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# *In silico* functional and structural characterisation of ferlin proteins by mapping disease-causing mutations and evolutionary information onto three-dimensional models of their C2 domains

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**Abstract**

Ferlins are C2 domain proteins involved in membrane fusion events, including membrane repair and synaptic exocytosis, and their deficiency can result in muscular dystrophy and deafness. We have undertaken a structural study of their C2 domains by sequence comparison and homology modelling to understand the function of these poorly characterised proteins and to predict the molecular impact of disease-causing mutations. We observe that non-conservative mutations affecting buried residues tend to result in detrimental phenotypes, likely because of decreased protein stability, whereas most variants with replacements in surface residues do not. The few cases of exposed residues altered in variants known to cause diseases are found in conserved areas of functional importance, including essential calcium-binding regions, as deduced by analogy to other characterised C2 domains. Furthermore, we report distinct features of some C2 domains in the two known ferlin subfamilies that correlates with the presence or absence of the DysF domains. Taken altogether, our results highlight potential targets for further experimental analyses to understand the function of ferlin proteins. We believe our modelling data will aid the diagnosis of diseases associated with ferlin mutations and the development of therapeutic strategies.


*Keywords:* C2 domain; Dysferlin; Muscular dystrophy; Otoferlin; Evolutionary analysis; Structural modelling

## 1. Introduction

Dysferlin, otoferlin and myoferlin collectively known as the ferlins are C2 domain-containing proteins encoded by a multiple gene family which shares homology with the *C. elegans* sperm vesicle fusion protein FER-1 [1,2]. Dysferlin and otoferlin have been implicated in genetic disease, namely muscular dystrophy and deafness, respectively [1]. All ferlins share a conserved architecture consisting of tandem C2 domains and a single C-terminal transmembrane segment (Fig. 1c). However, a subset of the ferlins (i.e. dysferlin, myoferlin and FER-1) contains an additional evolutionary conserved domain, designated DysF (Fig. 1c), of unknown function [3]. The ferlin proteins are predicted to play a role in membrane trafficking based on the presence of C2 domains and the findings that fer-1 mutants show defective membrane fusion of large membranous vesicles in developing sperm [4].

C2 domains have been identified in many proteins involved in membrane trafficking and signal transduction where they are capable of protein–protein interactions as well as calcium-mediated phospholipid binding [5–7]. C2 domains fold into an eight-stranded β-sandwich that can adopt two structural arrangements, type-I and type-II [5], related by a circular permutation (Fig. 1a). Many C2 domains contain a calcium-binding region (CBR) which is located in the loops at one end of the fold (Fig. 1a and b) where negatively charged residues, primarily aspartates, serve as ligands for two or three

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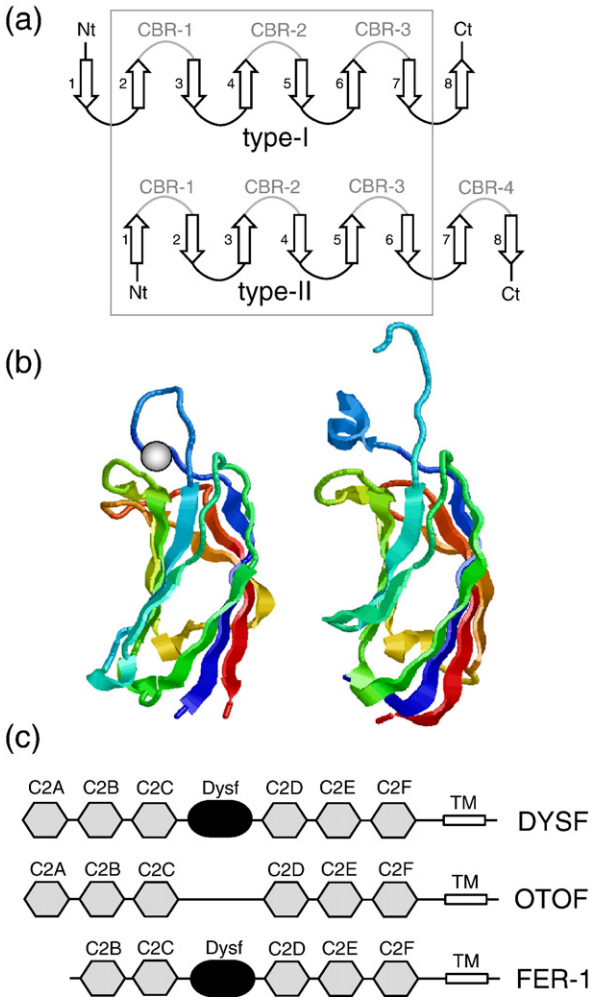


Fig. 1. C2 structural types, homology modelling templates and domain organization of ferlin proteins. (a) Scheme depicting the two topological types of the eight-stranded C2 domain fold in which the potential calcium-binding site is located in the loops at one end of the fold. The C2 core of six strands referred in the main text is shown enclosed in a square. (b) Ribbon representation of the two type-II C2 domains used as templates for the modelling, phospholipase C-δ1 (left) [33] and protein kinase C-ε (right) [34]. The colouring follows the sequence from the N- (blue) to C-terminus (red). A grey sphere indicates the calcium-binding site in one of the structures. (c) Diagram depicting the domain architecture of ferlin proteins: dysferlin-like (DYSF), otoferlin-like (OTOF) and worm ferlin (FER-1). The C2 domains are represented as hexagons and they are named according to their relative locations (DYSF and OTOF) or similarities (FER-1) between other ferlin proteins. For clarity, the DysF domain is shown as a single unit even though it adopts a complex nested structure [3].

calcium ions. C2 domains can exist individually or in association with other domains adopting structural, regulatory or major functional roles that seem to correlate with distinct conservation patterns present on their surface [8]. For example, C2 domains that bind and insert into membranes present highly conserved CBRs, including some aromatic residues [9]. In other C2 families where membrane binding occurs without membrane insertion, the CBR loops usually show conservation only of those residues directly interacting with calcium.

Recent data highlights that dysferlin and myoferlin have a role in muscle membrane fusion events. The membrane trafficking pathways associated with dysferlin are linked with cell membrane repair [10]. Following a membrane disruption dysferlin accumulates at the wound site in wild type muscle, but dysferlin-deficient muscle fibres are defective in resealing membrane disruptions [10]. Thus, a mechanism linked to defective sarcolemmal repair resulting from dysferlin mutations is thought to cause limb-girdle muscular dystrophy type 2B, Miyoshi myopathy or anterior distal muscular dystrophy [11,12] collectively termed the dysferlinopathies. Regardless of the type of dysferlin mutation, patients with dysferlinopathy show complete or partial deficiency of dysferlin. Myoferlin has not been implicated in a genetic disease as yet despite the fact that myoferlin null mice also show an abnormal muscle phenotype but linked to defects in myoblast fusion [13]. It has been proposed that dysferlin and myoferlin may function as $Ca^{2+}$ sensors to facilitate vesicle fusion via their C2 domains because (i) one of the major mechanisms operating in cell membrane repair involves $Ca^{2+}$-dependent lysosomal exocytosis a process that is regulated by a C2 domain-containing protein, synaptotagmin VII [14]; and (ii) biochemical studies of dysferlin and myoferlin have revealed calcium-dependent phospholipid binding of their respective C2A domains [13,15]. Further evidence that the ferlin proteins may function as $Ca^{2+}$ sensors have emerged from recent publications on FER-1 C2 domains and analysis of otoferlin null mice [16,17]. C2 domain mutants of FER-1 have been shown to alter the $Ca^{2+}$ sensitivity of the fusion of the large membranous vesicles with the worm spermatocyte plasma membrane leading to suggestions that FER-1 C2 domains function as $Ca^{2+}$ sensing units mediating the membrane fusion events between the large membranous vesicles and the spermatid plasma membrane [17]. Examination of otoferlin expression in the inner ear of the mouse has revealed association of otoferlin to ribbon-associated synaptic vesicles and interaction with the synaptic exocytosis protein machinery [16].

To understand better the distinct mechanisms by which dysferlin, myoferlin and otoferlin are involved in membrane fusion events we have undertaken a structural study of their C2 domains by means of sequence comparisons and homology modelling in order to determine if, like other C2 domain proteins, their functional specificity correlates with distinct conservation patterns present on their C2 surfaces. For this we first determined the molecular characteristics of ferlin C2 domains and examined the effect of pathogenic mutations and other sequence variants onto their modelled structures. We show that most disease-causing mutations in ferlin proteins correspond to structurally important residues whose replacement may result in decreased protein stability, whereas most mutations located on the surface of the C2 domains are in general not linked to a mutant phenotype. However, in contrast to a recent report [18], we present examples of detrimental ferlin mutations associated with surface residues