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Supplemental Data

Mutations in *DNMT3B* Modify Epigenetic Repression of the D4Z4 Repeat and the Penetrance of Facioscapulohumeral Dystrophy

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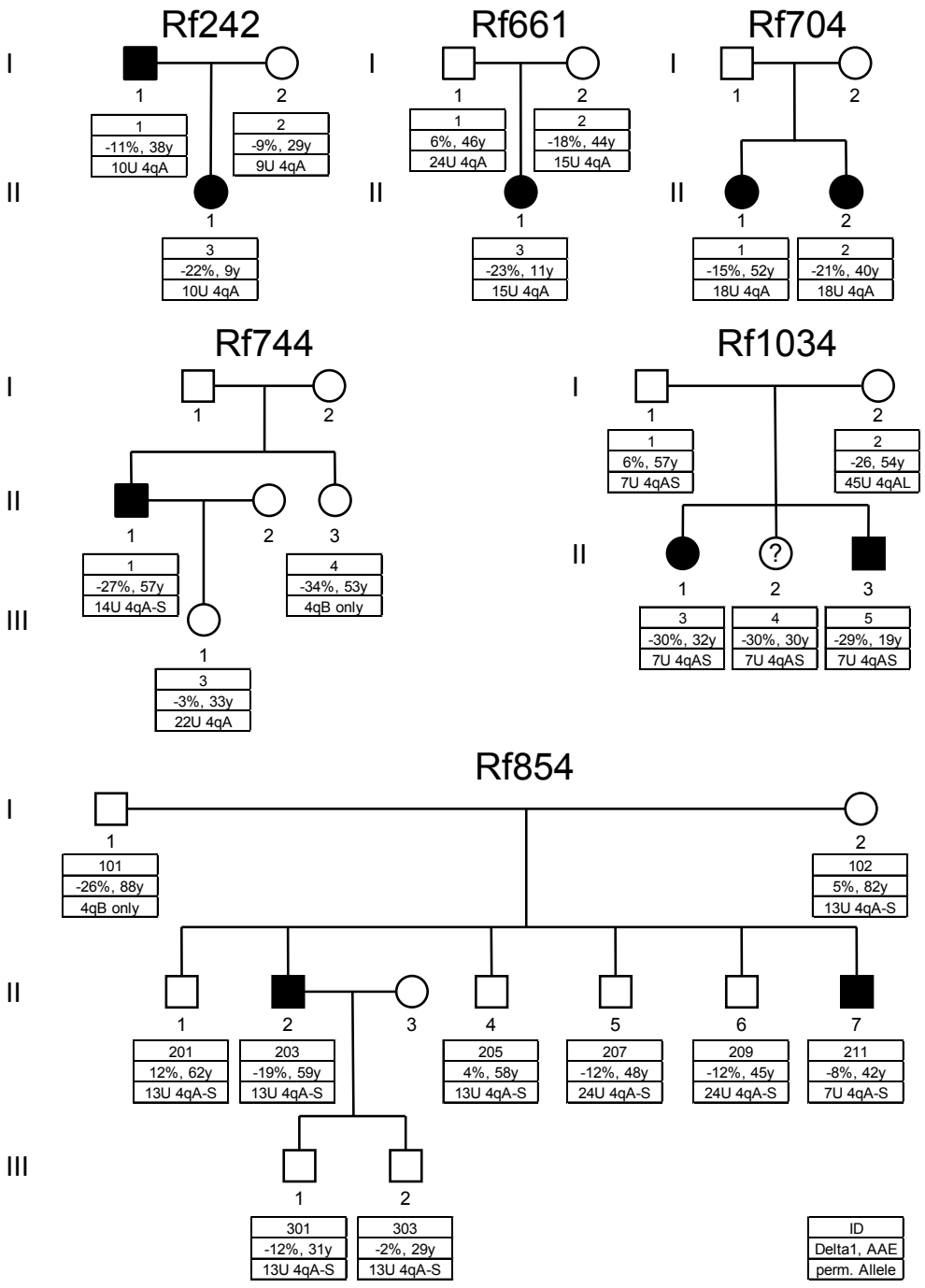
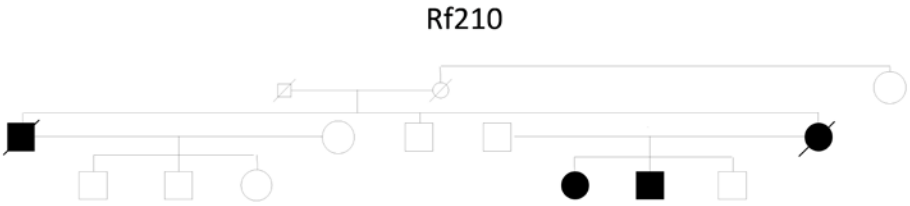


Figure {1. FSHD2 families without *SMCHD1* mutation

Families with evidence for hereditary D4Z4 hypomethylation that were tested negative for exonic *SMCHD1* and *DNMT3B* mutations. In all families, the Delta1 score was moderately to strongly reduced with possibility of dominant or recessive inheritance of D4Z4 hypomethylation. Family Rf854 was presented previously as FSHD1 (Rf854.211) and *SMCHD1* mutation negative FSHD2 family.[1] Key: ID = identifier, Delta1 score, AAE = age at examination, number of repeat units (U) on smallest permissive allele.



nr	201		301		303		306		202		209		211		316		317		319		212		104			
allele	a	b	a	a'	a	a'	b	a'	a'	a'	a	c	d	e	d	a	d	a	d	a	b	a	d'	c	position	dbSNP
	G	G	G	G	G	G	G	G	NA	NA	G	C	C	C	C	G	C	G	C	G	G	G	C	C	exon 1	rs2430853
	A	A	A	A	A	A	NA	NA	A	A	A	G	G	G	G	A	G	A	G	A	A	A	NA	NA	intron 1	rs555117
	A	A	A	A	A	A	NA	NA	A	A	A	A	G	G	G	A	G	A	NA	NA	A	A	NA	NA	intron 1	rs611187
	T	A	T	T	T	T	A	T	T	T	T	T	A	A	A	T	A	T	A	T	A	T	A	T	exon 14	rs8090988
	G	G	G	G	G	G	G	G	G	G	G	G	A	A	A	G	A	G	A	G	G	G	A	G	exon 14	rs635132
	A	A	A	A	A	A	A	A	A	A	A	G	A	A	A	A	A	A	A	A	A	A	A	G	exon 16	rs2276092
	T	T	T	T	T	T	T	T	T	T	T	A	A	A	A	T	A	T	A	T	T	T	A	A	exon 21	rs633422
	A	A	A	A	A	A	A	A	A	A	A	G	G	G	G	A	G	A	G	A	A	A	G	G	exon 28	rs521992
	A	A	A	A	A	A	A	A	A	A	A	G	A	G	A	A	A	A	A	A	A	A	A	G	exon 28	rs12327477
	A	A	A	A	A	A	A	A	A	A	A	A	G	A	G	A	G	A	G	A	A	A	NA	NA	intron 31	rs2304860
	A	A	A	A	A	A	A	A	A	A	A	G	A	G	A	A	A	A	A	A	A	A	NA	NA	intron 31	rs2304861
	T	T	T	T	T	T	T	T	T	T	T	C	C	C	C	T	C	T	C	T	T	T	C	C	exon 37	rs483547
	A	A	A	A	A	A	A	A	A	A	A	G	A	G	A	A	A	A	A	A	A	A	NA	NA	intron 43	rs3213926
Delta1	-29%		-8%		-6%		5%		-8%		8%		-2%		-8%		-8%		-29%		-30%		-2%			

Figure S2. Exclusion of *SMCHD1* in Rf210

Haplotype analysis in family Rf210 based on common SNPs defined different alleles. Segregation analysis showed that the common *SMCHD1* allele (allele a) found in individuals with D4Z4 hypomethylation (201, 212 and 319; Delta1 values -29% and -30%) was also found in individuals with normal methylation (301, 303, 209, 316 and 317). The position of the SNPs (NA means not analyzed) and dbSNP identifier are shown on the right. Delta1 methylation values are shown below.

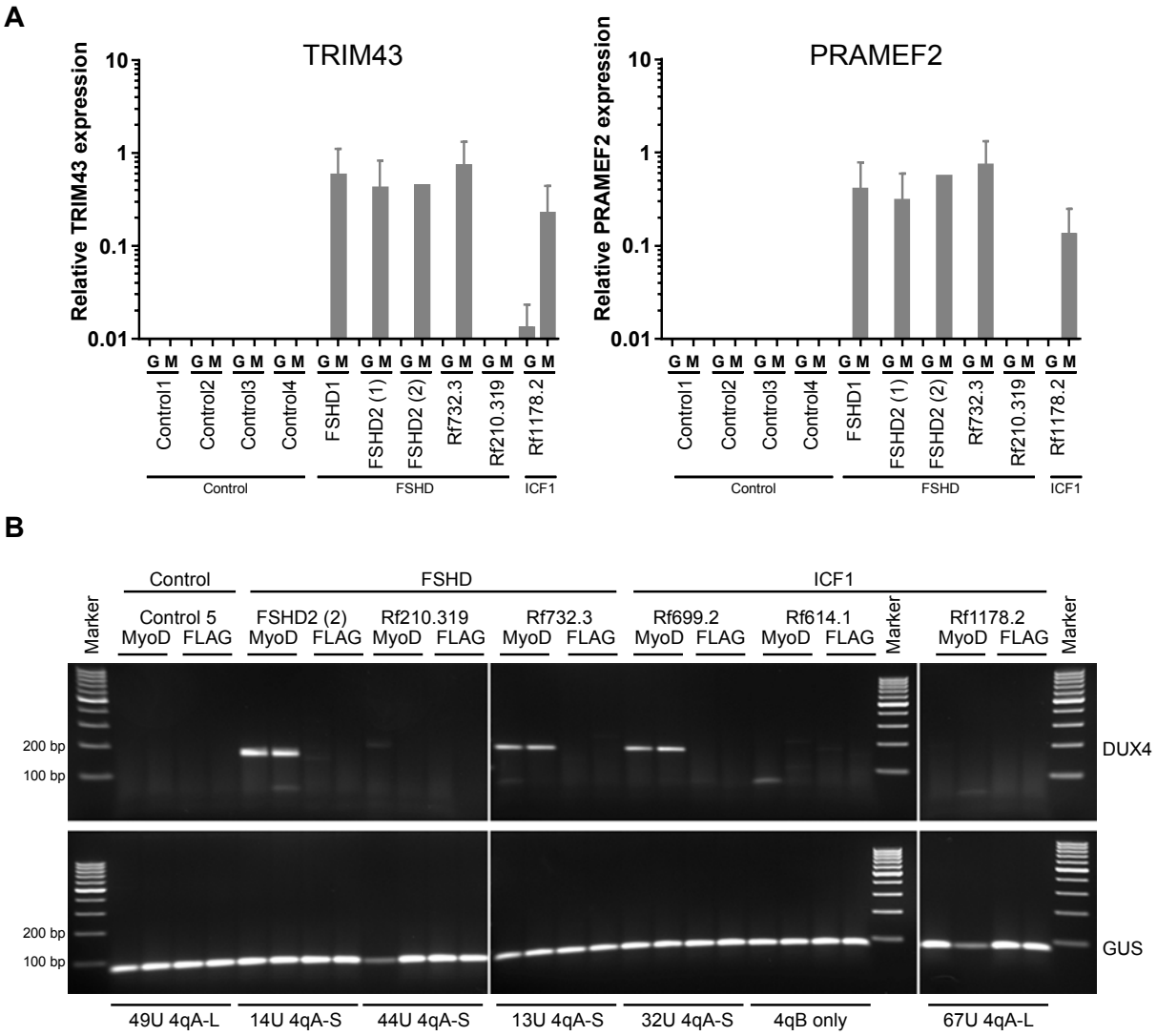
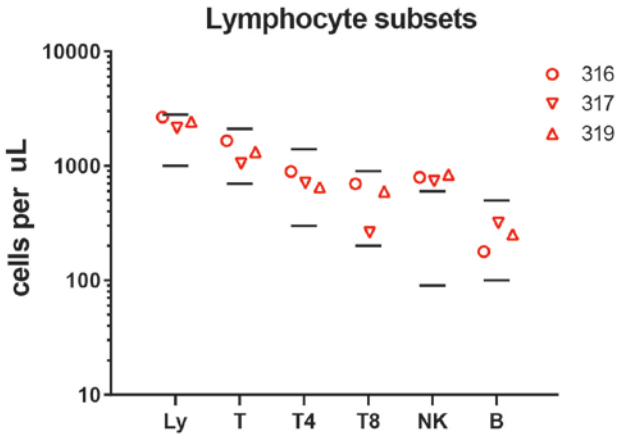


Figure S3. DUX4 target gene expression and DUX4 expression in FSHD and ICF1

(A) Expression of DUX4 target genes *TRIM43* and *PRAMEF2* by Q-PCR in GFP- (G) or MyoD- (M) transduced fibroblasts from controls, FSHD1, FSHD2, Rf210.319, Rf732.3 and ICF1 (Rf1178.2). All transductions were performed twice for each cell line, except for control 4 (1x transduced with GFP, 2x transduced with MyoD) and FSHD2 (2) (transduced 1x with GFP and 1x with MyoD). Mean expression values with standard deviations are shown relative to the reference genes *GUSB* and *RPL27*. (B) Gel of Q-PCR for DUX4 and GUS in Flag and MyoD transduced fibroblasts from control, FSHD2, Rf210.319, Rf732.3 and ICF1 (Rf699.2, Rf614.1, Rf1178.2). Technical duplicates are shown. The smallest D4Z4 repeat array on a FSHD permissive allele (4qA) in each individual is indicated below the gel. A PCR product for DUX4 is only detected in MyoD transduced fibroblasts from FSHD2, Rf732.3 and Rf699.2. The DUX4 RT-PCR is performed with primers for the most common DUX4-4A-S variant, but the primers do not recognize DUX4-4A-L. The fibroblast from Rf1178.2 carries a 4qA-L allele.

A



B

ID	IgG (g/L)	IgA (g/L)	IgM (g/L)
316	12.2	2.81	0.99
317	9.38	1.92	0.74
319	10.5	2.29	0.56
reference values	7-16	0.7-4	0.42-2.3

Figure S4. Immunological analysis

(A) The numbers of lymphocytes (Ly), T-cells (T), CD4+ and CD8+ T-cell subsets (T4 and T8), NK cells (NK) and B-cells (B) in blood for siblings from family Rf210. The range of the normal values is depicted and represents the 5th and 95th percentiles. (B) Serum levels of IgG, IgA and IgM for siblings from family Rf210 and the reference values.

		Chromosome position	Transcript position				4q allele 1		4q allele 2	
Nr	gender	Chr20(GRCh37)	NM_006892.3	AAE	ACSS	delta1	units	A/B	units	A/B
Rf210.101	M	NA	NA	NA	NA	NA	20	4A161L	29	4B168
Rf210.201	M	g.[31386354T>C];[=]	c.[1579T>C];[=]	63	111	-29	9	4A161S	20	4A161L
Rf210.301	M	WT	WT	38	0	-8	20	4A161L	71	4B168
Rf210.303	M	WT	WT	35	0	-6	9	4A161S	33	4B163
Rf210.306	F	WT	WT	33	0	5	20	4A161L	33	4B163
Rf210.202	F	WT	WT	60	0	-8	33	4B163	71	4B168
Rf210.209	M	WT	WT	55	0	8	17	4B163	29	4B168
Rf210.211	M	WT	WT	56	0	-2	23	4B168	44	4A161S
Rf210.316	F	WT	WT	30	67	-8	9	4A161S	44	4A161S
Rf210.317	M	WT	WT	40	50	-8	9	4A161S	23	4B168
Rf210.319	M	g.[31386354T>C];[=]	c.[1579T>C];[=]	37	0	-29	29	4B168	44	4A161S
Rf210.212	F	g.[31386354T>C];[=]	c.[1579T>C];[=]	52	135	-30	9	4A161S	29	4B168
Rf210.102	F	NA	NA	NA	NA	NA	9	4A161S	17	4B163
Rf210.104	F	WT	WT	70	0	-2	9	4A161S	15	4B163
Rf732.1	M	g.[31389159C>T];[=]	c.[2072C>T];[=]	74	0	-22	13	4A161S	24	4B168
Rf732.3	M	g.[31389159C>T];[=]	c.[2072C>T];[=]	45	89	-22	13	4A161S	15	4B163
Rf732.5	M	WT	WT	38	0	-10	13	4A161S	25	4B168
Rf732.2	F	WT	WT	68	0	1	15	4B163	25	4B168

Table S1. Detailed genotype and phenotype FSHD families

Table summarizing the clinical, D4Z4 methylation and genetic data from FSHD families Rf210 and Rf732. Column 1 shows the individual number, column 2 the gender (F= female, M = male), column 3 and 4 show the mutation in DNMT3B at the chromosome and transcript position, respectively, column 5 shows the age at examination (AAE), column 6 shows the age corrected clinical severity score (ACSS)[2,3], column 7 shows the Delta1 value for D4Z4 methylation at the *Fse*I site, column 8-11 show the D4Z4 array sizes, and haplotype (including S or L for 4qA-S or 4qA-L) of the 4q alleles. NA means not analyzed.

A

				4q allele 1		4q allele 2		
nr	gender	ACSS	Delta1	units	A/B	units	A/B	Pedigree
Rf201.309	M	143	-21	7	4A161S	27	4B163	
Rf242.3	M	NA	-22	10	4A161	105H2	4A157	see figure S2
Rf537.1	M	NA	-18	7	4A161S	37	4B163	
Rf584.2	F	19	-18	9	4A161S	32	4B168	
Rf661.2	F	NA	-18	15	4A161	20	4A161	see figure S2
Rf704.2	F	150	-21	18	4A161	48	4A161	see figure S2
Rf744.1	M	88	-27	14	4A161S	29	4B163	see figure S2
Rf838.1	F	100	-32	11	4A161L	58	4A161L	
Rf854.203	F	34	-19	13	4A161S	15	4B163	see figure S2
Rf901.1	F	49	-17	18	4B168	37	4A161S	
Rf946.2	M	63	-22	8	4A161S	27H1	4A166	
Rf982.1	M	127	-18	7	4A161S	20	4B163	
Rf1010.1	F	NA	-21	12	4A161S	51	4B163	
Rf1034.5	M	158	-29	7	4A161S	45	4A161L	see figure S2
Rf1049.1	F	54	-22	6	4A161S	37	4A161S	
Rf1093.1	F	NA	-17	11	4A161	13	4A161	
Rf1154.2	F	NA	-32	37	4A161	43	4B163	
Rf1239.1	M	140	-19	8	4A161	36	4A161	
Rf1449a.1	F	196	-27	7	4A161	36	4B168	
Rf1464.1	M	94	-19	14	4A161S	12	4B163	

B

nr	gender	ACSS	DR1 methylation	4qA allele
1	M	NA	23	>10
2	F	128	21	11A
3	F	159	24	8A
4	F	189	21	7A
5	F	97	17	8A
6	M	135	13	13A

Table S2. Overview of individuals screened for exonic *DNMT3B* mutations

(A) Table summarizing the clinical, D4Z4 methylation and genetic data from 20 FSHD cases screened for exonic mutations in *DNMT3B* at the LUMC, Leiden, the Netherlands. Column 2 shows gender (F= female, M = male), column 3 shows the age corrected clinical severity score (ACSS), column 4 shows the Delta1 score for D4Z4 methylation at the FseI site, column 5-8 show the sizes, SSLP size and haplotype (including S or L for 4qA-S or 4qA-L when available) of the 4q alleles. NA means not analyzed. (B) Table summarizing the clinical, D4Z4 methylation and genetic data from 6 cases screened for exonic mutations in *DNMT3B* at the NCNP, Tokyo, Japan. Column 4 shows the DR1 methylation percentage, column 5 shows the size of the shortest 4qA allele, size information of the other 4q allele and SSLP sizes are not available.

	DNMT3B mutations						
	Transcript position NM_006892.3		Protein position NP_008823.1		Hansen et al. 1999 (ref 4)	Hagleitner et al. 2008 (ref 5)	Weemaes et al, 2013 (ref 6)
Patient identifier	Allele 1	Allele 2	Allele 1	Allele 2			
Rf285.1	c.2421-11G>A	c.2421-11G>A	p.E806_R807insSerThrPro	p.E806_R807insSerThrPro	-	Patient 33	Patient 33
Rf285.2	c.2421-11G>A	c.2421-11G>A	p.E806_R807insSerThrPro	p.E806_R807insSerThrPro	Family 2	Patient 29	Patient 29
Rf286.2	c.2177T>G	c.2177T>G	Val726Gly	Val726Gly	Family 1	Patient 16	Patient 16
Rf614.1	c.2292G>T	c.2342_2343del	Arg764Ser	Ile781Lysfs*23	-	Patient 45	Patient 45
Rf699.2	c.1918G>C	c.1918G>C	Gly640Arg	Gly640Arg	-	-	Patient 50
Rf1178.2	c.1807G>A	c.2421-11G>A	Ala603Thr	p.E806_R807insSerThrPro	Family 3	Patient 7	Patient 7

Table S3. Overview of ICF1 patients included in this study

Column 1 shows the patient identifier in this study. Columns 2-5 show the positions of the DNMT3B mutations on the transcript and protein level. Columns 6-8 show the identifiers from the patients in previous studies.

DNMT3Bex2F	GGCAAGAGCATCACCCTAAG
DNMT3Bex2R	TTGTGGTGGAGGTTGTCAGAGA
DNMT3Bex3F	GACGGACTGAGAGCAAATCC
DNMT3Bex3R	CGTGATGAAAGCCAAAGACA
DNMT3Bex4F	GTGTGTTGTGATGAGTGACCCG
DNMT3Bex4R	GCTCCCCTAAGGAGCTATGC
DNMT3Bex5F	CAGGCCTCCAGTCACCTAAG
DNMT3Bex5R	AGCCACAACCAGTAGTGCAG
DNMT3Bex6F	TTCTTTTTGCCTAGGAGCCA
DNMT3Bex6R	GGTAACTGGTTTTTCCCCGT
DNMT3Bex7F	GCCTCTCCTCACTGGGATTT
DNMT3Bex7R	TTTGTCTTCAAAGGGAGGCA
DNMT3Bex8F	CACCTGGGACACACCTGTAG
DNMT3Bex8R	TCTCTTGCTTCATCCCTGC
DNMT3Bex9F	GGAATGTAGGCCCTGGCT
DNMT3Bex9R	GTGGCTGACTCTCCCAAGAA
DNMT3Bex10F2	AGGCTGAGGTGGGAGAATTG
DNMT3Bex10R2	GCAAAGAAATCAGAAGAAAGTGC
DNMT3Bex11-12F	CTGGTACCCAGGCATAGCAT
DNMT3Bex11-12R	AGGACAAGGCAGGCCTAGAG
DNMT3Bex13-14F	ACTGAGAGACCCCAGGCTTT
DNMT3Bex13-14R	GACTGCAGGAACGTAGGAGC
DNMT3Bex15F	TCCCTGTGGAAGTGGTAAGG
DNMT3Bex15R	TTCCAGAGCTTTCCAACACCC
DNMT3Bex16F	CAAGGTTTGAAGCCCTCTGA
DNMT3Bex16R	TAATCCCCAGGGACCTTTCT
DNMT3Bex17F	GCTGCTGTGTGCTCAGCATCATT
DNMT3Bex17R	GGAGGACTGGGGAAAAAGAC
DNMT3Bex18F	TGACCTCAGGTAATCCACCC
DNMT3Bex18R	CCAGTAACTTGGCCAGAAGC
DNMT3Bex19F	CCTGCTGGTCTCAGGGAATA
DNMT3Bex19R	GACCAAGAACGGGAAAGTCA
DNMT3Bex20F	GCCTCATCCATAGTCAGGGA
DNMT3Bex20R	CAGAGCCAGGTCTTTCT
DNMT3Bex21F	TGCCAGGATCATTTTCATCA
DNMT3Bex21R	TCACCAAGTGCATTTTTCCA
DNMT3Bex22F2	CAGCCCTGCCACTCTTCT
DNMT3Bex22R	TCTGCCCATTTGTGTTTTGA
DNMT3Bex23F	ACTGATGGGACTGAGGGATG
DNMT3Bex23R	ATGCCTTCAGGAATCACACC

¢able {4. *DNMT3B* primers used for screen for exonic *DNMT3B* mutations in this study

Target	Forward	Reverse
PRAMEF2	GCAAGTTAAGCCTGGAGACG	CCCTAGCAGCAAAGATGGAG
LEUTX	AAGGAGGAGACTCCCTCAGC	AAAGAGAGTGGAGGCCCAAG
TRIM43	ACCCATCACTGGACTGGTGT	CACATCCTCAAAGAGCCTGA
RPL27	CCCACATCAAGGAACTGGAG	TGTTGGCATCCAAGGTCATA
DUX4	TCCAGGAGATGTAACTCTAATCCA	CCCAGGTACCAGCAGACC
GUSB	CCGAGTGAAGATCCCCTTTTTA	CTCATTTGGAATTTTGCCGATT
MYOG	GCCAGACTATCCCCTTCCTC	GGGGATGCCCTCTCCTCTAA
MYH3	GATTGCAGGATCTGGTGGAT	CCTGCTGGAGGTGAAGTCTC

Table S5. Q-PCR primers used in this study

References:

1. Lemmers, R.J., Tawil, R., Petek, L.M., Balog, J., Block, G.J., Santen, G.W., Amell, A.M., van der Vliet, P.J., Almomani, R., Straasheijm, K.R., et al. (2012). Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. Nat. Genet. 44, 1370-1374.
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