Whole genome and global expression profiling of Dupuytren’s disease: systematic review of current findings and future perspectives

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ABSTRACT

Dupuytren’s disease (DD) is a common fibroproliferative disorder affecting the palmar fascia, which may lead to permanent contracture of the affected digit. Profiling studies investigating DD at whole-genomic, transcriptomic and proteomic levels have been carried out, from which large numbers of candidate genes potentially involved in DD have been reported. This review focuses on identifying genes reported by multiple studies or validated by multiple experimental techniques, as well as signalling pathways suggested to contribute to DD. Meta-analysis was also carried out on three microarray datasets. Twenty-one genes were found to be reported as dysregulated in multiple gene expression microarrays, seven of which have been further validated by other experimental methods. Sixty-four genes determined by meta-analysis correlate to those reported by published microarray studies. In addition, several pathways have been proposed to be involved in DD by whole-genome or global expression profiling. Further investigation in these genes and pathways, and correlating them to genotypes or environmental factors for DD, may aid in further elucidation of mechanisms involved in DD pathogenesis.

Dupuytren’s disease (DD) is one of the most common connective tissue disorders among Caucasians of northern European extraction.1 DD is a fibroproliferative disease affecting the palmar fascia, and may lead to permanent flexion contracture of the affected digits. In affected individuals, DD-like fibrosis can also be found over the knuckles (Garrod’s pads), feet (Lederhose’s disease) and penis ( Peyronie’s disease).2 3 While surgical intervention is the mainstay of treatment for DD, there is a high recurrence rate post-surgery.1 2 The aetiology of DD remains unknown, however, a strong genetic component has been suggested. The evidence supporting this assertion includes increased family history, high Caucasian prevalence and reports of common occurrence in twins.4 Several studies have attempted to identify the susceptibility genes to DD; however, to date no single gene has been confirmed to contribute to DD with a fully elucidated mechanism. Environmental factors, including trauma, alcohol, smoking and associated disease, have also been suggested to contribute to DD.1 5

The aim of this review is to summarise our current molecular understanding of DD, with a focus on findings from global gene expression studies by: (1) carrying out meta-analysis on microarray datasets available in public repositories; (2) comparing genes reported to be dysregulated by more than one global expression study or with more than one experimental technique; (3) comparing genes reported to be dysregulated and located within or adjacent to a genetic linkage in association with DD; and (4) signalling pathways suggested to be involved in DD by global expression profiling/meta-analysis.

CANDIDATE GENES IN META-ANALYSIS AND GLOBAL RNA EXPRESSION STUDIES

Eight global messenger RNA expression microarray studies5–12 and one microRNA profiling13 study were identified (see supplementary table S1, available online only). Four sets of microarray data on DD were identified at both the National Center for Biotechnology Information gene expression omnibus (GEO) and the European Bioinformatics Institute array express record ID for GEO: GSE4457, GSE2688, GSE1221 and GSE3155. Two of the datasets, GSE268812 and GSE4457,6 were identical; therefore, the two datasets (and associated studies) were considered as one in the analysis of this review. GSE122110 involved the use of two experimental platforms on the same biological samples; the dataset from the CodeLink platform was selected for meta-analysis as the authors presented a higher number of significantly dysregulated genes with the CodeLink platform in their publication.10 A total of 7072 unique UniGene ID was included in the meta-analysis (see supplementary text, available online only, and figure 1).

Twenty-one genes have been reported with consistent regulation by multiple gene expression profiling microarray studies (table 1 and figure 2). Nineteen of these 21 genes were assessed in the meta-analysis, 16 of which were also determined to show statistically significant dysregulation in DD with the same regulation (over/underexpression) (table 1). Eight of the genes reported by multiple microarray studies have also been validated by other gene-specific experimental methods (table 1), including ADAM metalloprotease domain 12 (ADAM12),14 collagen type I α 1 (COL1A2),15 matrix metalloprotease (MMP)2,16 periostin, osteoblast specific factor (POSTN),17 proteoglycan 4 (PRG4)18 and tenascin C (TNC) (table 1).14

There is only one array-based microRNA profiling study in DD. MicroRNA are post-transcriptional regulators that negatively regulate their mRNA targets.17 Mosakhani et al.15 compared the microRNA profiles in their study with the mRNA expression profiles in the study by Forsman et al.12 and showed concurrence on the downregulation of microRNA and upregulation of their target mRNA. The genes
highlighted by Mosakhani et al include: ADAM12, collagen, type V, alpha 2 (COL5A2), transforming growth factor β (TGFβ), v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB), POSTN, TNC, wingless-type MMTV integration site family, member (WNT)5A and Zic family member 1 (ZIC1) (table 1). Other than TGFβ1, all of these genes were found to be significantly upregulated in the meta-analysis (table 1).

A total of 322 genes (out of 7072 analysed) was determined to be significantly dysregulated in DD by the meta-analysis; 64 of the 322 genes demonstrated the same regulation as those reported by at least one gene expression microarray study (see supplementary table S2, available online only, and figure 1). Twenty-eight of these 64 genes share the same regulation as those seen in fibromatosis, based on the expression data available in the gene expression atlas (see supplementary table S2, available online only, and figure 1).

COMPARING GLOBAL RNA EXPRESSION WITH PROTEIN EXPRESSION

Investigation of both mRNA and protein levels are necessary and complimentary for the identification of biomarkers in human disease. Two whole-proteome studies have been carried out to identify differentially expressed proteins in DD. While O’Gorman et al identified three potential low molecular weight tissue protein markers that were positively associated with DD, the genes coding for these protein markers were not determined; therefore these findings were not included in this review. By comparing biospies of DD with internal palmar fascia, Kraljevic Pavelic et al have reported 44 genes showing five-fold upregulation in DD through two-dimensional gel electrophoresis. Of these genes, three were reported in mRNA expression profiling microarrays with consistent regulation; ARHGDIA, COL6A3 (meta-analysis) and TPM4. ARHGDIA is also overexpressed in Peyronie’s disease.

Several genes determined to be dysregulated by multiple gene expression microarrays have been validated at the protein level by immunohistochemistry, western blotting, or zymography, and include MMP2, COL1A2 and POSTN. POSTN demonstrates the highest consistency in its altered expression in DD found to be dysregulated in three mRNA microarray studies and the meta-analysis in this review (derived from data in Lee et al, Satish et al and Zhang et al), which also showed upregulation of POSTN. POSTN is an extracellular membrane protein that is found in collagen-rich connective tissues, and overexpression of POSTN may contribute to tumorigenesis by promotion of cancer cell survival, epithelial-mesenchymal transition, invasion and metastasis. Application of exogenous POSTN to fibroblasts derived from DD and control fascia was found differentially to regulate apoptosis, proliferation, alpha-smooth muscle actin expression and fibroblast populated collagen lattice contraction.

Protein staining for several extracellular matrix (ECM) proteins or growth factors in DD correlates to mRNA dysregulation reported in microarray studies. Howard et al demonstrated higher protein levels of fibronectin in DD, also supported by mRNA microarray studies. ECM surrounding alpha smooth muscle actin-positive cells in proliferative nodules demonstrate staining of type IV collagen, laminin and tenasin. Several immunohistological studies investigate specific ECM in DD. Pathological collagen deposition has been associated with DD. Collagens are triple helical proteins found in ECM, and there are more than 30 collagens and collagen-related proteins. Early studies have noted an increase in the ratio of type III to type I collagen going from control palmar fascia through uninvolved, mildly involved cords to nodules of DD.
Similarly, the high levels of tenascin protein observed in more aggressive forms of DD, which is in agreement with upregulation of tenasin C mRNA and downregulation of microRNA targeting TNC.\(^{9,13,14}\) Similarly, TGFB2 mRNA has been shown to be upregulated in DD,\(^{12}\) and shows intense intracellular localisation within myofibroblasts in the proliferative and involutional stages of the disease.\(^{26}\) Indeed, the addition of TGFB2 had a significant effect on the development of myofibroblasts, a cell type thought to contribute to DD contracture.\(^{26,38}\)

### CORRELATING GENE EXPRESSION WITH LINKAGE AND GENOME-WIDE ASSOCIATION STUDIES

Several chromosomal regions have been associated with DD from genetic linkage and association studies, including comparative genomic hybridisation, \(^{39,41}\) genome-wide family linkage, \(^{42}\) and case–control whole genome association studies.\(^{43,44}\) Genes within significantly associated loci were compared with the differentially expressed genes reported in the gene expression profiling studies. In this review, 2 genes, MAFB and protein kinase X-linked (PRKX), were found to be highlighted by both types of studies (figure 2). PRKX, which contains a single nucleotide polymorphism (SNP; rs17355275) that showed a positive association with DD,\(^{44}\) is PRKX – 1447. doi:10.1136/annrheumdis-2012-201295 RT-qPCR, reverse transcription quantitative PCR.

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**Table 1** Genes that have been reported by messenger RNA expression microarray studies

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene expression microarray studies</th>
<th>Upregulation or downregulation</th>
<th>Fold change</th>
<th>Meta-analysis (pfp/regulation/fold change)(^{8,10,12})</th>
<th>Dysregulated microRNA predicted to target the gene(^{15})</th>
<th>Downstream confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAM12</td>
<td>Multiple(^{9,10})</td>
<td>Up</td>
<td>4</td>
<td>&lt;0.001/Up/2.9</td>
<td>miR-1, miR-101, miR-103b, miR-204, miR-206, miR-29c, miR-30b</td>
<td>RT-qPCR(^{14})</td>
</tr>
<tr>
<td>ADH1B</td>
<td>Multiple(^{10})</td>
<td>Down</td>
<td>−3.4 to −4</td>
<td>&lt;0.001/Down/−4.1</td>
<td>Western blot; RT-qPCR(^{23,65})</td>
<td></td>
</tr>
<tr>
<td>AKR1C2</td>
<td>Multiple(^{10})</td>
<td>Down</td>
<td>−2.3 to −11.8</td>
<td>0.003/Down/−1.9</td>
<td>RT-qPCR(^{14})</td>
<td></td>
</tr>
<tr>
<td>ALDH2</td>
<td>Multiple(^{10})</td>
<td>Down</td>
<td>−1.9 to −25.5</td>
<td>0.015/Down/−1.7</td>
<td>RT-qPCR(^{14})</td>
<td></td>
</tr>
<tr>
<td>ANGPTL7</td>
<td>Multiple(^{10})</td>
<td>Down</td>
<td>−2.6 to −4</td>
<td>&lt;0.001/Down/−1.9</td>
<td>RT-qPCR(^{14})</td>
<td></td>
</tr>
<tr>
<td>APP</td>
<td>Multiple(^{3,9})</td>
<td>Up</td>
<td>1.7 to 5.8</td>
<td>Not significant</td>
<td>miR-29c</td>
<td></td>
</tr>
<tr>
<td>ARL4C</td>
<td>Multiple(^{12})</td>
<td>Up</td>
<td>4</td>
<td>No assessed</td>
<td>miR-29c</td>
<td></td>
</tr>
<tr>
<td>CD01</td>
<td>Multiple(^{6,11})</td>
<td>Down</td>
<td>−2 to −4.8</td>
<td>&lt;0.001/Down/−2.2</td>
<td>RT-qPCR(^{14})</td>
<td></td>
</tr>
<tr>
<td>CLU</td>
<td>Multiple(^{10})</td>
<td>Down</td>
<td>−4 to −4.8</td>
<td>&lt;0.001/Down/−4.6</td>
<td>RT-qPCR(^{14})</td>
<td></td>
</tr>
<tr>
<td>COL1A1</td>
<td>Multiple(^{12})</td>
<td>Up</td>
<td>2 to 4.4</td>
<td>0.042/Up/1.9</td>
<td>miR-140-5p RT-qPCR(^{3,65})</td>
<td></td>
</tr>
<tr>
<td>COL1A2</td>
<td>Multiple(^{12})</td>
<td>Up</td>
<td>2 to 2.3</td>
<td>Not significant</td>
<td>miR-140-5p RT-qPCR(^{3,65})</td>
<td></td>
</tr>
<tr>
<td>COL1A4</td>
<td>Multiple(^{12})</td>
<td>Up</td>
<td>3 to 10.4</td>
<td>0.009/Up/2.4</td>
<td>miR-140-5p RT-qPCR(^{3,65})</td>
<td></td>
</tr>
<tr>
<td>COLS1A</td>
<td>Multiple(^{9})</td>
<td>Up</td>
<td>2 to 4</td>
<td>&lt;0.001/Up/2.7</td>
<td>miR-29c</td>
<td></td>
</tr>
<tr>
<td>COLS2A</td>
<td>Multiple(^{9,12})</td>
<td>Up</td>
<td>2 to 4</td>
<td>&lt;0.001/Up/2.9</td>
<td>miR-29c</td>
<td></td>
</tr>
<tr>
<td>LAMB1</td>
<td>Multiple(^{12})</td>
<td>Up</td>
<td>2.9 to 3</td>
<td>Not significant</td>
<td>miR-29c</td>
<td></td>
</tr>
<tr>
<td>LRRC17</td>
<td>Multiple(^{9,12})</td>
<td>Up</td>
<td>3 to 4</td>
<td>&lt;0.001/Up/4.4</td>
<td>miR-29c</td>
<td></td>
</tr>
<tr>
<td>MMP2</td>
<td>Multiple(^{9,12})</td>
<td>Up</td>
<td>3 to 29</td>
<td>0.012/Up/1.8</td>
<td>miR-29c</td>
<td></td>
</tr>
<tr>
<td>POSTN</td>
<td>Multiple(^{9,11})</td>
<td>Up</td>
<td>3 to 62.8</td>
<td>&lt;0.001/Up/5.0</td>
<td>miR-29c</td>
<td></td>
</tr>
<tr>
<td>PRG4</td>
<td>Multiple(^{9,10})</td>
<td>Down</td>
<td>−3 to −137.5</td>
<td>Not assessed</td>
<td>miR-1229, miR-1238, miR-494, miR-494</td>
<td></td>
</tr>
<tr>
<td>RGS3</td>
<td>Multiple(^{12})</td>
<td>Up</td>
<td>3</td>
<td>&lt;0.001/Up/2.5</td>
<td>miR-1229, miR-1238, miR-494, miR-494</td>
<td></td>
</tr>
<tr>
<td>TNC</td>
<td>Multiple(^{9,12})</td>
<td>Up</td>
<td>2 to 5.2</td>
<td>&lt;0.001/Up/1.9</td>
<td>miR-130b, miR-29c, miR-296-5p, miR-301b, miR-654-5p</td>
<td></td>
</tr>
<tr>
<td>TGFβ1</td>
<td>Single(^{6})</td>
<td>Up</td>
<td>n/a</td>
<td>Not significant</td>
<td>miR-130b, miR-29c, miR-301b, miR-654-5p</td>
<td></td>
</tr>
<tr>
<td>MAFB</td>
<td>Single(^{12})</td>
<td>Up</td>
<td>n/a</td>
<td>0.022/Up/1.6</td>
<td>miR-130b, miR-29c, miR-301b, miR-654-5p</td>
<td>miR-140-5p RT-qPCR(^{3,65})</td>
</tr>
<tr>
<td>WNT5A</td>
<td>None</td>
<td>n/a</td>
<td>n/a</td>
<td>0.047/Up/1.4</td>
<td>miR-22, miR-30b, miR-494, miR-494</td>
<td>miR-140-5p RT-qPCR(^{3,65})</td>
</tr>
<tr>
<td>ZIC1</td>
<td>None</td>
<td>n/a</td>
<td>n/a</td>
<td>0.003/Up/3.3</td>
<td>miR-101</td>
<td></td>
</tr>
</tbody>
</table>

*pfp, estimated percentage of false positive predictions. This is an equivalent to the false discovery rate or adjusted p values.

RT-qPCR, reverse transcription quantitative PCR.

†MicroRNA that are both dysregulated in Dupuytren’s disease and predicted (using TargetScan) to target the genes indicated in the first column are as given by Mosakhani \(^{13}\) RT-qPCR, reverse transcription quantitative PCR.
inheritance pattern has not been thought to be x-linked; a case with polyfribromatosis including keloid scars and DD was reported to be x-linked.47

The possible involvement of MAFB, a transcription factor that serves as a regulator of commitment and lineage determination of haematopoietic cells, has been extensively studied in DD.6 13 46 47 MAFB is downstream of a SNP (rs8124695, showing a linkage) that has been shown to be positively associated with DD.48 Both previous microarray studies and meta-analysis carried out in this review indicate upregulation of MAFB in DD at the mRNA level.6 12 In addition, microRNA (miR-130b, miR-29c and miR-301b) predicted to target MAFB are downregulated in DD.13 Furthermore, Lee et al have observed that while all control fascia are negative for MAFB, half of the DD tissues showed positive immunohistochemical staining for MAFB. Uprogulation of MAFB is also observed in fibromatosis (gene expression atlas).18

While no gene copy number variations were found in DD by Kaur et al,39 increased DNA copy numbers at chromosomes 7p14.1 and 14q11.2 have been reported in DNA extracted from nodules, when compared with DNA from blood of the same patient or external controls.40 41 Secreted frizzled-related protein (SFRP)4 and MMP14 are found within 450 kB of these copy number alterations, and significantly higher levels of SFRP4 and MMP14 expression were found in DD nodules.40 In addition, SNP (rs16879765) that was shown in a genome-wide association study to be significantly associated with DD, is adjacent to SFRP4.43 SFRP4 is a Wnt antagonist and inhibitor of the Wnt-β-catenin pathway;50 its expression is associated with reduced scar size after ischaemic injury51 and restricted tumour growth.52

Genetic variations in the regulatory regions of genes can potentially result in altered protein levels.53 Polymorphisms within microRNA binding sites in the 3′ untranslated region of candidate genes for colorectal cancer have been linked to colorectal cancer formation.54 By comparing SNP reported to be significantly associated with DD and microRNA dysregulated in DD, a SNP (rs4730775)43 is found to be located within a predicted target site for miR-299-3p,53 a microRNA found to be exclusively detected in the majority of DD fascia (compared with the minority that shows an expression profile resembling the controls).13 This SNP locates within the 3′ untranslated region.

Figure 2 Summary of genes that have been implicated in Dupuytren’s disease pathogenesis by multiple genome-wide association or global expression studies. The figure indicates genes that have been reported by multiple genome-wide association studies or global expression studies (yellow block); these genes have been reported in DNA and RNA studies, multiple DNA studies, multiple RNA studies and RNA and protein studies (joined by solid lines). Out of these genes, those that have also been confirmed by other gene-specific experimental approaches are indicated in the light blue box (joined by dotted lines).

Figure 3 Studies carried out on the major signalling pathways that have been proposed to contribute to Dupuytren’s disease (DD). From molecular aberrances observed in DD, three pathways have been suggested in the literature to be involved in DD pathogenesis, including the Akt signalling pathway, Wnt/β-catenin signalling pathway and TGFβ pathways. In addition, functional analysis and inhibition studies carried out for genes within, or downstream of, the TGFβ pathway. The study type (whether the observed aberrances are based on DNA, RNA, protein or inhibition/functional studies) is indicated by the colours, green, purple, blue and red. Molecules that have been studied by different methods (for example, TGFBR1 has been proposed to be aberrant at both DNA and RNA levels) have been labelled by two colours.
of \( \text{WNT2} \); however, the expression of \( \text{WNT2} \) has not previously been reported to be abnormal. Further experiments investigating microRNA, associated SNP and protein expression for \( \text{WNT2} \) in the same group of patients would be required before determining a functional role.

Finally, a higher level of \textit{iroquois homeobox protein 6} (\textit{IRX6}) mRNA has been observed in DD nodules and fat tissue adjacent to DD.\(^1\) \(^{14}\) \textit{IRX6} locates within a critical chromosomal region, where positive genetic linkage was previously reported in a Swedish family.\(^4\)\(^2\)

### PATHWAYS AND DD

Several signalling pathways have been suggested to be involved in DD in published global gene expression or genome-wide association studies, including TGF-\( \beta \) signalling,\(^12\) Akt signalling\(^21\) and Wnt signalling\(^43\) (see supplementary table S3, available online only, and figure 3). In addition, genes identified to demonstrate different regulation in this review (from published global gene expression studies and meta-analysis) have been used for pathway enrichment analysis using the KEGG orthology-based annotation system (KOBAs) 2.0, from which pathways associated with integrin from multiple databases were significant (table 2). These signalling pathways are not mutually exclusive from each other; activation of one pathway may induce another, or cross-talk between two pathways may occur (figure 4).\(^5\)\(^5\)\(^9\)

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**Table 2** Pathway enrichment of genes reported by messenger RNA microarray, meta-analysis and whole proteome studies

<table>
<thead>
<tr>
<th>Term name</th>
<th>Database</th>
<th>Term ID</th>
<th>No of genes dysregulated in DD</th>
<th>Background no of genes</th>
<th>Corrected p value</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta_1 ) Integrin cell surface interactions</td>
<td>PID curated</td>
<td>Integrin(_1) pathway</td>
<td>17</td>
<td>60</td>
<td>3.80E-05</td>
<td>CD14, CD81, COL1A1, COL1A2, COL5A1, COL5A2, COL6A1, COL6A2, FN1, LAMB3, LAMB1, PLAU, PLAU, TGF_B1, THBS2, TNC</td>
</tr>
<tr>
<td>Muscle contraction</td>
<td>Reactome</td>
<td>REACT:17044</td>
<td>11</td>
<td>49</td>
<td>0.000427365</td>
<td>TPM1, TPM2, NEB, TPM4, MYL12, MYL3, SORBS1, MYL6, VM, MYH6, AAT6</td>
</tr>
<tr>
<td>ECM receptor interaction</td>
<td>KEGG PATHWAY</td>
<td>hsa04512</td>
<td>16</td>
<td>85</td>
<td>0.000427365</td>
<td>CD36, COL1A1, COL1A2, COL4A1, COL4A2, COL5A1, COL5A2, COL6A1, COL6A3, FN1, LAMB1, LAMB3, THBS2, TNC, TNXB</td>
</tr>
<tr>
<td>Protein digestion and absorption</td>
<td>KEGG PATHWAY</td>
<td>hsa04974</td>
<td>15</td>
<td>81</td>
<td>0.000865334</td>
<td>COL1A4, COL1A5, COL1A8, COL1A1, COL1A2, COL4A1, COL4A2, COL4A5, COL5A1, COL5A2, COL6A1, COL6A3, CPA3, EGN</td>
</tr>
<tr>
<td>Developmental biology</td>
<td>Reactome</td>
<td>REACT:111045</td>
<td>32</td>
<td>392</td>
<td>0.001614686</td>
<td>ABL1M1, ADIPQ1, CD36, CDON, CTNN1, COL1A1, COL1A2, COL4A1, COL4A5, COL5A1, COL5A2, COL6A1, COL6A3, COL6A5, CTNN1A1, DL1G1, FABP4, KCNN3, LAMB1, LPL, MYL12B, MYL6, NCAM1, PUN1, PLXNC1, RHOA, SEMA6A6, SLIT2, SFTBN1, STIP1, TCF7</td>
</tr>
<tr>
<td>Phenylalanine degradation IV (mammalian, via side chain)</td>
<td>BioCyc</td>
<td>PWY-6318</td>
<td>4</td>
<td>5</td>
<td>0.015637937</td>
<td>ALDH2, ALDH3A2, MAGA, HPD</td>
</tr>
<tr>
<td>Amoebiasis</td>
<td>KEGG PATHWAY</td>
<td>hsa05146</td>
<td>15</td>
<td>108</td>
<td>0.01734527</td>
<td>CD14, COL1A1, COL1A2, COL4A1, COL4A2, COL4A5, COL5A1, COL5A2, COL6A1, COL6A3, FN1, GNAS, HSPB1, LAMB1, LAMB3, PRRX, TGF_B2,</td>
</tr>
<tr>
<td>Glycogen breakdown (glycogenolysis)</td>
<td>PID reactome</td>
<td>500680</td>
<td>4</td>
<td>11</td>
<td>0.029934763</td>
<td>PYGM, PHK2, GYY2, PHKG1</td>
</tr>
<tr>
<td>Integrin signalling pathway</td>
<td>PANTHER</td>
<td>P00034</td>
<td>18</td>
<td>157</td>
<td>0.029934763</td>
<td>ACTG1, ARF1, COL1A4, COL1A5, COL1A1, COL1A2, COL4A1, COL4A2, COL4A5, COL5A1, COL5A2, COL6A1, COL6A3, COL7A1, COL8A1, COL9A3, FN1, RHODA, COL1A1, COL1A2, ED1_3, FN1, LAMB1, PLAU, PLAU, TGF_B1, THY1</td>
</tr>
<tr>
<td>( \beta_3 ) Integrin cell surface interactions</td>
<td>PID curated</td>
<td>integrin3(_3) pathway</td>
<td>9</td>
<td>38</td>
<td>0.031254944</td>
<td>CD14, CD81, COL1A1, COL1A2, COL5A1, COL5A2, COL6A1, COL6A2, FN1, LAMB3, LAMB1, PLAU, PLAU, TGF_B1, THBS2, TNC</td>
</tr>
</tbody>
</table>

\( \text{DD} \), Dupuytren’s disease; ECM, extracellular matrix; PID, Pathway Interaction Database

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**Figure 4** Potential molecular/environmental involvement in the pathogenesis of Dupuytren’s disease (DD). Both genetic and environmental factors have been proposed to contribute to DD development. Four signalling pathways have been suggested to be involved in DD, the Akt, TGF-\( \beta \), integrin and Wnt signalling pathways. Interactions have been suggested to occur between these pathways (references as indicated on the arrows between the pathways). In addition, unknown signalling pathways can also be involved in DD pathogenesis. The different severity of DD may have resulted from different levels of contribution from environmental and genetics factors, thereby triggering different activated/suppressed signalling pathways.
INTEGRIN SIGNALLING PATHWAY

Integrins constitute a family of transmembrane receptors that mediate attachment between the (intracellular) cytoskeleton and (extracellular) ECM, allowing transmission of signals from outside to inside of the cells, and vice versa. Cross-talk between integrin and growth factors, such as TGFβ and insulin-like growth factor (IGF) receptors (IGFIR), have been implicated in downstream molecules of TGFβ have previously been reported, which include the SMAD pathway and the extracellular signal-regulated kinase (ERK) MAP (mitogen-activated protein) kinase pathways. TGFβ has been shown to induce bimodal proliferation in connective tissue cells through regulation of platelet-derived growth factor (PDGF), which has been implicated in DJD. Treatment with inhibitors of TGFβ, the PDGF receptor and the MAP kinase pathway result in the decreased expression of fibrotic and proliferation markers.

Different isoforms of TGF-β have been reported to have both profibrotic and antifibrotic effects. TGFβ1 is thought to be profibrotic, while TGFβ3 is antifibrotic. However, the profibrotic or antifibrotic effects of TGFβ2 remain contradictory. 

β-CATENIN AND WNT SIGNALLING PATHWAY

Aberrant levels of β-catenin have been noted in DD, which is also supported by microRNA profiling studies. Varallo et al. showed high levels of β-catenin protein in the diseased fascia, whereas almost no expression is found in the control fascia. Neither genotypes nor DD recurrence could be associated with β-catenin overexpression.

β-Catenin is a central component of the canonical Wnt signalling pathway, which influences cell survival and proliferation. It has been suggested that the overexpression of β-catenin may be as a result of alterations in the Wnt signalling pathway. O’Gorman et al. investigated the gene expression of 13 Wnt molecules using microarray data and competitive reverse transcription PCR analysis and concluded that changes in Wnt expression were unlikely to be the cause of β-catenin dysregulation. However, O’Gorman et al. acknowledge that this does not rule out the potential involvement of altered Wnt signalling in DD pathogenesis; other genes involved in the Wnt pathway may be dysregulated, such as SFRP1, which demonstrated a consistently reduced level of expression in DD as previously reported.

A recent genome-wide association study has revealed six different DD risk loci containing genes, which are involved in the Wnt signalling pathway, and include WNT4, WNT2, R-spondin 2 (RSPO2), WNT7B, SFRP4 and sulphatase 1 (SULT1A1). In addition, microRNA predicted to target WNT5A and ZIC1 have been shown to be downregulated in DD cord, which coincides with the overexpression of WNT5A and ZIC1 mRNA observed in the meta-analysis in this review as well as analysis of microRNA data of Forsman et al. Compared with Mosakhani et al., higher levels of WNT5A have been observed in the β-catenin-accumulating involutional zones in comparison with proliferative and residual zones. ZIC1 and β-catenin co-expression have been described in DD myofibroblasts. Receptor tyrosine kinase-like orphan receptor 2 (ROR2), which was demonstrated.
to be upregulated in DD at both protein and mRNA levels, was shown to be involved in osteoclastogenesis,5 8 and is considered a potential biomarker for leiomyosarcoma and gastrointestinal stromal tumours.59 In addition, WNT3A-RO2 signalling has been suggested to be necessary for the expression of MMP13 during the development of cartilaginous tissue; MMP13 is also overexpressed in DD.60 51 Androgen receptors (AR), which interact with β-catenin and promote Wnt signalling at the chromatin level,92 have been reported to be elevated and co-localise with α-smooth muscle actin in DD cultures and DD tissue.80 Testosterone or dihydrotestosterone, which is converted from testosterone, activates AR; the involvement of AR may explain a male predominance that has been seen in DD.93 AR mRNA levels, however, were reported to be significantly underexpressed by Satish et al10 and the meta-analysis carried out here. While implication of the Wnt and β-catenin signalling pathways in the aetiology of DD has been reported by several studies through different techniques (figure 3), detailed analysis of the downstream components and targets of the Wnt pathway in vitro would be required to elucidate its involvement in DD.94

CONCLUSIONS AND FUTURE PERSPECTIVES

Whole genome association studies and global gene expression studies that have investigated polymorphisms, transcript or protein expression across the entire genome have provided data regarding genes that may be involved in DD, through altered gene expression or genotype associations. The initial analysis of these data created large lists of affected genes of potential interest, some with unknown biological function and relevance to DD. Interpreting these data continues to be a challenging task, and targets of the Wnt and β-catenin signalling pathways in the aetiology of DD has been shown to be involved in osteoclastogenesis, 5 88 and is to be upregulated in DD at both protein and mRNA levels,53 93 94 and targets of the Wnt pathway in vitro would be required to elucidate its involvement in DD.94

IN BRIEF

- Dupuytren’s disease (DD) is a common fibrosing disorder of connective tissue that affects the palmar fascia, typically causing the development of fibrotic nodules and contractures of the fingers.
- Several factors have been identified as potential biomarkers for DD, including matrix metalloproteinases (MMPs), fibronectin, and the androgen receptor (AR).
- A detailed analysis of the downstream components and targets of the Wnt pathway in vitro would be required to elucidate its involvement in DD.

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Whole genome and global expression profiling of Dupuytren's disease: systematic review of current findings and future perspectives

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