**Supplementary Table 1.**

List of natural missense mutations, and of the associated FD phenotype, overlapping positions of the selected *GLA* PTCs.

|  |  |  |  |
| --- | --- | --- | --- |
| **Mutation** | **Coding DNA** | **Exon** | **Phenotype** |
| p.C52R | c.154T>C | 1 | Classic |
| p.C52G | c.154T>G | 1 | not reported |
| p.C52S | c.155G>C | 1 | Classic |
| p.C52Y | c.155G>A | 1 | Classic |
| p.C52W | c.156C>G | 1 | not reported |
| p.C56G | c.166T>G | 1 | Classic |
| p.C56F | c.167G>T | 1 | Classic |
| p.C56Y | c.167G>A | 1 | Classic |
| p.W204C | c.612G>C | 4 | not reported |
| p.Q321E | c.961C>G | 6 | Classic |
| p.Q321R | c.962A>G | 6 | Classic |
| p.Q321L | c.962A>T | 6 | not reported |
| p.Q321H | c.963G>C | 6 | not reported |
| p.W340R | c.1018T>C | 7 | Classic |
| p.W340S | c.1019G>C | 7 | Classic |
| p.R342Q | c.1025G>A | 7 | Classic |
| p.R342L | c.1025G>T | 7 | not reported |
| p.R342P | c.1025G>C | 7 | not reported |
| p.Q386P | c.1157A>C | 7 | Classic |
| p.Q416P | c.1247A>C | 7 | not reported |

**Supplementary Table 2.**

Oligonucleotides used to introduce nonsense and missense changes into the cDNA encoding wild-type α-Gal (reference sequence NM\_000169.2). The modified oligonucleotide(s) (bold) and the resulting triplet (underlined) are indicated.

|  |  |
| --- | --- |
| **Primer** | **Sequence (5'→3')** |
| GLA C52X F | GAGCGCTTCATGTG**A**AACCTTGACTGCC |
| GLA C56X F | GTGCAACCTTGACTG**A**CAGGAAGAGCCAG |
| GLA Q119X F | CCTCATGGGATTCGC**T**AGCTAGCTAATTATG |
| GLA W204X F | GTACTCCTGTGAGTG**A**CCTCTTTATATG |
| GLA W209X F | CCTCTTTATATGTG**A**CCCTTTCAAAAGC |
| GLA Q321X F | GTAATTGCCATCAAT**T**AGGACCCCTTGGGC |
| GLA Q330X F | GCAAGCAAGGGTAC**T**AGCTTAGACAGGGAG |
| GLA Q333X F | GGTACCAGCTTAGA**T**AGGGAGACAACTTTG |
| GLA W340X F | CTTTGAAGTGTG**A**GAACGACCTCTCTC |
| GLA R342X F | GAAGTGTGGGAA**T**GACCTCTCTCAGGCTTAG |
| GLA Q357X F | GCTATGATAAACCGG**T**AGGAGATTGGTGGAC |
| GLA Q386X F | CCTGCTTCATCACA**T**AGCTCCTCCCTGTG |
| GLA Y397X F | GAAGCTAGGGTTCTA**G**GAATGGACTTCAAG |
| GLA Q416X F | GGCACTGTTTTGCTT**T**AGCTAGAAAATACAA |
| GLA C56C F | GTGCAACCTTGAC**C**GCCAGGAAGAGCCAG |
| GLA C56W F | GTGCAACCTTGACTG**G**CAGGAAGAGCCAG |
| GLA Q119K F | CATGGGATTCGC**A**AGCTAGCTAATTATG |
| GLA Q119Y F | CATGGGATTCGC**T**A**C**CTAGCTAATTATG |
| GLA W204C F | GTACTCCTGTGAGTG**T**CCTCTTTATATGTGG |
| GLA W204R F | GTGTACTCCTGTGAG**C**GGCCTCTTTATATG |
| GLA W209C F | GCCTCTTTATATGTG**T**CCCTTTCAAAAGCCC |
| GLA W209R F | GGCCTCTTTATATG**C**GGCCCTTTCAAAAG |
| GLA Q321K F | GCCATCAAT**A**AGGACCCCTTG |
| GLA Q321Y F | GCCATCAAT**T**A**C**GACCCCTTGG |
| GLA R342C F | GAAGTGTGGGAA**T**G**C**CCTCTCTCAGGC |
| GLA R342W F | GAAGTGTGGGAA**T**G**G**CCTCTCTCAGGC |

**Supplementary Figure 1.**

**Functional comparison of untagged and MYC-tagged wild-type rGal.**

Representative activity assay on cell lysates from HEK293 cells expressing wild-type untagged (black circles) or MYC-tagged (white circles) recombinant α-Gal. Cell lysates, normalized for total protein content, were serially diluted and assayed towards the 4-methylumbelliferone-α-D-galactopyranoside fluorogenic substrate.

RFU, Relative Fluorescence Units.

