

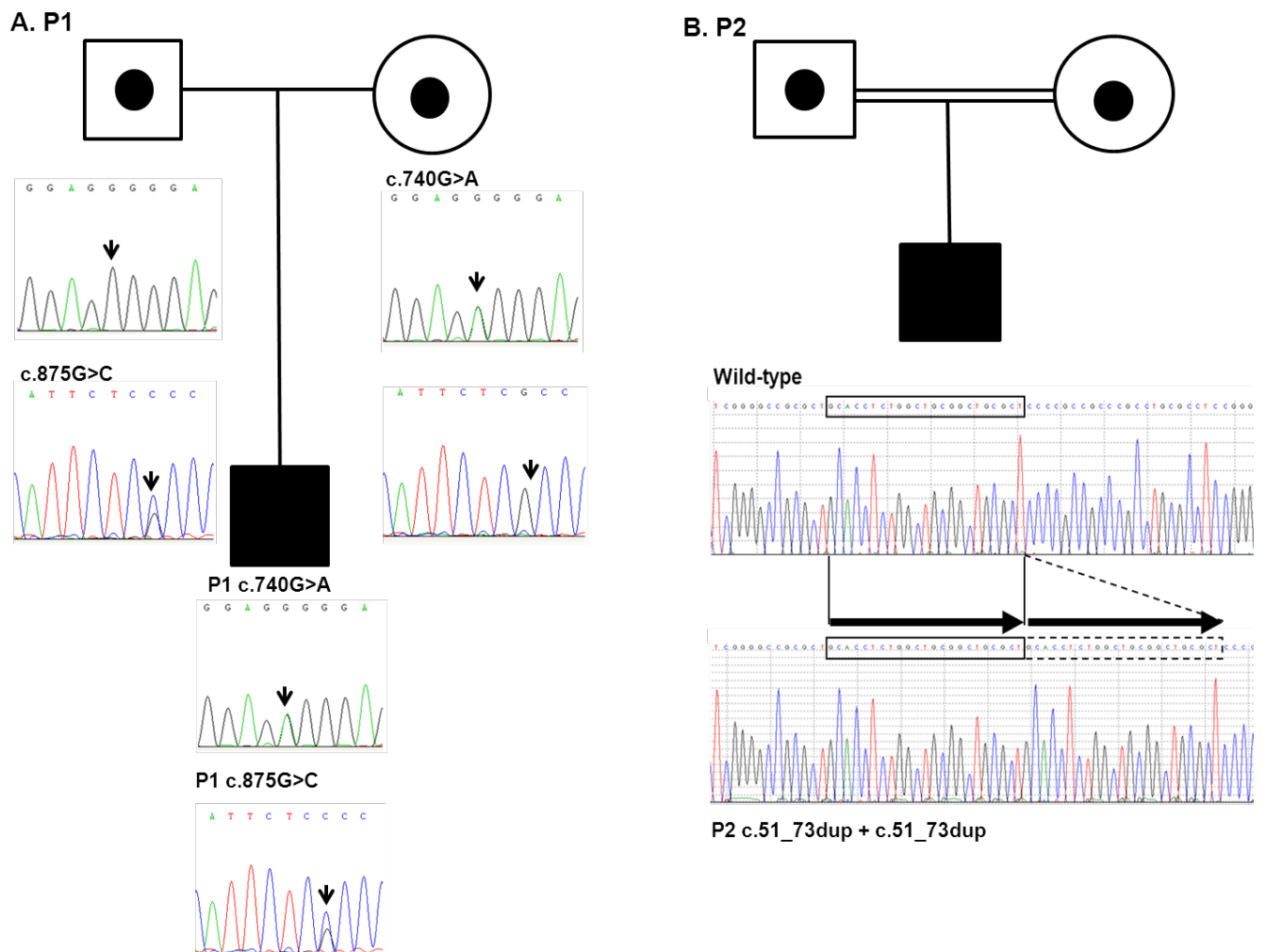
Supplemental Information

Mutations in *B3GALNT2* Cause

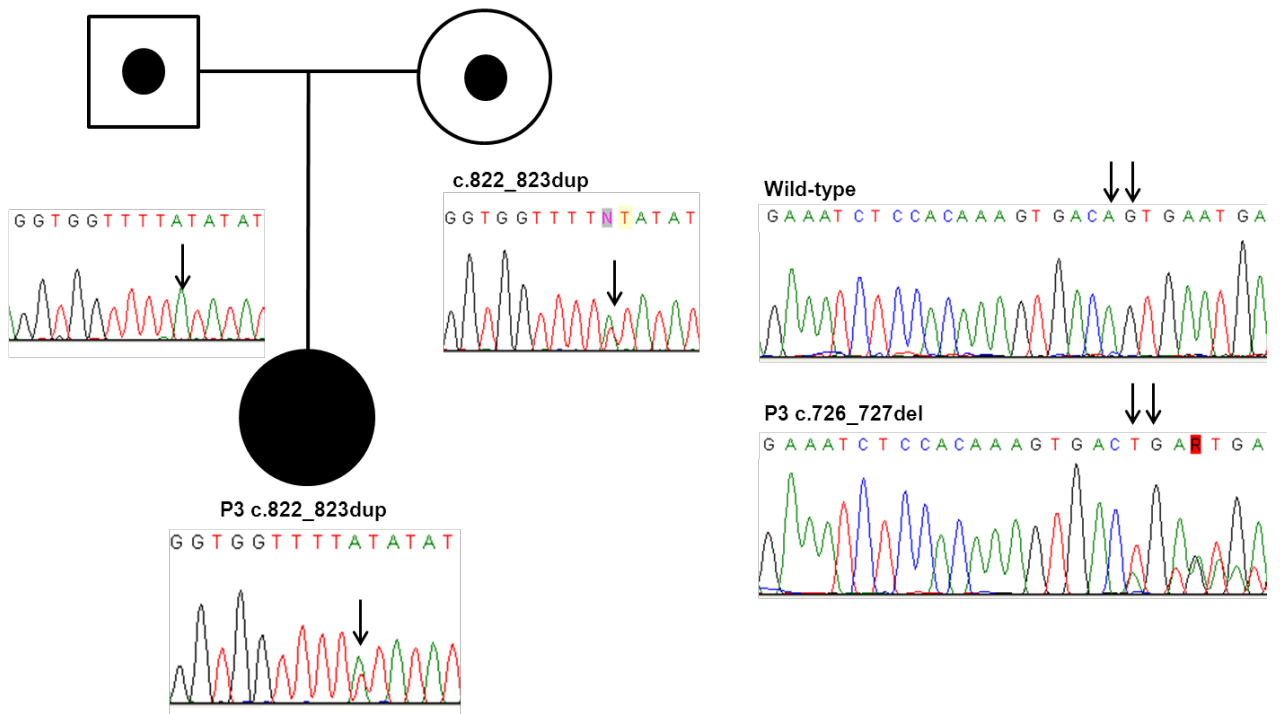
Congenital Muscular Dystrophy

and Hypoglycosylation of α -Dystroglycan

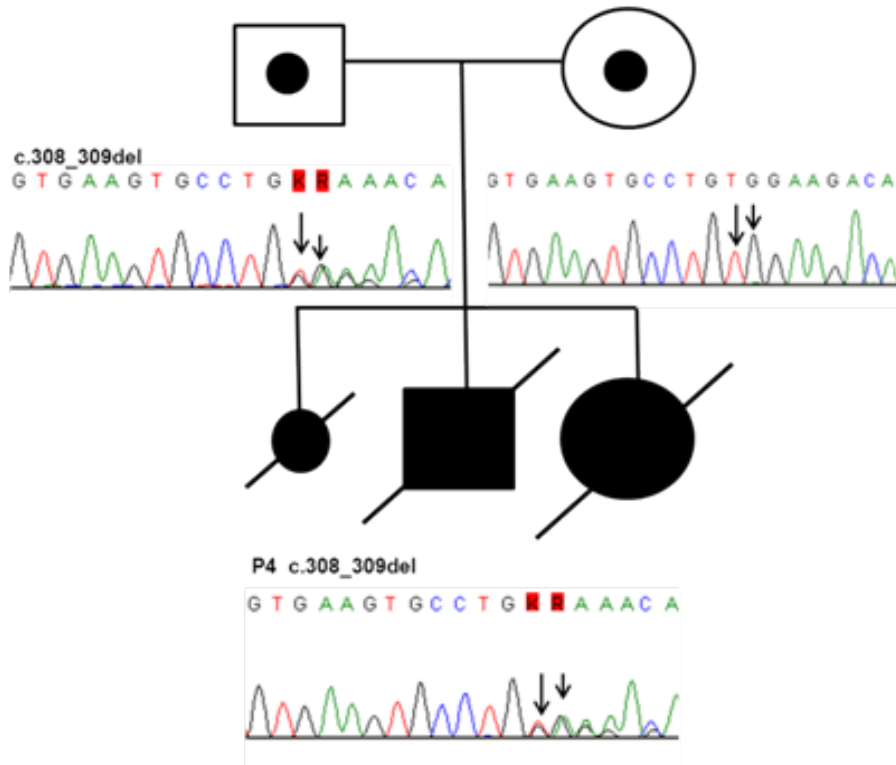
Elizabeth Stevens, Keren J. Carss, Sebahattin Cirak, A. Reghan Foley, Silvia Torelli, Tobias Willer, Dimira E. Tambunan, Shu Yau, Lina Brodd, Caroline A. Sewry, Lucy Feng, Goknur Haliloglu, Diclehan Orhan, William B. Dobyns, Gregory M. Enns, Melanie Manning, Amanda Krause, Mustafa A. Salih, Christopher A. Walsh, Matthew Hurles, Kevin P. Campbell, M. Chiara Manzini, UK10K Consortium, Derek Stemple, Yung-Yao Lin, and Francesco Muntoni



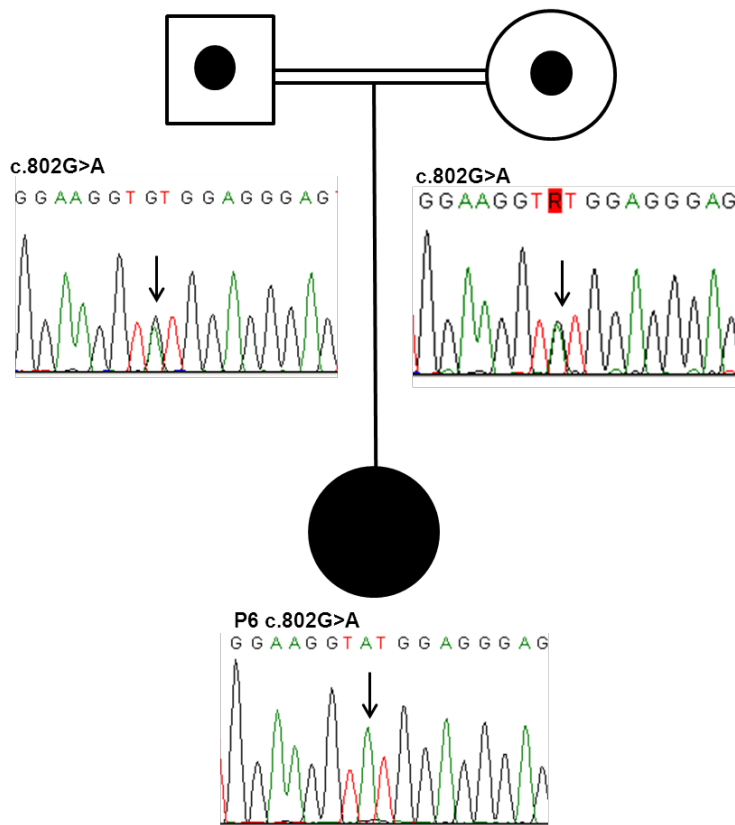
C. P3



D. P4



E. P6



F. P7

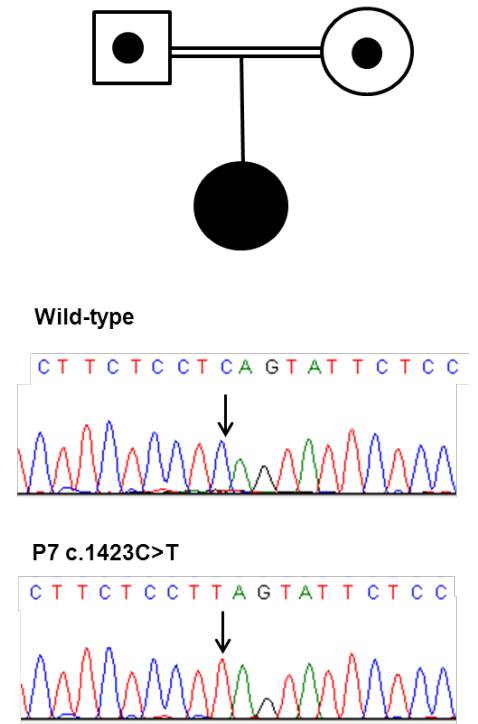


Figure S1. **Pedigree and Chromatograms from Sanger sequencing.** A. P1 has two heterozygous missense mutations (c.740G>A and c.875G>C). B. P2 has a homozygous duplication (c.51_73dup +c.51_73dup). C. P3 is heterozygous for a deletion and a frame shift mutation (c.726-727del and c.822-823dup). Paternal DNA was not available. D. P4 is heterozygous for a missense mutation and a frameshift mutation (c.775T>G and c.308-309del). Sequence chromatograms were only available for the deletion. E. P6 has a homozygous missense mutation (c.802G>A). F. P7 has the homozygous stop mutation c.1423C>T. Parental DNA was not available. No DNA was available for P5.

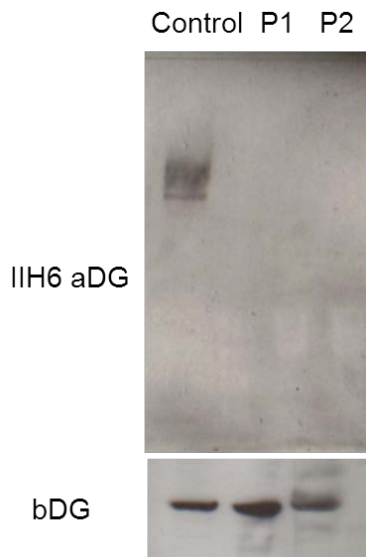


Figure S2. **Immunoblotting analysis of fibroblast cell lysate from control and *B3GALNT2* affected individuals, P1 and P2.** The membrane was incubated with the anti α -DG IIH6 and anti β -DG primary antibodies. P1 and P2 patient fibroblasts were negative for α -DG IIH6 expression.

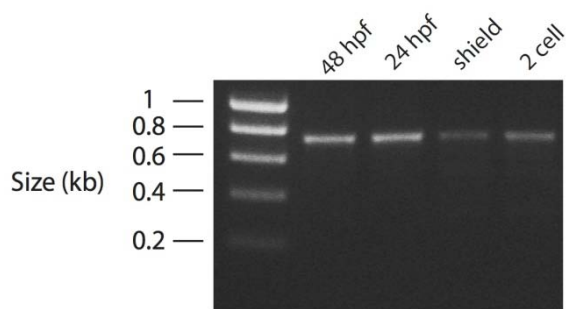


Figure S3. **Expression of zebrafish *b3galnt2*.** RT-PCR shows expression of *b3galnt2* at 4 early stages of zebrafish embryonic development.

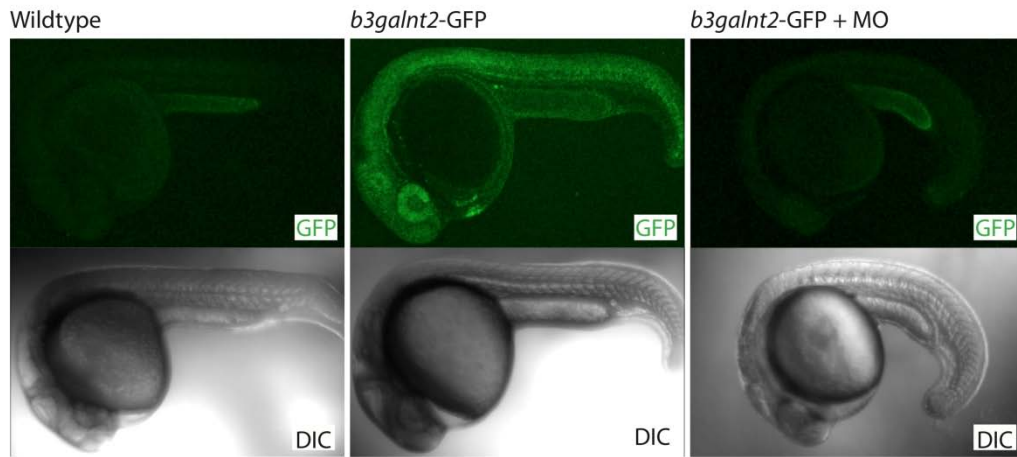


Figure S4. **The *b3galnt2* morpholino inhibits expression of recombinant GFP-tagged *b3galnt2* RNA.** *b3galnt2*-GFP embryos express wildtype recombinant GFP-tagged *b3galnt2* RNA (25 pg). This is suppressed when coinjected with morpholino (*b3galnt2* TB 4 ng coinjected with *p53* TB 2 ng). All images at 24 hpf.

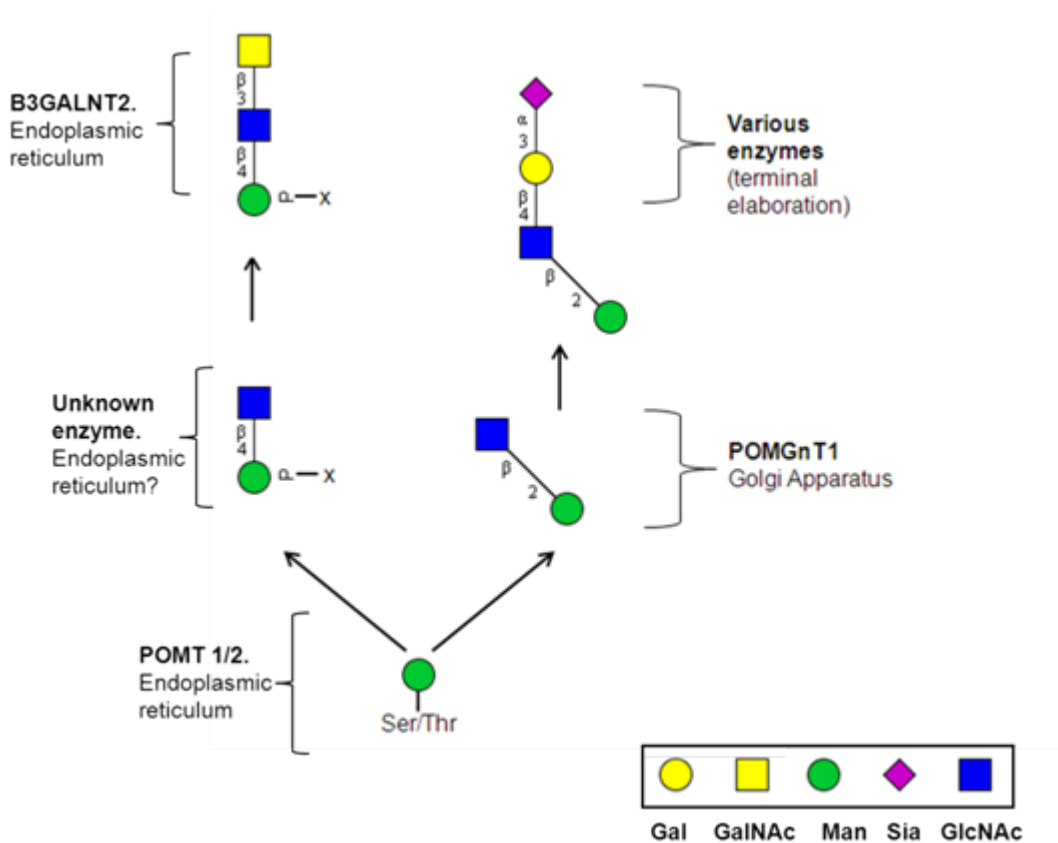


Figure S5. **B3GALNT2 is a glycosyltransferase which synthesises the carbohydrate structure GalNAcβ1-3GlcNAc.** α -DG is heavily *O*-mannosylated in its mucin-like domain. *O*-mannosylation occurs in the endoplasmic reticulum (ER) through the addition of a mannose onto either serine or threonine by POMT1 and POMT2. It can then be modified by a β 1,2 linked GlcNAc or a β 1,4 GlcNAc with POMGnT1 in the Golgi apparatus and an unknown beta-1,4-N-acetylglucosaminyltransferase, respectively. B3GALNT2 can then modify this β 1,2 linked GlcNAc with β 1,3 linked GalNAc. As B3GALNT2 localises to the ER, this unknown beta-1,4-N-acetylglucosaminyltransferase is hypothesised to also localise to the ER. The final produce is the unique trisaccharide GalNAc-b1,3-GlcNAc-b1,4-Man. This trisaccharide is believed to contain a LARGE dependent elongation of a 6-*O*-phosphoryl modification on the mannose residue.

Plasmid Construction and Mutagenesis

Table S1. Primers used to clone *B3GALNT2* into pcDNA 3.1/V5-HIS TOPO

B3GALNT2-V5
Fwd: ATGCGAAACTGGCTGGTGC
Rev: TCTTGCTTGACATCGAC

Table S2. Primers used to introduce point mutations into *B3GALNT2* pcDNA 3.1/V5-HIS TOPO. The location of the mutation is highlighted in bold.

B3GALNT2-V5 (G247E)	B3GALNT2-V5 (R292P)	B3GALNT2-V5 (V268M)
Fwd: GACAGTGAATG ATGGAGAGGG AGTTCTCAGAG TCA	Fwd: CTTACACAACCT TCATTCTCCCCT CAAAGACTTATT GATC	Fwd: TGCCTCATGA ATTCTTGGAA GGTATGGAGG GAGTTG
Rev: TGACTCTGAGA ACTCCCTCTCC ATCATTCACTG TC	Rev: GATCAATAAGTC TTTGAGGGGGAG AATGAAGGTTGT GTAAG	Rev: CAACTCCCTCC ATACCTTCCA AGAATTCATG AGGCA

Table S3. Validation of flow cytometry method to assess functional α -DG glycosylation using controls and pathological controls. MFI= mean fluorescence intensity of α -DG I1H6 by flow cytometry, N= number of repeat experiments (each fibroblast cell line was analysed three times), Std. Dev.= standard deviation. The P value is the result of an unpaired T-test comparing each fibroblast cell line to the MFI value of C1's fibroblasts.

Identity	Gene	Mutation	Phenotype	MFI	N	Std. Dev.	P
Control 1	n/a	Wild type	n/a	75.28	3	2.1	N/A
Control 2	n/a	Wild type	n/a	79.98	3	5.7	Ns
Pathological control 1	<i>RYR1</i>	Heterozygous p.S71Y, p.N2283H	Myopathy	77.15	3	1.7	Ns
Pathological control 2	<i>GAA</i>	Information unavailable, experimental finding.	Glycogen storage disease II	72.1	3	5.7	Ns
Known dystroglycanoapthy case 1	<i>POMT1</i>	homozygous c.2179-2180delTC (p.Ser727fs)	MEB	29.0	3	5.3	0.0002
Known dystroglycanopathy case 2	<i>FKRP</i>	homozygous 1023G>A (p.Trp341X)	MDC1C	39.2	3	4.51	0.0004