SCIENTIFIC ARTICLE

Molecular Phenotypic Descriptors of Dupuytren's Disease Defined Using Informatics Analysis of the Transcriptome

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Purpose Dupuytren's disease (DD) is a fibroproliferative disorder of unknown etiopathogenesis, which may cause progressive, permanent contracture of digits. Previous studies provide compelling evidence that genetic alterations play an important role. Macroscopically affected areas demonstrate phenotypic differences between the two structurally distinct fibrotic elements in DD (ie, the nodule and the cord). In this study, we set out to (1) compare gene expression profiles between DD and transverse carpal fascia of control subjects (external control); (2) profile DD cords and nodules from the palm against the unaffected transverse palmar fascia (internal control); and (3) identify biologically important candidate genes from the transcriptome profiles.

Methods RNA samples from DD nodules (n = 4), cords (n = 4), and internal control (n = 4) as well as external control (n = 4) from unaffected individuals were subjected to differential gene expression profile analysis. Changes of more than 2-fold in DD groups and controls were recorded. Quantitative reverse transcriptase–polymerase chain reactions were performed to validate 16 implicated genes, which included developmental control genes, matrix metalloproteinases, and apoptotic genes.

Results Several genes associated with DD formation were common across all 6 pairwise analyses. Genes markedly upregulated shared common expression levels across all pairwise analysis studies. Pairs involving the DD nodule arrays were notably distinguishable from all other permutations. The majority of genes dysregulated in the DD cords demonstrated an increase in fold change when compared with the DD nodule tissues. Key collagens, collagenases, metalloproteinases, and inhibitors were identified. Genes involved in cytoskeleton development and lipid metabolism were markedly dysregulated. Confirmations of these alterations were obtained in quantitative reverse transcriptase–polymerase chain reaction.

Conclusions These data demonstrate a gradation in expression of certain genes in DD tissue phenotypes compared with control fascia. Transcriptome profiling is predictive not only of disease but also of disease phenotype. These results indicate a number of important candidate genes associated with DD formation, which may provide clues for molecular mechanisms involved in DD pathogenesis. *(J Hand Surg 2008;33A:359–372. Copyright © 2008 by the American Society for Surgery of the Hand.)*

Key words Cord, Dupuytren's disease/contracture, gene expression profiling, nodule, Skoog's fibers, transcriptomics, transverse palmar fascia.

Dupuytren's disease (DD) is a nodular palmar fibromatosis that may cause permanent flexion contracture of the metacarpophalangeal and proximal interphalangeal joints of the digits. DD is a progressive and irreversible disorder with a high rate of recurrence after surgical excisional treatment.[1–3] It is often a familial disorder and is highly prevalent in individuals of Northern European extraction. Familial and epidemiologic studies provide compelling evidence that genetic alterations play an important role in DD development. DD is

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considered to be one of the most common heritable disorders of connective tissue in Caucasians (Online Mendelian Inheritance in Man [OMIM] 126900).[3]

DNA microarrays allow differential analysis of expression of multiple genes,[4] which permits establishment of gene expression variations segregating and associating with DD phenotypes and control tissue samples. Gene expression analysis, using high-density microarrays, has shown itself to be a powerful tool for diagnosing and classifying disease subtypes. The aim of this study was to compare gene expression profiles between different areas of diseased tissue of DD patients and the corresponding healthy tissue, both from the same patients and from healthy individuals not affected with DD. Gene profiling studies have the potential to help in diagnostic as well as prognostic staging of the disease. The *theranostic* (therapy tailored to prognostic staging before treatment) ability of gene profiling may help in predicting the rate of recurrence of DD postoperatively and aid in offering adjuvant therapy as and when required. In addition, as the genes depict activity, disease activity may lead to clues in further identifying biomarkers involved in disease pathogenesis.

MATERIALS AND METHODS

Dupuytren disease patients (n = 4 men) were entered into the study. The age range was between 37 and 70 years with a mean age of 57 years (SD = 11). Cases were all unrelated Caucasians from the northwest region of England. A full medical history using a proforma and clinical examination of both hands was performed for each case.

All cases had a confirmed diagnosis of DD preoperatively with the presence of characteristic Dupuytren nodules in the palm of the hand(s) with contracture of the metacarpophalangeal joint as well as the proximal interphalangeal joint. Control cases (n = 4 men) were all ethnically matched, healthy Caucasians (mean age, 46 years; range, 24 to 76 y; SD, 12.5) selected from patients having simple carpal tunnel decompression with no family history of DD or evidence of other neoplastic or fibrotic disease. The local and hospital ethical committees had given approval for the study protocol and proformas. Written consent was obtained from all individuals.

Source of Biopsy Tissue Specimens

Tissue biopsies were obtained from each DD patient having fasciectomy: nodules from the palm (n = 4), cords from the palm (n = 4); each nodule and cord was obtained from the same individual. Transverse palmar ligament (internal control; n = 4) was also obtained from each individual having fasciectomy. Normal palmar fascia tissue biopsies (n = 4) were also obtained from control subjects having carpal tunnel decompression. These tissue biopsies were regarded as external controls. Biopsies were harvested at the time of surgery. Tissue was carefully excised to include merely the diseased or the normal fascia without the adjacent adipose/connective tissue. Tissue was then placed immediately into an RNA Later (Ambion, Warrington, UK) solution to help prevent RNA degradation. The samples were then stored at −80°C until ready for RNA extraction.

RNA Extraction and Purification

Tissue biopsies were removed from RNA Later and homogenized in Trizol reagent (Invitrogen, Paisley, UK). Aliquots of the homogenized sample were placed in three 1.5-mL Eppendorf tubes and centrifuged at 12,000 rpm for 10 minutes. A maximum of 700 μL of the RNA mixture was added to an RNeasy minicolumn (QIAgen, Crawley, UK), and the manufacturer's protocol was followed.

RNA Quantification and Quality Analysis

RNA was quantitated and quality checked using a NanoDrop ND-1000 UV-visible spectrophotometer (Labtech International, Ringmer, UK). RNA integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies UK Limited, Stockport, Cheshire, UK).

Affymetrix Microarray Analysis

RNA (10 μg) was reverse transcribed into cDNA using the Superscript cDNA synthesis kit (cat. no. 11917-010; Invitrogen) according to the manufacturer's guidelines. cDNA was purified using a GeneChip (Affymetrix, Santa Clara, CA). Aliquots of labeled cRNA (20 μg) were fragmented and then hybridized to a Human Genome U133A GeneChip array for 16 hours, rotating at 60 rpm at 45°C in a GeneChip Hybridization Oven 640 (Affymetrix). Each chip was washed and stained on a GeneChip Fluidics Station 450 (Affymetrix) and scanned on a GeneChip Scanner 450 (Affymetrix) using standard recommended protocols.

Microarray Data Analysis Overview

The Affymetrix array data analysis was performed using GeneSifter microarray analysis tool (VizX Labs, Seattle, WA).[5] Relative changes in gene expression were evaluated by the fold-change (FC), as determined from the Robust Multiarray Average (RMA)[6] normalized data reported by GeneSifter. A transcript displaying expression greater than a 2-fold change from the mean in at least 1 array was used as a cut-off level in this study (nonbiased analysis). The gene expression profile in terms of fold change from the mean was compared using the Benjamini and Hochberg multiple test correction.[7] Results with a p value ≤.05 at the 95% confidence level were considered significant. Individual thresholds were further applied (p value <.01 to .05) to obtain a filtered set of statistically significant genes (Fig. 1). Genes and arrays were clustered according to their expression patterns using Cluster software,[8] and heat maps were produced with Java TreeView.[9] Principal component analysis[10] on RMA normalized data was performed to test the quality of the array data using the Singular Value Decomposition option in maxdView 1.0.5[11] to confirm correlation between clusters in related gene array chips.

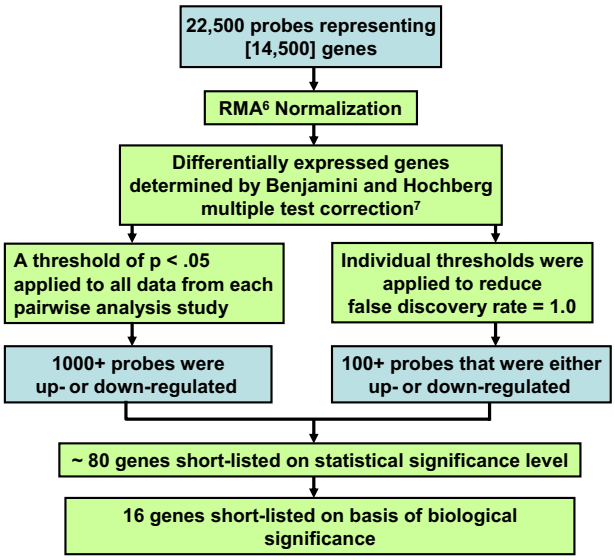

FIGURE 1: Flowchart representing the steps taken to analyze gene expression data. RMA, robust multiarray average.

Microarray Data Analysis with R-Bioconductor Tools Suites

The Affymetrix CEL files were also uploaded and analyzed with the R-Bioconductor tools suite[12] using MADAT[13] (MicroArray Data Analysis Tool: a front-end Java application of the R-Bioconductor packages) to compare and contrast the significant candidate genes. The results from MADAT corroborated with those obtained from GeneSifter, and statistically significant regulated genes were present in both data analysis studies.

Statistical Analysis

The Affymetrix HGU133A GeneChip array contains approximately 22,500 probe sets representing 14,500 different transcripts. After clustering analyses of array data, a series of *t*-tests were applied to find a subset of genes that were significantly differentially regulated. Pairwise comparisons (6 in total) between the different types of tissue were made (ie, DD nodule tissues vs external control, DD nodules vs internal control, DD cord vs external control, DD cord vs internal control, DD nodule vs DD cord, and internal control vs external control).

Data Filtering and Mining

Normalized and multiple corrected probe sets showing fold change $>$ 2 in expressions having a p value $<$.05 were filtered to provide a subset of genes to study for biological relevance using GeneSifter,[5] NetAffx,[14] Gene Ontology,[15] and DAVID (Database for Annotation, Visualization and Integrated Discovery).[16]

From Gene List to the Biological Function

GeneSifter produced an ontology summary report for each selected gene. The filter conditions applied were set at a threshold of $p < .05$ and a $>$ 2-fold change, returning a maximum of 100 genes. Individual thresholds were further applied to obtain a filtered set of statistically significant genes. Results with a p value in the range .001 to .055 (95% to 99% confidence level) were considered significant. NetAffx[14]-Affymetrix Identifiers were converted to annotated gene names using the NetAffx Batch Query tool on the Affymetrix Web site. Some genes were represented by multiple probe sets; redundancy was removed by taking the highest expression values. DAVID[16]-Affymetrix Identifiers were converted to gene list in DAVID. The genes derived from this high-throughput study were grouped based on functional similarity. This was performed using the Functional Classification Tool, which generated a gene-to-gene similarity matrix based on shared functional annotation using more than 75,000 terms from 14 functional annotation sources. The clustering algorithm classified highly related genes into functionally related groups.

Assay Design for Quantitative Reverse Transcriptase–Polymerase Chain Reaction

Exiqon Probefinder software was used to design assays for quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) using the Probelibrary locked nucleic acid probes (Roche Diagnostics, Penzberg, Germany) for specificity; primer sequences were then subjected to a Basic Local Alignment Search Tool search to ensure only unique sequences were used. Assays were successfully designed for selected candidate genes. Both a disintegrin and metalloproteinase domain 12 (ADAM12) and bone morphogenetic protein 1 (BMP1) have splice variants, for these transcripts assays were designed across conserved exon-exon boundaries present in all splice variants so that one assay detected all variants (Table 1). RNA was reverse transcribed into cDNA using SuperScript II RNase H$^-$ Reverse Transcriptase kit (Invitrogen), and the manufacturer's protocol was followed.

RESULTS

Gene Expression Analysis

A total of 8 Dupuytren's disease tissue samples and 8 normal palmar fascia biopsy samples (4 transverse palmar ligament from DD cases acting as internal control and 4 palmar fascia from unaffected individuals acting as external control) were analyzed using the Affymetrix oligonucleotide arrays. The hierarchical clustering method was applied in the analysis of control palmar fascias and DD tissue subjects. Genes were clustered by applying two-way clustering where on one axis (horizontal) are the samples of controls (internal and external controls combined) and DD tissue cases (nodules, cords) and on the other axis (vertical) are the genes. The heat map and clustering algorithms identified subsets of DD genes that behave similarly. The results of agglomerative hierarchical clustering of all samples are demonstrated in Figure 2. Presence of large contiguous patches of color represent groups of genes that share similar expression patterns over

TABLE 1: A List of Selected Candidate Genes on Which Reverse Transcriptase–Polymerase Chain Reaction Was Performed to Validate the Microarray Findings*

Gene Name	Chromosome Position	Gene Function
ADAM12 A disintegrin and metalloproteinase domain 12	(10q26.3)	Regulation of adipogenesis and myogenesis
ADAMTS3 A disintegrin-like and metalloproteinase with thrombospondin type 1 motif, 3	(4q21)	Member of Adamts family, associated with arthritis
ADH1B Alcohol dehydrogenase	(4q22)	Alcohol metabolism
BMP5 Bone morphogenetic protein 5	Chr.6	Growth and patterning of skeletal tissue
CD36 CD36 antigen (collagen type I)	7q11.2	Leukocyte differentiation antigen, cell adhesion molecule
COL18A1 Collagen XVIII, alpha-1 polypeptide	21q22.3	Endostatin, potent antiangiogenic protein
COL4A2 Collagen IV, alpha-2 polypeptide	13q34	Collagen of basement membrane
COL1A1 Collagen I, alpha-1 polypeptide	17q21.31-q22	Major collagen of skin, tendon, and bone
COL5A1 Collagen V, alpha-1 polypeptide	9q34.2-q34.3	Control of fibrillogenesis
COL6A1 Collagen VI, alpha-1 polypeptide	21q22.3	Cell migration and differentiation, microfibrillation
FRZB Frizzled-related protein	2q31-q33	Induction of apoptosis susceptibility, tumor suppressor gene
MMP3 Matrix metalloproteinase 3	11q23	Degrade the major components of ECM
MMP11 Matrix metalloproteinase 11 (stromelysin 3)	22q11.2	ECM remodeling in embryonic development, tissue repair, and tumor progression
MMP14 Matrix metalloproteinase 14 (membrane-inserted)	14q11-q12	Degrade various components of the extracellular matrix
NOV Nephroblastoma overexpressed gene	8q24.1	Binds insulin-like growth factors
THY1 Thy-1 T-cell antigen	11q23.3	Major cell surface glycoprotein characteristic to T cells

*Assays were designed for the 16 target genes and 1 endogenous control.

multiple conditions. Branch lengths represent the degree of similarity between the genes. Coregulated and functionally related genes were statistically grouped into clusters. Larger groups of clustered genes were examined when we observed a strong tendency for these genes to share common roles in cellular processes.

There is a clear separation of control samples and DD disease tissue. In contrast with normal samples, DD tissue samples also show an additional level of heterogeneity. There were 2 distinct clusters identified among the DD samples separating it into cord and nodule. The dendrogram in Figure 3 clearly demonstrates the differential agglomerative hierarchical profiling of disease versus control. The disease separates itself into 2 distinct clusters starting with the nodule samples and is followed by the cord samples. The other end of the dendrogram clusters the control (external) tissue followed by clusters from the fascia (internal control) tissue.

Pairwise analyses studies were performed on all tissue samples including every possible permutation. Principal

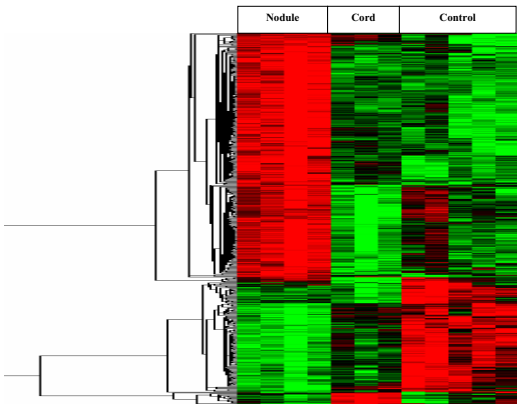

FIGURE 2: Hierarchical clustered display of data from the control palmar fascias tissue and DD disease tissue (nodules and cords). Genes were clustered by applying 2-way clustering where on one axis (horizontal) are the samples of controls and DD tissue subjects and on the other axis (vertical) are the genes. Genes that are upregulated appear in red; those that are downregulated appear in green; black indicates approximately the same gene expression as the mean for that gene across all samples.

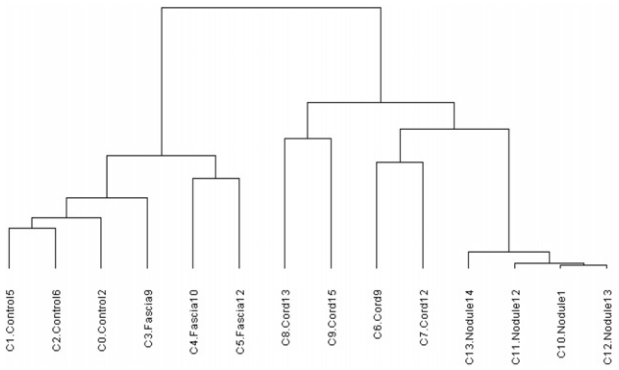

FIGURE 3: Cluster diagram for microarray chips. The dendrogram clearly demonstrates the differential agglomerative hierarchical profiling of disease versus control. The disease separates itself into 2 distinct clusters starting with the nodule samples followed by the cord samples. The other end of the dendrogram clusters the control (external) tissue followed by clusters from the fascia (internal control) tissue.

component analysis on the RMA normalized data confirmed a correlation between clusters in related arrays. The cluster diagram distinguished the data from each sample set separating the nodule tissue arrays from the cord, transverse palmar fascia (internal control), and normal tissue (external control) arrays. Similarly, the cord tissue arrays clustered together, as did the tissue from the transverse palmar fascia and normal control tissues.

Genes were compared for their presence across the statistically and biologically significant transcript lists retrieved from 6 pairwise analysis studies (ie, transcripts over- and underexpressed when DD tissue samples were analyzed against the external control and also between the DD sample subsets). Tables 2 through 7 and Figure 4 represent transcripts that statistically demonstrated major alterations in expression in the pairwise analysis study. The major upregulated genes are denoted in the tables in normal font only, while the downregulated genes are both underlined and italicized. The major dysregulated genes that commonly recurred across all pairwise analysis studies are in boldface. Analysis of these major recurring dysregulated transcripts dictated to a narrow selection criterion of candidate genes (Table 1).

From the highest selected genes listed in Tables 2 through 7, 19 genes (12 upregulated and 7 downregulated) were recurrently dysregulated in DD nodules when compared with both the internal and external controls (Tables 2 and 3). A similar comparison was made between the differentially expressed genes obtained from the pairwise analysis study in which both DD nodules and DD cords were compared against the external controls (Tables 2 and 5). Sixteen genes (8 upregulated and 8 downregulated genes) were recurrent and more than 2-fold dysregulated. The acquired dysregulated genes common to the DD cord tissues compared against internal control (DD fascia) and external (normal) controls included collagen, type I, alpha 1 (COL1A1), alcohol dehydrogenase (ADH1B), periostin, osteoblast specific factor (POSTN), tenascin C (TNC), cytochrome P450, family 4, subfamily B, polypeptide 1 (CYP4B1), and tenascin XB (TNXB) (Tables 5 and 6).

In addition, altered gene expression profiles that may affect smooth and striated muscle contraction were observed (for nodule and cord) for myosin (MYL2), tropomyosin 4 (TPM4), troponin T type 1 (TNNT1) and titin (TTN). The collagens COL1A1, COL5A1, and COL5A2 were more than 4-fold upregulated in the diseased nodule tissue when compared against the external control and more than 3-fold compared with adjacent palmar fascia tissue (internal control).

As depicted by the histogram in Figure 4, pairs involving the nodule arrays were particularly distinguishable from all other permutations. Genes significantly upregulated shared common expression levels across all pairwise comparisons. Nineteen dysregulated genes were common to DD nodule tissues when compared against the 2 controls. Sixteen common genes were dysregulated when both the DD nodules and DD cords tissues were compared with the external controls. Six genes were established as commonly dysregulated in both cases in which the DD cords were compared with the internal controls and the external control. We also compared the 2 dysregulated DD tissue types (nodule and cord) with each other. Table 4 demonstrates that the majority of genes dysregulated in the cords show an increase in fold change when compared with nodule tissue. In the case in which the 2 controls were compared (DD fascia compared with external control tissue), a 2-fold increase in 6 genes is reported (Table 7).

TABLE 2: Expressions of Major Regulated Genes in the DD Nodules Compared With External Control Tissue From Pairwise Analysis Study*

Gene Name	Chromosome	p Value	Regulated	FC
Procollagen C–endopeptidase enhancer 2	3	.002	Down	−6
Myocilin	1	.001	Down	−5
Periostin, osteoblast specific factor	13	.003	Up	5
Clusterin	8	.015	Down	−5
Collagen, type I, alpha 1	17	.002	Up	4
ADP-ribosylation factor–like 4C	2	.001	Up	4
Cysteine and glycine-rich protein 2	12	.002	Up	4
5-Nucleotidase domain containing 2	3	.001	Up	4
Nebulin	2	.062	Down	−4
Leucine rich repeat containing 17	7	.001	Up	4
Proteoglycan 4	1	.002	Down	−4
Tenascin C (hexabrachion)	9	.001	Up	4
Collagen, type V, alpha 1	9	.002	Up	4
Hemoglobin, alpha 2	16	.002	Down	−4
Alcohol dehydrogenase IB (class I), beta polypeptide	4	.007	Down	−4
Angiopoietin-like 7	1	.033	Down	−4
Glutathione peroxidase 3	5	.004	Down	−4
Aldehyde dehydrogenase 1 family, member A1	9	.002	Down	−4
Lysyl oxidase-like 2	8	.001	Up	4
ADAM metallopeptidase domain 12	10	.001	Up	4
Collagen, type V, alpha 2	2	.002	Up	4
Calponin 3, acidic	1	.022	Up	4
Matrix metallopeptidase 3 (stromelysin 1, progelatinase)	11	.002	Down	−4
Cadherin 11, type 2, OB-cadherin (osteoblast)	16	.005	Up	3
Aggrecan	15	.004	Up	3
Laminin, beta 1	7	.004	Up	3
Myristoylated alanine-rich protein kinase C substrate	6	.011	Up	3
Matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)	16	.042	Up	3
Collagen, type IV, alpha 2	13	.001	Up	3
Collagen, type VI, alpha 1	—	.019	Up	3
Collagen, type XVIII, alpha 1	21	.003	Up	3
Frizzled-related protein	2	.001	Down	−3
TIMP metallopeptidase inhibitor 3	22	.003	Down	−3
Thy-1 cell surface antigen	11	.001	Up	3

*External control tissues were excised from patients having simple carpal tunnel decompression with no family history of DD. FC is the fold change. The upregulated genes are denoted in normal font only, and the downregulated genes are both underlined and in italic face. The genes that are commonly recurring across all pairwise analyses are in boldface.

TABLE 3: Expressions of Major Regulated Genes in the DD Nodules Compared With Internal Control Tissue From Pairwise Analysis Study*

Gene Name	Chromosome	p Value	Regulated	FC
Alcohol dehydrogenase IB (class I), beta polypeptide	4	.003	Down	−5
Periostin, osteoblast specific factor	13	.002	Up	4
Myocilin	1	.009	Down	−4
Procollagen C–endopeptidase enhancer 2	3	.012	Down	−4
Apolipoprotein D	3	.009	Down	−4
ATP-binding cassette, subfamily A (ABC1), member 8	17	.006	Down	−4
ADAM metallopeptidase domain 12	10	.002	Up	4
Leptin receptor	1	.003	Down	−4
ADP-ribosylation factor–like 4C	2	.003	Up	4
Periplakin	16	.005	Down	−4
Cysteine and glycine-rich protein 2	12	.005	Up	4
Collagen, type I, alpha 1	-	.004	Up	4
Lysyl oxidase–like 2	8	.005	Up	4
Tenascin C (hexabrachion)	9	.007	Up	3
Hemoglobin, alpha 1	16	.007	Down	−3
Aldehyde dehydrogenase 1 family, member A1	9	.010	Down	−3
Aggrecan	15	.004	Up	3
Collagen, type V, alpha 2	2	.008	Up	3
Clusterin	8	.040	Down	−3
Glypican 3	X	.005	Down	−3
Glutathione peroxidase 3	5	.008	Down	−3
5-Nucleotidase domain containing 2	3	.006	Up	3
Adiponectin, C1Q and collagen domain containing	3	.024	Down	−3
Microfibrillar associated protein 5	12	.015	Down	−3
Matrix metallopeptidase 14	14	.004	Up	3
Sorbin and SH3 domain containing 1	10	.007	Down	−3
CD36 molecule (thrombospondin receptor)	7	.011	Down	−3
Leucine rich repeat containing 17	7	.003	Up	3
Regulator of G-protein signaling 3	9	.003	Up	3
Collagen, type V, alpha 1	9	.013	Up	3
TIMP metallopeptidase inhibitor 3	22	.007	Down	−3

*Internal control tissues were excised from the unaffected transverse palmar fascia from the palms of patients affected by DD. FC is the fold change. The upregulated genes are denoted in normal font only, and the downregulated genes are both underlined and in italic face. The genes that are commonly recurring across all pairwise analyses are in boldface.

DISCUSSION

Cellular and biological processes depend on the complex interactions between many different genes and their products. In this study, transcriptome profiling has enabled the investigation of the expression levels of thousands of genes associated with DD formation simultaneously. Transcriptome profiling accounted for raw expression estimates and identified transcripts that were differentially expressed between controls and DD tissue samples, together with profiles of differentially expressed transcripts between the subsets of DD tissues.

Landmark genes identified in previous studies[17–21] to be involved in DD were also validated in this study. In addition, other genes not previously described in the study

TABLE 4: Expressions of Major Regulated Genes in the DD Nodules Compared With DD Cord Tissue From Pairwise Analysis Study

Gene Name	Chromosome	p Value	Regulated	FC
Clusterin	8	.009	Down	−3
Collagen, type VI, alpha 1	21	.001	Up	3
Myocilin	1	.001	Down	−3
5-Nucleotidase domain containing 2	3	.001	Up	3
Collagen, type I, alpha 1	—	.001	Up	3
Collagen, type VI, alpha 2	21	.001	Up	3
ADP-ribosylation factor–like 4C	2	.002	Up	3
Procollagen C–endopeptidase enhancer 2	3	.002	Down	−3
Angiopoietin-like 7	1	.018	Down	−3
ADAM metallopeptidase domain 12	10	.001	Up	3
Angiopoietin-like 2	9	.001	Up	3
Leucine rich repeat containing 17	7	.001	Up	3
Mesoderm	7	.002	Up	3
Cysteine and glycine-rich protein 2	12	.001	Up	3
Microfibrillar-associated protein 2	1	.002	Up	3
Fatty acid binding protein 5	8	.003	Up	3
Sushi-repeat-containing protein, X-linked 2	X	.001	Up	2
Collagen, type V, alpha 1	9	.001	Up	2
Laminin, beta 1	7	.001	Up	2
Procollagen-proline, 2-oxoglutarate 4-dioxygenase	17	.015	Up	2
Tropomyosin 4	19	.001	Up	2
Apolipoprotein D	3	.001	Down	−2
Tumor necrosis factor, alpha-induced protein 6	2	.002	Up	2
Zyxin	7	.001	Up	2
PDZ and LIM domain 7	5	.001	Up	2
Collagen, type IV, alpha 2	13	.003	Up	2
SMAD family member 1	4	.001	Up	2
Transforming growth factor, beta 1	19	.003	Up	2
Matrix metallopeptidase 14	14	.001	Up	2
Alcohol dehydrogenase IB (class I), beta polypeptide	4	.013	Down	−2
Collagen, type V, alpha 2	2	.001	Up	2
Lysyl oxidase–like 2	8	.001	Up	2

The upregulated genes are denoted in normal font only, and the downregulated genes are both underlined and in italic face. The majority of genes dysregulated in the cords show an increase in fold change when compared with nodule tissue.

SMAD, Small Mothers Against Decapentaplegic (SMADs are a class of proteins that modulate the activity of transforming growth factor beta ligands).

of DD were regulated significantly (Tables 2 through 7) and were of particular interest because of their prominent role in tissue development, cellular differentiation, apoptosis, mitochondrial cytoskeletal development, and lipid metabolism. The current study was similar to previous ones in the context of using high-throughput techniques;

TABLE 5: Expressions of Major Regulated Genes in the DD Cords Compared With External Control Tissue From Pairwise Analysis Study*

Gene Name	Chromosome	p Value	Regulated	FC
Periostin, osteoblast specific factor	13	.054	Up	3
Collagen, type I, alpha 1	17	.024	Up	3
Procollagen C–endopeptidase enhancer 2	3	.023	Down	−3
Alcohol dehydrogenase IB (class I), beta polypeptide	4	.049	Down	−3
Proteoglycan 4	1	.026	Down	−3
Four and a half LIM domains 1	X	.052	Down	−3
Cytochrome P450, family 4, subfamily B, polypeptide 1	1	.032	Down	−3
Hemoglobin, beta	11	.026	Down	−3
Matrix metallopeptidase 3 (stromelysin 1, progelatinase)	11	.033	Down	−3
Nephroblastoma overexpressed gene	8	.048	Up	3
Hemoglobin, alpha 1	16	.045	Down	−3
B-cell CLL/lymphoma 6 (zinc finger protein 51)	3	.025	Down	−2
Glutathione peroxidase 3 (plasma)	5	.056	Down	−2
Collagen, type V, alpha 2	2	.049	Up	2
Hemoglobin, alpha 2	16	.052	Down	−2
Cysteine dioxygenase, type I	5	.023	Down	−2
Tenascin C (hexabrachion)	9	.044	Up	2
Versican	5	.023	Down	−2
Collagen, type I, alpha 2	7	.058	Up	2
Cadherin 11, type 2, OB-cadherin (osteoblast)	16	.049	Up	2
Frizzled-related protein	2	.023	Down	−2
Tenascin XB	6	.051	Down	−2
Cartilage acidic protein 1	10	.050	Down	−2
Coagulation factor C homolog, cochlin	14	.052	Down	−2
Aldehyde dehydrogenase 1 family, member A1	9	.059	Down	−2
Collagen, type V, alpha 1	9	.052	Up	2
Cysteine and glycine-rich protein 2	12	.033	Up	2
Lysyl oxidase–like 2	8	.052	Up	2
Heat shock 70kDa protein 1A	6	.052	Down	−2
Myocilin	1	.052	Down	−2

CLL, chronic lymphocytic leukemia.
*External control tissues were excised from patients having simple carpal tunnel decompression with no family history of DD. FC is the fold change. The upregulated genes are denoted in normal font only, and the downregulated genes are both underlined and in italic face. The genes that are commonly recurring across all pairwise analyses are in boldface.

however, it differed as it highlighted individual genes within this network of interactions. This advance was possible as a more targeted approach in defining and using control tissue and a larger number of genes was included in the comparisons. Previously, comparison of palmar fascia of DD patients was made with palmar fascia of healthy individuals[17]

TABLE 6: Expressions of Major Regulated Genes in the DD Cords Compared With Internal Control Tissue From Pairwise Analysis Study*

Gene Name	Chromosome	p Value	Regulated	FC
Alcohol dehydrogenase IB (class I), beta polypeptide	4	.052	Down	−4
Periostin, osteoblast specific factor	13	.055	Up	3
Gelsolin (amyloidosis, Finnish type)	9	.052	Down	−3
Thioredoxin interacting protein	1	.053	Down	−3
Cytochrome P450, family 4, subfamily B, polypeptide 1	1	.052	Down	−2
Sorbin and SH3 domain containing 1	10	.052	Down	−2
Tryptase alpha/beta 1	16	.051	Down	−2
Four and a half LIM domains 1	X	.051	Down	−2
Leptin receptor	1	.026	Down	−2
Phosphatidic acid phosphatase type 2B	1	.054	Down	−2
EGF-containing fibulin-like extracellular matrix protein 1	2	.027	Down	−2
Glypican 3	X	.052	Down	−2
Glutamate–ammonia ligase (glutamine synthetase)	1	.059	Down	−2
Periplakin	16	.026	Down	−2
Odd-skipped related 2	8	.052	Down	−2
CD36 molecule (thrombospondin receptor)	7	.053	Down	−2
Spectrin, beta, non-erythrocytic 1	2	.052	Down	−2
Collagen, type I, alpha 1	17	.052	Up	2
Tryptase beta 2	16	.051	Down	−2
ADP-ribosylation factor 1	1	.052	Down	−2
Tenascin C (hexabrachion)	9	.053	Up	2
Microfibrillar associated protein 5	12	.056	Down	−2
Protein kinase, cAMP-dependent, regulatory, type I, alpha	17	.055	Down	−2
MAP kinase interacting serine/threonine kinase 2	19	.052	Down	−2
Phosphatidic acid phosphatase type 2B	1	.052	Down	−2
Tenascin XB	6	.051	Down	−2
Angiotensin II receptor, type 1	3	.026	Down	−2
Transforming growth factor, beta receptor III	1	.008	Down	−2

EGF, epidermal growth factor; MAP, mitogen-activated protein kinase.
*Internal control tissues were excised from the unaffected transverse palmar fascia from the palms of patients affected by DD. FC is the fold change. The upregulated genes are denoted in normal font only, and the downregulated genes are both underlined and in italic face. The genes that are commonly recurring across all pairwise analyses are in boldface.

and of patients with Peyronie's disease and Dupuytren's contracture,[18] with a considerably reduced density of microarray genes. This higher number of genes is found because we were inclusive rather than exclusive.

Differential alterations in expression between DD nodule and cord tissue were observed in pathways associated with focal cell adhesion, apoptosis, and inflammation. Among the dysregulated transcripts, marked enrichment was observed in those directly involved in developmental processes including cell growth, proliferation, differentiation, regulation of cell death, biological cell adhesion, localization, extracellular matrix-receptor interaction, and cell communication.

TABLE 7: Expressions of Major Regulated Genes in the Internal Control Compared With External Control Tissue From Pairwise Analysis Study*

Gene Name	Chromosome	p Value	Regulated	FC
Odd-skipped related 2	8	.027	Up	2
Insulin-like growth factor II	—	.049	Up	2
Thy-1 cell surface antigen	11	.009	Up	2
Myosin, light chain 9, regulatory	20	.040	Up	2
Actin, alpha 2, smooth muscle	10	.022	Up	2
Laminin, beta 1	7	.020	Up	2
Fibroblast growth factor 9 (glia-activating factor)	13	.040	Down	-1

*External control tissues were excised from patients having simple carpal tunnel decompression with no family history of DD. Internal control tissues were excised from the unaffected transverse palmar fascia from the palms of patients affected by DD. FC is the fold change. The upregulated genes are denoted in normal font only, and the downregulated genes are both underlined and in italic face.

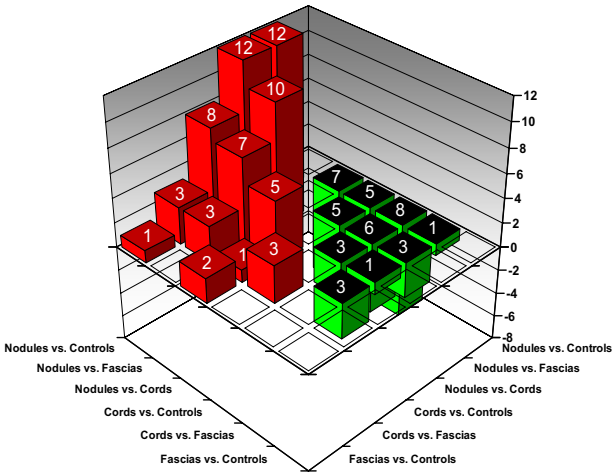

FIGURE 4: The histogram represents the number of common dysregulated genes from each pairwise analysis study. Red bars demonstrate the number of major common upregulated genes. Green bars demonstrate the number of major common downregulated genes. External control: control tissues harvested from normal palmar fascia from the distal transverse carpal ligament. Internal control: fascia tissues harvested from the transverse palmar fascia from the Dupuytren patient.

Individual genes that had altered expression levels confirmed results of earlier studies,[17–20] as well as identified all affected pathways not previously demonstrated. In the current study, however, the genes were discovered in the context of the pathways as a pathway-oriented approach was applied. From the same perspective, new pathways were found that were also altered in expression level including proteolysis, cytoskeletal development, lipid metabolism, and inflammation. The network diagram in Figure 5 demonstrates some of the major genes in DD nodule tissues retrieved from gene expression data and their involvement in biological themes and pathways.

A marked enrichment in genes associated with anatomic (epidermis and ectoderm) structure development was observed in the DD tissue; these include the 3-fold upregulated cadherin 11 (CDH11); post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (DlgA), and zonula occludens-1 protein (zo-1) (PDZ); and Lin11, Isl-1, and Mec-3 (LIM) domain 4 and 7 (PDLIM4 and PDLIM7). Previous studies have documented the expression of upregulated CDH11 in osteoblastic cell lines during differentiation, suggesting a specific function in bone development and maintenance. These genes play a major role in ossification and are particularly involved in the conversion of fibrous tissue into bone or a bony substance.

Expressions of key collagenases and genes with collagenolytic-like activity including downregulated matrix metalloproteinases (MMP3 [3-fold] and MMP27 [2-fold]) were observed in the DD nodules when compared with both controls. However, the expressions of MMP2, MMP14, and MMP11 (FC > 3) increased in the DD nodule. MMP19 (2-fold) expression also increased. Tissue inhibitor of metalloproteinase 1 (TIMP1) expression was increased notably in DD nodules compared with external control only, whereas TIMP3 was expressed at lower levels. Pairwise analysis study of the 2 diseased cases (DD nodule and DD cord) show marked downregulation of ADAM12 in the nodule tissues. Previous studies have documented that these genes also play an important role in normal wound healing, scarring, Peyronie's disease, and in other fibrotic processes.[18]

These results imply that DD is associated with a stimulation of collagen gene expression at the transcriptional and translational levels together with a reduction in the rate of collagenolytic activity in upregulated inhibitors such as ADAM metallopeptidase domains (ADAM12 and ADAM19). These genes were 4-fold upregulated in DD nodules when compared with both controls. A disintegrin-like and metalloproteinase with thrombospondin type 1 motif, 2 (ADAMTS2) and TIMP were notably expressed in DD nodule tissues compared with external controls only.

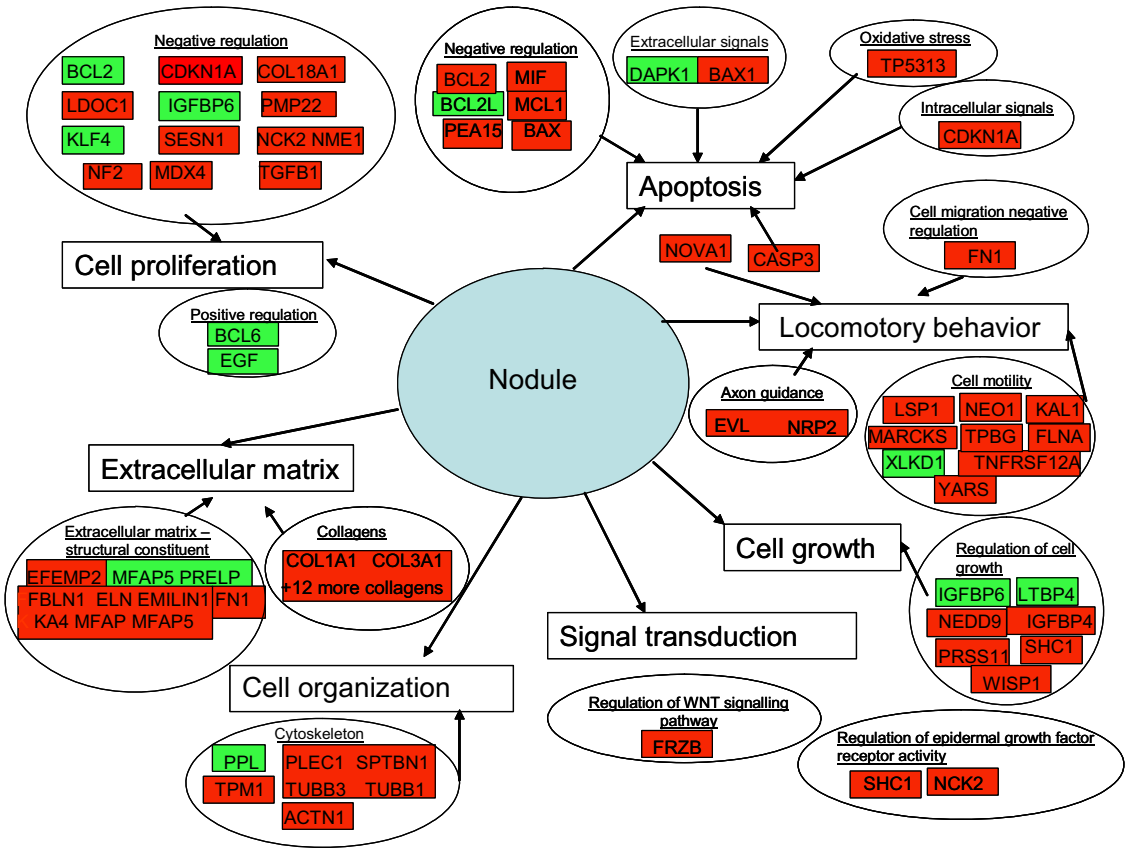

FIGURE 5: Network diagram representing some of the major genes expressed in DD nodule tissues from gene expression data and their involvement in biological processes. ACTN1, actinin, alpha 1; BAX, BCL2-associated X protein; BAX1, BCL2-associated X protein (promoter); BCL2, B-cell CLL/lymphoma 2; BCL2L, BCL2-like 1; BCL6, B-cell CLL/lymphoma 6; CASP3, caspase 3; CDKN1A, cyclin-dependent kinase inhibitor 1A; COL18A1, collagen, type XVIII, alpha 1; COL1A1, collagen, type I, alpha 1; COL3A1, collagen, type III, alpha 1; DAPK1, death-associated protein kinase 1; EFEMP2, EGF-containing fibulin-like extracellular matrix protein 2; EGF, epidermal growth factor; ELN, elastin; EMILIN1, elastin microfibril interfacer 1; EVL, Enah/Vasp-like; FBLN1, fibulin 1; FLNA, filamin A, alpha (actin binding protein 280); FN1, fibronectin 1; FRZB, frizzled-related protein; IGFBP4, insulin-like growth factor bindig protein 4; IGFBP6, insulin-like growth factor binding protein 6; KAL1, Kallmann syndrome 1 sequence; KLF4, Kruppel-like factor 4; LDOC1, leucine zipper, down-regulated in cancer 1; LSP1, lymphocyte-specific protein 1; LTBP4, latent transforming growth factor beta binding protein 4; MARCKS, myristoylated alanine-rich protein kinase C substrate; MCL1, myeloid cell leukemia sequence 1; MFAP, microfibrillar-associated protein 2 precursor; MFAP5, microfibrillar associated protein 5; MIF, macrophage migration inhibitory factor; NCK2, noncatalytic region of tyrosine kinase, beta; NEDD9, neural precursor cell expressed, developmentally down-regulated 9; NEO1, neogenin homolog 1; NF2, neurofibromin 2; NME1, non-metastatic cells 1; NOVA1, neuro-oncological ventral antigen 1; NRP2, neuropilin 2; PEA15, phosphoprotein enriched in astrocytes 15; PLEC1, plectin 1, intermediate filament binding protein 500kDa; PMP22, peripheral myelin protein 22; PPL, periplakin; PRELP, proline/arginine-rich end leucine-rich repeat protein; PRSS11, also known as HTRA1, HtrA serine peptidase 1; SESN1, sestrin 1; SHC1, SHC (Src homology 2 domain containing) transforming protein 1; SPTBN1, spectrin, beta, non-erythrocytic 1; TGFB1, transforming growth factor, beta 1; TNFRSF12A, tumor necrosis factor receptor superfamily, member 12A; TPBG, trophoblast glycoprotein; TPM1, tropomyosin 1 (alpha); TUBB1, tubulin, beta 1; TUBB3, tubulin, beta 3; WISP1, WNT1 inducible signaling pathway protein 1; XLKD1, also known as LYVE1, lymphatic vessel endothelial hyaluronan receptor 1; YARS, also known as TYRRS, tyrosyl-tRNA synthetase.

Simplified categorizations of regulated key players (upregulated/downregulated genes) in DD are those in which upregulated accumulations of disorganized collagen fibers occur and downregulation of metalloproteinases (or upregulation of their inhibitors) and genes controlling skeletal, tissue development, cell proliferation, differentiation, and apoptosis occur.

Highly differentially expressed and statistically significant genes were compared with their known gene expression profiles, biological themes, and purported functions in DD and other fibrotic tissue diseases. Previous studies have documented the associations of the key players in DD to be present or associated in other fibrotic tissue disorders involving abnormal proliferation of tissue. We found commonality across several fibrotic diseases that expressed dysregulation of these genes, including fibrosis of the liver,[20–23] lungs,[24] cardiac muscle,[25] and skin,[26,27] but particularly in liver fibrosis, in which the potential for matrix degradation is present.[27] POSTN is confirmed to be markedly upregulated in Peyronie's disease from studies of

differential gene expression.[18] COL5A2, ADAM12, and cysteine and glycine-rich protein 2 (CSRP2) were reported as 4-fold upregulated, and procollagen C–endopeptidase enhancer 2 (PCOLCE2) and MMP3 were more than 4-fold downregulated in our study. This is also the case in liver fibrosis. Angiotensin II was more than 2-fold downregulated in DD cord tissues compared with internal control. This is an important mediator of renal and cardiac vascular fibrosis through the increase of transforming growth factor (TGF)-β, in a process that appears to be counteracted by nitric oxide.[28] These alterations were confirmed in 16 sample tissues by qRT-PCR (Table 1). These fibrotic genes have been identified as being possible candidate regulatory genes. COL5A1 and COL18A1 were as expected from the microarrays upregulated in both the nodule and cord tissue when compared with other tissues. MMP14, ADAM12, and BMP1 had shown an increased expression in nodules only when compared with other tissues. MMP3 and BMP5 were upregulated in external control cases confirming gene expression levels were highest in the controls and least in the disease tissues.

This study demonstrated a unique approach to the analysis of DD. We compared profiles of genes differentially expressed in both disease tissues (nodules and cords) with external and internal controls. All DD tissue samples were excised at the same time course of patients having fasciectomy. Nevertheless, a significant difference is observed between the major dysregulated genes retrieved from DD nodules tissues (Tables 2 and 3) and of those recovered from the DD cords tissues (Tables 5 and 6); although all DD tissue subjects were removed at 1 time point, we observed different expression. These data demonstrate a gradation in expression of certain genes in DD tissue phenotypes (cords and nodules) compared with external control fascia. Transcriptome (mRNA transcripts present in a cell at a given time) profiling is predictive not only of disease but also of disease phenotype. These results indicate a number of important candidate genes associated with DD formation. This work may provide clues for molecular mechanisms involved in DD and may help to develop strategies for therapy and prophylaxis.

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