SMCHD1 mutations cause FSHD type 2 and act as modifiers of disease severity

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INTRODUCTION

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant muscular disorder.
Symptoms start in the second decade of life and show a mostly slow progression. The typical distribution is depicted in figure 1. Disease severity varies widely from asymptomatic carriers to wheelchair dependency.
The more common form FSHD1 is associated with a contraction of the D4Z4 repeat array on a FSHD-permissive chromosome 4 genetic background. The copy number variation ranges from 11 to 150 copies in the normal population and 1 to 10 in FSHD patients.
Recently, a digenic inheritance has been reported to cause the rarer form FSHD2.

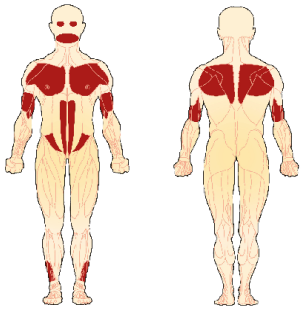

Figure 1: Typical distribution of muscular weakness in FSHD.
The first symptoms appear in the muscles of the face, shoulder girdle and upper arm, later on affecting also the pelvic girdle and foot extensors. Source: Muscular Dystrophy Association (MDA) 2011.

These patients do not show the D4Z4 repeat contraction but also carry the FSHD-permissive chromosome 4 allele and a heterozygous loss-of-function mutation in the *SMCHD1* gene on chromosome 18.
As a common pathogenic mechanism, both FSHD types share a state of hypomethylation of the D4Z4 repeat array. In FSHD1, chromatin decondensation and hypomethylation are effects of the repeat contraction. In FSHD2, haploinsufficiency of the SMCHD1 protein results in hypomethylation of D4Z4. An open chromatin structure of the D4Z4 locus results in activation of the *DUX4* gene whose expression is highly cytotoxic and causes skeletal muscle cell death.

PATIENTS and METHODS

In this study, we screened patients from 95 unrelated families (40 with a contraction in D4Z4 (group A), 55 without contraction on D4Z4 (group B)), all showing the typical clinical FSHD phenotype. We screend for the three components of FSHD2: (1) presence of a permissive haplotype, (2) mutations in the *SMCHD1* gene and (3) the methylation status of the D4Z4 repeat array.

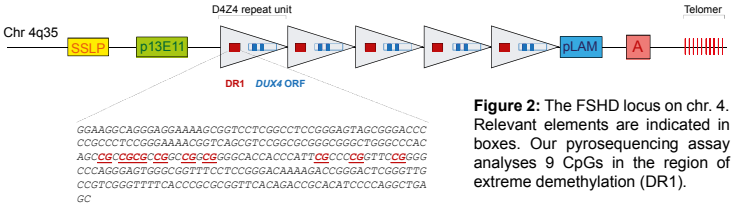

Figure 2: The FSHD locus on chr. 4. Relevant elements are indicated in boxes. Our pyrosequencing assay analyses 9 CpGs in the region of extreme demethylation (DR1).

RESULTS

We identified ten different mutations in *SMCHD1* in eleven index patients. Nine mutations were novel and one was already reported by Lemmers *et al* (2012). Figure 3 gives an overview of the mutations found.

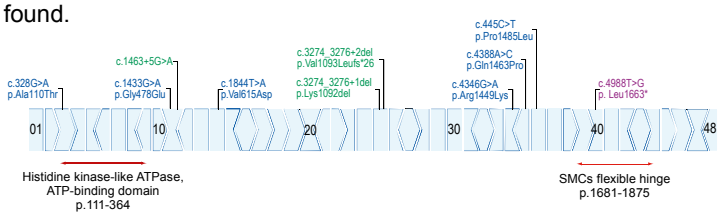

Figure 3: *SMCHD1* gene on chr. 18q11. Detected mutations are indicated at the position of the corresponding exons. Functional domains are indicated with arrows.

Mutations were ranging from missense mutations and splice mutations to a nonsense mutation. Mutations are spread all over the gene, not sparing the functional domains. There is a mutational hotspot in the 3' region of exon 25. All mutations were confirmed by Sanger sequencing and predicted as pathogenic by the bioinformatics tools of Alamut (interactive biosoftware).

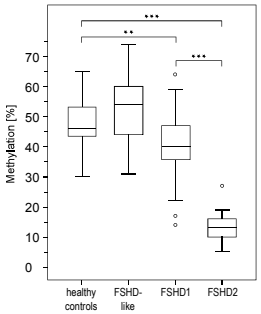

Figure 4: Methylation levels in D4Z4 determined by pyrosequencing of the DR1 region.

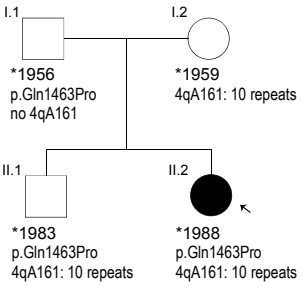

Figure 5: Pedigree of a FSHD1+2 family. Example for the digenic inheritance of the genetic components.

The methylation status of the D4Z4 repeat array for FSHD2 patients (13.3 % ± 5.9) was found significantly lower than for healthy controls (47.7 % ± 8.1) and also lower than for FSHD1 patients (40.2 % ± 11.6). Results are presented as box plots in figure 4.
In addition to the mutation in *SMCHD1*, two of the patients showed a contracted D4Z4 allele and therefore are positive for FSHD1 and 2.

CONCLUSION

Comparing the phenotype of patients, all FSHD2 patients were relatively mildly affected while patients with FSHD1+2 were much more severely affected than expected from their D4Z4 copy number. Hence haploinsufficiency of the remaining intact *SMCHD1* allele seems to be the pathomechanism underlying FSHD2.
Our findings confirm the digenic inheritance of FSHD2 with mutations in *SMCHD1* and hypomethylation of D4Z4.

In cases of "double trouble" with FSHD1 and 2, *SMCHD1* mutations seem to act as a modifier of disease severity.
With *SMCHD1* mutations found in 16.4 % of phenotypic FSHD patients (group A), FSHD2 cannot be considered to be excessively rare. Hence, we suggest including sequencing of *SMCHD1*, haplotyping and methylation analysis in the workflow of molecular FSHD diagnostics.

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