European Journal of Surgical Oncology 45 (2019) 691–698

Contents lists available at ScienceDirect

European Journal of Surgical Oncology

journal homepage: www.ejso.com

# Wnt targets genes are not differentially expressed in desmoid tumors bearing different activating β-catenin mutations

Milea J.M. Timbergen [a, d], Matthijs L. Janssen [a], Cornelis Verhoef [a], Dirk J. Grünhagen [a], Frederic Chibon [b, c], Marcel Smid [d], Stefan Sleijfer [d], Erik A.C. Wiemer [d, *]

[a] Department of Surgical Oncology, Erasmus MC Cancer Institute, Erasmus Medical Center Rotterdam, the Netherlands
[b] Department of Pathology, Institut Claudius Regaud, IUCT-Oncopole, Toulouse, France
[c] INSERM U1037, Cancer Research Center of Toulouse (CRCT), Toulouse, 31037, France
[d] Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus Medical Center Rotterdam, the Netherlands

ARTICLE INFO

*Article history:*
Received 31 August 2018
Received in revised form
17 September 2018
Accepted 24 September 2018
Available online 18 October 2018

*Keywords:*
Desmoid-type fibromatosis
Wnt/ β-catenin signaling
Desmoid tumor
CTNNB1 mutation

ABSTRACT

*Introduction:* Sporadic desmoid-type fibromatosis (DTF) is a rare soft tissue tumor of mesenchymal origin. It is characterized by local invasive growth and unpredictable growth behavior. Three distinct mutations involving the *CTNNB1* (β-catenin) gene have been identified in the vast majority of DTF tumors, which cause activation of the Wnt signaling pathway and impact prognosis. This study examines whether the different *CTNNB1* mutants (T41A, S45F) occurring in DTF tumors differentially affect Wnt signaling activity, which might explain the different disease course between DTF patients harboring different *CTNNB1* mutations.

*Materials and methods:* Real-time polymerase chain reaction (RT-PCR) on 61 formalin fixed paraffin embedded DTF samples with known *CTNNB1* status was used to measure the relative mRNA expression level of Wnt target genes *AXIN2*, *DKK1* and *CCND1*. Additionally, publicly available mRNA expression data retrieved from the Gene Expression Omnibus of 128 DTF samples were used for an unsupervised cluster analyses based on the expression of a selection of Wnt targets.

*Results:* No statistically significant difference in relative expression levels of Wnt target genes *AXIN2*, *DKK1* and *CCND1* was identified between either *CTNNB1* wild-type, S45F or T41A mutated DTF samples. Moreover, the hierarchical cluster analyses using selected Wnt targets did not discriminate between different *CTNNB1* mutation types.

*Conclusions:* No differences in the expression levels of Wnt target genes were observed between the different *CTNNB1* mutation types in DTF tumors. Further studies are needed to decipher the mechanism accounting for the diverse disease courses between DTF patients with different *CTNNB1* variants.



## Introduction

Sporadic desmoid-type fibromatosis (DTF) is a soft tissue tumor characterized by local invasive growth and unpredictable growth behavior with phases of progression, stable disease and even spontaneous regression [1–4]. The incidence in the Dutch population is approximately five cases per million people per year [5]. Affected patients are mostly females, with a peak incidence between the second and fourth decade of life [6–9]. Sporadic DTF has a mesenchymal origin, arising in musculoaponeurotic structures and can develop at practically any location in the body [10]. The most common localization is the abdominal wall whereas the predominant extra-abdominal localizations are the trunk and the proximal part of the extremities [7,11]. Treatment includes conservative management via active surveillance, surgical resection, radiotherapy or systemic therapy. The latter option comprises non-steroidal anti-inflammatory drugs (NSAIDs), anti-hormonal agents such as tamoxifen, low dose chemotherapy such as a combination of methotrexate with either vinblastine or vinorelbine, and tyrosine kinase inhibitors such as imatinib, sorafenib, or nilotinib [12]. No recommendations about the sequence of existing systemic

* Corresponding author. Dept. of Medical Oncology, Erasmus MC Cancer Institute Erasmus Medical Center, Room Be422, Wytemaweg 80, 3015 CN Rotterdam, the Netherlands.
*E-mail address:* e.wiemer@erasmusmc.nl (E.A.C. Wiemer).

https://doi.org/10.1016/j.ejso.2018.09.019
0748-7983/

**Table 1**
Patients and tumor characteristics of the Dutch and French cohort. The subgroups includes tumors harboring a T41A, S45F or a wild-type *CTNNB1* mutation.

| Clinicopathological characteristics | | Dutch cohort | | | | French cohort | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total group (n = 64) | | Subgroup (n = 61) | | Total group (n = 128) | | Subgroup (n = 93) | |
| | | Number of patients (%) | | | | Number of patients (%) | | | |
| Gender | Male | 23 | (35.9%) | 22 | (36.1%) | 45 | 35.2% | 36 | 38.7% |
| | Female | 41 | (64.1%) | 39 | (63.9%) | 79 | 61.7% | 56 | 60.2% |
| | Unknown | – | – | – | – | 4 | 3.1% | 1 | 1.1% |
| Median age in years at diagnosis (minimum age – maximum age) | | 35 | (4–79 years) | 34 | (4–79 years) | 38 | (1–74 years)[a] | 37 | (1–73 years)[b] |
| Mutation type | p.Thr41Ala | 38 | 59.4% | 38 | (62.3%) | 45 | 35.2% | 45 | (48.4%) |
| | p.Ser45Phe | 12 | 18.8% | 12 | (19.7%) | 34 | 26.6% | 34 | (36.6%) |
| | wild-type | 11 | 17.2% | 11 | (18%) | 14 | 10.9% | 14 | (15.1%) |
| | other | 3 | 4.7%* | – | – | 12 | 9.4%** | – | |
| | Unknown | – | – | – | – | 23 | 18% | – | |
| Tumor localization | Extra-abdominal | 38 | 59.4% | 38 | (62.3%) | 86 | 67.2% | 66 | (71%) |
| | Abdominal wall | 24 | 37.5% | 21 | (34.4%) | 24 | 18.8% | 20 | (21.5%) |
| | Intra-abdominal | 2 | 3.1% | 2 | (3.3%) | 12 | 9.4% | 6 | (6.5%) |
| | Unknown | – | – | – | – | 6 | 4.7% | 1 | (1.1%) |
| Median tumor size on radiology in mm (minimum size – maximum size | | 45 | (10–135 mm)[c] | 45 | (10–135 mm)[d] | 70 | (5–300 mm)[e] | 70 | (5–300 mm)[f] |

[a] n = 117, n = 11 missing value.
[b] n = 90, n = 3 missing value.
[c] n = 47, n = 17 missing value.
[d] n = 44, n = 17 missing value.
[e] n = 107, n = 21 missing value.
[f] n = 84, n = 9 missing value.
*n = 3, 133T > C (S45P).
**n = 6, 133T > C (S45P), n = 1, c.113_149delCTAC; n = 1, c.132_191del; n = 1, c.133delinsAAGG; n = 1, c.97_102del; n = 1, c.105_107del; n = 1, c.[98C > T(+)99T > G].

**Table 2**
Relative expression values of Wnt target genes *AXIN2, CCND1 and DKK1* for the Dutch and French subgroups (tumors harboring a T41A, S45F or wild-type *CTNNB1* gene mutation.

| | | | |
|---|---|---|---|
| A. Dutch cohort, subgroup data (n = 61)[a,b] | | Median (IQR) | p-value[c] (p < 0.05, 95% CI) |
| | *AXIN2* | 1.48 (1.12) | 0.273 |
| | *CCND1* | 0.61 (0.86) | 0.029[d] |
| | *DKK1* | 0.02 (0.04) | 0.319 |
| B. French cohort, subgroup data (n = 93)[a] | | Median using log2 transformed data (IQR) | p-value[c] (p < 0.05, 95% CI) |
| | *AXIN2 (224498_x_at)* | 4.80 (1.48) | 0.058 |
| | *CCND1* (208711_s_at) | 5.89 (0.80) | 0.486 |
| | *DKK1* (204602_at) | 5.20 (1.17) | 0.652 |

a T41A, S45F and wild-type CTNNB1 tumors included.
b compared to house keeper gene Peptidylprolyl Isomerase A.
c calculated p-values using the Kruskal Wallis test.
d non-significant after Bonferoni correction for multiple testing.
IQR - interquartile range.
AXIN2 - Axin 2.
CCND1 - Cyclin D1.
DKK1 - Dickkopf 1.
CI - confidence interval.

treatment options can be given yet, although the recent results of a randomized phase 3 trial suggest a possible role of sorafenib in symptomatic patients [13]. Recurrence rates after treatment are high with a 5-year local recurrence rate of 49% after surgery [9].

The *CTNNB1* (β-catenin) gene is mutated in the vast majority of sporadic DTF tumors [9,14–22]. Normally, β-catenin acts as a key mediator in the Wingless (Wnt) signaling pathway by operating as a transcriptional activator through binding in the nucleus to

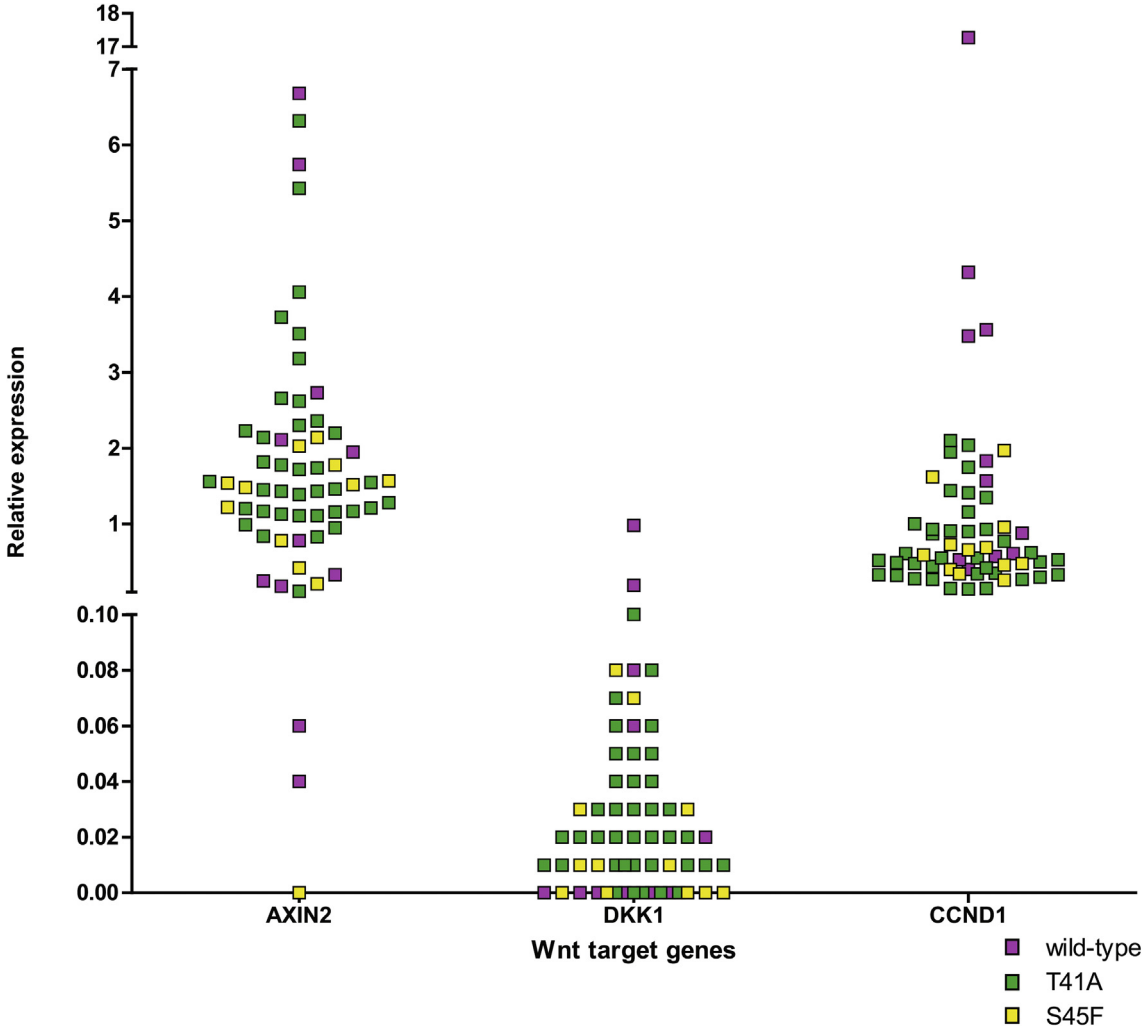
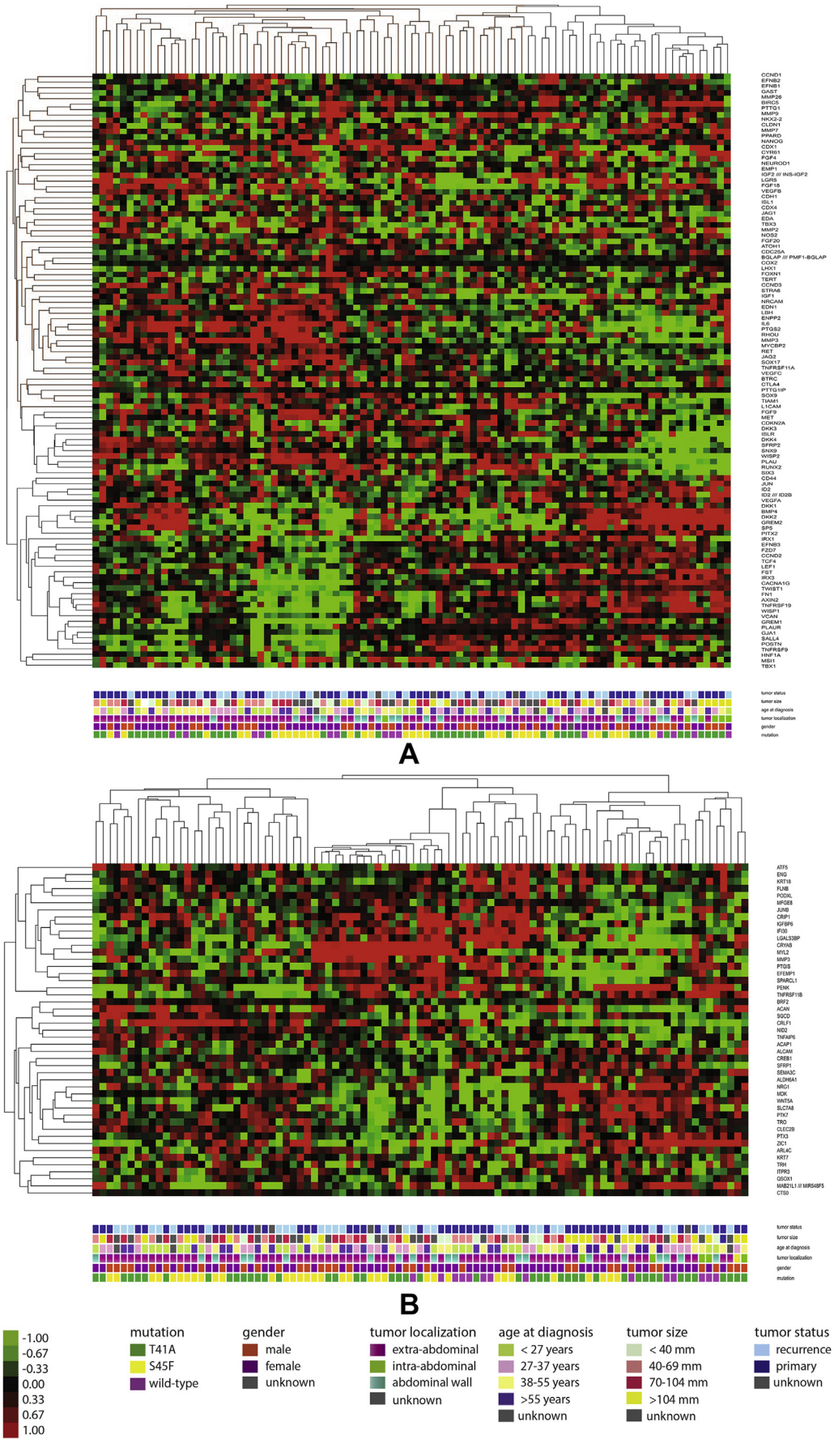



**Fig. 1.** Dot plot depicting the individual data relative expression levels measured with realtime qPCR of Wnt target genes *AXIN2*, *DKK1* and *CCND1* in DTF harboring either a S45F or T41A *CTNNB1* mutation or wild-type DTF.


A
B
-1.00
-0.67
-0.33
0.00
0.33
0.67
1.00
mutation
T41A
S45F
wild-type
gender
male
female
unknown
tumor localization
extra-abdominal
intra-abdominal
abdominal wall
unknown
age at diagnosis
< 27 years
27-37 years
38-55 years
>55 years
unknown
tumor size
< 40 mm
40-69 mm
70-104 mm
>104 mm
unknown
tumor status
recurrence
primary
unknown

members of the TCF/LEF transcription factor family. The *CTNNB1* mutations, found in DTF, mainly affect two codons in exon 3; substituting threonine at position 41 with alanine (T41A) and replacing serine at position 45 with either phenylalanine (S45F) or proline (S45P) [8,9]. These mutations prevent its phosphorylation, poly-ubiquitination and subsequent proteasomal degradation. Instead, β-catenin is stabilized and translocated into the nucleus where it drives transcription of Wnt target genes. The Wnt signaling cascade is involved in several biological processes like embryonic development and maintenance and regeneration of adult cells [23,24]. The aberrant activation of the Wnt signaling pathway, as in DTF, is observed in various malignancies like colorectal cancer, breast cancer and non-small cell lung cancer, and is considered to be a driver of tumorigenesis [14,25–28]. The *CTNNB1* genotype-phenotype relation has been extensively investigated in liver cancer, providing evidence that different *CTNNB1* mutations are linked to different levels of β-catenin activation. The S45 mutation leads to a weak activation and T41 mutations are associated with a moderate activation which may yield differences in clinical behavior [29]. Desmoid tumors that harbor a S45F mutation exhibit a higher recurrence rate after primary resection than wild-type (WT) and T41A mutant tumors [9,20,30,31] and were shown to be more resistant for the NSAID meloxicam [32]. However, others report conflicting results and could not reveal an impact of *CTNNB1* mutations on outcome [18,22]. The molecular mechanisms, underlying a *CTNNB1* genotype-phenotype relationship, are not known, although Hamada et al. reported that S45F desmoid cells have a stronger nuclear β-catenin staining in a preclinical model and observed an upregulation of Wnt target genes *AXIN2* and *CCND1* compared to WT and T41A cells [33].

Here we examined whether the *CTNNB1* mutants, encountered in DTF, differentially affect Wnt signaling activity in human desmoids and whether this may explain the variable clinical behavior between the different *CTNNB1* mutants.

## Materials and methods

### *Dutch and French dataset*

A Dutch, formalin fixed paraffin embedded (FFPE) DTF cohort (n = 64), with *CTNNB1* mutation status determined with Sanger sequencing, previously described by van Broekhoven et al. was available for this study [20]. Samples were derived from patients with sporadic DTF who underwent surgical excision of their tumor. Clinical characteristics included: gender, tumor localization (extra-abdominal, abdominal wall or intra-abdominal), age at diagnosis and tumor size in millimeters.

Additionally, the clinical and molecular data from a French, fresh frozen DTF sample set (n = 128), containing a total of 54613 probe sets, previously described by Salas et al. were accessed through the Gene Expression Omnibus (series matrix file, accession number GSE58697) [34]. This file contains data normalized using the GCRMA algorithm. The data on the *CTNNB1* mutations status of these tumors were kindly provided by dr. Frederic Chibon, Cancer Research Center of Toulouse, France. Clinical data included: gender, age at the time of diagnosis, tumor size in millimeters, tumor localization, and tumor status (primary or recurrent). The patient and tumor characteristics of both sample sets are listed in Table 1.

### *RNA isolation and RT-qPCR*

Total RNA was extracted from two to four tissue sections (20 μm) of DTF FFPE tumor samples using the RecoverAll™ total nucleic acid isolation kit (Ambion/Life Technologies) according to the manufacturer's recommendations. Total RNA quality and concentration were determined using a Nanodrop-2000 (Isogen Life Science). The 260nm/280 nm ratio was ≥1.80 for all RNA preparations. The mRNA expression of *AXIN2*, *CCND1* and *DKK1* was assessed by RT-qPCR. In brief: 50 ng RNA was reverse transcribed with RevertAid™ H Minus (Thermo Fischer Scientific, EP0452) to generate cDNA. The resulting 4 μL cDNA was subsequently pre-amplified for 15 cycles in a final volume of 8 μL using Taqman PreAmp mastermix (Thermo Fisher Scientific) in combination with 100-fold diluted primer-probe combinations for *AXIN2*, *CCND1*, Dickkopf 1 (*DKK1)* and Peptidyl-prolyl Isomerase A (*PPIA),* (Thermo Fisher Scientific; Assay Ids Hs00610344_m1, Hs00765553_m1, Hs00183740_m1 and Cat # 4333763F, respectively). Next, for each sample, four individual - gene transcript specific - real time PCRs were carried out in a Mx3000P (Agilent) for 40 cycles using SensiFast Probe Lo-ROX master mix (BioLine) according the manufacturer's recommendation and the same four primer-probe combinations. A 10 ng/μL down to 0.04 ng/μL serially diluted RNA sample isolated from MCF7 cells and expressing all targets was used in each experiment to monitor the efficiencies of the RT-qPCRs and a minus RT sample as a negative control. All targets were amplified with an equal efficiency (98%–110%). Finally, levels of the target genes were normalized on the stable expressed PPIA mRNA levels using the delta Cq normalization method.

### *Statistical analysis of AXIN2, CCND1 and DKK1 expression in WT and CTNNB1 DTF mutants*

The relative expression values of *AXIN2*, *CCND1* and *DKK1 were* measured by RT-PCR on FFPE samples, in the Dutch DTF cohort. Median values and the maximum interquartile range (IQR) were calculated. Because of non-normally distributed data, a Kruskal Wallis test was performed to identify differences in ranked expression levels of *AXIN2, CNND1* and *DKK1* (p-value < 0.05, confidence interval 95%) between DTF with various *CTNNB1* mutation types (T41A, S45F) and DTF with no mutations in exon 3 of *CTNNB1* (WT). A Bonferoni correction was used to correct for multiple testing.

To validate the results of the RT-PCR data, the most variable probes of *AXIN2, CCND1 and DKK1*, were selected based on the highest IQR using log2 transformed data of the total French cohort. Because of a non-normal distribution, a Kruskal Wallis test was performed to identify differences in ranks of expression levels of *AXIN2, CNND1* and *DKK1* (p-value < 0.05, confidence interval 95%) between DTF with various *CTNNB1* mutation types (T41A, S45F)

**Fig. 2.** Hierarchical clustering based on Wnt target mRNA expression in desmoid tumors does not discriminate wild-type and *CTNNB1* S45F and T41A mutants. (A) Heat map depicting an unsupervised hierarchical cluster analysis of 93 desmoid-type fibromatosis samples harboring T41A or S45F *CTNNB1* mutations and wild-type tumors using the mRNA expression levels of selected mammalian Wnt target genes. Red and green represent relative high and low expresion levels, respectively. Tumor status (primary or recurrent); tumor size (0–49 mm, 50–99 mm, > 99 mm); age at diagnosis presented in categories based on the median and the 25th and 75th percentiles (age < 27 years, 37-37 years 38–55 years and >55 years); tumor localization (intra-abdominal, extra-abdominal and abdominal wall); gender and *CTNNB1* mutation status are indicated.
(B) Heat map depicting an unsupervised hierarchical cluster analysis of 93 desmoid-type fibromatosis samples harboring T41A, S45F *CTNNB1* mutations and wild-type tumors using selected the mRNA expression levels of a set of putative mesenchymal Wnt target genes. Red and green represent relatively high and low expression levels, respectively. Tumor status (primary or recurrent); tumor size (0–49 mm, 50–99 mm, > 99 mm); age at diagnosis presented in categories based on the median and the 25th and 75th percentiles (age < 27 years, 37-37 years 38–55 years and >55 years); tumor localization (intra-abdominal, extra-abdominal and abdominal wall); gender and *CTNNB1* mutation status are indicated.

and DTF with no mutations in exon 3 of *CTNNB1* (WT).

*Selection of Wnt target genes*

To rule out that the limited set of Wnt genes analyzed so far caused a selection bias affecting the outcome of our analyses, the number of Wnt targets genes was increased. Wnt target genes used in a hierarchical unsupervised cluster analyses using, transcript expression data from DTF tumors, were selected using the mammalian Wnt target genes listed on the Wnt homepage (https://web.stanford.edu/group/nusselab/cgi-bin/wnt/target_genes) as a reference. [35] Supplemental Table 1 summarizes the mammalian Wnt target genes that were selected, the corresponding Affymetrix probe sets and alias terms (https://www.ncbi.nlm.nih.gov/gene/). Since DTF tumors are of mesenchymal origin, they may express Wnt target genes partly different from those observed in epithelial cancers. Therefore, unsupervised hierarchical cluster analyses were repeated using a list of putative mesenchymal Wnt target genes in desmoids published by Denys et al. [36] Gene names, corresponding Affymetrix probe sets and alias terms are shown in Supplemental Table 2.

*Hierarchical cluster analysis*

For hierarchical cluster analysis, the retrieved normalized mRNA expression data of the 128 DTF samples of the French cohort [26] were log2 transformed to minimize outliers. Next, Wnt target genes were selected based on their names and alias terms as described in paragraph 2.4. In case of multiple probes representing a single gene, the probe with the highest variability, based on the maximum IQR, using the entire dataset, was selected for further analyses. An unsupervised hierarchical cluster analysis was performed using median centered expression data with Cluster 3.0 for Windows (Human Genome Center, University of Tokyo, 2002) and Java Treeview, version 1.1.6rv, for visualization. The clustering was based on the centered correlation as a distance metric using average linkage.

*Ethical approval*

This study was part of a protocol entitled “Translational research on soft tissue sarcomas” which was assessed by the Medical Ethics Committee of the Erasmus MC, Rotterdam, the Netherlands (MEC-2016-213).

## Results

*No difference in AXIN2, CCND1 and DKK1 expression between wild-type and CTNNB1 mutated desmoids*

To examine whether different *CTNNB1* mutations in desmoid tumors cause differential expression of Wnt target genes, the transcript levels of *AXIN2*, *CCND1* and *DKK1* were determined by RT-PCR. In total, 64 FFPE samples from the Dutch cohort were available for the RT-PCR analysis. The cohort included 23 males (35.9%) and 41 females (64.1%). Mutational status was as follows; 11 tumors (17.2%) were WT for exon 3, 38 (59.4%) tumors harbored a T41A mutation, 12 (18.8%) tumors harbored a S45F mutation and 3 (4.7%) tumors harbored a S45P mutation. Because of the low prevalence, the desmoids with the S45P mutation were not included in the RT-PCR analysis leaving a total of 61 patients. The tumor and patient characteristics are summarized in Table 1. The relative expression values of *AXIN2, CCND1* and *DDK1* transcripts were measured and the median expression levels and the maximum IQR were calculated (Table 2). A Kruskal-Wallis test shows that the mean rank of the relative expression levels was not statistically significantly different between groups for *AXIN2* and *DKK1* (Table 2). The mean rank of the relative expression level for *CCND1* showed a significant difference between groups, however failed significance after Bonferoni correction for multiple testing between mutation groups (Table 2). A dot plot depicting the relative expression levels of individual samples of AXIN2, CCND1 and DKK1 is shown in Fig. 1.

Next, mRNA expression data from the French cohort were used to validate these findings. The patient and tumor characteristics of this cohort are listed in Table 1. Ninety-three desmoid samples; *CTNNB1* WT (n = 14); T41A (n = 45) and S45F (n = 34) were selected for subsequent analysis. First, the Affymetrix probe sets for *AXIN2 (222696_at, 224176_at, 222695_s_at, 224498_x_at*) *CCND1* (208712_at, 208711_s_at) and *DKK1* (204602_at) were identified and the probes that displayed the most variable expression values after log 2 transformation, based on the highest IQR, were selected for further analysis (*224498_x_at*, 208711_s_at and 204602_at). A Kruskal-Wallis test showed no significantly differences between the mean ranks of the groups with T41A or S45F mutations and WT *CTNNB1* (Table 2).

*Hierarchical cluster analyses based on Wnt target gene expression does not discriminate between wild-type and CTNNB1 mutant desmoids*

The total dataset contained 54613 probes, of which 107 probes were selected as mammalian Wnt targets and 47 were selected as mesenchymal Wnt targets. The hierarchical clustering using mammalian Wnt targets (Fig. 2A) did not discriminate WT and mutated DTF (T41A and S45F) tumors, nor showed a discriminative pattern based on sex, age at the time of diagnosis, tumor size or tumor status (primary or recurrent tumor). An additional hierarchical clustering, using expression data from all 128 desmoid samples (including WT tumors as well as T41A, S45F, S45P mutated tumors, rare mutations classified as “other”, and tumors with an unknown mutation type), showed similar results (supplemental Figure 1A). Cluster analyses using putative mesenchymal Wnt targets [36] in a subgroup containing samples that are *CTNNB1* WT, T41A and S45F (Fig. 2B) and the cluster analysis using all samples (supplemental Figure 1B), also showed that the DTF samples did not cluster according to *CTNNB1* mutational status, neither did the observed cluster appear to be driven by clinicopathological parameters like sex, age at time of diagnosis, tumor size or tumor status (primary or recurrent tumor).

## Discussion

This study evaluated whether the potentially more aggressive clinical behavior of S45F mutated DTF, compared to T41A mutants and WT desmoids, could be explained by a differential Wnt signaling activity. As aberrant Wnt/β-catenin signaling plays a pivotal role in the initiation and development of DTF, variable activity of Wnt signaling may affect cancer-related biological processes like cancer stem cell maintenance and invasiveness in a different manner [37–39]. We therefore examined and compared Wnt target expression levels using different independent DTF sample sets that included DTF designated as WT as well as T41A and S45F *CTNNB1* mutants. No evidence was found that WT tumors, T41A and S45F tumors have a differential activation of Wnt/β-catenin signaling. This finding contrasts with Hamada et al. who reported a significant increase of *AXIN2*, *CCND1* and *c-MYC* transcript levels in S45F DTF isolated single cell lines compared to human skin fibroblasts and WT and T41A cells [33].

A study conducted by Meneghello et al. showed upregulated

(two to six fold) mRNA expression of *AXIN2* measured by RT-PCR but also reported that desmoid cells had downregulated mRNA expression of *CCND1* (two-four fold) when comparing DTF samples with connective tissue of non-desmoid patients [17]. In addition, Jilong et al. compared β-catenin mutated tumors with WT tumors and found that *CCND1* was expressed more frequently in β-catenin mutated DTF [16]. Saito et al. found a statistically significant correlation between nuclear staining of β-catenin and *CCND1* overexpression [16,40]. Although the literature on Wnt target expression in DTF is limited and somewhat contradictory, both AXIN2 and CCND1 seem to be overexpressed in DTF. Interestingly, by studying genotype-phenotype correlations of *CTNNB1* mutations in liver cancer, others have found that T41 mutations were associated with moderate activity whereas S45 mutations led to a weak β-catenin activation which could be compensated by S45 mutant allele duplication [29].

None of our hierarchical cluster analyses, based on the expression of different Wnt target gene sets, discriminated *CTNNB1* mutated (S45 and T41) from WT DTF samples, neither were S45F and T41A clearly separated. A possible confounder in our analyses is that the WT group, now defined by the absence of mutations in exon 3 of *CTNNB1*, needs to be screened more thoroughly for *CTNNB1* mutations. It was demonstrated that a fair part of the DTF, designated as WT by Sanger sequencing, contain low frequency *CTNNB1* mutations that are only detected by whole exome sequencing or contain novel intra-genic deletions of the *CTNNB1* gene [19,41]. Moreover, in the WT group, other genomic alterations occurred like *APC* loss, chromosome 6 loss and *BMI1* mutations which are all linked to Wnt/β-catenin activation [19]. In our analyses, we could not distinguish between WT and S45F, andT41A mutant DTF corresponding to observations of Crago et al. [19] In contrast, Colombo et al. reported that *CTNNB1*-mutated and WT DTF had different gene expression profiles [42]. In our cluster analyses, distinct clusters of samples were discerned based on the expression levels of putative Wnt target genes that were not related to *CTNNB1* mutational status. Unfortunately, we were not able to identify the origin of the cluster pattern as it could not be explained by factors like sex, age at the time of diagnosis, tumor size and tumor status (primary or recurrent tumor). Other clinicopathological factors, not included in this study, for example immune cell infiltration could potentially explain the observed cluster pattern and could be the subject of future studies. Interestingly in this respect is a recent publication from Colombo et al. describing that T41A and S45F mutated DTF displayed a different expression of inflammation related genes [42].

No indications were found in this study for a differential Wnt/β-catenin signaling activity between the T41A and S45F mutants thereby failing to explain the reported different clinical behavior of the two DTF mutant groups. A more thorough investigation into the molecular consequences of different *CTNNB1* mutations is necessary, focusing on protein-protein interactions and identifying their genomic binding sites. Irrespective of the specific *CTNNB1* mutation present in DTF, it is clear that Wnt/β-catenin signaling is activated which plays an essential role in carcinogenesis. Inhibition of this pathway may provide a vulnerability in DTF that can therapeutically be exploited. However, drugs should operate at the level of β-catenin, interacting with transcription co-factors or target specific key downstream factors to avoid serious adverse effects [43,44]. Additionally, other signaling pathways like Notch1 and Hedgehog have also been implicated to play a role in DTF development and may be therapeutically targeted by small molecules [45,46]. Future studies are needed to gain more insight into the role of these additional signaling pathways, the cross-talks between each pathway and their contribution to the DTF tumorigenesis.

## Declarations of interest

None.

## Conclusion

Our study demonstrated no difference between either WT, S45F or T41A mutated DTF tumors regarding the investigated Wnt target gene expression levels. Apparently a differential Wnt/β-catenin signaling activity does not determine the observed differences in clinical behavior between S45F and T41A DTF mutants.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejso.2018.09.019.

## References

[1] Fiore M, Rimareix F, Mariani L, Domont J, Collini P, Le Pechoux C, et al. Desmoid-type fibromatosis: a front-line conservative approach to select patients for surgical treatment. Ann Surg Oncol 2009;16:2587–93.
[2] Bonvalot S, Ternes N, Fiore M, Bitsakou G, Colombo C, Honore C, et al. Spontaneous regression of primary abdominal wall desmoid tumors: more common than previously thought. Ann Surg Oncol 2013;20:4096–102.
[3] Colombo C, Miceli R, Le Pechoux C, Palassini E, Honore C, Stacchiotti S, et al. Sporadic extra abdominal wall desmoid-type fibromatosis: surgical resection can be safely limited to a minority of patients. Eur J Canc 2015:186–92.
[4] Shields CJ, Winter DC, Kirwan WO, Redmond HP. Desmoid tumours. Eur J Surg Oncol 2001;27:701–6.
[5] van Broekhoven DL, Grunhagen DJ, den Bakker MA, van Dalen T, Verhoef C. Time trends in the incidence and treatment of extra-abdominal and abdominal aggressive fibromatosis: a population-based study. Ann Surg Oncol 2015;22:2817–23.
[6] Sorensen A, Keller J, Nielsen OS, Jensen OM. Treatment of aggressive fibromatosis: a retrospective study of 72 patients followed for 1-27 years. Acta Orthop Scand 2002;73:213–9.
[7] Phillips SR, A'Hern R, Thomas JM. Aggressive fibromatosis of the abdominal wall, limbs and limb girdles. Br J Surg 2004;91:1624–9.
[8] Al-Jazrawe M, Au M, Alman B. Optimal therapy for desmoid tumors: current options and challenges for the future. Expert Rev Anticancer Ther 2015;15:1443–58.
[9] Lazar AJ, Tuvin D, Hajibashi S, Habeeb S, Bolshakov S, Mayordomo-Aranda E, et al. Specific mutations in the beta-catenin gene (CTNNB1) correlate with local recurrence in sporadic desmoid tumors. Am J Pathol 2008;173:1518–27.
[10] Wu C, Amini-Nik S, Nadesan P, Stanford WL, Alman BA. Aggressive fibromatosis (desmoid tumor) is derived from mesenchymal progenitor cells. Cancer Res 2010;70:7690–8.
[11] Merchant NB, Lewis JJ, Woodruff JM, Leung DH, Brennan MF. Extremity and trunk desmoid tumors: a multifactorial analysis of outcome. Cancer 1999;86:2045–52.
[12] Kasper B, Baumgarten C, Garcia J, Bonvalot S, Haas R, Haller F, et al. An update on the management of sporadic desmoid-type fibromatosis: a European consensus initiative between Sarcoma PAtients EuroNet (SPAEN) and European organization for research and Treatment of Cancer (EORTC)/Soft Tissue and bone Sarcoma group (STBSG). Ann Oncol 2017;28:2399–408.
[13] Gounder MM, Mahoney MR, Van Tine BA, Ravi V, Attia S, Deshpande HA, et al. Phase III, randomized, double blind, placebo-controlled trial of sorafenib in desmoid tumors (Alliance A091105). J Clin Oncol 2018;36. 36:suppl; abstr 11500.
[14] Alman BA, Li C, Pajerski ME, Diaz-Cano S, Wolfe HJ. Increased beta-catenin protein and somatic APC mutations in sporadic aggressive fibromatoses (desmoid tumors). Am J Pathol 1997;151:329–34.
[15] mini Nik S, Hohenstein P, Jadidizadeh A, Van Dam K, Bastidas A, Berry RL, et al. Upregulation of Wilms' tumor gene 1 (WT1) in desmoid tumors. Int J Canc 2005;114:202–8.
[16] Jilong Y, Jian W, Xiaoyan Z, Xiaoqiu L, Xiongzeng Z. Analysis of APC/beta-catenin genes mutations and Wnt signalling pathway in desmoid-type fibromatosis. Pathology 2007;39:319–25.
[17] Meneghello C, Ousghir B, Rastrelli M, Anesi L, Sommariva A, Montesco MC, et al. Nuclear GSK-3beta segregation in desmoid-type fibromatosis. Histopathology 2013;62:1098–108.
[18] Mullen JT, DeLaney TF, Rosenberg AE, Le L, Iafrate AJ, Kobayashi W, et al. β-catenin mutation status and outcomes in sporadic desmoid tumors. Oncologist 2013;18:1043–9.
[19] Crago AM, Chmielecki J, Rosenberg M, O'Connor R, Byrne C, Wilder FG, et al. Near universal detection of alterations in CTNNB1 and Wnt pathway regulators in desmoid-type fibromatosis by whole-exome sequencing and genomic analysis. Genes Chromosomes Cancer 2015;54:606–15.

[20] van Broekhoven DL, Verhoef C, Grunhagen DJ, van Gorp JM, den Bakker MA, Hinrichs JW, et al. Prognostic value of CTNNB1 gene mutation in primary sporadic aggressive fibromatosis. Ann Surg Oncol 2015;22:1464–70.
[21] Tejpar S, Nollet F, Li C, Wunder JS, Michils G, dal Cin P, et al. Predominance of beta-catenin mutations and beta-catenin dysregulation in sporadic aggressive fibromatosis (desmoid tumor). Oncogene 1999;18:6615–20.
[22] Domont J, Salas S, Lacroix L, Brouste V, Saulnier P, Terrier P, et al. High frequency of beta-catenin heterozygous mutations in extra-abdominal fibromatosis: a potential molecular tool for disease management. Br J Canc 2010;102:1032–6.
[23] Enzo MV, Rastrelli M, Rossi CR, Hladnik U, Segat D. The Wnt/beta-catenin pathway in human fibrotic-like diseases and its eligibility as a therapeutic target. Mol Cell Ther 2015;3:1.
[24] Peifer M, Polakis P. Wnt signaling in oncogenesis and embryogenesis–a look outside the nucleus. Science 2000;287:1606–9.
[25] Willert K, Nusse R. Beta-catenin: a key mediator of Wnt signaling. Curr Opin Genet Dev 1998;8:95–102.
[26] Nusse R, Clevers H. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. Cell 2017;169:985–99.
[27] Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. Oncogene 2017;36:1461–73.
[28] Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature 2005;434:843–50.
[29] Rebouissou S, Franconi A, Calderaro J, et al. Genotype-phenotype correlation of CTNNB1 mutations reveals different ss-catenin activity associated with liver tumor progression. Hepatology 2016;64:2047–61.
[30] Garvey PB, Booth JH, Baumann DP, Calhoun KA, Liu J, Pollock RE, et al. Complex reconstruction of desmoid tumor resections does not increase desmoid tumor recurrence. J Am Coll Surg 2013;217:472–80.
[31] Colombo C, Miceli R, Lazar AJ, Perrone F, Pollock RE, Le Cesne A, et al. CTNNB1 45F mutation is a molecular prognosticator of increased postoperative primary desmoid tumor recurrence: an independent, multicenter validation study. Cancer 2013;119:3696–702.
[32] Hamada S, Futamura N, Ikuta K, et al. CTNNB1 S45F mutation predicts poor efficacy of meloxicam treatment for desmoid tumors: a pilot study. PloS One 2014;9.
[33] Hamada S, Urakawa H, Kozawa E, Arai E, Ikuta K, Sakai T, et al. Characteristics of cultured desmoid cells with different CTNNB1 mutation status. Cancer Med 2016;5:352–60.
[34] Salas S, Brulard C, Terrier P, Ranchere-Vince D, Neuville A, Guillou L, et al. Gene expression profiling of desmoid tumors by cDNA microarrays and correlation with progression-free survival. Clin Canc Res 2015;21:4194–200.
[35] Nusse R, Lim X. The Wnt homepage, Wnt Target genes. https://web.stanford.edu/group/nusselab/cgi-bin/wnt/target_genes. [Accessed 06 March 2018].
[36] Denys H, Jadidizadeh A, Amini Nik S, Van Dam K, Aerts S, Alman BA, et al. Identification of IGFBP-6 as a significantly downregulated gene by beta-catenin in desmoid tumors. Oncogene 2004;23:654–64.
[37] Cheon SS, Cheah AY, Turley S, Nadesan P, Poon R, Clevers H, et al. beta-Catenin stabilization dysregulates mesenchymal cell proliferation, motility, and invasiveness and causes aggressive fibromatosis and hyperplastic cutaneous wounds. Proc Natl Acad Sci U S A 2002;99:6973–8.
[38] Nishita M, Enomoto M, Yamagata K, Minami Y. Cell/tissue-tropic functions of Wnt5a signaling in normal and cancer cells. Trends Cell Biol 2010;20:346–54.
[39] Duchartre Y, Kim YM, Kahn M. The Wnt signaling pathway in cancer. Crit Rev Oncol Hematol 2016;99:141–9.
[40] Saito T, Oda Y, Tanaka K, Matsuda S, Tamiya S, Iwamoto Y, et al. beta-catenin nuclear expression correlates with cyclin D1 overexpression in sporadic desmoid tumours. J Pathol 2001;195:222–8.
[41] Colombo C, Urbini M, Astolfi A, Collini P, Indio V, Belfiore A, et al. Novel intragenic large deletions of CTNNB1 gene identified in WT desmoid-type fibromatosis. Genes Chromosomes Cancer 2018;57:495–503.
[42] Colombo C, Belfiore A, Paielli N, De Cecco L, Canevari S, Laurini E, et al. beta-Catenin in desmoid-type fibromatosis: deep insights into the role of T41A and S45F mutations on protein structure and gene expression. Mol Oncol 2017;11:1495–507.
[43] Valenta T, Degirmenci B, Moor AE, Herr P, Zimmerli D, Moor MB, et al. Wnt ligands secreted by subepithelial mesenchymal cells are essential for the survival of intestinal stem cells and gut homeostasis. Cell Rep 2016;15:911–8.
[44] Zimmerli D, Hausmann G, Cantu C, Basler K. Pharmacological interventions in the Wnt pathway: inhibition of Wnt secretion versus disrupting the protein-protein interfaces of nuclear factors. Br J Pharmacol 2017;174:4600–10.
[45] Shang H, Braggio D, Lee YJ, Al Sannaa GA, Creighton CJ, Bolshakov S, et al. Targeting the Notch pathway: a potential therapeutic approach for desmoid tumors. Cancer 2015;121:4088–96.
[46] Ghanbari-Azarnier R, Sato S, Wei Q, Al-Jazrawe M, Alman BA. Targeting stem cell behavior in desmoid tumors (aggressive fibromatosis) by inhibiting hedgehog signaling. Neoplasia 2013;15:712–9.