Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant muscular disorder with a wide clinical variability. Contractions of the D4Z4 macrosatellite repeat on chromosome 4q35 are the molecular basis of the pathophysiology. Recently, in a subset of patients without D4Z4 repeat contractions, variants in the SMCHD1 gene have been identified that lead to hypomethylation of D4Z4 and thus DUX4 transcription, which causes FSHD type 2. In this study, we have screened 55 FSHD1-negative and 40 FSHD1-positive patients from unrelated families for potentially pathogenic variants in SMCHD1 by next-generation sequencing (NGS). We identified variants in SMCHD1 in 11 index patients, including missense, splice site and non-sense mutations. We developed a pyrosequencing assay to determine the methylation status of the D4Z4 repeat array and found significantly lower methylation levels for FSHD2 patients than for healthy controls and FSHD1 patients. Two out of eleven SMCHD1 mutation carriers had moderately contracted D4Z4 alleles thus these patients are suffering from FSHD1 and 2. 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. Our findings confirm the role of SMCHD1 mutations in FSHD2 and as a modifier of disease severity. With SMCHD1 variants found in 16.4% of phenotypic FSHD patients without D4Z4 repeat contractions, the incidence of FSHD2 is rather high and hence we suggest including sequencing of SMCHD1, haplotyping and methylation analysis in the workflow of molecular FSHD diagnostics.