Correction of Pseudoexon Splicing Caused by a Novel Intronic Dysferlin Mutation

Supporting Information

Figure S1.

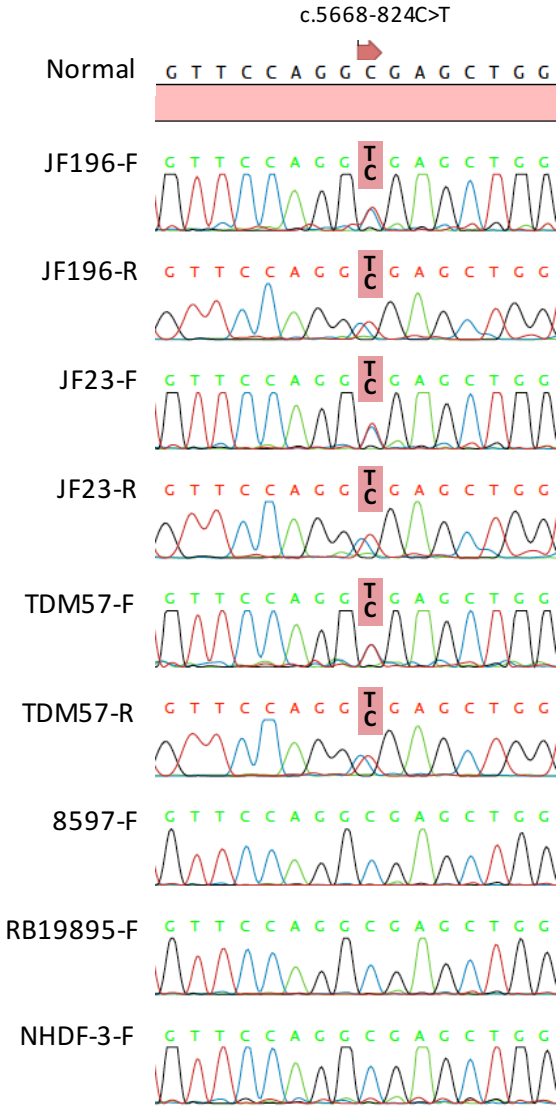

Figure S1. Analysis of genomic DNA from patients JF196, JF23 and TDM57 revealed that they are heterozygous for the c.5668-824C>T mutation deep within *DYSF* intron 50i. Genomic DNAs from patients' fibroblasts were amplified and sequenced using primer sets that tiled through 50i (Supplementary Table 1), revealing this mutation in these dysferlinopathy patient cells but not in fibroblasts from unrelated patients (8597: patient with other dysferlin mutations[18]; RB19895: ALS

patient) or normal human dermal fibroblasts (NHDF-3). Samples shown here were amplified using primers PE50.1-F and PE50.1-R then sequenced in the forward (F) and reverse (R) directions as indicated.

Figure S2.

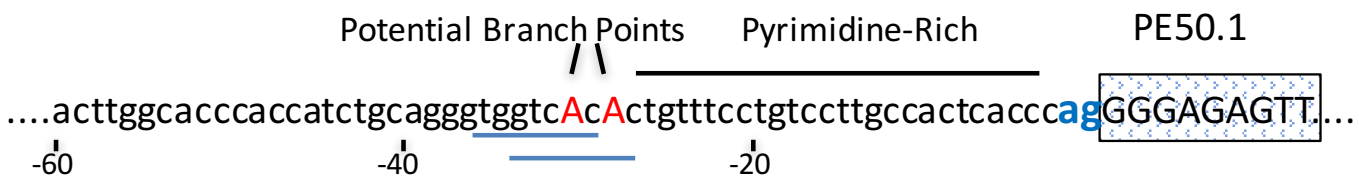

Figure S2. The intronic sequence upstream of pseudoexon PE50.1 contains additional consensus sites required for mRNA splicing. These include a splice acceptor sequence (ag) at the 5' end of PE50.1, an adjacent pyrimidine-rich region and two potential lariat branch point consensus sequences (underlined) that could be used to promote splicing. These sequences, in the presence of c.5668-824 C>T mutation, likely allow PE50.1 to be spliced between exons 50 and 51.

Table S1. Primers (forward (F) and reverse (R)) used to generate overlapping amplicons that span dysferlin intron 50i, and an additional primer set (PE50.1-F, PE50.1-R) useful for genotyping.

Primer Name	Sequence (5' – 3')
DYSF50i.1F	TGGGAGGTGAAGGCAACTT
DYSF50i.1R	TGGAAAAGGGGTGGATGC
DYSF50i.2F	TCATGGCACTACCGTGGTC
DYSF50i.2R	GGAGGGTGATGGCTGTGG
DYSF50i.3F	GCCTATGGTCACCGTCCA
DYSF50i.3R	AATCGGGCCAGCAGAATC
DYSF50i.4F	GTCCCACCCGGCATTAAA
DYSF50i.4R	TTGGGGCAGATGCAACCT
DYSF50i.5F	GAAAGCATGGGCCGTTTG
DYSF50i.5R	AATGGAGCCACCCCAAAT
DYSF50i.6F	AGAGAGGGTTACCCGGCAGT
DYSF50i.6R	GTTTCTGTCTCCGCCTTCG
DYSF50i.7F	GGGAGAAGGTGGCTGGAA
DYSF50i.7R	CTGACGTGCAGGGTGTGC
DYSF50i.8F	GCGGCTTTGAACCACCAC
DYSF50i.8R	AGCTCCCGAACCGAAGAC
DYSF50i.9F	CCAACCCAGGCAGCAGTC
DYSF50i.9R	CAGCTCGCCTGGAACAAG
DYSF50i.10F	CAGTCCCACACCGCTCAG
DYSF50i.10R	TATGCGTTGGCCCTCTACTG
DYSF50i.11F	CAGCTGCCAGGGTTTGAG
DYSF50i.11R	GGATGCAAGGAAGCAAGGT
DYSF50i.12F	CCCTTGGGGACATCCTACTC
DYSF50i.12R	ACTTGCGTCCCTCGCTTAC
PE50.1-F	CCCGGCACTCAGGACTTG
PE50.1-R	TGCTGGGAAGTTCCGTCTC

Table S2. AONs targeting human exonic splicing enhancer sequences (ESE) in DYSF PE50.1. AONs for *in vitro* studies are 2'-O-methyl RNA with full-length phosphorothioate backbones.

AON	**AON Sequence (5' - 3')**	**Target Sequence (sense strand) (5' - 3')**
DYSF50.1 AON1	AGUCUUGUUCUCUGUUUUCUCA	TGAGAAAACAGAGAACAAGACT
DYSF50.1 AON2	ACUGAGGCUUCAUGGAGCAAC	GTTGCTCCATGAAGCCTCAGT
DYSF50.1 AON3	GGUCAUCUUGGGCUUCCUCCCAC	GTGGGAGGAAGCCCAAGATGACC

Table S3. Primers (forward (F) and reverse (R)) used in quantitative PCR assays to analyze *DYSF* mRNA expression.

Target detected	**PCR Primer Name**	**Sequence (5' - 3')**
Exon 50-PE50.1 (Mutant)	DYSF50/51-QF DYSF50/PE50.1-QR.1	CGTGCATTATCGTTCCCTGG CAGCGCAGTCTTGTTCTCTG
Exon 50-51 (Normal)	DYSF50/51-QF DYSF50/51-QR.1	CGTGCATTATCGTTCCCTGG AAGGCATCCTTCTTGGCA
Exon 13-14 (Common, Normal)	DYSF 13/14-QF DYSF 13/14-QR	CTTTGCGGGGAAAATGCTGT TCATTGTGAGTCAGGCGGTC