTNNB1</i> mutation status |                  |                | <i>P</i> <sup>a</sup> |
|-----------------|-------------------------------|------------------|----------------|-----------------------|
|                 | T41A<br>(n = 43)              | S45F<br>(n = 10) | WT<br>(n = 51) |                       |
| Age (y)         |                               |                  |                | .84                   |
| <40             | 22 (46%)                      | 4 (8%)           | 22 (46%)       |                       |
| $\geq$ 40       | 21 (37%)                      | 6 (11%)          | 29 (52%)       |                       |
| Sex             |                               |                  |                | .58                   |
| Male            | 13 (41%)                      | 5 (16%)          | 14 (43%)       |                       |
| Female          | 30 (42%)                      | 5 (7%)           | 37 (51%)       |                       |
| Tumor size (cm) |                               |                  |                | .29                   |
| <8              | 25 (42%)                      | 3 (5%)           | 32 (53%)       |                       |
| $\geq$ 8        | 18 (41%)                      | 7 (16%)          | 19 (43%)       |                       |
| Tumor location  |                               |                  |                | .88                   |
| Abdominal wall  | 7 (39%)                       | 1 (6%)           | 10 (55%)       |                       |
| Extremity       | 12 (43%)                      | 1 (4%)           | 15 (53%)       |                       |
| Head and neck   | 4 (33%)                       | 3 (25%)          | 5 (42%)        |                       |
| Trunk           | 20 (44%)                      | 5 (11%)          | 21 (45%)       |                       |

<sup>a</sup>  $\chi^2$  Test.

**Table 3** Patient characteristics vs nuclear staining of  $\beta$ -catenin

| Variables       | Positivity         |                  |                      |                    | <i>P</i> <sup>a</sup> |
|-----------------|--------------------|------------------|----------------------|--------------------|-----------------------|
|                 | Absent<br>(n = 17) | Weak<br>(n = 16) | Moderate<br>(n = 47) | Strong<br>(n = 24) |                       |
| Age (y)         |                    |                  |                      |                    | .92                   |
| <40             | 6 (12%)            | 8 (17%)          | 22 (46%)             | 12 (25%)           |                       |
| $\geq$ 40       | 11 (20%)           | 8 (14%)          | 25 (45%)             | 12 (21%)           |                       |
| Sex             |                    |                  |                      |                    | .83                   |
| Male            | 7 (22%)            | 5 (16%)          | 12 (37%)             | 8 (25%)            |                       |
| Female          | 10 (14%)           | 11 (15%)         | 35 (49%)             | 16 (22%)           |                       |
| Tumor size (cm) |                    |                  |                      |                    | .85                   |
| <8              | 11 (18%)           | 10 (17%)         | 24 (40%)             | 15 (25%)           |                       |
| $\geq$ 8        | 6 (14%)            | 6 (14%)          | 23 (52%)             | 9 (20%)            |                       |
| Tumor location  |                    |                  |                      |                    | .79                   |
| Abdominal wall  | 2 (11%)            | 1 (5%)           | 10 (56%)             | 5 (28%)            |                       |
| Extremity       | 3 (11%)            | 4 (14%)          | 16 (57%)             | 5 (18%)            |                       |
| Head and neck   | 1 (9%)             | 4 (33%)          | 4 (33%)              | 3 (25%)            |                       |
| Trunk           | 11 (24%)           | 7 (15%)          | 17 (37%)             | 11 (24%)           |                       |

<sup>a</sup>  $\chi^2$  Test.

performed using SPSS ver.24 (SPSS, Chicago, IL), and *P* < .05 was considered statistically significant.

## 3. Results

### 3.1. Patient characteristics

The mean age at the time of diagnosis was 42.3 years (range, 8-80 years). There were 32 male and 72 female pa-

**Table 4** Patient characteristics vs cytoplasmic staining of  $\beta$ -catenin

| Variables       | Positivity        |                 |                      |                    | <i>P</i> <sup>a</sup> |
|-----------------|-------------------|-----------------|----------------------|--------------------|-----------------------|
|                 | Absent<br>(n = 9) | Weak<br>(n = 6) | Moderate<br>(n = 25) | Strong<br>(n = 64) |                       |
| Age (y)         |                   |                 |                      |                    | .79                   |
| <40             | 6 (12%)           | 2 (4%)          | 10 (21%)             | 30 (63%)           |                       |
| $\geq$ 40       | 3 (5%)            | 4 (7%)          | 15 (27%)             | 34 (61%)           |                       |
| Sex             |                   |                 |                      |                    | .58                   |
| Male            | 2 (6%)            | 2 (6%)          | 11 (35%)             | 17 (53%)           |                       |
| Female          | 7 (10%)           | 4 (6%)          | 14 (19%)             | 47 (65%)           |                       |
| Tumor size (cm) |                   |                 |                      |                    | .53                   |
| <8              | 6 (10%)           | 5 (8%)          | 11 (19%)             | 38 (63%)           |                       |
| $\geq$ 8        | 3 (7%)            | 1 (2%)          | 14 (32%)             | 26 (59%)           |                       |
| Tumor location  |                   |                 |                      |                    | .87                   |
| Abdominal wall  | 2 (11%)           | 0 (0%)          | 3 (17%)              | 13 (72%)           |                       |
| Extremity       | 2 (7%)            | 0 (0%)          | 10 (36%)             | 16 (57%)           |                       |
| Head and neck   | 0 (0%)            | 1 (8%)          | 3 (25%)              | 8 (67%)            |                       |
| Trunk           | 5 (11%)           | 5 (11%)         | 9 (19%)              | 27 (59%)           |                       |

<sup>a</sup>  $\chi^2$  Test.

**Table 5** *CTNNB1* mutation status and strong staining of  $\beta$ -catenin

| $\beta$ -Catenin positivity | <i>CTNNB1</i> mutation status |                  |                | <i>P</i> <sup>a</sup> |
|-----------------------------|-------------------------------|------------------|----------------|-----------------------|
|                             | T41A<br>(n = 43)              | S45F<br>(n = 10) | WT<br>(n = 51) |                       |
| Nuclear                     |                               |                  |                | <.01                  |
| Strong                      | 5                             | 8                | 11             |                       |
| Absent, weak, moderate      | 38                            | 2                | 40             |                       |
| Cytoplasmic                 |                               |                  |                | <.01                  |
| Strong                      | 34                            | 10               | 20             |                       |
| Absent, weak, moderate      | 9                             | 0                | 31             |                       |

<sup>a</sup>  $\chi^2$  Test.

tients. The location of tumors was the abdominal wall in 18 cases, an extremity in 28 cases, head and neck in 12 cases, and trunk in 46 cases. Shoulder and pelvic girdles were defined as trunk region in this study. The mean tumor size was 7.9 cm (range, 2-20 cm; Table 1).

### 3.2. Mutation status of *CTNNB1*

We performed genotyping of *CTNNB1* exon 3 for all the cases using tumor specimens and 5 cases of non-DF soft tissue tumors as a control group (Supplementary Table S1). Replacement of threonine by alanine in codon 41 (T41A) was detected in 43 (41%) of the 104 cases. Replacement of serine by phenylalanine in codon 45 (S45F) was identified in 10 (10%) of the 104 cases. Fifty-one cases (49%) of the 104 did not have mutations in exon 3 (wild type [WT]). There were no positive cases of hotspot mutation of *CTNNB1* in the control group, which was consistent with the previous report investigating 260 DFs and 191 DF mimics that hotspot mutation of *CTNNB1* was specific for diagnosis of DF [7]. There were no significant correlations between mutation status of *CTNNB1* and age ( $P = .84$ ), sex ( $P = .58$ ), tumor size ( $P = .29$ ), or tumor location ( $P = .88$ ; Table 2).

### 3.3. Immunohistochemical findings

Of the 104 cases evaluated, nuclear positivity of  $\beta$ -catenin staining was absent in 17, weak in 16, moderate in 47, and strong in 24, and cytoplasmic positivity was absent in 9, weak

**Table 6** *CTNNB1* mutation status and intracellular predominance of  $\beta$ -catenin staining

| Intracellular predominance | <i>CTNNB1</i> mutation status |                  |                | <i>P</i> <sup>a</sup> |
|----------------------------|-------------------------------|------------------|----------------|-----------------------|
|                            | T41A<br>(n = 43)              | S45F<br>(n = 10) | WT<br>(n = 51) |                       |
| Cytoplasmic                | 31                            | 2                | 17             | <.01                  |
| Other                      | 12                            | 8                | 34             |                       |

<sup>a</sup>  $\chi^2$  Test.**Table 7** *CTNNB1* mutation status and stainability of  $\beta$ -catenin in 2 different antibodies

| Nuclear $\beta$ -catenin positivity | <i>CTNNB1</i> mutation status |                 |                | <i>P</i> <sup>a</sup> |
|-------------------------------------|-------------------------------|-----------------|----------------|-----------------------|
|                                     | T41A<br>(n = 42)              | S45F<br>(n = 9) | WT<br>(n = 44) |                       |
| Dako antibody                       |                               |                 |                | <.05                  |
| Strong                              | 5                             | 7               | 8              |                       |
| Moderate                            | 23                            | 2               | 21             |                       |
| Weak                                | 9                             | 0               | 5              |                       |
| Absent                              | 5                             | 0               | 10             |                       |
| Novocastra antibody                 |                               |                 |                | .06                   |
| Strong                              | 2                             | 3               | 5              |                       |
| Moderate                            | 17                            | 2               | 6              |                       |
| Weak                                | 14                            | 3               | 13             |                       |
| Absent                              | 9                             | 1               | 20             |                       |

<sup>a</sup>  $\chi^2$  Test.

in 6, moderate in 25, and strong in 64. The ratio of the cases with nuclear stainability equal to or more than weak was 84%, and the percentage of the cases with cytoplasmic stainability equal to or more than weak was 91%. No significant correlations were found between nuclear staining positivity and patient characteristics (Table 3), or cytoplasmic staining positivity (Table 4). Next, the relationship between staining positivity of  $\beta$ -catenin and *CTNNB1* mutation status was evaluated. There were no significant correlations between mutation statuses of *CTNNB1* and nuclear or cytoplasmic staining positivity dividing the positivity into strong/moderate/weak and absent groups (nuclear,  $P = .24$ ; cytoplasmic,  $P = .91$ ). However, dividing the positivity into strong and moderate/weak/absent groups, strong nuclear staining was significantly higher in cases with S45F than in cases with T41A or WT ( $P < .01$ ; Table 5). The proportion of the cases with strong cytoplasmic staining was significantly higher in cases with T41A and S45F than in those with WT ( $P < .01$ ; Table 5). Next, we evaluated whether stainability was more intense in the nucleus or cytoplasm. The proportion of cases with staining predominant in the cytoplasm was significantly higher in cases with T41A mutation than in those with S45F or WT ( $P < .01$ ; Table 6).

Among 17 cases in which nuclear immunostaining were absent, hotspot *CTNNB1* mutation was observed in 5 cases (29.4%). On the other hand, among 51 cases negative for *CTNNB1* mutation, 39 cases (76%) showed positive nuclear immunostaining. Eighty-seven cases were nuclear positive for  $\beta$ -catenin, and among them, *CTNNB1* hotspot mutation was negative in 39 cases. Because positivity often differs according to the antibodies used for immunohistochemistry, we additionally evaluated the nuclear  $\beta$ -catenin stainability using a different antibody (dilution 1:50; Novocastra, Newcastle upon Tyne, UK mouse monoclonal antibody, NCL-B-CAT). Ninety five cases were subjected to immunohistochemical staining using both antibodies. Thirty cases (32%) showed negative nuclear staining for  $\beta$ -catenin using Novocastra antibody, and among these cases, *CTNNB1* mutation was

observed in 10 cases (33%; Table 7). Identical results were obtained with this different antibody (Novocastra) to those with Dako antibody, including the relationship between staining pattern and *CTNNB1* mutation status (Supplementary Tables S2-S5).

#### 4. Discussion

The results of this study revealed that not a few cases (16%) showed negative nuclear staining of  $\beta$ -catenin with a clinical and pathological diagnosis of DF. Moreover, among these cases, 29.4% had positive *CTNNB1* mutation. *CTNNB1* mutation has been reported to be highly prevalent in sporadic DF [7,8,12,17]. Le Guellec et al [7] identified the common *CTNNB1* mutations associated with sporadic DF by direct sequencing in 260 cases of typical DF and in 191 cases of spindle cell lesions, which can morphologically “mimic” DF. They reported that *CTNNB1* mutations were observed in 88% of sporadic DF but not in any other lesions. Amary et al [12] also analyzed 133 cases including 76 DFs and 18 superficial fibromatoses, in addition to a further 39 fibromatosis mimics. They reported that mutations were detected in 87% of 76 DFs but in none of the other lesions studied. Several reports investigated exon 3 *CTNNB1* mutations in carcinoma [18-25]. The same hotspot mutations as DF were observed in 6% of these carcinomas [7]. However, from a morphologic point of view, differentiating histologically such neoplasms and carcinomas from DF is not diagnostically problematic. Occasionally, spindle cell tumors and sarcomatous lesions need to be differentiated from DF. As reported by Le Guellec et al and Amary et al, no *CTNNB1* mutations were observed in these suspiciously similar tumors, suggesting that *CTNNB1* mutation analysis is specific and useful for the diagnosis of DF. In the Japanese clinical setting, *CTNNB1* mutation analyses are not performed in most institutions, including even specialist soft tissue tumor centers. Some of the institutions send specimens of cases with DF suspected to our institution (Nagoya University) to conduct *CTNNB1* mutation analysis in the present status. Considering that large pathology laboratories have already performed *CTNNB1* mutation analysis in the United States and European countries, centralized system for the analysis should be established.

There is also evidence that immunohistochemical detection of  $\beta$ -catenin can be helpful in the diagnosis of DF [6,10-16]. In these studies, in the cases diagnosed as DF, the positive rate of immunohistochemical staining of  $\beta$ -catenin was reported as 82% to 100%, which was consistent with the result of our study. Based on these results, immunohistochemistry is considered to show relatively high sensitivity for the diagnosis of DF. On the other hand, several spindle cell tumors other than DF exhibited positive staining for  $\beta$ -catenin. Ng et al [10] reported that a high level of positive nuclear  $\beta$ -catenin staining (>25% of cells) was observed in tumors including solitary fibrous tumor (6/15 cases), endometrial stromal sarcoma

(4/10 cases), synovial sarcoma (16/58 cases), fibrosarcoma (1/3 cases), and clear cell sarcoma (1.3 cases). Amary et al [12] reported that 72% of the fibromatosis mimics showed nuclear positivity for  $\beta$ -catenin. Several other studies also described that immunohistochemical detection of nuclear  $\beta$ -catenin was not a reliable test for distinguishing DF from benign or malignant fibroblastic lesions [7,11,12,26,27]. These reports indicate that immunohistochemistry for  $\beta$ -catenin is not a specific test for DF. In addition, considering that stainability for  $\beta$ -catenin is dependent on the antibody used and that the protocols of immunohistochemical staining differed in individual studies, the results of the immunohistochemical staining cannot be simply adopted for diagnosis. In the present study, nuclear accumulation of  $\beta$ -catenin was observed in 82% (Dako) of the cases, but only 67% of them showed positive  $\beta$ -catenin expression in the nucleus using the different anti- $\beta$ -catenin antibody (Novocastra). Furthermore, the presence of cases with negative nuclear  $\beta$ -catenin even in the cases with positive *CTNNB1* mutation suggested that immunohistochemical staining of  $\beta$ -catenin might be a less sensitive test for the diagnosis of DF than *CTNNB1* mutation analysis. Although immunohistochemical staining of  $\beta$ -catenin cannot be simply adopted for the diagnosis of DF, it would be helpful combined with characteristic findings of clinical features, images of magnetic resonance, and general microscopic findings.

Few reports have evaluated the immunohistochemical staining of  $\beta$ -catenin in the cytoplasm of DF. Andino et al [28] reported that all of 4 cases of pleuropulmonary DF showed strong nuclear and cytoplasmic staining. Bhattacharya et al [11] reported that all 21 cases showed focal to diffuse cytoplasmic staining. Ng et al [10] also reported that cytoplasmic  $\beta$ -catenin staining was seen commonly in 17 cases of DF. Similarly, Abraham et al [13] described that almost all of 33 cases (97%) of fibromatoses of the breast showed prominent cytoplasmic  $\beta$ -catenin staining. In the report of Andino et al, the cytoplasmic staining property was evaluated in 4 cases, with all of them showing positive results in the cytoplasm. In this study, the cytoplasmic staining was evaluated in 4 grades as well as the nucleus in all 104 cases, and we showed that the percentage of cases with strong cytoplasmic staining was significantly higher in T41A or S45F than in WT. The intracellular staining intensity was compared between the nucleus and the cytoplasm, indicating that a significantly higher number of cases in T41A showed stronger cytoplasmic staining than that of the nucleus in T41A than compared with those in S45F or WT.

In normal fibroblasts,  $\beta$ -catenin staining is limited to the cytoplasm and/or cell membranes. However, in DF, the Wnt signaling pathway is activated or the  $\beta$ -catenin degradation pathway is inactivated, leading to  $\beta$ -catenin accumulation in the cytoplasm and/or nucleus. In this study, the staining pattern of the nucleus and cytoplasm differed between *CTNNB1* mutation statuses. Phosphorylation of S45 is thought to be the initial process in the degradation of  $\beta$ -catenin, suggesting that S45F mutation may block the first step of phosphorylation, thereby precluding subsequent phosphorylation [29]. Extent of phosphorylation may vary among cases depending on the specific

codon in which mutation occurs. We speculate that this difference in phosphorylation may cause the differential staining pattern of  $\beta$ -catenin seen among cases.

There are few reports on the relationship between *CTNNB1* exon 3 mutation status and  $\beta$ -catenin staining property in sporadic DF. Amarty et al [12] reported that all 76 cases of DF showed positive staining of  $\beta$ -catenin (66 cases of *CTNNB1* mutation–positive DF included). However, they evaluated staining properties only as positive or negative. Hamada et al [9] evaluated immunohistochemical staining of  $\beta$ -catenin with positivity divided into 4 grades in 33 cases of DF and demonstrated that the nuclear expression of  $\beta$ -catenin correlated significantly with *CTNNB1* mutation status. However, their study analyzed only 33 cases, and cytoplasmic staining was not analyzed. A previous study from the laboratory of Lazar et al [8] reported the differential staining intensity and pattern in different *CTNNB1* mutation status. They analyzed 117 cases of DF with positive *CTNNB1* mutation. Results showed that cases with T41A mutations had stronger staining intensity of  $\beta$ -catenin compared with those with S45F mutations, which is not consistent with the results of the present study. A possible reason of the discrepant results would be that they determined staining scores using tissue microarray, which was composed of small cores of samples, whereas specimens analyzed in the present study were obtained mainly from incisional biopsy. Larger specimens would well reflect the characteristics of tumors. Thus, no definitive conclusion could be drawn. The present multi-institutional study focused on more than 3 times as many cases as in the previous study, making the present results more convincing.

The present study, however, had several limitations. It contained many WT cases. Recently, Crago et al [30] performed whole-exome sequencing on 8 cases of WT DF diagnosed with direct sequencing (Sanger method) and reported that 5 of 8 cases with WT actually had the mutation in the Wnt-related gene (3 in *CTNNB1*, 2 in adenomatous polyposis coli). Our present cases might similarly have included several with WT determined by the Sanger method, which would be shown to harbor mutations of Wnt-related genes if evaluated by whole-exome sequencing. Another possible reason might be that inadequate fixation in formalin may cause a poor quality of extracted DNA and false-negative results of *CTNNB1* mutation status for the cases analyzed using FFPE samples. Actually, 1 of the participating 6 institutions, where specimens are generally fixed in formalin for a prolonged time, showed a considerable number of WT cases.

## 5. Conclusion

From the results of this study, staining pattern of  $\beta$ -catenin might be affected by *CTNNB1* mutation status. Although immunohistochemical staining of  $\beta$ -catenin is useful and intriguing for the diagnosis of DF, we should

know the limitation of this tool (high sensitivity but not high specificity) for the diagnosis of DF. *CTNNB1* mutation analysis should be used in combination for the most accurate diagnosis of DF.

## Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.humpath.2018.09.018>.

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