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Dermatofibrosarcoma protuberans with a novel *COL6A3-PDGFD* fusion gene and apparent predilection for breast

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Abstract

Dermatofibrosarcoma protuberans is a locally aggressive superficial mesenchymal neoplasm. It typically occurs in adulthood, and has been reported to have a slight male predilection. Tumors have a characteristic histopathologic appearance, including: storiform architecture, infiltrative “honeycomb” growth within subcutaneous adipose tissue, and immunoreactivity for CD34. Virtually all molecularly characterized cases to date have been found to harbor a *COL1A1-PDGFB* fusion product. Following identification of an index patient with a novel *COL6A3-PDGFD* fusion gene, we undertook a molecular investigation, using a combination of RNA sequencing and fluorescence in situ hybridization (FISH), to assess the prevalence of *PDGFD* rearrangement in dermatofibrosarcoma protuberans (N = 63). Three additional patients were found to have balanced *PDGFD* rearrangements. Interestingly, all 4 tumors arose on the breast of females. As a result, we subsequently examined 16 additional cases of primary breast dermatofibrosarcoma protuberans, identifying 2 additional tumors with *PDGFD* rearrangement. The morphology and immunophenotype of all 6 cases was analogous to those with the canonical *COL1A1-PDGFB* fusion; none of the cases showed fibrosarcomatous transformation. This study illustrates that the *COL6A3-PDGFD* fusion product is rare in dermatofibrosarcoma protuberans, and associated with an apparent predilection for breast. An awareness of this variant is important for pathologists, as it will not be detected using conventional reverse transcription polymerase chain reaction or FISH-based diagnostic assays for dermatofibrosarcoma protuberans.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Keywords

breast; COL1A1; COL6A3; dermatofibrosarcoma protuberans; PDGFB; PDGFD

1 | INTRODUCTION

Dermatofibrosarcoma protuberans is a fibroblastic neoplasm originating in the dermis and/or superficial subcutis.¹ It may arise throughout the body, but most commonly involves the trunk and extremities.² Tumors typically occur in early-to-mid adulthood, although it is known to have a wide range of involvement^{2,3}; it is reported to have a slight male predilection.^{2,3} The clinical behavior is generally characterized by local aggressiveness.¹ Complete surgical resection, with widely negative margins, is generally curative.²

Morphologically, most tumors are composed of spindle cells in a prominent storiform/whorled pattern.^{2,3} Peripherally, there is often infiltration of subcutaneous adipose tissue yielding a characteristic “honeycomb” pattern. Tumors tend to spare adnexal structures. The cells are monomorphic and generally have only minimal mitotic activity.² Multiple morphologic variants exist, including, for example, those associated with melanin pigmentation (Bednar tumor),⁴ giant cells,^{5,6} prominent myxoid stroma,⁷ and myoid differentiation.⁸ A subset of cases may undergo fibrosarcomatous transformation (fibrosarcoma ex-dermatofibrosarcoma protuberans) which is associated with frequent local recurrence (58%), as well as metastasis (15%)⁹; in addition to areas of conventional dermatofibrosarcoma protuberans, these tumors tend to have a fascicular-herringbone architecture, and increased cellularity and mitotic activity.⁹ Additionally, a pleomorphic variant of dermatofibrosarcoma protuberans has recently been reported.¹⁰ Most tumors exhibit diffuse immunoreactivity for CD34,¹¹ but this may be diminished-to-absent in a proportion of cases with fibrosarcomatous transformation¹²; myoid differentiation is highlighted by smooth muscle actin.⁸ Virtually all molecularly characterized cases to date have been reported to have a *COL1A1-PDGFB* fusion gene. The concomitant chimeric protein causes autocrine activation of the PDGFRB protein-tyrosine kinase, which leads to disinhibited cell proliferation¹³; consequently, in advanced cases, this provides a rationale for targeted therapy using tyrosine kinase inhibitors, such as imatinib.

Clinical diagnostic testing for *PDGFB* rearrangement in dermatofibrosarcoma protuberans is generally performed by fluorescence in situ hybridization (FISH), since the wide variability in *COL1A1* genomic breakpoints makes routine testing by the reverse transcriptase polymerase chain reaction (RT-PCR) impractical. Recently, targeted next-generation sequencing—particularly in the context of limited sampling, and rare or novel fusion products—has emerged as a reliable and useful molecular diagnostic technique. Herein, we report an index case of dermatofibrosarcoma protuberans with a typical morphology and immunoprofile; however, diagnostic RNA sequencing (RNA-Seq) identified a novel *COL6A3-PDGFD* fusion gene. We subsequently investigated a series of cases of dermatofibrosarcoma protuberans to characterize the prevalence of this novel fusion variant.

2 | MATERIALS AND METHODS

2.1 | Cases

A novel *COL6A3-PDGFD* fusion transcript was identified by RNA-Seq in the course of routine diagnostic testing of the index tumor (Patient A1); consequently, where feasible, subsequent cases that were morphologically and immunophenotypically compatible with dermatofibrosarcoma protuberans—encountered at Mount Sinai Hospital (RNA-Seq) and Memorial Sloan Kettering Cancer Center (FISH) over approximately a 12-month period—were assessed to determine fusion gene status (Cohort A). As a result of these initial findings, a retrospective archival review was then performed at each of the three participating institutions for cases of dermatofibrosarcoma protuberans specifically arising from the breast and/or upper chest (Cohort B). This study was performed following institutional Research Ethics Board approval.

2.2 | Immunohistochemistry

Formalin-fixed paraffin-embedded tissue blocks were cut at 4 µm and stained for CD34, desmin, smooth muscle actin, S100, and keratin (AE1/AE3) using standard techniques (Supporting Information Table S1). Appropriate on-slide positive controls were used throughout. Scoring of tumors was based on the percentage of positive cells (0: no staining; 1+: <5%; 2+: 5%–25%; 3+: 26%–50%; 4+: 51%–75%; and 5+: 76%–100%).

2.3 | RNA sequencing

Total RNA was extracted from formalin-fixed paraffin-embedded tissue scrolls (3–4 per case) using the ExpressArt FFPE Clear RNA Ready kit (Amsbio, Cambridge, MA); it was assessed using the RNA 6000 Nano Bioanalyzer Kit (Agilent, Mississauga, ON) and quantitated using the Qubit RNA HS Assay Kit (ThermoFisher Scientific, Mississauga, ON). An input of 20–100 ng total RNA and the TruSight RNA Fusion Panel were used to prepare the RNA-seq libraries (Illumina, San Diego, CA), following manufacturer's instructions and as previously described.¹⁴ Sequencing of each sample was performed with 76 base-pair paired-end reads on an Illumina MiSeq at 8 samples per flow cell (~3 million reads per sample). The results were then analyzed using the STAR and BOWTIE2 aligners, and Manta and JAFFA fusion callers, respectively.^{15,16}

The expression profile of the *COL6A3-PDGFD* positive tumors were compared with 34 conventional *COL1A1-PDGFB* positive tumors available on the same RNA-Seq platform (Table 1); the latter had a morphology and immunophenotype typical of dermatofibrosarcoma protuberans. In addition, an unsupervised hierarchical clustering was performed, which further included a large number of other sarcoma types that has been tested on the same platform (these cases constituted routine clinical cases of sarcoma diagnostically evaluated for the presence of an underlying fusion gene event). The mRNA levels of genes of interest were also examined, including *PDGFB*, *PDGFRA*, and *PDGFRB*, among others.

2.4 | Fluorescence in situ hybridization

Fluorescence in situ hybridization for *PDGFB* and *PDGFD* was performed as previously described in detail (Supporting Information Table S2).¹⁷ Custom bacterial artificial chromosome (BAC) clone probes flanking the target genes were designed using the UCSC genome browser (<http://genome.ucsc.edu>), and obtained from BAC-PAC sources of Children's Hospital of Oakland Research Institute (Oakland, CA; <http://bacpac.chori.org>). DNA from each BAC probe was labeled with fluorochromes by nick translation. Formalin-fixed paraffin-embedded tissue sections were deparaffinized, pretreated, and then hybridized with the denatured probes. Following overnight incubation, the slides were rinsed, stained with 4',6-diamidino-2-phenylindole (DAPI), mounted, and analyzed using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany).

3 | RESULTS

3.1 | Dermatofibrosarcoma protuberans with the novel *COL6A3-PDGFD* fusion variant has a predilection for breast

Following discovery of a novel *COL6A3-PDGFD* fusion variant (Patient A1), 62 subsequent cases of dermatofibrosarcoma protuberans were molecularly investigated (N = 63; RNAseq = 43, FISH = 20) over approximately a 1-year interval (Cohort A). The average patient age was 43 years (range, 16–83). There were 37 females and 26 males (ratio, 1.4:1). Common sites of involvement included: back (N = 9), abdomen (N = 7) and groin (N = 5), chest (N = 7) and breast (N = 7), upper arm (N = 7), and thigh (N = 6) (Table 1). Fifteen cases had fibrosarcomatous transformation, and two were pigmented.

Of the 63 tumors examined, suitable RNA and/or DNA were obtained in all cases (100%). Four (6.3%) tumors, including the index patient, contained *PDGFD* rearrangement; in 3 cases testing was performed by RNA-Seq, which revealed a novel *COL6A3-PDGFD* fusion variant. Fifty-eight patients (92%) possessed conventional *PDGFB* rearrangement; in the samples tested by RNA-Seq, all were found to contain the canonical *COL1A1-PDGFB* fusion product (Table 1). A single tumor lacked any known fusion gene by RNA-Seq.

Having observed that all four cases with *PDGFD* rearrangement arose on the breast, FISH for both *PDGFB* and *PDGFD* was subsequently performed on a second cohort of dermatofibrosarcoma protuberans specifically arising on the breast or upper chest (Cohort B). A total of 15 cases were identified. All were females; and, the average age was 41 years (range, 26–79). Two additional cases were found to harbor *PDGFD* rearrangement (13.3%); 12 patients possessed conventional *PDGFB* rearrangement (80%) and one patient was negative for both *PDGFB* and *PDGFD* rearrangement. Overall, based on the combined cohorts, a total of six patients were found to have the variant *PDGFD* fusion partner; each case assessed by RNA-Seq identified *COL6A3* as the fusion partner. All *PDGFD* gene rearrangements detected by FISH had a balanced pattern, with no associated losses or gains/amplifications being noted.

3.2 | Dermatofibrosarcoma protuberans with the *COL6A3-PDGFD* fusion variant has typical histologic and immunohistochemical features, but often lacks dermal involvement

All six tumors containing *PDGFD* rearrangement occurred in females, with an average age of 39 years (range, 22–58)—all arose on the breast. Clinically, none were reported to be protuberant and the average size was 1.5 cm (range, 1.0–2.1 cm). Morphologically, most of the cases were centered in subcutaneous fat. Epidermis was represented in only a single case, and noted to be widely uninvolved; a single case involved dermal collagen (Patient B2); and, a single case infiltrated around breast glandular epithelium (Patient A2). A single case contained a prominent ectatic thin-walled vasculature (Patient B1). In each of the tumors the morphology was similar to those with conventional *PDGFB* rearrangement. The tumors were comprised of sheets of spindle cells with a prominent storiform pattern, and frequently contained peripheral interdigitation amongst lobules of adipose tissue yielding the so-called “honeycomb” pattern. The cytoplasm was scant and pale eosinophilic to amphophilic. The nuclei were ovoid, and variably elongated and monomorphic; mitotic activity averaged 0.8 per 10 HPFs (range, 0–2 per 10 HPFs). None of the cases showed fibrosarcomatous transformation, pigmentation or myoid nodules.

The immunophenotype was likewise similar to conventional dermatofibrosarcoma protuberans. All of the cases diffusely (5+) expressed CD34 (Figure 1); they were negative for desmin, smooth muscle actin, S100, and keratin.

The two cases lacking a fusion gene were likewise morphologically and immunohistochemically typical of dermatofibrosarcoma protuberans.

3.3 | Dermatofibrosarcoma protuberans with the *COL6A3-PDGFD* fusion variant clusters together with *COL1A1-PDGFB* fusion positive tumors, and shows upregulation of *PDGFRB* expression

Among the six cases positive for the *PDGFD* rearrangement, three were tested by RNA-Seq, which showed breakpoints in *COL6A3* exon 42 (Patients A1 and A3) and exon 43 (Patient A2) (NCBI Reference Sequence: NM_004369.3) which were fused to *PDGFD* exon 6 (NM_025208.4) (Figure 2A,B). Rearrangement of *PDGFD* was independently confirmed by fluorescence in situ hybridization in all six cases (Figure 1). No unbalanced *PDGFD* rearrangements or amplifications were noted.

By unsupervised hierarchical clustering using RNA-Seq data, the three cases with the *COL6A3-PDGFD* fusion clustered amongst the larger group of dermatofibrosarcoma protuberans with the typical *COL1A1-PDGFB* fusion, and away from other soft tissue sarcomas available on the same targeted RNA sequencing platform (Figure 2C). Specifically, transcripts were selected above a 20 RPKM threshold expression (calculated by mrQuantifier), followed by ward.D2 hierarchical cluster analysis and visualized on Dendroscope. The *PDGFD* gene was not one of the genes represented in our RNA sequencing panel, thus we were not able to evaluate the *PDGFD* mRNA expression in our cohort. As expected, *PDGFB*-positive tumors showed overexpression of *PDGFB* (Figure 2D), while *PDGFD*-fusion positive tumors did not. However, both fusion-variants showed

increased mRNA expression of *PDGFRB*, while no significant increase of *PDGFRA* was present (Figure 2D) compared with other sarcoma types.

4 | DISCUSSION

Dermatofibrosarcoma protuberans is a relatively common superficial sarcoma, which involves diverse anatomic sites and patients across a broad age range. Tumors have a characteristic clinical and histopathologic appearance. Moreover, to date, almost all molecularly characterized cases have been found to possess a *COL1A1-PDGFB* fusion gene. Herein, we report a novel *COL6A3-PDGFD* fusion variant in dermatofibrosarcoma protuberans, which appears to have a predilection for breast.

Darier and Ferrand,¹⁸ in 1924, are credited with first describing dermatofibrosarcoma protuberans, although earlier reports of the lesion appear to exist.¹⁹ Tumors have long been recognized for their distinct clinical presentation and locally aggressive course^{2,3}; the latter initially prompted debate as to whether this merited designation as a true malignancy.³ While the characteristic storiform morphology, adnexal-sparing, and “honeycomb” pattern of subcutaneous infiltration has long-been appreciated,^{2,3} it was not until the early 1990s that immunoreactivity for CD34 was recognized as distinguishing dermatofibrosarcoma protuberans from related neoplasms.¹¹ Early cytogenetic studies described a supernumerary ring chromosome in a subset of cases²⁰; this subsequently led to the identification of the t(17;22)(q22; q13) event.^{21–23} Ultimately the *COL1A1-PDGFB* fusion gene was cloned, which fairly consistently pairs exon 2 of *PDGFB* with numerous potential exons of *COL1A1*.¹³ This fusion product has been identified in 85%–96% of cases.^{24–30} The incidence appears to increase with the application of more sensitive detection assays; nevertheless, this still implies that a subset of cases may have an alternate fusion gene. This is supported by cytogenetic studies which raise the possibility of other translocation events, including: t(2;17)(q33;q25),³¹ t(9;22)(q32;q12.2),³² t(5;8)(q13–14;p21),³³ and t(X;7)(q21.2;q11.2).³⁴ None of these fusions encompass *PDGFD* or *COL6A3*, suggesting these may represent additional potential fusion partners in dermatofibrosarcoma protuberans. Recently an alternative *PDGFB* fusion partner was identified—*COL1A2-PDGFB* in a tumor that was otherwise typical of dermatofibrosarcoma protuberans.²⁹

In the routine work-up of a case of dermatofibrosarcoma protuberans using RNA-Seq we discovered a novel *COL6A3-PDGFD* fusion variant. We proceeded to test a cohort of 63 patients, identifying *PDGFD* rearrangement in four (6.3%) tumors, while 58 (92%) were confirmed to possess the canonical *PDGFB* rearrangement (Table 1). Rather surprisingly, all four patients with the variant fusion were women, and each tumor originated from the breast as a non-protuberant, but palpable, subcutaneous mass. As a result, a second cohort of patients—specifically, those with dermatofibrosarcoma protuberans occurring on the breast/chest—was investigated to determine whether this anatomic site may be enriched for the variant *PDGFD* fusion. A total of 15 additional cases were examined, with two additional cases found to harbor *PDGFD* rearrangement (13.3%) (Table 1); 12 contained conventional *PDGFB* rearrangement (80%); and, one patient was negative for both *PDGFB* and *PDGFD* rearrangement. All 6 patients with the *PDGFD* variant fusion were female (100%) and all tumors were located on the breast (100%), predominantly the left (83%). Three of these

cases were tested by RNA-Seq, which established *COL6A3* as the *PDGFD* fusion partner. Each of these six cases was morphologically typical of dermatofibrosarcoma protuberans, though dermal involvement could only be documented in a single case. All cases had prominent storiform growth, infiltration of subcutaneous adipose tissue with a “honeycomb” pattern, and minimal nuclear atypia and mitotic activity; all of the cases exhibited diffuse CD34 expression (Figure 1). In addition to facilitating discovery of the variant *COL6A3-PDGFD* fusion gene, RNA-Seq in this series identified two cases with *PDGFB* rearrangement that were found to be negative by conventional FISH testing (Patients A6 and A7). This suggests a possible limitation to FISH testing in a minority of cases.

The *COL1A1-PDGFB* fusion is sufficiently prevalent in dermatofibrosarcoma protuberans that it has led to the suggestion that, in many cases, the absence of this event implies an alternative diagnosis.³³ Variant fusions are nevertheless recognized, and with the report of a case with a variant *COL1A2-PDGFB* fusion it was proposed that constitutive expression of PDGFB is the fundamental mechanism of tumorigenesis in dermatofibrosarcoma protuberans.²⁹ To our knowledge, the *COL6A3-PDGFD* fusion gene has not previously been characterized, and its relationship to the conventional *COL1A1-PDGFB* fusion gene is unclear. *COL6A3* encodes collagen type VI alpha 3 chain, a microfibrillar component of the extracellular matrix, which is widely present in most connective tissues.³⁵ Fusions involving *COL6A3* are known to occur in other neoplasms, notably tenosynovial giant cell tumor, which frequently contains a *COL6A3-CSF1* fusion³⁶; in this context, the role of *COL6A3* is believed to be that of a strong promoter.³⁷ *PDGFD* is a member of the platelet-derived growth factor family, a family of disulfide-bonded dimeric isoforms, also known as a mesenchymal growth factor^{38,39}; recently, it has been proposed to have an important role in so-called “epithelial mesenchymal transition.”⁴⁰ The chimeric proteins arising from *COL6A3-PDGFD* and *COL1A1-PDGFB* fusion genes both retain the PDGF domain^{13,41}; and, similar to PDGFB,⁴² PDGFD binds and activates PDGFR- β ^{38,43}. This suggests that, despite a different ligand arising with the variant PDGFD chimera, it may nevertheless result in a similar pattern of autocrine activation via PDGFRB receptor tyrosine kinase signaling as conventional PDGFB. Moreover, by RNA-Seq, tumors with *COL6A3-PDGFD* fusions show upregulated mRNA expression of *PDGFRB*.

In summary, we report six novel cases of dermatofibrosarcoma protuberans containing *PDGFD* rearrangement; RNA-Seq testing in three cases identified *COL6A3* as the fusion partner. Whether *COL6A3* represents the fusion partner in all *PDGFD* variants, or whether other genes, such as *COL1A1* or *COL1A2*, are capable of pairing with *PDGFD* remains to be determined. All cases occurred on the breast of females; nevertheless, in view of the limited size of our initial cohort, this anatomic and sex restriction is perhaps merely coincidental. In each case the morphology and immunophenotype was indistinguishable from those with the conventional *COL1A1-PDGFB* fusion gene. The only potential difference being relatively infrequent dermal involvement—the tumors appeared largely centered within the subcutis or superficial breast parenchyma. By targeted RNA-Seq the tumors clustered within the same genomic group as dermatofibrosarcoma protuberans containing the conventional *COL1A1-PDGFB* fusion, suggesting a similar expression profile. With sufficient tissue the diagnosis of dermatofibrosarcoma protuberans is generally straightforward, and predicated on the basis of morphology and immunoreactivity with

CD34. In some instances, however, as with the case in limited sampling and/or unusual variants, the diagnosis can be more challenging. Rigorous molecular characterization facilitates both the accurate diagnosis of these neoplasms, as well as an improved understanding of the molecular and biological mechanisms of their tumorigenesis.³³ Furthermore, similar to dermatofibrosarcoma protuberans with conventional *PDGFB* fusions, the variant *PDGFD* fusion group also showed up-regulation of *PDGFRB*, which suggests that therapeutic targeting with tyrosine kinase inhibitors, such as imatinib, might be an option in locally advanced or metastatic lesions.^{44–46}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Representative images from dermatofibrosarcoma protuberans with *COL6A3-PDGFD* fusion gene product: (A) spindle cell neoplasm with relatively well-demarcated peripheral border, and tracking along fibrous septa (patient A4). (B) Tumor infiltrating adipose tissue with a “honeycomb” pattern with involvement, and sparing of glandular epithelium (patient A2). (C) Sheets of spindle cells with a prominent storiform pattern (patient A1). (D) Cells with ample eosinophilic cytoplasm, and bland elongated ovoid nuclei (patient A1). (E) Immunoreactivity for CD34 (patient A2). (F) Fluorescence *in situ* hybridization showing break-apart signal for *PDGFD* (arrows; patient A1) [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 2.

COL6A3-PDGFD fusion structure and molecular correlates. (A) Diagrammatic representation of *COL6A3* gene on 2q37.3 fused to *PDGFD* gene on 11q22.3. Red box and curved arrow depict 2 fusion transcript variants in which *COL6A3* exons 42 and 43, respectively, are fused with exon 6 of *PDGFD*. (B) Representative sequences from RNAseq data demonstrating the fusion of *COL6A3* exon 42 was fused to exon 6 of *PDGFD*, with emphasis on the protein domains projected to be retained in the fusion oncoprotein. (C) Unsupervised hierarchical clustering of RNAseq data showing that the 3 *COL6A3-PDGFD* fusion positive tumors (blue) cluster together with the 34 cases of dermatofibrosarcoma protuberans (green) with the classic *COL1A1-PDGFB* fusion, and away from other sarcoma types (gray) studied on the same targeted RNAseq platform. (D, left) box plot showing high levels of expression of *PDGFB* mRNA in the control group (green) compared with the study group (blue) and other sarcomas (gray); (D, center) *PDGFRA* levels are not increased in any

tumor category, while (D, right) *PDGFRB* mRNA is upregulated in both PDGFD (blue) and PDGFB (green) fusion positive DFSP compared with other sarcoma types (gray) [Color figure can be viewed at wileyonlinelibrary.com]

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TABLE 1

Patient summary

Patient	Age	Sex	Location	Diagnosis	RNA-Seq	FISH
A1	58	F	Breast, L	DFSP	<i>COL6A3-PDGFB</i>	<i>PDGFB</i>
A2	29	F	Breast, L	DFSP	<i>COL6A3-PDGFB</i>	<i>PDGFB</i>
A3	36	F	Breast, L	DFSP	<i>COL6A3-PDGFB</i>	<i>PDGFB</i>
A4	22	F	Breast, L	DFSP		<i>PDGFB</i>
A5	67	M	Lung [*]	F-DFSP	<i>COL1A1-PDGFB</i>	
A6	26	F	Abdomen	F-DFSP	<i>COL1A1-PDGFB</i>	Negative
A7	16	M	Groin	DFSP	<i>COL1A1-PDGFB</i>	Negative
A8	35	M	Shoulder	DFSP		<i>PDGFB</i>
A9	23	F	Shoulder	DFSP		<i>PDGFB</i>
A10	61	F	Groin	DFSP		<i>PDGFB</i>
A11	49	M	Neck	F-DFSP		<i>PDGFB</i>
A12	50	F	Back	DFSP		<i>PDGFB</i>
A13	45	F	Groin	DFSP		<i>PDGFB</i>
A14	28	F	Back	DFSP		<i>PDGFB</i>
A15	32	F	Foot	F-DFSP		<i>PDGFB</i>
A16	34	F	Back	F-DFSP		<i>PDGFB</i>
A17	28	F	Thigh	DFSP		<i>PDGFB</i>
A18	34	M	Scalp	DFSP		<i>PDGFB</i>
A19	71	F	Groin	F-DFSP		<i>PDGFB</i>
A20	74	M	Thigh	F-DFSP		<i>PDGFB</i>
A21	20	F	Hand	P-DFSP		<i>PDGFB</i>
A22	29	M	Back	F-DFSP		<i>PDGFB</i>
A23	19	M	Hand	F-DFSP		<i>PDGFB</i>
A24	44	F	Abdomen	DFSP	<i>COL1A1-PDGFB</i>	
A25	83	F	Back	DFSP	<i>COL1A1-PDGFB</i>	
A26	43	F	Breast	DFSP	<i>COL1A1-PDGFB</i>	
A27	69	M	Thigh	DFSP	<i>COL1A1-PDGFB</i>	
A28	43	F	Scalp	DFSP	<i>COL1A1-PDGFB</i>	

Patient	Age	Sex	Location	Diagnosis	RNA-Seq	FISH
A29	56	M	Chest	F-DFSP	<i>COL1A1-PDGFB</i>	
A30	36	F	Back	DFSP	<i>COL1A1-PDGFB</i>	
A31	25	M	Thigh	DFSP	<i>COL1A1-PDGFB</i>	
A32	33	M	Chest	DFSP	<i>COL1A1-PDGFB</i>	
A33	50	M	Back	DFSP	<i>COL1A1-PDGFB</i>	
A34	47	F	Thigh	DFSP	<i>COL1A1-PDGFB</i>	
A35	61	M	Chest	DFSP	<i>COL1A1-PDGFB</i>	
A36	79	M	Chest	F-DFSP	<i>COL1A1-PDGFB</i>	
A37	39	M	Chest	DFSP	<i>COL1A1-PDGFB</i>	
A38	34	M	Abdomen	DFSP	<i>COL1A1-PDGFB</i>	
A39	39	F	Arm	DFSP	<i>COL1A1-PDGFB</i>	
A40	34	M	Abdomen	DFSP	<i>COL1A1-PDGFB</i>	
A41	35	F	Shoulder	P-DFSP	<i>COL1A1-PDGFB</i>	
A42	29	F	Axilla	DFSP	<i>COL1A1-PDGFB</i>	
A43	17	F	Breast	DFSP	<i>COL1A1-PDGFB</i>	
A44	37	F	Abdomen	DFSP	<i>COL1A1-PDGFB</i>	
A45	67	F	Buttock	DFSP	<i>COL1A1-PDGFB</i>	
A46	49	M	Thigh	DFSP	<i>COL1A1-PDGFB</i>	
A47	37	F	Neck	DFSP	<i>COL1A1-PDGFB</i>	
A48	41	F	Groin	DFSP	<i>COL1A1-PDGFB</i>	
A49	49	M	Shoulder	F-DFSP	<i>COL1A1-PDGFB</i>	
A50	61	M	Abdomen	DFSP	<i>COL1A1-PDGFB</i>	
A51	53	M	Sacrum	DFSP	<i>COL1A1-PDGFB</i>	
A52	53	M	Buttock	F-DFSP	<i>COL1A1-PDGFB</i>	
A53	35	F	Arm	DFSP	<i>COL1A1-PDGFB</i>	
A54	51	M	Scalp	DFSP	<i>COL1A1-PDGFB</i>	
A55	28	F	Back	DFSP	<i>COL1A1-PDGFB</i>	
A56	52	F	Back	DFSP	<i>COL1A1-PDGFB</i>	
A57	54	F	Chest	DFSP	<i>COL1A1-PDGFB</i>	
A58	70	M	Arm	F-DFSP	<i>COL1A1-PDGFB</i>	
A59	39	F	Sacrum	DFSP	<i>COL1A1-PDGFB</i>	

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Patient	Age	Sex	Location	Diagnosis	RNA-Seq	FISH
A60	35	F	Abdomen	DFSP		<i>PDGFB</i>
A61	35	M	Chest	DFSP		<i>PDGFB</i>
A62	26	F	Hand	DFSP		<i>PDGFB</i>
A63	51	F	Breast	F-DFSP	Negative	
B1	51	F	Breast	DFSP		<i>PDGFD</i>
B2	38	F	Breast	DFSP		<i>PDGFD</i>
B3	40	F	Breast	F-DFSP		<i>PDGFB</i>
B4	79	F	Breast	DFSP		<i>PDGFB</i>
B5	31	F	Breast	F-DFSP		<i>PDGFB</i>
B6	45	F	Breast	DFSP		<i>PDGFB</i>
B7	48	F	Chest wall	DFSP		<i>PDGFB</i>
B8	38	F	Breast	DFSP		<i>PDGFB</i>
B9	44	F	Breast	DFSP		<i>PDGFB</i>
B10	36	F	Breast	DFSP		<i>PDGFB</i>
B11	40	F	Breast	DFSP		<i>PDGFB</i>
B12	31	F	Breast	DFSP		<i>PDGFB</i>
B13	26	F	Breast	DFSP		<i>PDGFB</i>
B14	34	F	Breast	DFSP		<i>PDGFB</i>
B15	36	F	Chest wall	DFSP		Negative

Cohort A: Prospective molecular testing of patients diagnosed with dermatofibrosarcoma protuberans over approximately 1 year. Cohort B: Retrospective molecular testing of patients diagnosed with dermatofibrosarcoma protuberans arising specifically on the breast or upper chest over approximately one decade. Novel variant highlighted in bold.

Abbreviations: DFSP, dermatofibrosarcoma protuberans; F, fibrosarcomatous; P, pigmented; F, female; L, left; M, male; FISH, fluorescence in situ hybridization.

*Primary leg with lung metastasis.