How does the interaction between the Filamin A repeat 10 domain and F-actin lead to severe OPD skeletal disorders?

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Abstract

The cytoskeleton network allows cells to differentiate, divide, and move in response to the external environment creating a mechanoprotection system against cell stress. The actin cytoskeleton is stabilised and tightly regulated by various actin-binding proteins, one of which are the family of Filamin (*FLN*) proteins that crosslink F-actin into three-dimensional networks. Filamins also link the actin cytoskeleton to the cellular membrane through interactions with transmembrane proteins and function as a molecular scaffold for signalling molecules. In addition to an actin binding domain, each monomer contains a rod region of 24 immunoglobulin-like repeat domains with dimerisation of the monomers occurring at repeat 24. The human filamin family contains three *FLN* isoforms; *FLNA*, *FLNB,* and *FLNC* which are differentially expressed where *FLNA* is identified as the dominant isoform located on the X-chromosome essential for mammalian development.

Mutations in Filamin A (*FLNA*) have been identified to cause distinctly different human diseases affecting the central nervous system, vascular system, or skeletal muscles; however, the molecular mechanisms of FLNA leading to these diseases remain unclear. Mutations cluster in distinct *FLNA* domains, suggesting their functional importance for mediating correct functions. Mutations in the *FLNA* repeat 10 domain are correlated with severe forms of the skeletal disorders Otopalatodigital syndrome spectrum disorders (OPD) thought to be due to an altered or gain-of-function phenotype. The aim of this study was to provide an insight into the biochemical properties of *FLNA* repeat 10 domain by better understanding how mutations in this domain lead to OPD.

Initially, recombinant wildtype (Wt) and mutant (V1249A and A1188T) FLNA repeat 10 domain proteins (FLNAR10) were purified then compared by *in vitro* biochemical studies to investigate secondary structure, stability, and affinity towards F-actin. The FLNAR10 protein was revealed to have relatively weak binding affinity towards F-actin, consistent with being an additional contributor in the filamin protein to bind F-actin. Mutations in the FLNAR10 protein exhibited a slight increase in affinity towards F-actin, accompanied by a slight reduction of thermostability in comparison to the Wt protein, but no significant changes in the secondary structure were observed. This slight increase in the affinity of the mutant FLNA repeat 10 proteins towards F-actin is

consistent with a gain-of function mechanism for the disease phenotype. Overall, these results contribute towards a better understanding of the FLNA function, providing further evidence towards a gain-of function mechanism for OPD.

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× g	Multiples of gravitational force
Å	Angstrom (10^{-10}m)
A1188T	FLNA repeat 10 mutation Alanine to Threonine leading to FMD
A_{280}	Absorbance at 280 nm
aa	Amino acid
ABC	Ammonium bicarbonate
ABD	Actin binding domain
AEX	Anion Exchange Chromatography
Ala	Alanine
Amp	Ampicillin
ATP	Adenosine-5´-triphosphate
BLAST	Base local alignment search tool
bp	Base pair
CD	Circular Dichroism
cDNA	Complementary DNA
CV	Column Volumes
CH	Calponin homology domain
C-terminal	Carboxyl terminal
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide Triphosphate
dsDNA	Double-stranded Deoxyribonucleic acid

DTT	Dithiothreitol
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium Bromide
F-actin	Filamentous actin
FLN	Filamin
FLNA	Filamin A
FLNAR10	Filamin A repeat 10 protein
FMD	Frontometaphyseal dysplasia
G-actin	Globular (monomeric) actin
His_6	Hexa-histidine tag
HPLC	High-performance liquid chromatography
Ig	Immunoglobulin
IPTG	Isopropyl-β-D-thio-galactoside
K_d	Dissociation constant
kb	Kilobase pairs (of DNA)
LB	Luria Bertani media
LB-Amp	Luria Bertani media with 100 μg/ml ampicillin
mAU	Milli absorbance units
MeCN	Acetonitrile
Min	Minute
MNS	Melnick–Needles syndrome
MOPS	3-(N-morpholino) propanesulfonic acid

MW	Molecular Weight
N-terminal	Amino terminal
OD	Optical density at 600 nm
OPD	Otopalatodigital spectrum malformation disorders
OPD1	Otopalatodigital syndrome type 1
OPD2	Otopalatodigital syndrome type 2
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDB	Protein data bank
PH	Periventricular nodular heterotopia
pH	Negative decadal logarithm of proton concentration
KOAc	Potassium Acetate
RPM	Revolutions per minute
rTEV	Recombinant tobacco etch virus protease
S1199L	FLNA repeat 10 mutation Serine to Leucine leading to FMD
SEC	Size exclusion chromatography
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
TAE	Tris-acetate-EDTA buffer
TEMED	Tetramethylethylenediamine
TFA	Trifluoroacetic acid
Thr	Threonine
Tm	Melting temperature

TPCK	Tosyl phenylalanyl chloromethyl ketone
Tris	Tris-(hydroxymethyl)-aminomethane
U	Units
UV	Ultraviolet
V1249A	FLNA repeat 10 mutation Valine to Alanine leading to MNS
Val	Valine
v/v	Volume/volume ratio
Wt	Wild-type
w/v	Weight/volume ratio
w/w	Weight/weight ratio