Proteomic comparison of clubfoot and Dupuytren disease - are there any similarities?

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ABSTRACT

Clubfoot and Dupuytren's disease (DD) belong to fibroproliferative diseases of unclear origin, and the prevalence of their relapses is very high. Although these two diseases seem different (occurring at various ages), they may have much in common (hypoxia, angiogenesis, fibrosis). The proteomic analysis compared samples from pathologically contracted tissues of both diseases with controls. Most proteins with significantly higher concentrations in pathological tissue from most analyses were common to both disorders. Enrichment analysis of significantly upregulated proteins from both comparisons yielded significant protein connections and protein-protein interactions. However, some differences were found in the protein level (specific proteins significantly upregulated in pathological tissues) and altered levels of collagen crosslinking. However, more similarities than differences were found between these diseases. This proteomic comparison of clubfoot and DD was performed for the first time, and these findings contribute to a better characterization of their etiology.

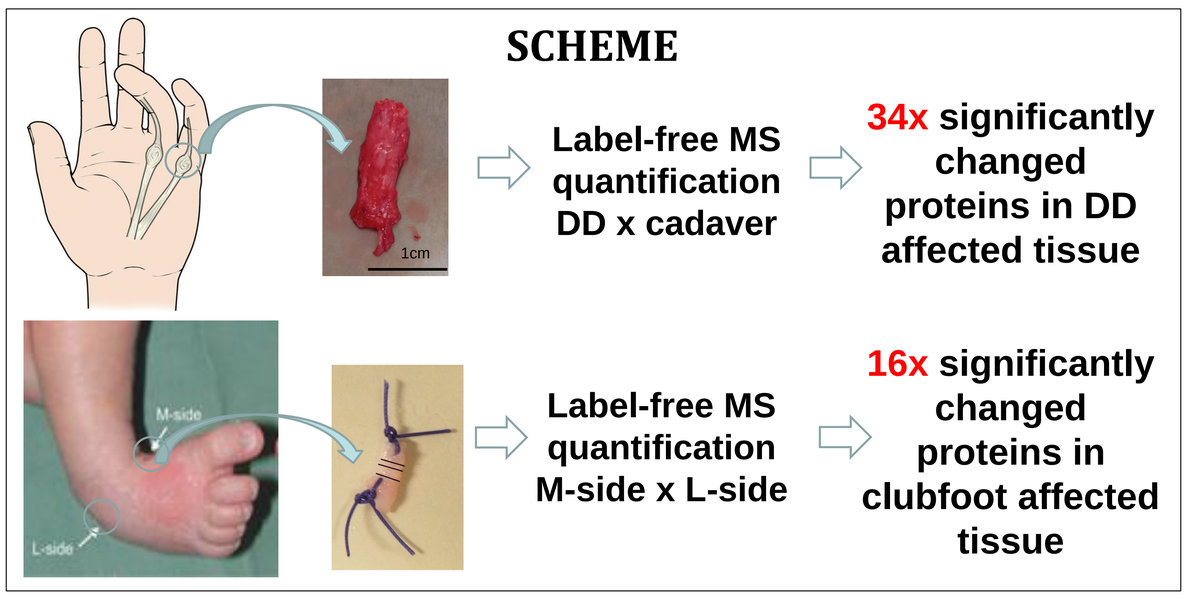
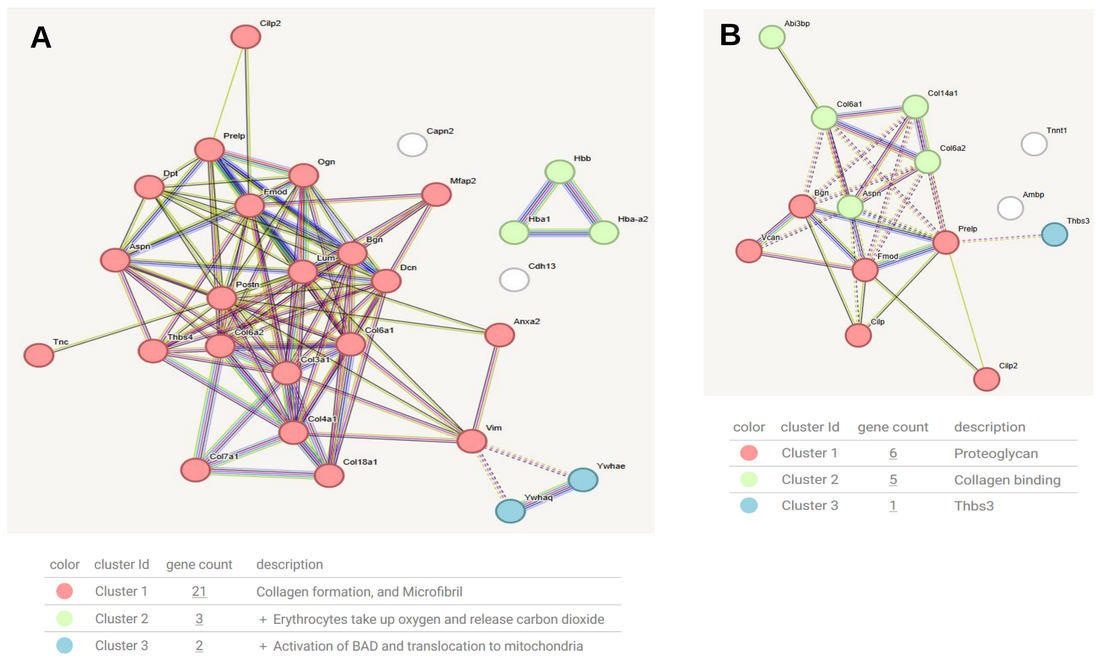


Figure 1: STRING analysis of significantly upregulated proteins in DD nodules (A) clubfoot affected tissue (B).



color	cluster Id	gene count	description
	Cluster 1	21	Collagen formation, and Microfibril
	Cluster 2	3	+ Erythrocytes take up oxygen and release carbon dioxide
	Cluster 3	2	+ Activation of BAD and translocation to mitochondria

color	cluster Id	gene count	description
	Cluster 1	6	Proteoglycan
	Cluster 2	5	Collagen binding
	Cluster 3	1	Thbs3

EXPERIMENTAL

Clubfoot samples: The samples of pathological contracted tissue (clubfoot relapses) were obtained from the area between the medial malleolus, navicular bone, and sustentaculum tali at the medial side of the foot (medial side: M-side), and the control samples will be obtained from normal tissue at the lateral surface of the calcaneocuboid joint (lateral side: L-side), as in our earlier study (Eckhardt 2019).

DD samples: Tissue samples from contracted palmar fascia (nodules) were obtained from DD patients undergoing surgical treatment. Control samples were obtained from physiological palmar fascia from cadavers.

Liquid chromatography coupled with mass spectrometry (LC-MS): All samples (n=9 for DD nodules, n=9 for cadavers, n=16 for clubfoot M-side, n=13 for L-side (control clubfoot) were about 5 mg dry weight. Samples were washed ,homogenized, treated with 4M guanidine, delipidated, and digested by trypsin. All samples were purified by Stage Tips and analyzed by mass spectrometry label-free quantification (Thermo Orbitrap Ascend (Thermo Scientific)). High-performance liquid chromatography (HPLC) analysis of collagen crosslinks: The HPLC analysis was performed at HPLC Agilent 1100 using protocol from Chromsystems (c.n. 48000): “Instruction manual for the HPLC analysis of Crosslinks in urine”. The concentration of trivalent collagen crosslinks (pyridinoline (PYR)) obtained by HPLC was measured in nmol/mg (mg - the dry weight of the sample).

CORRESPONDENCE

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INTRODUCTION

Clubfoot is a complex deformity characterized by plantar flexion, adduction, and inversion of the foot, an augmented midfoot arch, hindfoot varus, and equinus. Clubfoot and Dupuytren's disease belong to fibroproliferative diseases with unclear origins, and their relapses are very common. As different as these two diseases seem (completely different temporal occurrence), they may have much in common (hypoxia and fibrosis). Are there the same significantly altered proteins in clubfoot and Dupuytren's disease?

RESULTS AND DISSCUSION

In total, we detected significant increases in 34 protein concentrations in DD nodules in comparison with palmar fascia and 16 clubfoot-affected tissue (M-side) in comparison to non-affected tissue (L-side). Some of them were common to both diseases (Table 1). Most of these proteins are incorporated into tissue remodeling and/or fibrosis. Enrichment analysis of significantly differentially expressed proteins using the STRING and KEGG databases revealed similar associations and protein-protein interactions in both comparisons (Figure 1,2). However, some differences were found in the protein level (specific proteins significantly upregulated in pathological tissues – Figure 1). In KEGG pathways enrichment, the most represented pathways were “ECM-receptor interaction” in both cases. Interestingly, laminin concentration is reduced in DD, and conversely, vitronectin concentration is reduced in affected clubfoot tissue (Figure 2). Notably, collagen crosslinking significantly differed between affected tissue and controls in clubfoot. In contrast, the concentrations of collagen crosslinks in Dupuytren's disease nodules were almost identical to palmar fascia controls (Figure 3).

Table 1: List of the samesignificantly upregulated proteins in DD and clubfoot affected tissue

Dupuytren x Control		Clubfoot x Control	
Significant to DD		Significant to clubfoot	
gene name	p-value	gene name	p-value
ASPN	0,02	ASPN	0,03
COL6A1	0,02	COL6A1	0,02
COL6A3	0,02	COL6A3	0,02
TGF-βip	0,01	TGF-βip	0,02
COL6A2	0,02	COL6A2	0,02
PRELP	0,003	PRELP	0,049
FMOD	0,003	FMOD	0,01
BGN	0,02	BGN	0,01
CILP2	0,03	CILP2	0,01

(FDR-adjusted p-value in green)

Figure 3: Concentration of collagen crosslink (pyridinoline (PYR)) in DD nodules and palmar fascia

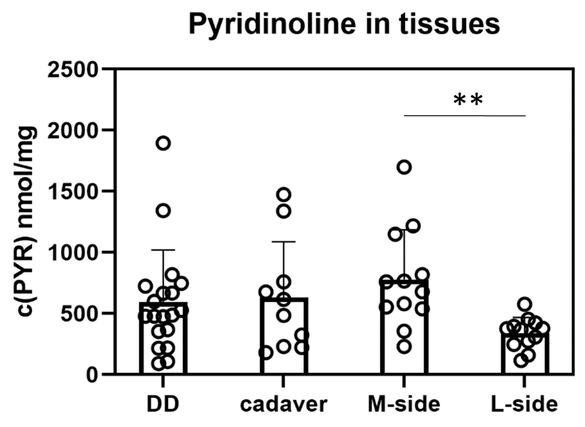
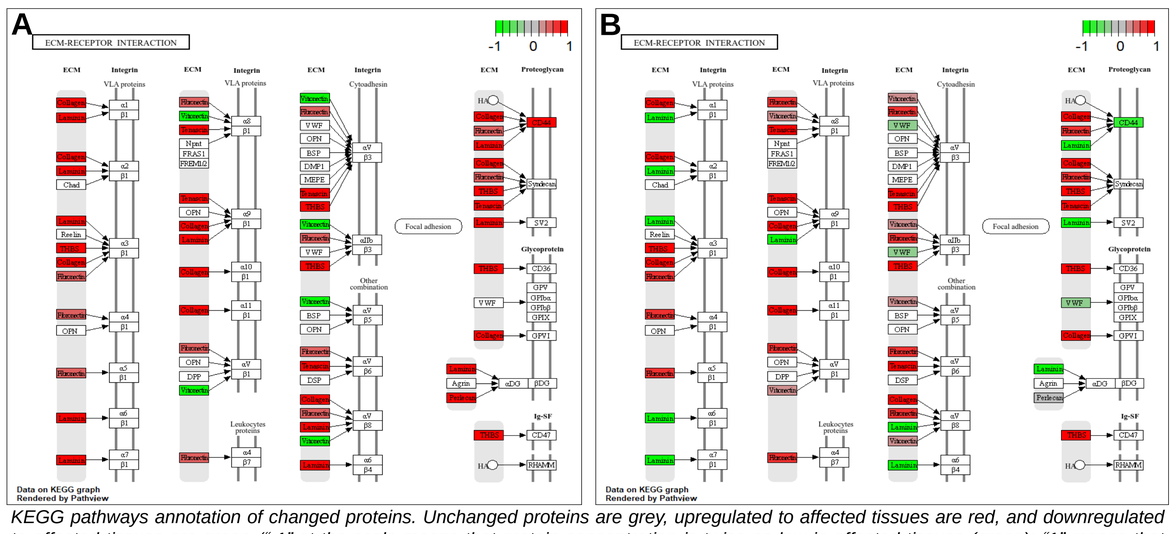


Figure 2: KEGG analysis of significantly upregulated proteins in DD nodules (A) clubfoot affected tissue (B).



KEGG pathways annotation of changed proteins. Unchanged proteins are grey, upregulated to affected tissues are red, and downregulated to affected tissues are green (“-1” at the scale means that protein concentration is twice as low in affected tissues (green); “1” means that protein concentration is twice as high in affected tissues (red)).

CONCLUSION

The proteomic profiles of clubfoot and Dupuytren's disease show more similarities than differences in individual protein expression and pathway enrichment. These findings suggest possible similarities in their etiology, which could help search for an adequate, currently lacking drug.

REFERENCE

Eckhardt A. 2019 J Orthop Res. Mar;37(3):769-778. doi: 10.1002/jor.24211.

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