A novel start codon variant in *SMCHD1* from a Chinese family causes facioscapulohumeral muscular dystrophy type 2

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To the Editor: Facioscapulohumeral muscular dystrophy type 2 (FSHD2) is an epigenetic myopathy caused by variants in genes encoding chromatin regulators, such as *SMCHD1*: these variants lead to derepression of the D4Z4-encoded *DUX4* retrogene in skeletal muscle.[1] The core phenotype of FSHD is progressive muscle weakness in such body parts as the face, shoulder girdle, and upper limbs. Additionally, FSHD may affect the axial muscles and produce bent spine syndrome. Several studies have reported that FSHD2 is associated with causative variants in *SMCHD1*,[1,2] but to the best of our knowledge, no Chinese FSHD2 patient has been reported. In this report, we presented a Chinese FSHD2 family with D4Z4 hypomethylation and identified a novel start codon variant (c.1 A>G) in *SMCHD1*. This study was approved by the Ethics Committee for Medical Research of the First Affiliated Hospital of Fujian Medical University (No. 2016 [17]). Informed consent was obtained from each participant and parent of the participant younger than 18 years of age.

Four patients from a family [Figure 1A] were suspected of having FSHD2. The proband (II.3) was a 47-year-old man. He was initially diagnosed with ankylosing spondylitis (AS) with muscle weakness in the bilateral upper limbs and lower back pain at the age of 26 years. At 36 years of age, he developed marked muscle dystrophy, exercise intolerance, and a stooped posture in a standing position. When he came to our hospital, we reconsidered the diagnosis of AS because of the normal results of HLA-B27 and X-ray examination of the sacroiliac joint. Physical examination showed core signs of FSHD [Supplementary Figure 1, http://links.lww.com/CM9/A481], including facial muscle weakness and left-right asymmetry of scapular winging. Additionally, his trunk muscle was also involved in presenting a "bent spine." Thus, the patient was classified as category D1 according to the clinical comprehensive evaluation form (CCEF) and received an FSHD clinical score (CS) of 7 points. Electromyography (EMG) results showed typical myopathic changes and slight spontaneous activity. Muscle 3.0-T magnetic resonance imaging (MRI) showed atrophy and mild fatty infiltration [Supplementary Figure 2, http://links.lww.com/CM9/A481]. At 34 years of age, he underwent muscular biopsy. Histopathology showed mild dystrophic changes with mild-moderate variation in fiber size, perivascular lymphocyte infiltrates, and interstitial fibrosis [Supplementary Figure 3, http://links.lww.com/CM9/A481]. The proband recalled that his mother (I.2) developed facial and bilateral upper limb muscle weakness, but detailed clinical data were lacking because she committed suicide in her 50s. The 42-year-old patient II.6 was the proband's half-sister. Physical examination also showed core FSHD signs including facial muscle weakness and scapula winging [Supplementary Figure 1, Supplementary Table 1, http://links.lww.com/CM9/A481]. She was classified as category A2 of the CCEF and received an FSHD CS of 8 points. The results of EMG showed typical myopathic changes. Muscle MRI showed relatively severe atrophy and moderate fatty infiltration [Supplementary Figure 4, http://links.lww.com/CM9/A481]. Her recent histopathology showed an end-stage appearance with marked variation in fiber size, endomysial fibrosis, and endomysial adipose tissue infiltration [Supplementary Figure 5, http://links.lww.com/CM9/A481]. Additionally, this patient II.6 developed impaired vision in her left eye with a visual index of 40 cm at 42 years of age. Because both the results of serum aquaporin-4 and myelin oligodendrocyte glycoprotein-immunoglobulin G were negative, and MRI of the spinal cord showed no changes associated with long transverse myelitis, she received a second diagnosis of acute optic neuritis. The diagnosis of retinal exudative retinopathy was excluded because of a normal result of fluorescence

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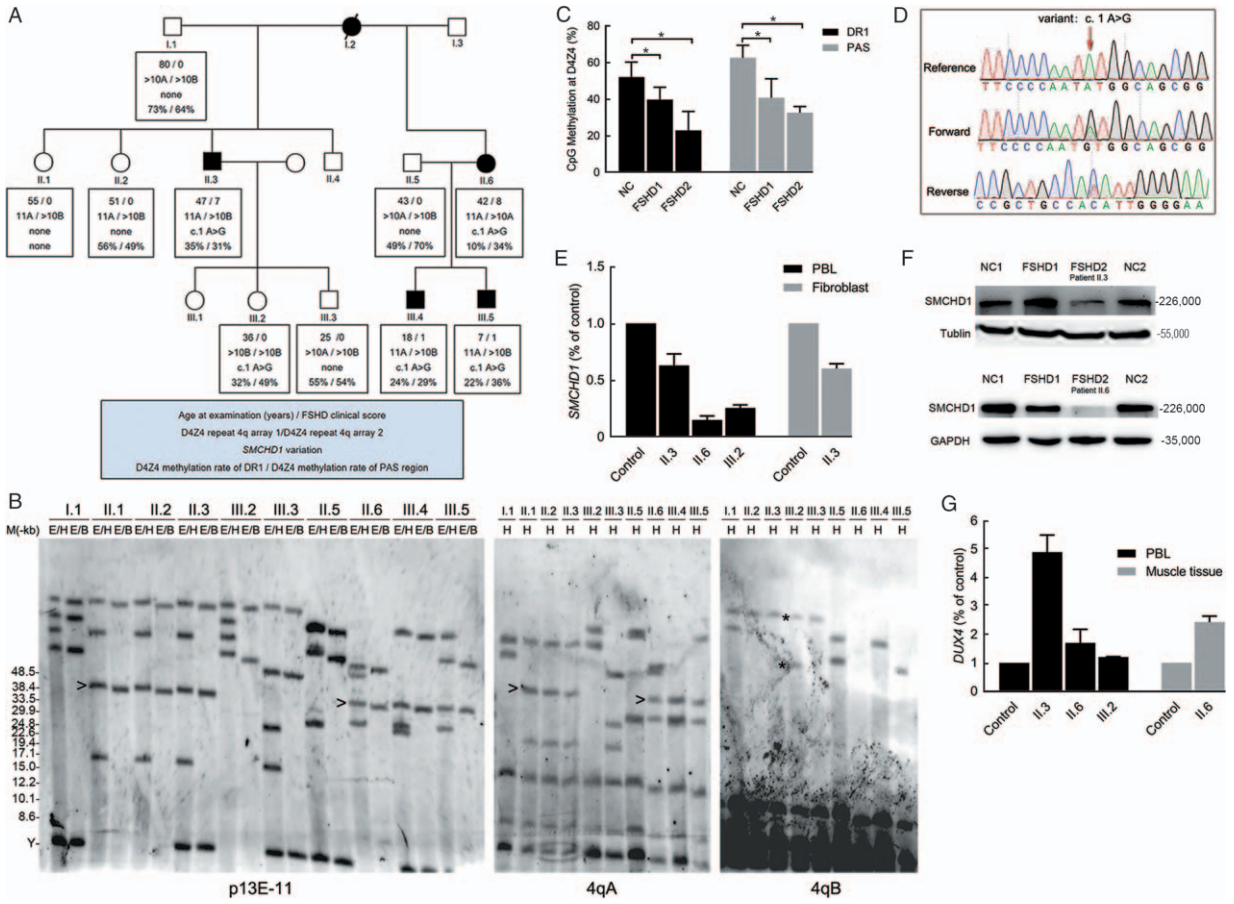

Figure 1: Pedigree, molecular analysis, and functional analysis of the FSHD2 family. (A) Pedigree of the FSHD2 family. For each individual, age at examination, FSHD clinical score, D4Z4 repeat 4q arrays, *SMCHD1* variation, and individual D4Z4 methylation level at DR1 and PAS region are shown. Detailed information is presented in the lower blue box. Note that the mother (I.2) of patients II.3 and II.6 had died and the father of patient II.6 declined to give blood samples. (B) Hybridization image of PFGE-based Southern blotting analysis of the FSHD2 family. The proband (II.3) and his two sisters (II.1 and II.2) carried two non-contracted D4Z4 arrays: one was 11 units with the 4q35A haplotype (arrow), and the other one was >10 units with the 4q35 B haplotype. Patient II.6 (the half-sister of proband) carried two non-contracted D4Z4 arrays: one was 11 units with the 4q35A haplotype (arrow), and the other one was >10 units with the 4q35 A haplotype. Patient III.2 (the daughter of the proband) carried two non-contracted D4Z4 arrays, both of which were >10 units with the 4q35B haplotype (asterisk). "M" meant marker (CHEF DNA Size Standard); "Y" meant Y chromosome; "E/H," "E/B," and "H" meant DNA were digested with *Eco*RI/*Hind*III, *Eco*RI/*Hind*III/*Bln*I, and *Hind*III, respectively. (C) Histogram bars represent the percentage of methylated CpG at the D4Z4 region of DR1 (black) and PAS (gray). NC indicates normal controls. Datasets were compared with the Kruskal-Wallis non-parametric test and brackets identify groups where values are significantly different based on *post hoc* Dunn comparison and Bonferroni correction ($^{*}P < 0.001$). (D) Sanger sequencing verified the heterozygous nucleotide variant (c.1 A>G) in *SMCHD1* in the FSHD2 family. (E) qPCR analysis indicated that the *SMCHD1* variant carriers had lower *SMCHD1* mRNA levels in PBLs (II.3, II.6, and III.2) and fibroblasts (II.3). Bars represent the mean ± SEM in a representative experiment. (F) The *SMCHD1* variant carriers of II.3 and II.6 showed reduced DUX4 protein levels in fibroblasts (upper panel) and muscle tissue (lower panel), respectively. (G) qPCR analysis indicated that the *SMCHD1* variant carriers had higher *DUX4* mRNA levels in PBLs (II.3, II.6, and III.2) and muscle tissue (II.6). Bars represent the mean ± SEM in a representative experiment. FSHD1: Facioscapulohumeral muscular dystrophy type 1; FSHD2: Facioscapulohumeral muscular dystrophy type 2; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; NC: Normal control; PBL: Peripheral blood leukocyte; qPCR: Quantitative polymerase chain reaction; SEM: Standard error of the mean.

fundus angiography. Both of patient II.6's two sons (III.4 and III.5) were also affected by FSHD. The older son of III.4 (18 years old) presented mild facial muscle weakness and clavicular flattening without limitation of upper limb muscle abduction. He was classified as category B2 and received an FSHD CS of 1 point. The younger son of III.5 (7 years old) presented mild scapular winging at rest without impairment of facial muscle [Supplementary Figure 1, http://links.lww.com/CM9/A481]. Creatine kinase was elevated two-fold. III.5 was classified as category B1 and received an FSHD CS of 1 point. Southern blotting images [Figure 1B] of this family showed that the proband (II.3) had two D4Z4 arrays in chromosome 4q: the first array was 11 units (41 kb) with the 4A161PAS haplotype, and the second array was >10 units with the 4B163 haplotype. The first array was also detected in his two asymptomatic biological sisters (II.1 and II.2) and in his symptomatic half-sister (II.6), suggesting that this array was inherited from their mother (I.2). Further supporting this possibility, this array was transmitted from patient II.6 to her two symptomatic sons (III.4 and III.5). The daughter of the proband (III.2) was asymptomatic, carrying two 4q D4Z4 arrays, both of which were >10 units with the 4B163 haplotype. The D4Z4 methylation levels of five family members, namely, II.3, II.6, III.2, III.4, and III.5 (individual D4Z4 methylation is presented in Figure 1A), were at least 2 SD below the average levels[1] in both the DR1 (52 ± 8%) and polyadenylation signal (PAS) (64 ± 7%) regions in the general population. Almost complete hypomethylation was observed in FSHD1 and FSHD2 patients compared with normal controls in both regions, and no significant difference was observed between FSHD1 and FSHD2 patients [Figure 1C]. Interestingly, a novel heterozygous variant (c.1 A>G) in *SMCHD1* [Figure 1D]

was demonstrated in the five family members with D4Z4 hypomethylation (II.3, II.6, III.2, III.4, and III.5). This variant was absent in 500 controls of non-FSHD and had not been reported previously. According to the guidelines for the interpretation of sequence variants,[3] the c.1 A>G of *SMCHD1* was considered as a loss-of-function mutation, which was strong evidence for pathogenicity. Cosegregation analysis indicated that the novel *SMCHD1* variant was initially inherited from the patient I.2 in addition to the D4Z4 array of 11 units with the 4qA haplotype. The mRNA level of *SMCHD1* was decreased in peripheral blood leukocytes (PBLs) from the three variant carriers, that is, II.3, II.6, and III.2 as well as in fibroblasts from II.3 compared with normal controls [Figure 1E], and the protein expression level of SMCHD1 was also decreased, both in fibroblasts from II.3 and in muscle tissue from II.6 compared with normal controls [Figure 1F]. DUX4 mRNA was increased in PBLs from II.3, II.6, and III.2 as well as in muscle tissue from II.6 compared with normal controls [Figure 1G]. The DUX4 transcript of NM_001306068.3 was detected in patient II.6 [Supplementary Figure 6, http://links.lww.com/CM9/A481], and the 4A161L haplotype was relatively highly expressed in muscle tissue [Supplementary Figure 7, http://links.lww.com/CM9/A481]. These data indicated that the defective expression of *DUX4* may be result from loss of function of the novel *SMCHD1* variant.

The distinctive atypical features of the proband (II.3) were an initial onset of lower back pain, as well as a unique and heretofore unexplained phenotype of the bent spine, which were inconsistent with the core phenotypes of FSHD. Pain has been reported in other FSHD studies. Due to the "bent spine," the proband was classified as category D1 according to CCEF. These results indicated that those individuals who presented minor signs suggestive of or atypical phenotypes of FSHD required particular attention in genetic analysis and should be followed up in evaluating the risk of disease onset and expression. Patient II.6 (half-sister of proband) exhibited unexplained phenotypes of acute optic neuritis, which are possibly related/unrelated to FSHD. In addition to FSHD2, pathogenic *SMCHD1* variants were reported in clinically unrelated Bosma arhinia microphthalmia syndrome[4] that typically presents severe hypoplasia or absence of the external nose. However, there were no reports regarding acute optic neuritis in either FSHD or pathogenic *SMCHD1* variant carriers. Thus, acute optic neuritis in patient II.6 may be only a coincidental syndrome and thus unable to be ascribed to FSHD2. Muscle MRI of patients II.3 and II.6 demonstrated imaging changes consistent with asymmetric atrophy, and the manifestation of fat infiltration in the II.6 MRI was consistent with her histology. Thus, muscle MRI may be useful to select a muscle for biopsy and could also be used in FSHD longitudinal studies with the advantage of being without risk, pain-free, and not limited by age and disease severity. The digenic inheritance pattern has been reported to explain the causal mechanism of FSHD2, that is, putative dominant-negative *SMCHD1* mutations and haploinsufficiency mutations.[1,5] In our FSHD2 patients, the c.1 A>G variant was located on the start codon of *SMCHD1*, which led to decreased expression of SMCHD1 and subsequently increased expression of DUX4. In combination with this loss of function in *SMCHD1* mutation, our observation of D4Z4 hypomethylation in FSHD2 patients provides evidence for a haploinsufficiency mechanism in FSHD2.

Our study first identified four FSHD2 patients from a Chinese family based on the strict criteria: a novel heterozygous variant (c.1 A>G) in the start codon of the *SMCHD1* gene that cosegregated with D4Z4 hypomethylation, as well as an intermediate size for the D4Z4 array of 11 units on the 4qA chromosome.

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Conflicts of interest

None.

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