

## Abstract

Collagen VI is a ubiquitous heterotrimeric protein of the ECM with a complex assembly process and a multiplicity of functions. In skeletal muscle, collagen VI provides both structural integrity to the endomysium and basement membrane surrounding muscle fibres and also provides signalling cues to underlying satellite cells in the growth and regeneration of skeletal muscle. Mutations in collagen VI lead to a spectrum of congenital myopathies, from the relatively mild Bethlem Myopathy to the severe Ullrich Congenital Muscular Dystrophy.

The multi-subunit composition of collagen VI contains predominantly von Willebrand Factor type A domains (VWA), which are protein protein interaction domains found in a range of ECM proteins. The  $\alpha 3(\text{VI})$  chain in particular has up to ten VWA domains at its N terminus joined end to end. Here, it is demonstrated that the flexibility between these domains, thus far only experimentally hinted at, is indeed present. The flexibility between adjacent domains ranges from an elastic stiff spring type in distal tandem domains (after N4) to a highly flexible linker between the N4-N3 domains, indicating a hinge-like mechanism in the latter region. Pathogenic mutations are uncommon in this region of the  $\alpha 3(\text{VI})$  chain, suggesting an integral role which is intolerant to variants that compromise these structural features.

Disease causing mutations are however common in the triple helical region and flanking VWA domains. The  $\alpha 3(\text{VI})$  N2 VWA domain is one such domain and the solved crystal structure was helpful to understand the pathomechanisms. The consequences of point mutations introduced into the single N2 domain depends on the location within the structure resulting in either misfolding, retention and triggering of ER stress or secretion into the ECM to form aberrant interactions. Tentative structure function relationships were thus explored.

These fundamental disease-causing mechanisms are also paralleled by mutations occurring in the triple helical region. A splice site mutation in COL6A1 (c.930+189C>T) leading to the inclusion of a pseudoexon and a 24 residue interruption to the Gly-Xaa-Yaa repeat, results in a rapidly progressive form of UCMD. The resulting mutant  $\alpha 1(\text{VI})$  chain is shown to be largely secreted as a non-triple helical form into the ECM of dermal fibroblasts, notwithstanding a degree of intracellular retention of high molecular weight aggregates which may trigger ER stress. The mutant  $\alpha 1(\text{VI})$  chain then accumulates in the ECM of skeletal muscle and that deposited by cultured dermal fibroblasts, where it exists in cluster-like formations which interact with wild-type collagen VI. This chain therefore seems to act primarily via a gain-of-misfunction, toxic pathomechanism, representing a novel disease paradigm for a collagen VI myopathy. Thus, biochemical characterisation of this mutation has proved valuable in the development of a promising oligonucleotide skipping based therapy for this form of UCMD.