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## Near Universal Detection of Alterations in *CTNNB1* and Wnt Pathway Regulators in Desmoid-Type Fibromatosis by Whole-Exome Sequencing and Genomic Analysis

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### Abstract

*CTNNB1* mutations or *APC* abnormalities have been observed in ~85% of desmoids examined by Sanger sequencing and are associated with Wnt/ $\beta$ -catenin activation. We sought to identify molecular aberrations in 'wild-type' tumors (those without *CTNNB1* or *APC* alteration) and to determine their prognostic relevance. *CTNNB1* was examined by Sanger sequencing in 117 desmoids; a mutation was observed in 101 (86%) and 16 were 'wild-type'. 'Wild-type' status did not associate with tumor recurrence. Moreover, in unsupervised clustering based on U133A-derived gene expression profiles, 'wild-type' and mutated tumors clustered together. Whole-exome sequencing of eight of the 'wild-type' desmoids revealed that three had a *CTNNB1* mutation that had been undetected by Sanger sequencing. The mutation was found in a mean 16% of reads (vs 37% for mutations identified by Sanger). Of the other five 'wild-type' tumors sequenced, two had *APC* loss, two had chromosome 6 loss, and one had mutation of *BMII*. The finding of low-frequency *CTNNB1* mutation or *APC* loss in 'wild-type' desmoids was validated in the remaining eight 'wild-type' desmoids; directed miSeq identified low-frequency *CTNNB1* mutation in four and comparative genomic hybridization identified *APC* loss in one. These results

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demonstrate that mutations affecting *CTNNB1* or *APC* occur more frequently in desmoids than previously recognized (111 of 117; 95%), and designation of ‘wild-type’ genotype is largely determined by sensitivity of detection methods. Even true *CTNNB1* wild-type tumors (determined by next-generation sequencing) may have genomic alterations associated with Wnt activation (chromosome 6 loss/*BM11* mutation), supporting Wnt/ $\beta$ -catenin activation as the common pathway governing desmoid initiation.

## Keywords

Desmoid; fibromatosis;  $\beta$ -catenin; *CTNNB1* mutation

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## INTRODUCTION

Desmoid-type fibromatosis represents a clonal proliferation arising from mesenchymal stem cell progenitors (Alman, et al. 1997a; Wu, et al. 2010). They are diagnosed in approximately 1000 patients in the United States each year. Desmoids have no metastatic potential, but can be locally aggressive, causing pain or intestinal obstruction and fistulization (Lewis, et al. 1999). For this reason, surgical resection has been the ‘gold standard’ of treatment. However, aggressive attempts at complete resection in many cases cause significant morbidity, and rates of local recurrence following surgery are as high as 70% in some series (Markhede, et al. 1986; Easter and Halasz 1989; Lopez, et al. 1990; Higaki, et al. 1995; Lewis, et al. 1999; Merchant, et al. 1999).

In the majority of desmoids, tumorigenesis is thought to be driven by disruptions of Wnt/ $\beta$ -catenin signaling.  $\beta$ -catenin, a transcription factor, is the final regulator in the canonical Wnt/ $\beta$ -catenin pathway, and desmoids frequently display nuclear staining of  $\beta$ -catenin (Ng, et al. 2005). In 85% of patients, the desmoid bears an activating mutation in the  $\beta$ -catenin gene, *CTNNB1*. Several activating mutations of *CTNNB1* are known, all of them in exon 3 (Huss, et al. 2013). In a small minority of patients, desmoids result from germline or sporadic loss of *APC* (Alman, et al. 1997b; Li, et al. 1998; Tejpar, et al. 1999). Because *APC* is a negative regulator of  $\beta$ -catenin stability, loss of *APC* leads to activation of  $\beta$ -catenin.

Because of the presence of *CTNNB1* or *APC* mutations, Wnt/ $\beta$ -catenin activation is thought to represent the central oncogenic event in most cases of desmoid-type fibromatosis. However, approximately 15% of desmoids lack known *APC* or *CTNNB1* disruption, so it is unclear what drives the formation of these so-called wild-type lesions (Tejpar, et al. 1999; Salas, et al. 2010). Recent reports suggest that patients with ‘wild-type’ desmoids have better outcomes than patients whose tumor harbors a defined mutation in *CTNNB1* (T41A, S45F, or S45P), but this report has not been universally validated (Lazar, et al. 2008; Colombo, et al. 2013; Mullen, et al. 2013).

In this study, we performed a genomic characterization of ‘wild-type’ desmoids to identify genetic drivers of tumorigenesis. We also compared the ‘wild-type’ desmoids with *CTNNB1*-mutant desmoids to assess heterogeneity in clinicopathologic characteristics between the

## METHODS AND MATERIALS

### Tissue Procurement, Immunohistochemistry, and Nucleic Acid Preparation

Tumor and adjacent normal fat or muscle tissue samples were collected from 117 individual patients after surgical resection of desmoid-type fibromatosis between 2002 and 2013. Clinicopathologic characteristics describing patients and tumors, therapeutic interventions, follow-up dates, and disease status were recorded in a prospectively maintained database. Patients with a recorded history of familial adenomatous polyposis were excluded from this analysis. All patients provided informed consent per a protocol (#02-060) that was reviewed and approved by the institutional review board at Memorial Sloan Kettering Cancer Center. Samples were snap-frozen and cryomolds prepared. Hematoxylin and eosin (H&E)—stained slides from each cryomold were reviewed by a sarcoma-specific pathologist (M.H.) and regions of normal tissue removed by macrodissection. This procedure allowed all analyses to be performed on samples composed of >90% tumor. DNA and RNA were prepared using RNeasy and DNeasy Mini Kits (QIAGEN).

### Gene Expression Array Preparation and Analysis

cDNA was synthesized in the presence of oligo(dT)<sub>24</sub>-T7 from Genset, and cRNA prepared using biotinylated CTP and UTP. cRNA was hybridized to HG U133A 2.0 mRNA expression arrays (Affymetrix). Results from these arrays were processed using the standard R/Bioconductor packages: gcRMA (based on Robust Multi-Array Average method) for quantitation and normalization and LIMMA empirical Bayes method for differential expression (Irizarry, et al. 2003; Smyth 2004). Unsupervised clustering was based on the 223 genes most variably expressed between each pairwise comparison; it was performed using the R function ‘dendrogram’ on one dimension.

### Sequencing

Bidirectional Sanger sequencing was performed as previously reported (Pratilas, et al. 2008; Janakiraman, et al. 2010). Briefly, *CTNNB1* exon 3 was amplified by PCR using primers with sequences GTAAAACGACGGCCAGTTCAGCTAACCCTGGCT and CAGGAAACAGCTATGACCTCCACAGTTCAGCATTTACCT and HotStart Taq (Kapa Biosystems). Templates were purified (AMPure, Agencourt Biosciences) and sequenced bidirectionally with Big Dye Terminator Kit v. 3.1 (Applied Biosystems). After removal of dye terminators (CleanSEQ, Agencourt Biosciences), reactions were run on ABI PRISM 3730xl sequencing apparatus (Applied Biosystems). Reads were assembled against the reference sequence using Consed 16.0 (Gordon, et al. 1998). Mutations were called by Polyphred 6.02b and Polyscan 3.0 and annotated with Genomic Mutation Consequence Calculator (Nickerson, et al. 1997; Chen, et al. 2007; Major 2007).

Whole-exome sequencing and data analysis were performed as previously described (Chmielecki, et al. 2013). Briefly, DNA (100 ng) from tumor and a normal muscle or fat sample from each patient was sheared. After end repair, samples were phosphorylated and ligated to barcoded sequence adaptors. Fragments between 200 and 350 bp underwent exonic hybrid capture with SureSelect v2 Exome bait (Agilent), then captured fragments were sequenced on Illumina HiSeq flowcells. The Firehose pipeline was used to manage

input and output files, and MuTect and MutSig algorithms were used to identify statistically significant somatic mutations. The CapSeg (Copy number from exome sequencing) was used to identify copy number alterations and dRanger to identify somatic fusions (Chmielecki, et al. 2013; Cibulskis, et al. 2013; Lawrence, et al. 2013).

#### 454 and MiSeq Validation of Gene Mutations

For 454 sequencing, genomic DNA extracted from tumor (DES1002) and normal control tissue from the same patient was used for PCR using primers with 454 tails (CGTATCGCCTCCCTCGCGCCATCAGTCTCAGGATATGAATTAGCTTATTTAGTTG and CTATGCGCCTTGCCAGCCCGCTCAGTACCCTCCACAAAGCACACACATATTAGTT). For MiSeq sequencing, biotinylated probes covering *CTNNB1* exon 3 were used to prepare samples. In both cases, PCR was performed using the KAPA HiFi HotStart DNA polymerase.

In 454 sequencing, libraries were hybridized to the 454FLX platform. Between 1500 and 2000 reads per amplicon were generated using a 454 FLX platform (Roche). Alignment and variant detection was alternatively carried out both with ssahaSNP (Sanger Institute) and with a pipeline that combined BWA alignment, Picard tools, and VarScan (Koboldt, et al. 2009) variant detection.

In MiSeq sequencing, libraries were hybridized to the Illumina MiSeq platform (IDT Xgen lockdown protocol). Results were analyzed using BWA alignment and the Haplotype mutation caller (GATK) (Ho, et al. 2013).

#### Array Comparative Genomic Hybridization (CGH) and Molecular Cytology

Genomic DNA was analyzed with Agilent 1M oligonucleotide arrays according to the manufacturer's instructions. A DNA reference set [Human genomic DNA from blood (buffy coat); Roche Applied Science] was applied in conjunction with DNA prepared from tumor to allow for competitive hybridization. Array CGH data were processed using a normalization method that corrects for GC artifacts and then segmented using the standard CBS segmentation algorithm. Gene-level copy number calls were determined using the R-package algorithm Copynumber (Nilsen, et al. 2012).

Fluorescent *in situ* hybridization (FISH) was performed with a 3-color probe comprising centromeric repeat plasmids for chromosomes 6, 7, and 17. Tissue sections were de-waxed and hybridized according to standard procedures. FISH signals were scored for a minimum of 100 nuclei.

#### Statistical Analysis

Clinicopathologic and immunohistochemical characteristics were compared across subgroups of desmoids defined according to underlying mutation using Fisher's exact test analysis on all samples. Univariate analysis of recurrence-free survival stratified based on mutation status was performed for patients undergoing R0 or R1 resection using Kaplan-

Meier analysis and log rank test; the corresponding multivariate analysis was performed using Cox regression analysis.

## RESULTS

### Sanger Sequencing of *CTNNB1* in Desmoid-type Fibromatosis

Sanger sequencing of *CTNNB1* exon 3 was performed on sporadic desmoids resected from 117 individual patients (Tables S1, S2). *CTNNB1* T41A (n=55; 46%), S45F (n=35; 29%), and S45P (n=8; 6.7%) mutations were identified in 98 patients (82%), a proportion similar to that in previous reports. Two patients had deletions in exon 3 of *CTNNB1* (H36del and A39-G48del) and another had a deletion (A39del) as well as T41A and T40A mutations; based on the structure of  $\beta$ -catenin, all these mutations are expected to be activating. The remaining 16 patients (13%) had no discernible mutation in *CTNNB1* exon 3 as assessed by Sanger sequencing ('wild-type' tumors; Figure S1).

### Clinicopathologic Comparison of 'Wild-type' and Mutated Desmoids

Patients with 'wild-type' tumors were similar to subsets of patients with each defined *CTNNB1* mutation (i.e., T41A, S45F, S45P, or deletion) in terms of age, gender, tumor size, and primary vs recurrent presentation status (all  $p>0.1$ ; Table S1). Mutation was, however, highly correlated with tumor site ( $p=0.006$ ). Tumors with S45F mutation were most often localized to the extremity (57%), T41A-associated tumors to the abdominal cavity (44%), and 'wild-type' tumors to the abdominal wall (38%).

Immunohistochemistry was used to examine  $\beta$ -catenin in 40 desmoids. Twenty-one (52%) had nuclear  $\beta$ -catenin staining (Figure S2). Nuclear staining was positive in 4 of 8 (50%) 'wild-type' tumors and in 17 of 32 tumors (53%) with known *CTNNB1* mutation (4 of 11 with S45 mutation, 12 of 18 with T41A mutation, 1 of 2 with S45P mutation, and not in the tumor with H36del). Frequency of nuclear staining did not differ significantly between subgroups of tumors with each unique mutation ( $p=0.44$ ) or between 'wild-type' tumors and those with all defined mutations (0.874), suggesting that  $\beta$ -catenin signaling is dysregulated in both mutated and 'wild-type' tumors.

Similar to results observed in clinicopathologic analyses, gene expression profiles did not clearly define differences between the *CTNNB1* mutant and 'wild-type' desmoid-type fibromatosis. RNA isolated from 45 tumors and 16 normal mesenchymal tissue samples (8 fat and 8 muscle) was analyzed using U133A 2.0 expression arrays. Unsupervised clustering demonstrated that all the desmoids clustered separately from normal tissue (Figure S3), but 'wild-type' tumors did not cluster separately from *CTNNB1* mutant tumors. Similarly, unsupervised clustering of the tumors alone did not separate the two tumor subsets (Figure 1).

Finally, patient outcomes were analyzed in tumors designated 'wild-type' based on Sanger sequencing and those with Sanger-identified *CTNNB1* mutations. Median follow-up (from the time of initial resection at Memorial Sloan Kettering) was 40 months. No significant differences in local recurrence rates were observed between patients with T41A, S45F, or S45P *CTNNB1* mutations as compared to those with none of these three mutations identified

by Sanger sequencing (Figure S4A). Patients with ‘wild-type’ tumors tended to have longer recurrence-free survival than those with S45F mutant tumors during early follow-up, but this association was not clearly sustained at later time points and did not reach significance in univariate analysis ( $p=0.17$ ). In a multivariate analysis that included mutation status and tumor site, the association between mutation status and recurrence-free survival was clearly non-significant ( $p>0.4$ ), whereas tumor site was strongly associated with recurrence-free survival (multivariate hazard ratio 3.56 for extremity vs non-extremity;  $p=0.013$ ) (Table 1). We also analyzed the subset of 80 patients who underwent resection for primary desmoids (Figure S4B; Table 1), and the results were nearly identical to those for the entire cohort. In particular, there continued to be no significant difference in recurrence-free survival for patients with T41A or S45F mutation compared to those with ‘wild-type’ tumors as defined by Sanger sequencing.

### Whole-exome Sequencing of Desmoid-type Fibromatosis

To further characterize the genomic events underlying initiation in ‘wild-type’ desmoids, whole-exome sequencing was performed on DNA from eight of the ‘wild-type’ tumors, eight of the tumors with *CTNNB1* point mutations, and one tumor with H36del mutation. On average 29 Mb was sequenced for each tumor, and 87% of exons were captured at a depth of 88x or greater. The number of non-synonymous mutations detected per tumor ranged from 4 to 29, with less than one mutation detected per Mb (Figure S5, Table S3). This corresponded to 249 somatically mutated genes, with 45 reaching statistical significance as determined by the MutSig algorithm ( $q<0.1$ ; Figure 2) (Lawrence, et al. 2013).

Surprisingly, the whole-exome sequencing detected mutations in *CTNNB1* in three of the eight ‘wild-type’ samples. In each case, the mutation was present at a low mutant allele frequency (10%, 10%, and 21%), presumably below the limit of detection for Sanger sequencing. In two other tumors, somatic mutations in the *APC* gene were identified. Thus, five of the eight ‘wild-type’ desmoids (62%) had *CTNNB1* or *APC* mutation (Table 2).

Of the three remaining ‘wild-type’ tumors analyzed by whole-exome sequencing, two had no somatic mutations that can be clearly linked to regulation of Wnt/ $\beta$ -catenin signaling, but one had a missense mutation affecting the *BMI1* gene (Q59E; 8.8% of reads). *BMI1* activates Wnt signaling by downregulation of the DKK family of proteins (Cho, et al. 2013). The mutation in *BMI1* was confirmed by 454 sequencing (3% of reads in tumor, but absent in normal blood from the same patient).

### Copy Number Alterations in ‘Wild-type’ Desmoid-type Fibromatosis

Whole-exome data on ‘wild-type’ and *CTNNB1* desmoids were analyzed to identify potential fusion proteins and copy number alterations that may affect tumorigenesis. No recurrent fusions were identified (Table S4), but a few recurrent copy number alterations were identified (Figure S6). In both of the wild-type tumors with identified mutation in *APC*, we also detected copy number loss in a stretch of chromosome 5 that encompasses *APC*, suggesting a ‘second hit’ contributing to desmoid initiation. In the two tumors with no definable mutation in genes regulating Wnt signaling (*CTNNB1*, *APC*, or *BMI1*), the analysis demonstrated chromosome 6 loss, an event previously associated with



medulloblastomas with activated Wnt signaling cascades. This finding was supported by both array CGH (Figure 3) and FISH analysis with probe directed to the centromere of chromosome 6, with chromosome 7 and 17 centromere probes as controls (Figure S7). In the tumor DES999, 89% of nuclei had 0 or 1 signals detected for chromosome 6, compared to 69% for chromosome 7 and 77% for chromosome 17. In the other tumor, DES955, the percentages of nuclei with 0–1 signals detected were 81%, 62%, and 61% for chromosomes 6, 7, and 17, respectively.

### Directed Genomic Analysis of Additional ‘Wild-type’ Desmoids

In light of the whole-exome results described above, we sequenced *CTNNB1* exon 3 using the MiSeq platform in eight additional ‘wild-type’ desmoid samples (i.e., the desmoids identified as ‘wild-type’ in our initial Sanger sequencing that were not analyzed by whole-exome sequencing). Average coverage of exon 3 in the region of common *CTNNB1* mutations was greater than 2300x. Consistent with our whole-exome analysis, four of the eight tumors had *CTNNB1* mutations: three T41A (6%, 16%, and 33% of reads) and one A39V (1% of reads). Of note, repeat Sanger sequencing performed on the sample with 33% mutated reads again failed to identify the mutation by this traditional method (Figure S8).

The four remaining samples were analyzed by array CGH to look for loss of *APC* or deletion of chromosome 6. One of these samples had loss of *APC*. Therefore, five of this set of eight ‘wild-type’ samples had genomic events affecting *CTNNB1* or *APC*. Taken together with whole-exome sequencing and MiSeq data, this result demonstrates that ten of 16 ‘wild-type’ samples in the study cohort and 111 of 117 total samples (95%) had *CTNNB1* mutation, *APC* mutation, and/or *APC* loss. In general, those tumors with *CTNNB1* mutation detected by whole exome sequencing or MiSeq had lower mutant allele frequencies than did those tumors with mutation detected by Sanger sequencing.

Having refined our knowledge of which tumors were *CTNNB1* mutant and which wild type, we re-analyzed the associations of *CTNNB1* mutation status, now defined by next-generation sequencing platforms, with tumor site and with patient outcome. Mutation status persisted in being highly correlated with site ( $p=0.0015$ ). Though only one patient with true wild-type genotype (defined by whole exome or MiSeq analysis) had a recurrence, *CTNNB1* wild-type genotype was not clearly associated with improved recurrence-free survival even on univariate analysis (hazard ratio 0.31 and  $p=0.26$  for wild-type vs S45F mutation; hazard ratio 0.77 and  $p=0.80$  for wild-type vs T41A mutation). This may reflect the rarity of the wild-type genotype and the consequent limited power of this analysis. In fact, it may not be feasible to characterize true ‘wild-type’ lesions as defined by next-generation sequencing since these tumors may be driven by a range of extremely rare genomic events.

## DISCUSSION

Although disruptions of the Wnt/ $\beta$ -catenin pathway are believed to underlie pathogenesis of most desmoids, in multiple studies approximately 15% of desmoids have shown no aberrations in *CTNNB1* or *APC* (Alman, et al. 1997b; Salas, et al. 2010). To determine what molecular events underlie pathogenesis in these ‘wild-type’ desmoids, we undertook a genomic analysis of 117 desmoids, with in-depth, multiplatform analysis being performed

on 16 with no mutation in *CTNNB1* as assessed by Sanger sequencing. Our gene expression and clinicopathologic results were consistent with the idea of a common oncogenic pathway in *CTNNB1* mutant and ‘wild-type’ desmoids as assessed by Sanger sequencing. When gene expression profiles were examined, the ‘wild-type’ tumors did not cluster separately from other desmoids, ‘wild-type’ lesions did not have a different clinical behavior from tumors with either a *CTNNB1* mutation, and a substantial number of ‘wild-type’ tumors stained for nuclear  $\beta$ -catenin, as do tumors with classic mutations in the Wnt pathway components. Of note, rates of IHC positivity were lower than those commonly reported in the literature, potentially because staining was done on a tissue microarray, and the small samples combined with heterogeneous staining observed for  $\beta$ -catenin may affect sensitivity of the stain.

*APC* loss, previously observed in sporadic desmoids, was identified in three of these ‘wild-type’ desmoids (Alman, et al. 1997b). Surprisingly, however, deep sequencing clearly demonstrated that *CTNNB1* mutation was present in seven of the ‘wild-type’ tumors, albeit generally at lower frequency than in the tumors in which a *CTNNB1* mutation was detected by traditional Sanger sequencing. One mutation, A39V, has not commonly been observed in desmoids. These results demonstrate that a larger proportion of desmoids than previously recognized have an activating *CTNNB1* exon 3 mutation or *APC* loss (95% in this study as compared to ~85% in previous reports). This finding has important implications for attempts to use mutation status to predict patient outcomes. A subset of recent studies has suggested that desmoids without mutations in *CTNNB1* have lower local recurrence rates than those with detectable mutation (Lazar, et al. 2008; Colombo, et al. 2013) though this has not been universally observed (Mullen, et al. 2013). Our study suggests that standard laboratory testing may not be accurate in defining ‘wild-type’ mutation status, and difference in methodologies used to detect mutations may also underlie variation between studies on the prognostic significance of ‘wild-type’ designation.

It should be noted that many of the desmoids we analyzed by next-generation sequencing, despite being prepared from cryomolds that were dissected to remove contaminating tissues, had *CTNNB1* mutation in only a small number of reads (e.g., 6%). This suggests a significant amount of intratumoral heterogeneity, which could represent infiltration of the tumors by normal stromal or immune cells. Given the relatively homogeneous histologic appearance of desmoid-type fibromatosis, however, and the fact that not all tumor cells within a section stain for  $\beta$ -catenin, it is also possible that our findings represent mosaicism within at least some desmoids. Similar findings have been observed in subsets of endochondromas and spindle cell hemangiomas (*IDH1* mutations) and osteochondroma (*EXT1* and *EXT2* mutations) (Bovee 2010; Pansuriya, et al. 2011). Some of the tumors with infrequent mutations detected by whole-exome sequencing or MiSeq showed significant heterogeneity in nuclear  $\beta$ -catenin, but this was also observed in many of the samples with mutations detected by Sanger sequencing (data not shown), so it is difficult to draw definitive conclusions from these data.

Wnt signaling in neoplasms can be affected by mutations other than the canonical abnormalities in *CTNNB1* and *APC* (e.g., *AXIN2* and *RNF43* mutations) (Liu, et al. 2000; Giannakis, et al. 2014). Although we detected no genomic changes in *AXIN2* or *RNF43*, we



did detect other aberrations that could disrupt Wnt/ $\beta$ -catenin signaling in three of the six ‘wild-type’ tumors without *CTNNB1* mutation or *APC* loss. One tumor had mutation of *BMII* (a regulator of the DKK family of Wnt inhibitors), and two had loss of chromosome 6, an event exclusively associated with subsets of medulloblastoma with known Wnt activation (Clifford, et al. 2006; Cho, et al. 2013). Thus, among 117 desmoids, only three were not shown to have genetic aberrations hypothesized to affect Wnt/ $\beta$ -catenin signaling. Because these three tumors had not undergone whole-exome sequencing, it remains possible that they had mutations in *APC*, *BMII*, or other regulators of the Wnt/ $\beta$ -catenin pathway.

Systemic therapies have been of little efficacy in patients with desmoid-type fibromatosis, and those, such as sorafenib and adriamycin, that can effect responses do so by unclear mechanisms. Although we lack clinically effective Wnt and  $\beta$ -catenin inhibitors, given the near universal finding genomic events altering Wnt/ $\beta$ -catenin signaling molecules, it is obvious that future studies should seek to understand the signaling aberrations induced by the Wnt/ $\beta$ -catenin pathway in desmoid-type fibromatosis. This may allow us to develop predictive markers for response to treatments already in clinical use or to develop novel therapeutics.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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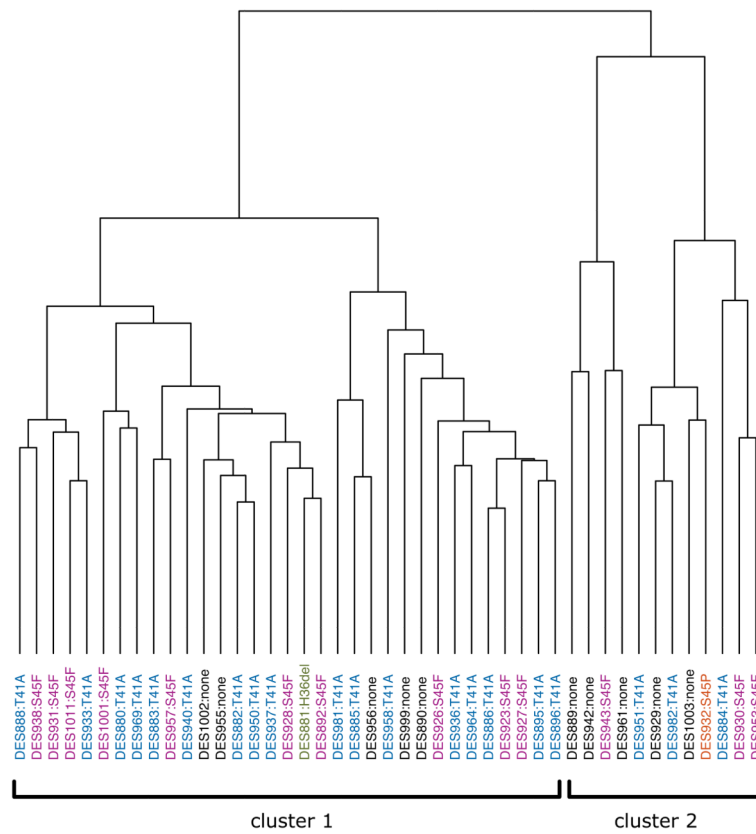
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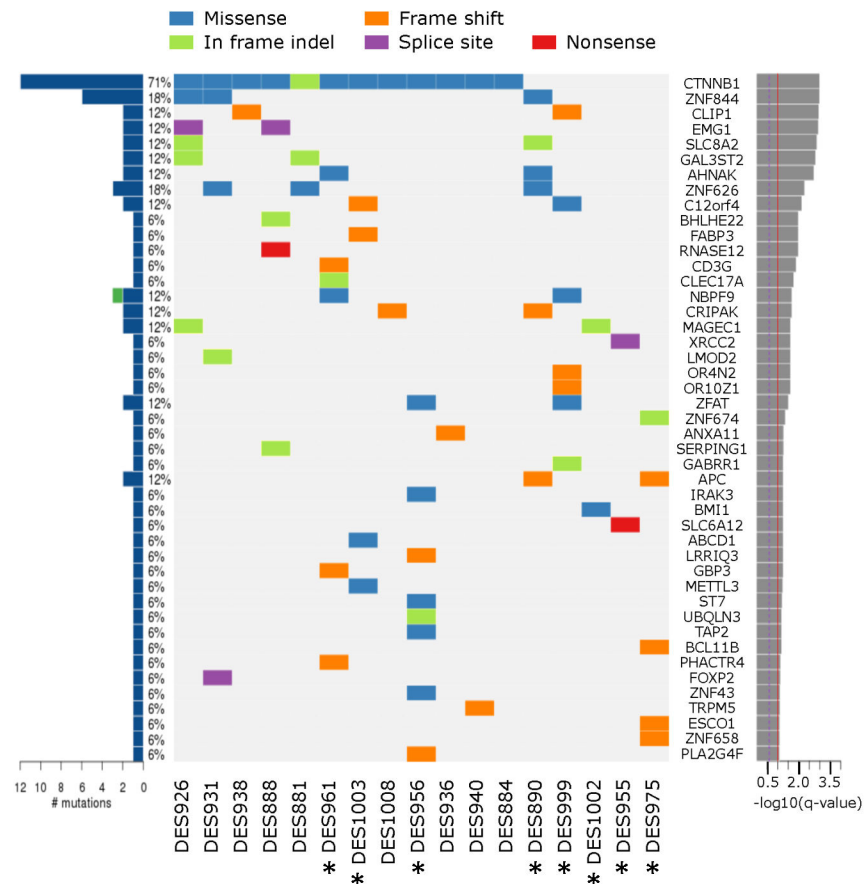
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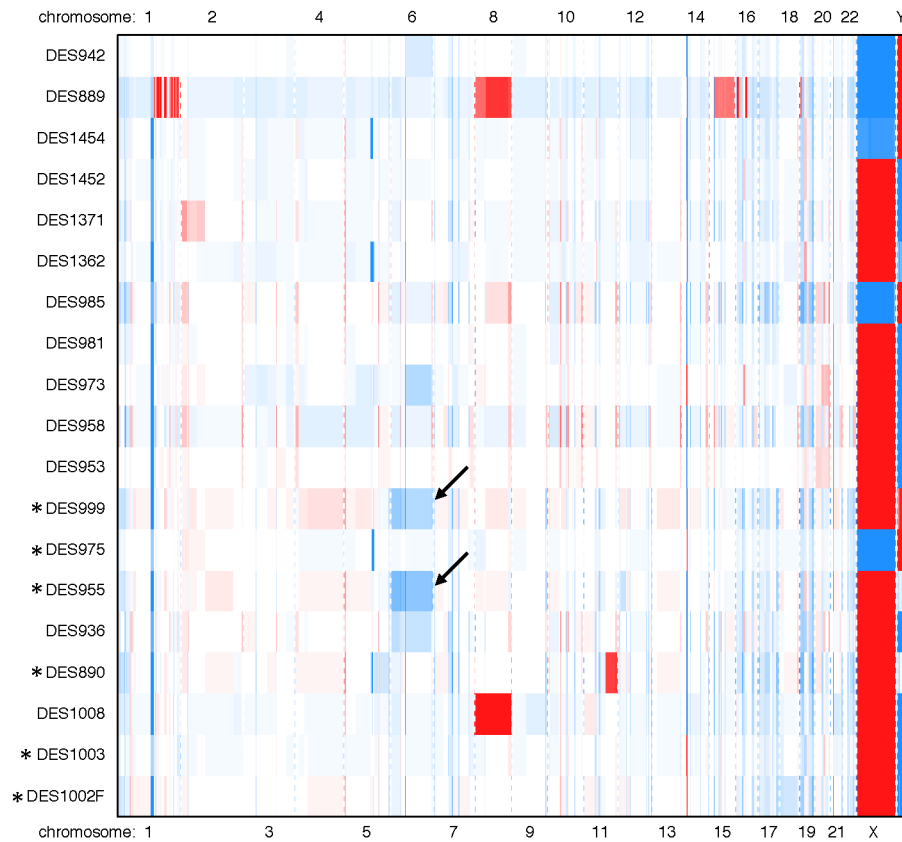
**Figure 1.** Unsupervised clustering of desmoid-type fibromatoses based on the 1% of genes whose expression was most variable between tumors. ‘Wild-type’ tumors are indicated with black text; tumors with *CTNNB1* detected by Sanger sequencing are indicated with colored text.



**Figure 2.**

Somatically mutated genes in desmoid tumors reaching statistical significance. Desmoids designated 'wild-type' by Sanger sequencing are annotated (\*). The bar chart at the left shows the number of mutations found and the percentage of tumors affected; blue indicates non-silent mutations and green denotes the one silent mutation found in these genes (in *NBPF9*). For *ZNF844*, six mutations were found in three tumors. The center panel shows the types of non-silent mutations in each tumor. The bar graph at right shows the statistical significance of each mutated gene (derived from MutSig), with the red line indicating the threshold for significance ( $q < 0.1$ ).





**Figure 3.**

Copy number alterations in desmoid tumors as identified from array CGH results by the R-package algorithm copynumber. Desmoids designated ‘wild-type’ by Sanger sequencing are annotated (\*). Blue indicates copy number loss and red indicates gain. Chromosome 6 loss was found in two tumors with no identifiable abnormality in *CTNNB1* or *APC* (DES955, DES999; see arrows) as well as in two tumors with *CTNNB1* mutation.

**Table 1**

Analyses of factors prognostic for local recurrence in desmoid-type fibromatoses.

	Univariate		Multivariate	
	Hazard ratio	p	Hazard ratio	p
<i>All patients</i>				
<i>Mutation (vs. none)*</i>				
T41A	0.76	0.64	0.92	0.89
S45F	2.15	0.17	1.59	0.41
<i>Site (vs. non-extremity)</i>				
Extremity	3.56	<0.001	2.79	0.013
<i>Patients with primary tumors only</i>				
<i>Mutation (vs. none)*</i>				
T41A	0.76	0.65	–	–
S45F	2.15	0.17	–	–

\* mutation status as defined by Sanger sequencing

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**Table 2**

Whole-exome sequencing of desmoids

Sample	<i>CTNNB1</i> mutation By Sanger	Whole-exome sequencing results (allele frequency)		
		<i>CTNNB1</i> mutation	<i>APC</i> mutation	Other event noted
DES1008	S45F	S45F (32%)	None	
DES931	S45F	S45F (32%)	None	
DES938	S45F	S45F (46%)	None	
DES926	S45F	S45F (38%)	None	
DES884	T41A	T41A (36%)	None	
DES888	T41A	T41A (33%)	None	
DES936	T41A	T41A (22%)	None	
DES940	T41A	T41A (54%)	None	
DES881	H36del	H36del (30%)	None	
DES1003	None	T41A (10%)	None	
DES956	None	T41A (21%)	None	
DES961	None	T41A (10%)	None	
DES890	None	None	I1918fs (61%)	<i>APC</i> loss
DES975	None	None	K1462fs (70%)	<i>APC</i> loss
DES955	None	None	None	Chr 6 loss
DES999	None	None	None	Chr 6 loss
DES1002	None	None	None	<i>BMI1</i> Q59E (8%)