

A novel *ATRX* mutation causes Smith-Fineman-Myers syndrome in a Chinese family

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Abstract. Smith-Fineman-Myers syndrome (SFMS) is a rare inherited disorder characterized mainly by mental retardation and anomalies in the appearance of patients. SFMS is caused by a mutation in the α -thalassemia/mental retardation syndrome X-linked (*ATRX*) gene and has an X-linked recessive pattern. In the present study, a novel *ATRX* mutation was identified, and the association between its genotype and the phenotype was explored in a Chinese Han family with SFMS. This study aimed to lay a foundation for prenatal diagnosis for this family. Briefly, genomic DNA was extracted from peripheral blood samples obtained from the family. High-throughput genetic sequencing was employed to detect the whole exome; subsequently, Sanger sequencing was performed to verify the candidate mutations. Clinical analysis of the proband was also accomplished. Consequently, a novel missense *ATRX* mutation was identified comprising a single nucleotide change of C to T, which caused an amino acid substitution at codon 172 in exon 7 (c.515C>T; p.Thr172Ile) of the proband. This mutation was found to co-segregate in the present SFMS pedigree and was located in a highly conserved region of the *ATRX* protein, thus suggesting that it may be a pathogenic mutation. Taken together, these findings provided novel information that may lead towards an improved understanding of the genetic and clinical features of patients with SFMS, thereby facilitating a more accurate prenatal diagnosis of SFMS.

Introduction

X-linked mental retardation (XLMR) syndrome is a highly heterogeneous disorder characterized by cognitive impairment and intellectual disability; this syndrome has an estimated prevalence of 1/600 in male patients worldwide (1). XLMR can be classified into two types: Non-syndromic XLMR and syndromic XLMR, according to the clinical features (1-3). The XLMR type predominantly includes Menkes syndrome, α -thalassemia with MR syndrome (4), Cabezas syndrome (5), fragile X syndrome (6), Holmes-Gang syndrome, Juberg-Marsidi syndrome, Carpenter-Waziri syndrome, Chudley-Lowry syndrome and Smith-Fineman-Myers syndrome (SFMS) (7), and its typical clinical symptoms include short stature, hypogonadism, an abnormally large fontanel, microcephaly, seizures and MR (7-9).

Smith *et al* (10) described two brothers in 1980 who exhibited symptoms of MR, hypotonia, shortness of stature and unusual facial appearance; however, these symptoms differed from those of previously described syndromes. This syndrome was designated as Smith-Fineman-Myers syndrome (SFMS; OMIM #309580) (10), a rare form of XLMR. SFMS is characterized by short stature, seizure, hypotonia, craniofacial anomalies, psychomotor retardation with dysphasia, growth delay and an intelligence level lower than that of boys of a similar age, in addition to the aforementioned symptoms. SFMS is an uncommon clinically heterogeneous disease with an X-linked recessive inheritance trait in which hemizygous males are affected, although heterozygous females appear normal. Among these clinical manifestations, the craniofacial changes mainly comprise microcephaly, macrostomia, hyperopia, micrognathia and a patulous lower lip. Affected patients may also exhibit dolichocephaly, foot deformities, hyperreflexia, hypogonadism and hypertelorism (11,12).

The α -thalassemia/MR syndrome X-linked (*ATRX*; OMIM #300032) gene is located on the X chromosome (Xq21.1), and is also known as *XH2* or *XNP*. This gene was named after α -thalassemia with MR syndrome (OMIM #301040), which is characterized by severe MR, genital abnormalities, characteristic facial traits and α -thalassemia (6,13-18). The *ATRX* gene is composed of 37 exons and spans 288,392 bp; this is a disease-causing gene associated with XLMR (19).

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Being a member of the SWI/SNF2 superfamily of helicases/ATPases with a length of $\leq 2,492$ amino acids, the *ATRX* protein consists of several domains, including a C2-C2 zinc-finger motif, plant homeodomain (PHD)-like motif and the SWI/SNF2 ATPase/helicase-like motifs, which are involved in regulation of transcription, DNA recombination and repair, and meiosis and mitosis via effects on chromatin remodeling (4,15-17,19-24). As well as SFMS (25), mutations in *ATRX* can give rise to several other syndromes, including Holmes-Gang syndrome (7), Carpenter-Waziri syndrome (26), Juberg-Marsidi syndrome (23) and Chudley-Lowry syndrome (20).

In the present study, a novel missense mutation in the *ATRX* gene was screened and identified by performing whole exome sequencing (WES) in combination with Sanger sequencing validation in a Chinese family with SFMS. The present study may provide support to elucidate the complex genotype-phenotype associations of SFMS and could enrich the *ATRX* pathogenic variant spectrum, which may facilitate an improved early molecular diagnosis and the timely treatment of children with SFMS.

Patients and methods

Patients. The subjects selected for the present study were from a Chinese Han family based in Shandong province. A 2-year-old male proband (IV1; Fig. 1) was diagnosed with SFMS based on clinical manifestations; laboratory investigations, including Bayley Scales of Infant Development of China Revision (27); and the results of molecular genetic detection at The Affiliated Hospital of Qingdao University. Notably, all other family members were identified to be asymptomatic. After having obtained written informed consent from the family, including parents or guardians of the minors, and 200 healthy control subjects (male:female ratio, 1:1.3, age range, 1-50 years), peripheral venous blood samples were collected from the proband, other individuals in his family and healthy controls. All of the clinical data were collected prior to clinical treatment of the patient. The present study was approved by the ethics committee of The Affiliated Hospital of Qingdao University.

DNA extraction. Blood samples (400 μ l) were collected from the family (the proband, the mother, the grandmother and three maternal granduncles) and the healthy controls, and DNA was extracted from peripheral venous blood using a Qiagen DNA extraction kit (Qiagen, Inc.), strictly according to the manufacturer's protocol.

Screening for mutations using WES. Genomic DNA was randomly fragmented using a Covaris S220 sonicator (Covaris, Inc.) with 200 cycles per burst and 80 sec processing time. T4 DNA polymerase and polynucleotide kinase (PNK; Vazyme Biotech Co., Ltd.) were added to the DNA fragments for the end-repair reaction, so as to obtain a 5'-blunt-end DNA short fragment library containing phosphoric acid groups. Polymerase chain reaction (PCR) amplification may be used to effectively enrich DNA fragments with ligated adapter molecules at both ends. To meet this purpose, an Agilent™ SureSelect Human All Exon

V6 kit (Agilent Technologies, Inc.) was used according to manufacturer's protocol for library hybridization with an exome array to capture the exons of the gene; subsequently, PCR using CAP-PCR Master Mix and CAP Hotstar Enzyme (Kapa Biosystems; Roche Diagnostics) was performed to amplify the exon DNA library as follows: An initial denaturation of 98°C for 30 sec, 12 cycles of denaturation at 98°C for 25 sec, annealing at 65°C for 30 sec, extension at 72°C for 30 sec, and a final extension of 72°C for 5 min. Finally, the fragments from the purified DNA library were sequenced on Illumina NextSeq 500 sequencer (Illumina, Inc) designed for paired-end 150 bp reads. All of the coding exon regions and exon-intron boundaries in *ATRX* were sequenced by WES. The concentration of DNA used was >10 ng/ μ l, the volume was >100 μ l, and the purity aimed for in terms of the DNA fragments was determined from measures of absorbance ($A_{260/280}>1.8$, and $A_{260/230}>1.2$).

Sanger sequencing validation. The *ATRX* variant of the proband discovered by WES was validated by Sanger sequencing samples of the proband, the mother, the grandmother, three maternal granduncles and 200 healthy controls. A pair of fragments covering the mutation locus was amplified by PCR in a total reaction volume of 20 μ l, including 2 μ l template DNA, 6 μ l ddH₂O, 10 μ l Master Mix (Tsingke Biological Technology Co., Ltd.), and 1 μ l forward and reverse primers respectively. The primer pair for exon 7 in *ATRX* was designed using Primer Premier version 5.0 software (PREMIER Biosoft) primer sequences: Forward, 5'-TGGAATTTGCCAAGGTTGTCA-3' and reverse, 5'-TCCGGTGTGCTCCCATAATC-3'). The reaction conditions were as follows: Initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec and extension at 72°C for 30 sec, followed by final extension at 72°C for 7 min. Amplified PCR products were analyzed by 1% agarose gel electrophoresis stained with GoldViewI (Beijing Zoman Biotechnology Co., Ltd.). If the target band was revealed to be clearly separated and strongly fluoresced following electrophoresis, the PCR product was purified and sequenced using an ABI 3730XL automated sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.). DNA sequences were analyzed using the BioEdit program (V7.0.1; <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) and compared with a reference sequence published on the National Center Biotechnology Information (NCBI) website (<https://www.ncbi.nlm.nih.gov/>). The detected mutation was predicted by bioinformatics software Polymorphism Phenotyping v2 (PolyPhen-2; <http://genetics.bwh.harvard.edu/pph2/>) and was identified in the Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk/ac/index.php>), 1000 Genomes (<http://browser.1000genomes.org/>), dbSNP (<https://www.ncbi.nlm.nih.gov/projects/SNP/index.html>) and ESP6500 (<https://evs.gs.washington.edu/EVS/>) databases. Finally, the conservative analysis of the mutation site was performed.

Results

Clinical phenotype. The general features and results of the clinical examination of the proband (proband IV1; male; age, 2 years; Fig. 1) are shown in Table I, including developmental

Table I. Clinical findings in the patient with Smith-Fineman-Myers syndrome.

Clinical phenotype	Patient (IV1)
Short stature	+
Psychomotor retardation	+
Severe dysphasia	+
Early hypotonia	+
Restlessness	+
Undescended testes	+
Microcephaly	+
Ocular hypertelorism	+
Micrognathia	+

delay, characteristic facial appearance and hypogonadism. These physical anomalies met the diagnostic criteria for SFMS. With the exception of the proband, the phenotypes of the other family members were all normal (data not shown).

Mutation analysis of ATRX. Following the screening of numerous mutations in the entire exome using WES, candidate genes were detected by WES, including *ATRX*, *FCN3*, *TAS1R1*, *FOXD3*, *F5*, *TTN*, *CACNA2D3*, *NAPRT1*, *VPS13A*, *HPS6* and *RBM20*. Notably, only mutated *ATRX*, which matched the clinical phenotype of the proband, was identified in the present study. In addition, *ATRX* is associated with SFMS, Holmes-Gang syndrome, Carpenter-Waziri syndrome, Juberg-Marsidi syndrome, Chudley-Lowry syndrome and α -thalassemia with MR syndrome, while the clinical symptoms of the proband were associated with SFMS and co-segregation analysis also excluded dominant inherited α -thalassemia with MR syndrome after comprehensive consideration.

ATRX mutational analysis of the proband revealed a missense mutation, which was absent from the Exome Aggregation Consortium (<http://exac.broadinstitute.org/>) and Genome Aggregation Database (<https://gnomad.broadinstitute.org/>). The mutation consisted of a substitution of thymine for cytosine at position 515, resulting in a change from threonine to isoleucine at codon 172 (c.515C>T or p.Thr172Ile) (Fig. 2). Polymorphism Phenotyping v2 (PolyPhen-2; <http://genetics.bwh.harvard.edu/pph2/>) analysis predicted that this mutation would exert a significantly damaging effect on the structure and function of ATRX due to a prediction score of 1.000 for the effect of an amino acid substitution on protein function. This specific mutation site was subsequently screened for in the other family members and in 200 healthy control subjects using Sanger sequencing. The results revealed that the boy's mother (III1; Fig. 1) and his maternal grandmother (II1; Fig. 1) possessed the heterozygous mutation, whereas the mutation was not possessed by three maternal granduncles (II3, II4 and II5; Fig. 1); likewise, the mutation was absent from the 200 unrelated normal individuals of identical ethnic origin (data not shown). Therefore, the co-segregation analysis of this pedigree indicated that this missense mutation was most likely to have been the pathogenic variant responsible for the SFMS pheno-

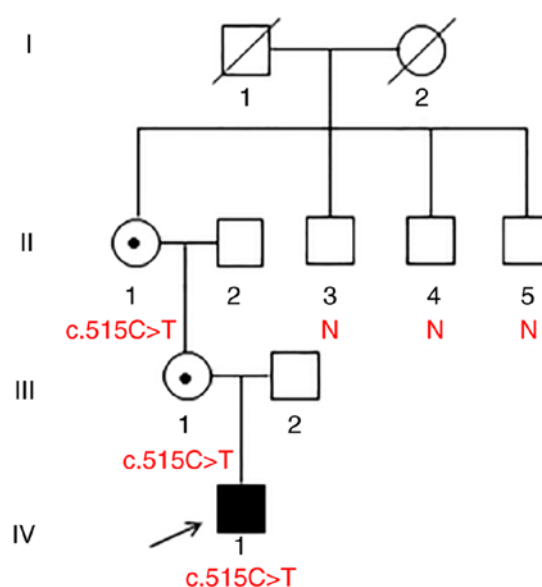


Figure 1. Pedigree of the family with Smith-Fineman-Myers syndrome. The arrowhead denotes the proband; black shape represents affected patient; white shapes represent unaffected family members; squares represent male family members; circles indicate female family members; dots indicate carriers; lines represent deceased family members.

type in this family. Additional genetic studies of the family history revealed that this mutated gene had been inherited from the proband's grandmother. Based upon searches of the Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk/ac/index.php>), 1000 Genomes (<http://browser.1000genomes.org/>), dbSNP (<https://www.ncbi.nlm.nih.gov/projects/SNP/index.html>) and ESP6500 (<https://evs.gs.washington.edu/EVS/>) databases, to the best of our knowledge, this mutation has not previously been detected, and is therefore considered a novel variant.

Bioinformatics analysis of the ATRX mutation. The *ATRX* sequences of various animal species, including *Homo sapiens*, *Bos taurus*, *Canis lupus familiaris*, *Mus musculus*, *Pan troglodytes*, *Pongo pygmaeus* and *Sus scrofa*, were obtained from the NCBI (<https://www.ncbi.nlm.nih.gov/>) and UniProtKB (<https://www.uniprot.org/>) websites, and multiple sequence alignment was performed using DNAMAN software (V6.0.3.99; Lynnon Corporation). The c.515C>T/p.Thr172Ile variant was revealed to be located in a highly conserved region of the ATRX protein (Fig. 3).

Discussion

In the present study, a Chinese pedigree affected by SFMS was investigated, and a novel missense mutation in the *ATRX* gene, c.515C>T/p.Thr172Ile, was subsequently detected and confirmed using WES followed by Sanger sequencing validation. This finding may expand our knowledge with regards to the clinical spectrum and pathogenic mutants associated with SFMS. That the mutation co-segregated with the syndrome in this family was supported by the finding that this mutational variant was not identified in 200 unrelated healthy controls of identical ethnic origin. In addition, PolyPhen-2 analysis

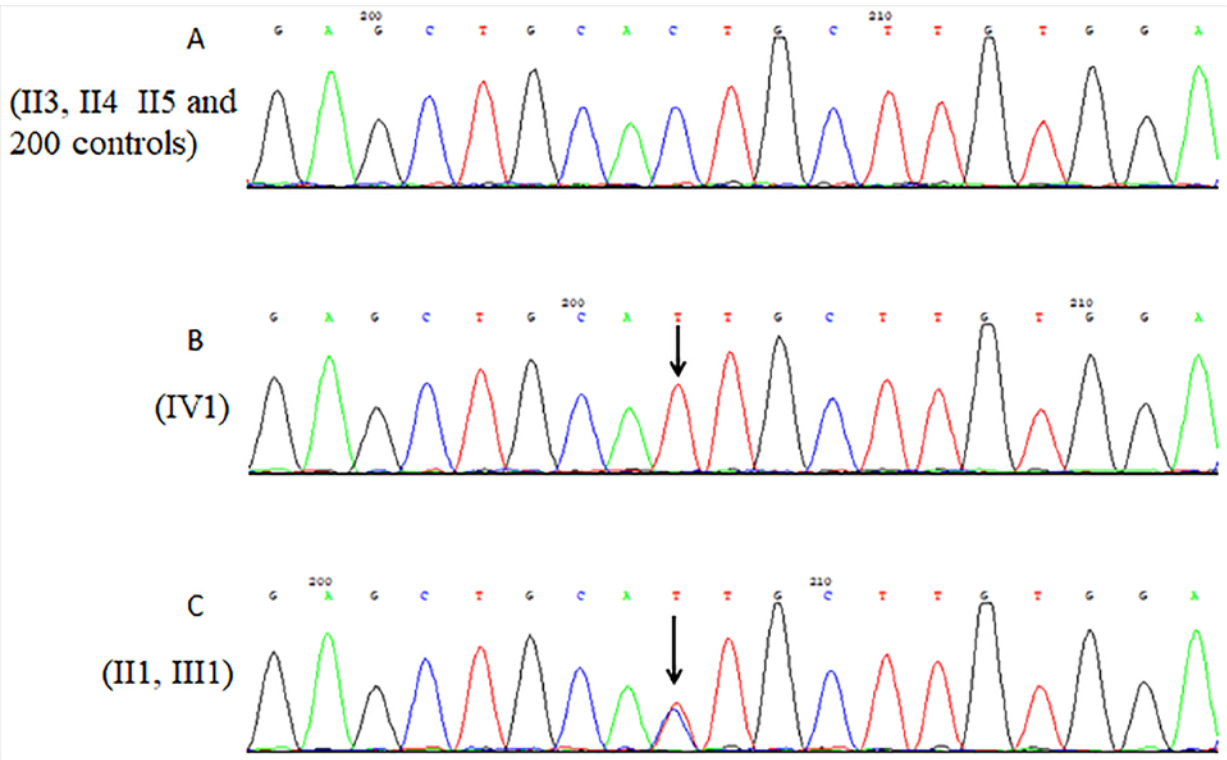


Figure 2. Sequence chromatograms. (A) Normal sequence (family members II3, II4 and II5); (B) hemizygous mutation, c.515C>T/p.Thr172Ile in exon 7, which was detected in the proband (IV1); and (C) heterozygous mutation, c.515C>T/p.Thr172Ile in exon 7, which was detected in the proband's mother (family member III1) and grandmother (family member III).

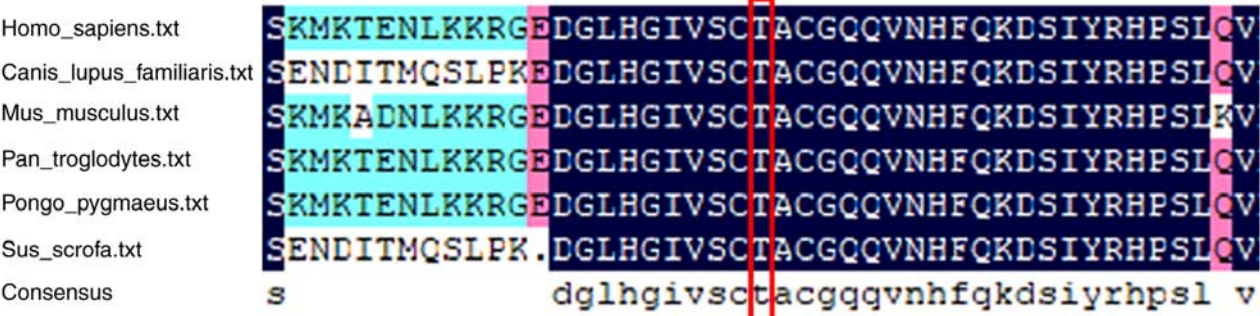


Figure 3. Multiple sequence alignment of the α -thalassemia/mental retardation syndrome X-linked protein from different species. The rectangular frame indicates the location of the mutation.

predicted that this mutation, located in a highly conserved region of *ATRX*, might result in a damaging impairment of *ATRX* protein function. Bioinformatics analysis and co-segregation analysis revealed that the c.515C>T mutation in *ATRX* is likely to give rise to the disease-causing variant of SFMS. However, it was impossible to draw definitive conclusions in this regard due to the absence of functional experiments.

SFMS is an uncommon congenital mental deficiency disorder that is governed by mutations in *ATRX* and is transmitted in an X-linked recessive manner. Since SFMS was first defined in 1980 by Smith *et al* (10), only a few cases have been reported. Patients with SFMS have specific similar clinical symptoms, including MR, characteristic facial appearance, short stature, microcephaly, micrognathia, failure to grow, seizure, hypotonia, genital abnormalities, abnormal speech,

cortical atrophy and cleft palate (19,28). Briefly, the assessment for SFMS, like any other form of MR, is mainly based on an inspection of the detailed family history, physical defects, clinical examinations and genetic testing. Currently, a correct diagnosis for many patients with SFMS, and those suffering from other forms of XLMR, remains problematic due to clinical and genetic heterogeneity, unknown mechanisms of pathogenesis, and the ever-growing number of novel mutants of causative genes. Furthermore, it is even more difficult to determine which gene is most likely to be associated with the affected individuals based only on clinical manifestations. Consequently, genetic detection and prenatal genetic screening are vitally important in the diagnosis of SFMS.

Mutations in the genes *ATRX/XNP*, *APIS2*, *GDII/OPHN2*, *UBE2A*, *OPHN1*, *MIDI*, *MECP2* and *ARX*, etc., have been reported to be associated with XLMR (1,6,19,25,29-31),

whereas patients with SFMS only possess mutations in *ATRX* (NM-000489.4). *ATRX* was originally considered to be involved in α -thalassemia with MR syndrome; this gene spans ~300 kb in chromosome Xq21.1, is comprised of 37 exons and serves an important role in sex differentiation (17). Subsequently, it was identified that *ATRX* is associated with other MR syndromes, including Holmes-Gang syndrome, Chudley-Lowry syndrome, Carpenter-Waziri syndrome, MR with spastic paraplegia and Juberg-Marsidi syndrome (4,7,19,20,23,25), and with tumorigenesis, including pancreatic neuroendocrine tumors, low-grade glioma, glioblastoma multiforme, osteosarcoma and adrenocortical tumors (32). *ATRX* is integral to the development of the brain, skeleton, genital organs and facial morphogenesis (14), suggesting that different mutations in *ATRX* may cause distinct symptoms, and varying severity under different conditions. Although a growing number of mutations have been reported in *ATRX* since it was revealed to be a pathogenic gene of XLMR, only a few mutations have been identified in SFMS (4,25).

The *ATRX* protein is a member of the SWI2/SNF2 family of chromatin-remodeling proteins, with a length of 2,492 amino acids (33). This protein has several functional domains that exert differing influences on associated proteins, a phenomenon which could account for the clinical phenotypic heterogeneity of patients with SFMS (20). The N-terminus comprises a cysteine-rich region considered to include a C2-C2 zinc finger and a PHD-like zinc finger domain, whereas the ATPase/helicase-like domain mainly controls transcriptional regulation, cell cycle regulation and mitotic chromosome segregation (17). The central portion of *ATRX* includes a predicted coiled-coil domain, a predicted nuclear localization signal and a stretch of 21 glutamic acid residues. A conserved region among different SNF2 proteins and a glutamine-rich region are located in the C-terminal sequence of *ATRX* (17,34).

ATRX mutations can result in alterations in the pattern of ribosomal DNA methylation, Y-specific repeats and subtelomeric repeats (14,19,35), when the mutational 'hot-spots' occur in the cysteine-rich region and the ATPase/helicase-like motifs (36). However, patients with mutations in the PHD-like zinc finger present with more severe phenotypes compared with those that have mutations in the helicase domain (37). In addition to the expression of α -globin and an impaired intellectual level, mutated *ATRX* has been reported to be associated with various degrees of urogenital developmental disabilities (17).

With the expanding scope of genetic testing in scientific research and clinical medicine, high-throughput sequencing technology has undergone rapid development, and the detection speed has been accelerated. At present, DNA sequencing technology is not only advancing genetic research, but is also being used for the detection of a variety of genetic diseases. The traditional methods of genetic diagnosis often fail to lead to the identification of the pathogenic gene, which means that patients do not receive a definite diagnosis, let alone the corresponding treatment or subsequent prenatal diagnosis. WES enables high-throughput sequencing to be performed after DNA capture and enrichment in the exon region of the genome using sequence capture technology, and genetic variations associated with changes in protein function can be directly detected. WES has the advantages of high-sequencing

depth, accurate mutation detection and a high accuracy of data. Although exons account for only 1% of the genome, >85% of Mendelian diseases are associated with this region (38). Since the majority of diseases are caused by mutations that may change the function of encoded proteins, and diseases are mostly associated with rare mutations, WES has currently established itself as the most cost-effective and reliable method for studying Mendelian genetic diseases.

In conclusion, recently, more cases associated with mutations in *ATRX* have been identified on the basis of severe MR with typical physical impairment (25,37). In the present study, since the family was relatively small and other family members did not exhibit similar phenotypes, it was difficult to clearly diagnose and identify the pathogenic gene; therefore, WES followed by Sanger sequencing validation was adopted to detect the defective gene. Consequently, a novel missense mutation was described that may have led to decreased *ATRX* activity in a Chinese proband with SFMS, and which is available for the accurate diagnosis of other patients, laying the foundation for genetic counseling and prenatal diagnosis. Therefore, this study may further broaden the phenotypic spectrum of SFMS associated with *ATRX* mutations, thereby providing the basis for further investigation of SFMS pathogenesis and gene therapy.

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Availability of data and materials

The datasets used and analyzed during the study are available from the corresponding authors on reasonable request.

Authors' contributions

LL performed the experiments and wrote the manuscript. JY, XZ, MH and WL made important contributions to data acquisition, analysis and interpretation. HL and SL designed the present study and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the ethics committee of The Affiliated Hospital of Qingdao University. Written informed consent was obtained for all patients, family members and volunteers; for minors, consent was obtained from their parents or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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