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Supplemental Data

The FSHD2 Gene *SMCHD1* Is a Modifier of Disease Severity in Families Affected by FSHD1

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Table S1: Genetic and epigenetic data of the three families.

Rf	Nr	LOVD-ID	*SMCHD1* genomic (GRCh37, NC_000018.9)	*SMCHD1* cDNA (NM_015295.2)	SMCHD1 protein (NP_056110.2)	Position	Type	Fsel %	Allele 4q-1	Allele 4q-2	Allele 10q-1	Allele 10q-2
Rf1021	**I-1**		None	-	_	_	_	37	**9U 4A161**	27U 4B168	9U 10A166	27U 10A166
	I-2	#30789	**g.2700849C>T**	**c.1580C>T**	**p.Thr527Met**	**Exon 12**	**Missense**	**24**	39U 4Q163	54U **4A161**	11U 10A166	21U 10A166
	II-1	#30790	**g.2700849C>T**	**c.1580C>T**	**p.Thr527Met**	**Exon 12**	**Missense**	**17**	**9U 4A161**	54U 4A161	11U 10A166	27U 10A166
	II-2		None	-	_	_	_	26	11U 4A161	18U 4A161	53U 10A166	54U 10A166
	III-1		None	-	_	_	_	45	11U 4A161	54U 4A161	11U 10A166	54U 10A166
	III-2	#30791	**g.2700849C>T**	**c.1580C>T**	**p.Thr527Met**	**Exon 12**	**Missense**	**10**	**9U 4A161**	18U 4A161	11U 10A166	53U 10A166
Rf1110	**I-1**	#30792	**g.2729409T>C**	**c.3048+2T>C**		**Intron24**	**Splice site**	**8**	**9U 4A161**	63U 4A161	15U 10A166	19U 10A166
	I-2		None	-	_	_	_	57	16U 4B163	22U 4B168	30U 10A166	43U 10A166
	II-1	#30793	**g.2729409T>C**	**c.3048+2T>C**		**Intron24**	**Splice site**	**10**	22U 4B168	63U **4A161**	19U 10A166	30U 10A166
	II-2		None	-	_	_	_	28	**9U 4A161**	16U 4B163	15U 10A166	30U 10A166
Rf1121	**II-1**	#30794	**g.2769710delA**	**c.4738delA**	**p.Ser1580fs**	**Exon 38**	**Deletion**	**9**	**9U 4A161**	59U 4A166	34U 10A164	34U 10A166

Detailed analysis of D4Z4 genotype on chromosomes 4 and 10 (columns Allele 4q-1, Allele 4q-2, Allelel 10q-1 and Allele 10q-2), D4Z4 methylation (column *Fse*I%) and *SMCHD1* mutations for all individuals of families Rf1021, Rf1110 and Rf1121. Variant and phenotype data were submitted to the SMCHD1 gene variant database (www.LOVD.nl/SMCHD1/) with patient accession numbers 30789 to 30794 (column LOVD-ID). The position of the mutation in the *SMCHD1* gene is given with respect to chromosome (GRCh37, NC_000018.9), transcript (NM_015295) and protein (NP_056110.2). The position of the SMCHD1 mutations and the type are also indicated.

The comprehensive genotype of the D4Z4 region on chromosomes 4 and 10 was based on analysis of the size of the repeat arrays by pulsed field gel electrophoresis analysis, single sequence length polymorphism (SSLP) analysis at the proximal end of the repeat arrays and determination of the distal variation A or B, as previously described[1] (see also the Fields Center for FSHD Research Website http://www.urmc.rochester.edu/fields-center/ for detailed protocols). The repeat lengths of the FSHD1-sized D4Z4 repeat arrays was confirmed using Southern blot analysis of genomic DNA digested with *Eco*RI and double digested with *Eco*RI and *Bln*I separated by conventional linear gel electrophoresis, using a 5 kb ladder as DNA size standard (Biorad, 170-3624). Methylation levels in the proximal D4Z4 repeat units of chromosomes 4q and 10q was performed as reported.[2]

Mutation analysis of the *SMCHD1* gene at DNA or RNA level was performed as described in our previous study.[3] Primer sequences are available on request.

Table S2: Genetic data FSHD1 myoblasts in SMCHD1 depletion experiment.

		Allele 4_1		Allele 4_2		Allele 10_1		Allele 10_2	
sample	M/F	units	haplotype	units	haplotype	units	haplotype	units	haplotype
FSHD1 a	M	6	4A161	11	4A168	16	10A166	23	10A176T
FSHD1 b	F	6	4A161	52	4B168	18	10A166	21	10A166
FSHD1 c	F	4	4A161	13	4B163	9	10A166	19	10A166

Detailed analysis of D4Z4 genotype (number of units and haplotype) on chromosomes 4 and 10 (columns Allele 4_1, Allele 4_2, Allele 10_1 and Allele 10_2) for three different myoblasts that have been used in the SMCHD1 depletion experiment. Gender of the myoblast is indicated in column M/F.

These unrelated FSHD1 myoblasts were obtained from the Fields Center and grown as previously described.[3] Genetic analysis identified an FSHD-permissive *DUX4*-PAS containing 4qA chromosome with a repeat size of 6 units (FSHD1a and FSHD1b), or 4 units (FSHD1c). Two individuals (FSHD1b and FSHD1c) carry a 4qB haplotype on the non-affected chromosome 4 and therefore *DUX4* expression is only possible from their FSHD1 allele.

Supplemental References

1. Lemmers, R.J., van der Vliet, P.J., van der Gaag, K.J., Zuninga, S., Frants, R.R., de Knijff, P., and van der Maarel, S.M. (2010). Worldwide population analysis of the 4q and 10q subtelomeres identifies only four discrete duplication events in human evolution. Am. J. Hum. Genet. *86*, 364-377.

2. de Greef, J.C., Lemmers, R.J., Camano, P., Day, J.W., Sacconi, S., Dunand, M., van Engelen, B.G., Kiuru-Enari, S., Padberg, G.W., Rosa, A.L., et al. (2010). Clinical features of facioscapulohumeral muscular dystrophy 2. Neurology *75*, 1548-1554.

3. 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.