A novel *BRF1* mutation in two middle-aged siblings with cerebellofaciodental syndrome

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To the Editor: Cerebellar–facial–dental syndrome (CFDS) is a rare and autosomal recessive (AR) neurodevelopmental disease, characterized by cerebellar hypoplasia and intellectual disability, facial dysmorphisms, short stature, microcephaly, and dental anomalies.[1] In 2015, Borck et observed three pairs of siblings with a previously undescribed pattern of abnormalities and established CFDS as a clinical entity. To date, a total of six CFDS families have been reported.[1-4] The age of the reported patients ranges from infancy to early adulthood. Since the rarity of CFDS, more study is required for evaluating its natural history and clinical characteristics. This paper would like to report a novel homozygous *BRF1* gene mutation in two middle-aged CFDS patients from Chinese, and to summarize its clinical and genetic characteristics, reinforcing the pathogenicity of *BRF1* gene mutations and expanding the manifestation spectrum of CFDS.

This study recruited an AR family with parental consanguinity including five members [Figure 1A]. All five subjects were evaluated by two qualified neurologists. Genomic DNA was extracted from peripheral blood leukocytes of all five individuals. This study was approved by the institutional ethics board of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (No. TJ-C20200129), and consents were obtained from all individuals involved in the study. Genomic DNA was enriched for exonic and adjacent splice site sequences with the Agilent SureSelect Human All ExonV6 kit, and libraries were run on an Illumina HiSeq 4000 Sequencer (Illumina Inc., San Diego, CA, USA) via a paired-end 150-bp protocol. The high-quality sequence reads were subsequently aligned to the human reference genome (GRCh37/hg19) using the BWA (version 0.7.8-r455) tool. Sambamba was used to mark duplicate reads. SAMtools were used for variant calling. Variant annotation and interpretation were conducted using ANNOVAR (http://www.openbioinformatics.org/annovar/).

We filtered for variants that met the following criteria: (1) Homozygous or compound heterozygous variants; (2) the frequency of variants was <1% in the databases of 1000 Genomes Project, esp6500, and gnomAD; (3) variants in exonic or splicing (±2 bp) region; (4) nonsynonymous variants; (5) variants were predicted to be harmful by at least two predictive software, including SIFT (http://sift.jcvi.org), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2), MutationTaster (http://www.mutationtaster.org), and CADD (http://cadd.gs.washington.edu) or predicted to affect splicing by software dbscSNV (http://asia.ensembl.org/info/docs/tools/vep/script/vep_plugins.html#plugins_existing).

Cosegregation was performed using polymerase chain reaction and Sanger sequencing. Candidate variants that cosegregated with the disease were sequenced in 200 normal chromosomes using polymerase chain reaction and Sanger sequencing. According to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines, variants were classified into "pathogenic," "likely pathogenic, ""uncertain significance," "likely benign," and "benign."

By performing whole exome sequencing in the proband, we detected 108,892 single-nucleotide polymorphisms and 12,293 indels and identified only one homozygous missense variant c.874C>A (p.P292T) in *BRF1* gene (NM_001519) that met the filtering criteria [Figure 1A]. This novel amino acid change is located at the same residue as the previously reported pathogenic variant c.875C>A (p.P292H) [Figure 1B]. This variant was absent in the databases of 1000 Genomes Project, esp6500, and gnomAD and was highly conserved among species. It was predicted to be disease causing by SIFT, PolyPhen-2, and Mutation Taster programs. Cosegregation analysis showed that the two affected siblings (the proband and her older brother) carried homozygous variant c.874C>A, the healthy consanguineous parents carried the heterozygous

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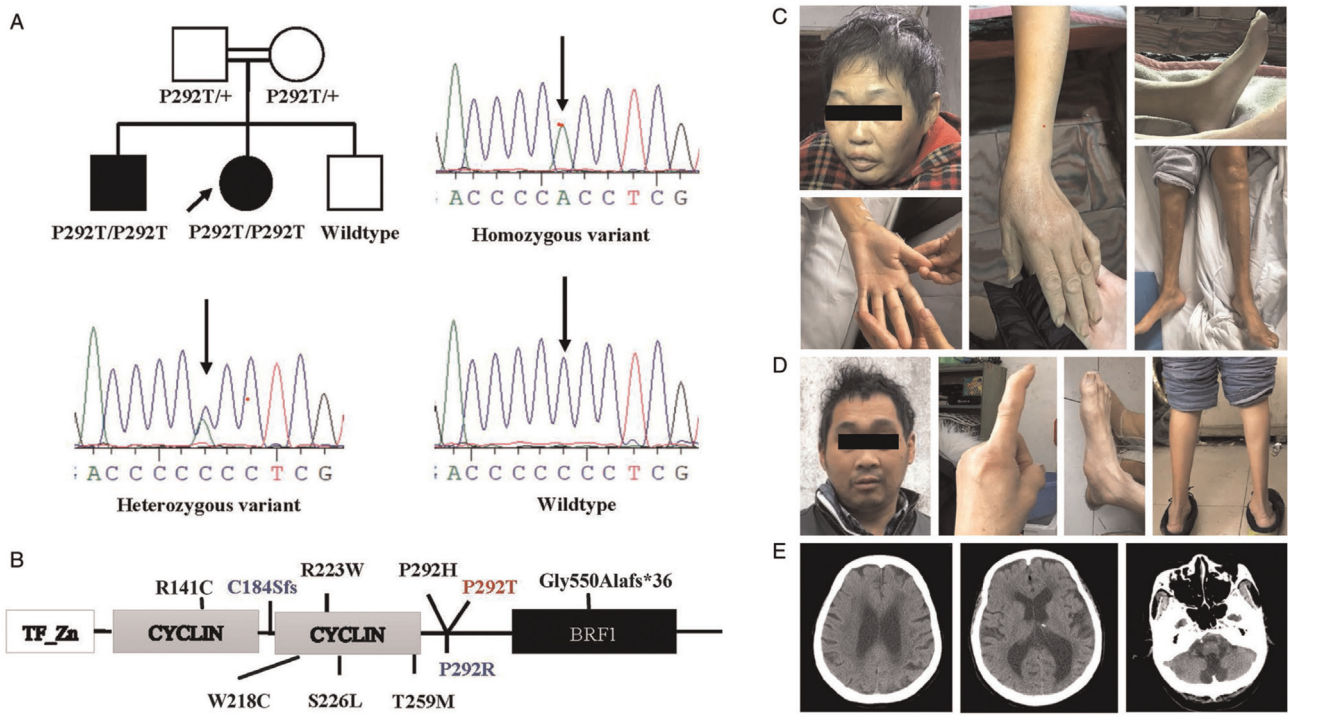

Figure 1: Genotypic and phenotypic characteristics of CFDS patients carrying the novel *BRF1* mutation c.874C>A (p.P292T). (A) This family with parental consanguinity included two affected members and three unaffected members. The proband and her older brother were homozygous for the variant, her parents were heterozygous, and her younger brother was wildtype. The proband was denoted by an arrow. (B) Location of novel and reported variants in *BRF1* (red: novel variant reported in this study). (C, D) Photos of CFDS patients showed that they had craniofacial dysmorphisms, dental anomalies, camptodactyly of interphalangeal joint, distal limbs muscle atrophy, and pes planus. (E) Brain CT of proband showed that brain atrophy, especially cerebellar vermis atrophy, bilateral periventricular leukomalacia, ventricular enlargement, and multiple scattered low-density lacunes. CFDS: cerebellar–facial–dental syndrome; CT: computed tomography.

variant, and the healthy sibling (the younger brother of the proband) was wildtype. This variant was absent in 200 normal chromosomes. According to the ACMG standards and guidelines, it was classified into a likely pathogenic variant (PM1, PM2, PM5, PP2, PP3).

The proband was a 41-year-old woman [Figure 1C]. She was referred to our hospital for acute cerebral infarction. She presented with acute-onset right limbs numbness and weakness, as well as headache, dizziness, and nausea. She was born at full term and showed no obvious developmental delay as an infant. She was noted to have intellectual disability and speech delay at preschool age and have muscle atrophy of distal lower limbs at age of ten. She had hypertension for 4 months and recurrent upper respiratory infections. Physical examination showed that she had positive signs of the nervous system (including intellectual disability, slurred speech, slender extremities with relatively truncal obesity, limbs weakness, sensory disturbance of right limbs, and right positive pyramidal sign [increased muscle tone, tendon hyperreflexia, and positive pathological signs]), craniofacial dysmorphisms (including sparse hair and eyebrows, especially the outer eyebrows, wave-shaped eyelids, flat nose, low-set ears, open-mouthed appearance, and micrognathia), dental anomalies (including prominent upper central incisors and early tooth loss), and skeletal anomalies (including short stature, long slender fingers, camptodactyly of the fifth finger, and pes planus). Microcephaly and scoliosis were not observed. Her Mini-Mental State Examination (MMSE) score is five. Hematological results showed that she had severe hyperhomocysteinemia (>50 μmol/L), hypokalemia (2.72 mmol/L, normal range: 3.5–5.1 mmol/L), and hyperthyroidism (FT3: 11.00 pmol/L, FT4: 55.20 pmol/L, thyroid-stimulating hormone: 0.01 μIU/mL). Her heart anatomy was normal. Ultrasonography and biopsy confirmed that she had thyroid carcinoma. Since she had irremovable metal dentures, computed tomography of the brain was performed instead of magnetic resonance imaging, which showed that brain atrophy, especially cerebellar vermis atrophy, bilateral periventricular leukomalacia, ventricular enlargement, and multiple scattered low-density lacunes [Figure 1].

The older brother of the proband was a 43-year-old male [Figure 1D]. He was prematurely born and showed obvious psychomotor impairment, including motor and speech delay. He learned to walk by the age of 2 years and to speak by the age of 3 years. He developed muscle atrophy of distal lower limbs at age of ten. Physical examination showed that he had positive signs of the nervous system (including intellectual disability and slender extremities with relatively truncal obesity), cranio-facial dysmorphisms (including sparse hair and eyebrows, especially the outer eyebrows, wave-shaped eyelids, low-set ears, flat nose, and micrognathia), dental anomalies (early tooth loss), and skeletal anomalies (including short stature, long slender fingers, camptodactyly of distal interphalangeal joint of index finger, and pes planus). Microcephaly and scoliosis were not observed. His MMSE score is 12. Clinical

manifestations of both patients are listed in [Supplementary Table 1, http://links.lww.com/CM9/A862].

This study identified a novel homozygous variant c.874C>A (p.P292T) of *BRF1* gene in two middle-aged siblings, characterized by cerebellar hypoplasia, intellectual disability, craniofacial dysmorphisms, dental anomalies, and skeletal anomalies. These symptoms were also present in all the previously reported CFDS patients.[1-4] Thus, these five symptoms may be the essential clinical features of CFDS. Besides these typical features, we observed some previously undescribed but common features in our patients, including distal limbs muscle atrophy, relatively truncal obesity, long slender fingers, finger joint contractures, early tooth loss, and pes planus. Microcephaly and scoliosis were common features in previously reported patients, but not observed in our patients. These indicated that CFDS was a clinically heterogeneous disease and might manifest differently at different ages. Given that BRF1 protein is highly expressed in the peripheral nerve, we inferred that *BRF1* variants might cause distal muscle atrophy and foot deformities by impairing the function of the peripheral nerve. Therefore, we suggested that an electromyogram should be performed in CFDS patients. Cerebellar hypoplasia, especially cerebellar vermis volume loss, ventricular enlargement, and thin corpus callosum were common radiological features and important diagnostic clues of CFDS.[1,2] The proband of our study had acute cerebral infarction, hyperhomocysteinemia, hypokalemia, hyperthyroidism, and thyroid carcinoma, which have never been previously reported. Whether these diseases are associated with CFDS remains unknown and needs further observation.

BRF1 gene is located to chromosome 14q32.33, and encodes BRF1 protein, which contains a conserved zinc ribbon domain and two cyclin domains. To date, a total of six *BRF1* variants have been reported. The variant c.874C>A (p.P292T) in our study was located at the same residue with the previously reported variant in two families, suggesting the amino acid P292 was a hot spot codon.[1,2]. BRF1 protein is ubiquitously expressed in brain tissues, including cerebellum, cerebellar hemisphere, anterior cingulate cortex, cortex, hippocampus, basal ganglia, and so on. Borck *et* al[1] found that suppression of *brf1* in zebrafish resulted in a significant reduction of brain size. Liko et al[5] found that loss of BRF1 decreased the tRNA levels and translation activity, increased apoptosis and necrosis, and reduced the size of multiple organs. Expression analysis in cell lines showed that BRF1 has a high expression in several kinds of human cancer cells, such as HeLa, PC3, and MCF7. Previous studies have demonstrated that elevated levels of BRF1 associate with poor prognosis in multiple human cancers, such as prostate cancer and breast cancer. Although recent studies have provided us insight into the function of BRF1, the mechanisms of CFDS remained largely unknown and needed further elucidation.

In conclusion, our study identified a novel homozygous variant of *BRF1* gene in two middle-aged CFDS siblings. In addition to the typical features, our study observed some previously unrecognized features, including distal limbs muscle atrophy, relatively truncal obesity, long slender fingers, finger joint contractures, early tooth loss, and pes planus, suggesting that CFDS patients may manifest differently at different ages. These results are essential for evaluating the natural history and clinical heterogeneity of CFDS, further confirming the pathogenicity of *BRF1* mutations and expanding the manifestation spectrum of CFDS.

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Conflicts of interest

None.

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