Supplementary data

Table S3 - Pathogenicity assessment of new missense variants

Gene location	DNA mutation	Protein effect	Grantham distance (a)	PolyPhen V2 score (b)	Phylogenetic conservation (c)	Human Splice Finder (d)	Population screening (e)	Remarks	Conclusion
Exon 5	c.323G>A	p.Gly108Asp	90	0.998	9/10 (*y*)	-	-	-	Probably pathogenic
Exon 8	c.595C>A	p.Pro199Thr	38	0.995	10/10	-	0/180	other reported mutations affecting the same residue	Pathogenic
Exon 8	c.637C>T	p.Leu213Phe	22	0.523	9/10 (*ce*)	-	-	-	Probably pathogenic
Exon 8	c.659G>C	p.Arg220Thr	71	1.000	10/10	-	-	-	Probably pathogenic
Exon 11	c.1241T>C	p.Phe414Ser	155	0.998	10/10	-	0/140	-	Pathogenic
Exon 11	c.1247A>G	p.His416Arg	29	1.000	10/10	NDSS (75.1%)	-	disruption of ligand binding site	Probably pathogenic
Exon 14	c.1600T>C	p.Trp534Arg	101	1.000	10/10	DSS: +2%	-	-	Probably pathogenic

Pathogenicity assessment was performed with the aid of the commercial software Alamut version 2.1 (Interactive Biosoftware, Rouen, France). Results corroborating pathogenicity are underlined. Variants described according to the reference sequence NM_000252.2, using HGVS nomenclature guidelines.

(a) - Grantham's distance (Grantham, 1974), compares wild type and mutated aminoacids considering physical and chemical parameters (volume, weight, polarity, and carbon-composition); range of values: 0-215 (higher value indicates larger difference).

(b) - PolyPhen version 2 (Adzhubei *et al.*, 2010). This software attributes a score to the impact of substitutions on the structure and function of protein; range of values: 0-1.000 (higher value more likely to be pathogenic).

(c) - Phylogenetic conservation analysis: performed using an alignment of myotubularin's orthologues from 10 different species, 10/10 means conserved residue in all species; 9/10 means conserved except in one specie: yeast (*y*) or *C. elegans* (*ce*).

(d) - Impact of variants on splice-sites scores using the Human Splice Finder (Desmet *et al.*, 2009); NDSS - new donor splice site; DSS - donor splice site.

(e) - Population screening performed in ethnically matched controls, N/Nt - indicates the number of control chromosomes with variant / total number of chromosomes tested.

References:

Grantham, R. Amino acid difference formula to help explain protein evolution. Science. 1974; 185:862-4.

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Desmet FO, Hamroun D, Lalande M, Collod-Béroud G, Claustres M, Béroud C. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res. 2009; 37(9):e67.