

# Identification of a novel pathogenic mutation of the *MYH3* gene in a family with distal arthrogryposis type 2B

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Received March 3, 2018; Accepted August 29, 2018

DOI: 10.3892/mmr.2019.10820

**Abstract.** Distal arthrogryposis (DA) type 2B (DA2B) is an autosomal dominant congenital disorder, characterized by camptodactyly, thumb adduction, ulnar deviation and facial features, including small mouth, down-slanting palpebral fissure and slight nasolabial fold. It has been reported that four genes are associated with DA2B, including troponin I, fast-twitch skeletal muscle isoform, troponin T3, fast skeletal, myosin heavy chain 3 (*MYH3*) and tropomyosin 2, which are all associated with embryonic limb morphogenesis and skeletal muscle contraction. In the present study, three affected family members and five unaffected individuals were identified through clinical and radiological assessment. Genomic DNA was obtained from the three patients, which then underwent whole-exome sequencing, and candidate mutations were verified by Sanger sequencing in all available family members and 100 healthy volunteers. Then, the spatial models of embryonic MYH were further constructed. In the clinic, the three patients recruited to the present study were diagnosed with DA2B. Mutation analysis indicated that there was a novel heterogeneous missense mutation c.2506 A>G (p.K836E) in the *MYH3* gene among the affected individuals, which was highly conserved and was not identified in the unaffected family members and healthy controls. Furthermore, protein modeling revealed that the altered position interacted with regulatory light chain. Thus, the present study identified a novel pathogenic mutation of the *MYH3* gene in a Chinese family with DA2B, which expanded the mutational spectrum

of *MYH3* and provided additional information regarding the association between mutation locations and different types of DA.

## Introduction

Distal arthrogryposis (DA), a group of autosomal dominant disorders with clinical and genetic heterogeneity, is characterized by congenital non-progressive contractures of the distal upper and/or lower limbs (1,2). Typically, the distinctive clinical features of DA include severe camptodactyly, overriding fingers, thumb adduction, ulnar deviation, hypoplastic or absent interphalangeal creases of hands, calcaneovalgus deformities, clubfoot and short stature (2). It should be noted that patients with DA usually exhibit normal levels of intelligence, without primary neurological or muscular diseases (2,3).

To date, the most common classification of DA was introduced by Bamshad *et al* (2) in 1996, who generalized DA into nine types (types 1 to 9) according to clinical features. In this classification system, only DA1 was not associated with extra abnormalities, whereas the other forms of DA had various additional phenotypic features (2). In 1997, Krakowiak *et al* (4) reported a large family containing 21 patients over 5 generations that were affected with an intermediate disorder between DA1 and DA2. They termed this novel type as DA type 2B (DA2B), which is also known as Sheldon-Hall syndrome, and altered the previous type DA2 to DA2A (4). Following this, Stevenson *et al* (5) reported on a family affected with plantar flexion contractures, which was defined as DA10 in 2006. On the basis of different causative genes, DA1 was further divided into DA1A and DA1B (6). The newest DA classification system was established by various physical and genetic features (Table I) (7). It should be noted that there are some overlapping features among the different types of DA, which has added difficulties to diagnosis.

DA2B, one of the most common types of DA, was first described by Krakowiak *et al* (4) in 1997. In addition to the common manifestations of DA, the typical phenotypes of DA2B also include several facial features such as a triangular face, micrognathia, down-slanting palpebral fissures, prominent nasolabial folds and a small mouth (2,8-10). In recent years, mutations in four genes have been successively reported to be responsible for DA2B. In 2003, Sung *et al* (11,12) determined that DA2B may be caused by mutations in the troponin I,

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**Abbreviations:** DA, distal arthrogryposis; DA2B, distal arthrogryposis type 2B; *MYH3*, myosin heavy chain 3; *TNNT3*, troponin T3, fast skeletal; RLC, regulatory light chain

**Key words:** myosin heavy chain 3, distal arthrogryposis type 2B, embryonic myosin heavy chain, mutation

fast-twitch skeletal muscle isoform (*TNNI2*; MIM 191043) and troponin T3, fast skeletal (*TNNT3*; MIM 600692) genes; this work established the foundation for studying the genetic mechanism underlying DA2B. Missense mutations in myosin heavy chain 3 (*MYH3*; MIM 160720), which encodes embryonic MYH, were then identified by Toydemir *et al* (9) in 2006. It has been hypothesized that mutations in the *MYH3*, *TNNI2* and *TNNT3* genes collectively account for ~50% of all cases of DA2B. In addition, Tajsharghi *et al* (3) identified a heterozygous mutation in the tropomyosin 2 (*TPM2*; MIM 190990) gene, further enriching the genetic background of DA2B. In general, these mutations cause DA2B by altering the adenosine triphosphatase (ATPase) activity of myosin or by influencing the interactions between myosin and other proteins (9,13,14).

To date, all of the previous cases of DA2B associated with *MYH3* have been caused by missense mutations, except for a 3-bp deletion reported in a small number of cases (9,15-18). Furthermore, mutations in *MYH3* have also been observed in DA1, DA2A and DA8 (9,16,19,20). In contrast to the other types of DA, all affected residues in DA2B are located within the head and neck domains of embryonic MYH (9,15-17), and it interacts with other proteins of the contractile apparatus including light chain, actin and troponin (14,21).

The present study identified a novel causative missense mutation of the *MYH3* gene in a Chinese family with DA2B. Consistent with previous studies (9,20), the mutation site identified in the present results was located in the neck domain of embryonic MYH.

## Materials and methods

**Patients.** A three-generation non-consanguineous family (Fig. 1) containing three DA2B patients, identified by two independent orthopedic surgeons, was investigated in the present study. The three patients were a 4-year-old boy (III<sub>2</sub>), a 31-year-old female (II<sub>2</sub>) and a 64-year-old male (I<sub>1</sub>). This family was recruited from the outpatient department of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China) when they sought treatment for the proband (III<sub>2</sub>). All of the 8 family members underwent systematic physical assessment and the proband was additionally subjected to X-ray examination. Written informed consent was obtained from all of the participants. The present study was approved by the Ethics Committees of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China).

**Whole-exome sequencing and mutation prediction.** Peripheral blood samples from all of the available family members (except II<sub>4</sub>) and 100 unrelated healthy donors aged 18-50 (50 males and 50 females) were collected and stored at -80°C until use in subsequent experiments. Genomic DNA was then extracted using the QuickGene DNA whole blood kit (Kurabo Industries Ltd., Osaka, Japan). The present study sequenced the whole-exome of the three affected individuals in order to determine the mutated gene that induces this disorder. The SureSelect Human All Exon 57Mb Kit (Agilent Technologies, Inc., Santa Clara, CA, USA) and the HiSeq 2000 platform (Illumina, Inc., San Diego, CA, USA) were used to capture the whole-exome sequence, following the manufacturer's protocol. Illumina base-calling software v1.7 was then applied

to convert the raw image files into 90-base-paired-end reads. BioAnalyzer 2100 (Agilent Technologies, CA, USA) was subsequently employed to analyze the bioinformatics and all of the mutations were validated.

All detected variants were filtered against six single-nucleotide polymorphism databases, including dbSNP144 (hgdownload.cse.ucsc.edu/goldenPath/hg19/database/snp144) (22), the HapMap Project (ftp.ncbi.nlm.nih.gov/hapmap) (23), the 1,000 Genomes Project (www.internationalgenome.org/), the YanHuang database (yh.genomics.org.cn/), the Exome Variant Server (evs.gs.washington.edu/EVS/) and the Exome Aggregation Consortium database (exac.broadinstitute.org/). Noncoding variants were initially excluded and the most likely pathogenic candidate mutations were presented in accordance with previous studies (3,9,11,12). The present study further checked the conservation of altered amino acid residues and predicted the risk of missense mutations by utilizing Polymorphism Phenotyping version 2 (Polyphen-2; genetics.bwh.harvard.edu/pph2/) and Protein Variation Effect Analyzer (PROVEAN; provean.jcvi.org/index.php/).

**Sanger sequencing.** The DNA sequence for the *MYH3* gene was obtained from NCBI gene database (Gene ID: 4621). The two mutation regions of the *MYH3* gene that were identified following whole-exome sequencing were amplified using a standard polymerase chain reaction protocol (24). The primers were designed using Primer-3 software (bioinfo.ut.ee/primer3-0.4.0/). Direct sequencing was then performed using the BigDye Terminator Cycle Sequencing Ready Reaction Kit, version 3.1 (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA), and the sequences were analyzed with an ABI Prism 3130 automated sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.). Mutations were checked using Polyphred program (droog.mbt.washington.edu/poly\_get.html).

**Protein spatial model construction.** The wild type ribbon structure of embryonic MYH was initially constructed using DeepView and SWISS-MODEL (swissmodel.expasy.org/repository/uniprot/P11055). In addition, to illustrate the position of the pathogenic missense mutation p.K836E, the amino acid residue substitution was further incorporated into the sequence and the mutant protein structure was constructed. Then, the interactive protein associated with the altered position was added to the structure.

## Results

**Clinical features.** The primary phenotypes of the 4-year-old (III<sub>2</sub>) proband and his mother (II<sub>2</sub>) were highly consistent (Fig. 2), including bilaterally medium camptodactyly, thumb adduction, evident ulnar deviation and hypoplastic interphalangeal creases of the hands (Fig. 2A, B, D and E). As for facial features, only slight nasolabial folds and small mouths without down-slanting palpebral fissures were observed (Fig. 2C and F).

Notably, the proband's grandfather (I<sub>1</sub>) had a normal left hand, while his right hand exhibited similar manifestations to those of the other aforementioned patients (Fig. 2G). Furthermore, the facial features of I<sub>1</sub> were also

Table I. Classification of DA.

Type	Subtype	Characteristic phenotype	Relative genes	OMIM number
DA1	DA1A	Camptodactyly, tight clenched fists neonatally	TPM2, TNNI2, TNNT3, MYH3	108120
	DA1B	Camptodactyly, ulnar deviation of the fingers	MYBPC1	614335
DA2	DA2A	H-shaped chin dimple, 'Whistling' appearance	MYH3	193700
	DA2B	Intermediate between DA1 and DA2A	TNNI2, TNNT3, MYH3, TPM2	601680
DA3	/	Short stature, cleft palate	PIEZO2	114300
DA4	/	Severe scoliosis	/	609128
DA5	/	Ocular abnormalities, ptosis, ophthalmoplegia	PIEZO2	108145
DA6	/	Sensorineural deafness	/	108200
DA7	/	Trismus	MYH8	158300
DA8	/	Multiple pterygia	MYH3	178110
DA9	/	Congenital contractural arachnodactyly	FBN2	121050
DA10	/	Congenital plantar contracture	/	187370

OMIM, Online Mendelian Inheritance in Man; DA, distal arthrogryposes.

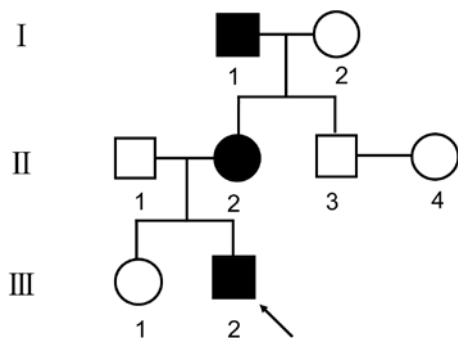


Figure 1. Pedigree of the distal arthrogryposis type 2B family. Filled symbols indicate the affected individuals (I<sub>1</sub>, II<sub>2</sub>, III<sub>2</sub>) and open symbols indicate the unaffected ones (I<sub>2</sub>, II<sub>1</sub>, II<sub>3</sub>, II<sub>4</sub>, III<sub>1</sub>). II<sub>4</sub> was not available for DNA sequencing due to individual reluctance.

asymmetric, including the prominent right nasolabial fold, the down-slanting palpebral fissure and the relatively small mouth (Fig. 2H). In addition, short statures were observed in all three of the affected family members when compared with their peers. All unaffected individuals in this family had normal hands and medium statures without any characteristic facial appearances.

**Mutation analysis.** Following systematic screening, >100 potentially disease-causing candidate single nucleotide variants among the three patients remained. Considering the function of the genes and the results of previous studies (9,20), the present study focused on the novel heterozygous missense mutation c.2506A>G (p.K836E) in exon 22 of the *MYH3* gene. The Polyphen-2 and PROVEAN scores of the mutation c.2506A>G (p.K836E) were 0.785 and -3.277, respectively, which indicated a 'deleterious' function. Notably, another missense mutation c.5263A>G (p.K1755E) of *MYH3* was identified in the proband's genomic DNA; however, it was not observed in the other two patients. According to the single-nucleotide polymorphism (SNP) databases, c.5263A>G

(p.K1755E) was recorded as an SNP (rs546653497) with a rather low minor allele frequency (MAF; 0.0002). However, c.2506A>G (p.K836E) was not observed in the SNP database and its MAF was far <1% (0.00%) in the control population; thus, the present study identified it as a mutation.

To confirm the two mutations of the *MYH3* gene revealed through whole-exome sequencing, the present study performed Sanger sequencing for all of the available family members and the 100 healthy donors. In accordance with the results of whole-exome sequencing, the mutation c.2506A>G (p.K836E) was identified in the three affected individuals (Fig. 3A); however, not in the available unaffected family members and the 100 healthy donors (Fig. 3B). The p.K1755E SNP was only identified in the proband and his father, and not in the other available family members (Fig. 3C and D). In addition, the mutation c.2506A>G (p.K836E) also affected highly conserved amino acid residues in other species (Fig. 3E).

**Protein structural model.** According to the spatial ribbon structure of the protein, the mutant p.K836E is located in the neck, and spatially, on the surface of embryonic MYH, adjacent to several previous mutation sites in DA2B (Fig. 4A-C) (9,16). Although there is no apparent difference between the wild-type protein and the mutant one, the changed region would still tightly interact with the regulatory light chain (RLC) (Fig. 4B). In addition, the SNP p.K1755E is located at the tail far away from the other mutation sites associated with DA (Fig. 4C).

## Discussion

DA2B is an autosomal dominant inherited congenital disorder, resulting from mutations in genes associated with embryonic limb morphogenesis (10). On account of the similar clinical features, DA2B, DA1 and DA2A are occasionally considered to be a phenotypic continuum of one syndrome (25). Due to this, DA2B is difficult to diagnose, as its clinical symptoms, including camptodactyly, overriding fingers, thumb adduction, ulnar deviation, facial features, and hypoplastic interphalangeal

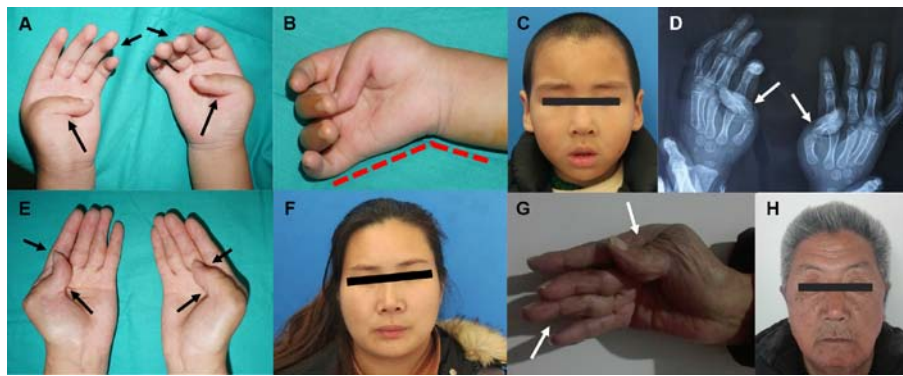


Figure 2. Clinical and radiological presentations of the affected family members. (A) Images of the proband's hands showing camptodactyly and thumb adduction (black arrows). (B) Evident ulnar deviation (red lines). (C) Proband's facial features including slight nasolabial folds and a small mouth. (D) Corresponding radiograph of the proband's hands, presenting camptodactyly and thumb adduction (white arrows). (E) Hands of the proband's mother (II<sub>2</sub>) also presented camptodactyly and thumb adduction (black arrows). (F) The facial features of the proband's mother (II<sub>2</sub>) were similar to the proband. (G) The right hand of the proband's grandfather (I<sub>1</sub>) presented evident camptodactyly and thumb adduction (white arrows). (H) The facial features of I<sub>1</sub> were asymmetric, including more apparent right nasolabial fold and right down-slanting palpebral fissure.

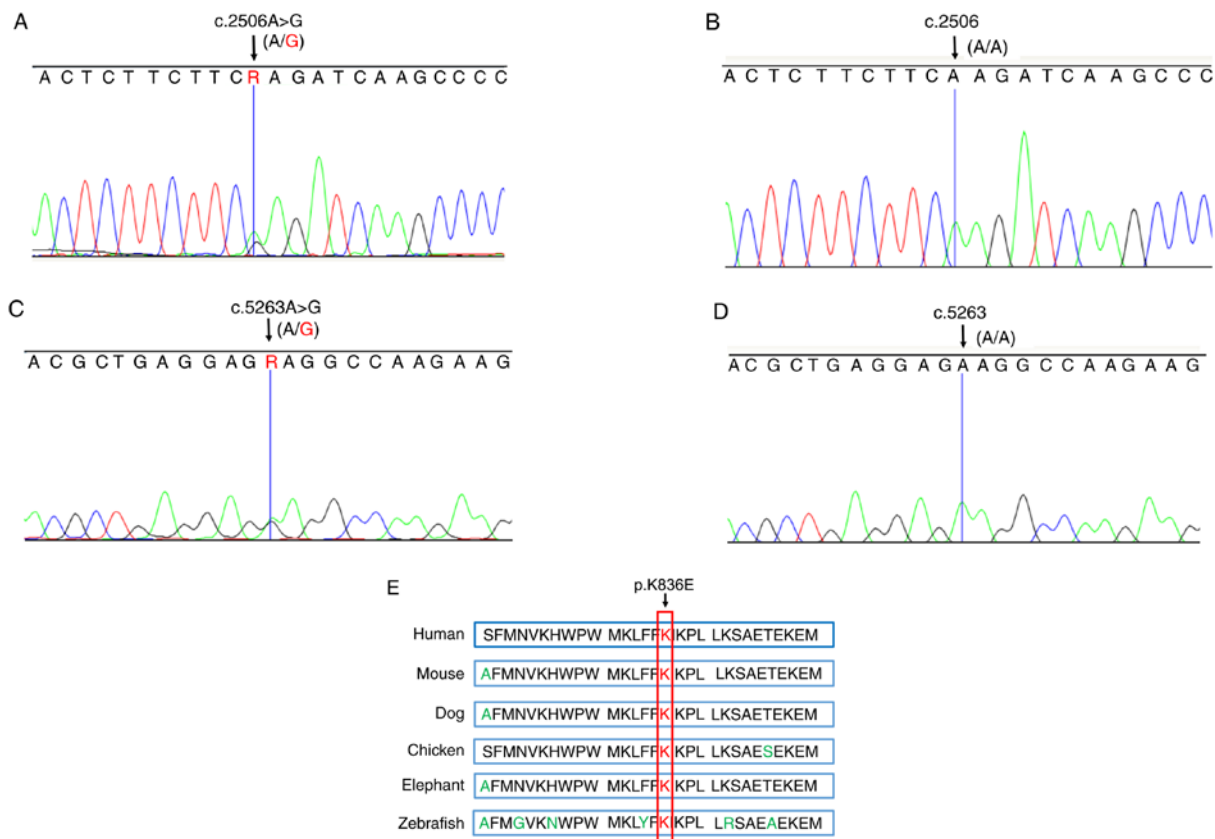


Figure 3. Mutation sites. (A) The novel heterozygous mutation site c.2506A>G (black arrows) resulting from an A/A to A/G mutation in the affected family members. (B) Corresponding sequence in the unaffected individuals. (C and D) The other myosin heavy chain 3 mutation c.5263A>G (black arrows) was detected in the proband and his father; however, not in the other individuals. (E) The pathogenic heterozygous missense mutation c.2506A>G p.K836E (red letter) occurred in a highly conserved position, as shown by the comparison between humans and 5 other species.

creases of hands, are also present in DA1 and DA2A (2,10). The affected individuals in the present study exhibited typical limb manifestations including medium camptodactyly, thumb adduction, evident ulnar deviation and slight facial features without scoliosis. Therefore, the diagnosis of DA2B was clear.

Notably, only the unilateral limb and face of I<sub>1</sub> were affected, while the other two patients exhibited a symmetric appearance. In addition, all of the siblings of I<sub>1</sub> and their offspring were not

affected (data not shown). The intra-familial difference may be a result of various factors such as incomplete penetrance and variable expressivity, which are common in congenital developmental diseases. These phenomena are usually based on differing susceptibilities due to discrepancies in gene background and the uterine environment during the embryonic period. Furthermore, the asymmetric phenotype of I<sub>1</sub> may also be explained by p.K836E as a *de novo* mutation, occurring during the embryonic development

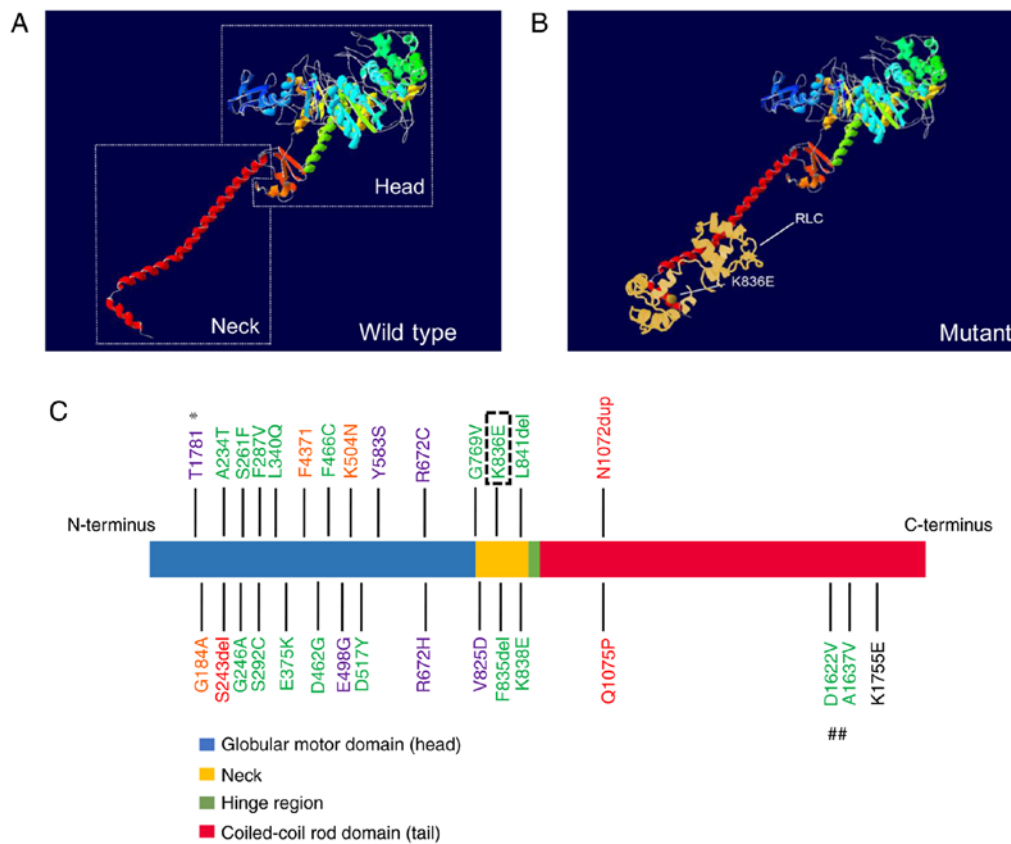


Figure 4. Protein modeling of embryonic myosin heavy chain. (A) Wild type ribbon structure of the globular motor domain and neck of embryonic myosin heavy chain. (B) There was no evident difference between the wild type and mutant protein; however, the substitution site of p.K836E in the present study was located to the region interacting with regulatory light chain. (C) The stylized structure of the protein is composed of a globular motor domain, a short neck, a hinge region and a coiled-coil rod domain. Previous mutations associated with DA1 (orange), DA2A (purple), DA2B (green) and DA8 (red) are marked at the corresponding positions of the protein sequence. The pathogenic substitution (p.K836E) in the present study is framed by a black rectangle. The substitution K1755E in black is the other mutation identified in the present study. \*, DA2A and DA2B can be caused by T178I; #, the two mutations are now considered to be polymorphisms according to large databases; DA, distal arthrogryposis.

period, affecting the right half of his body with a certain number of reproductive cells, which may have enabled him to transmit the pathogenic mutation to his descendants (18). However, this hypothesis of chimerism was hard to confirm in the present study as tissues from different parts of the patient's body were not available due to individual reluctance and ethical concerns.

In recent years, it has been reported that a total of four genes are associated with DA2B, including *TNNI2*, *TNNT3*, *MYH3* and *TPM2*, all of which encode the fast-twitch skeletal muscle myofibers complex and participate in skeletal muscle contraction (3,9,11,12). The *MYH3* gene, which is mapped to chromosome 17p13.1, is composed of 41 exons, 39 of which are responsible for protein coding (26). Embryonic MYH is the product of the *MYH3* gene, which consists of 1,940 amino acids, and serves a vital role in the embryonic morphogenesis of the limbs and face (9). The embryonic MYH is divided into a globular motor domain (amino acid residues 1-~767), a short neck (~767-~847) and a long coiled-coil rod domain (~847-1,940) attached to the neck by a hinge (~847) (9). When variants occur in the protein coding regions of the *MYH3* gene, the spatial structure of embryonic MYH may be altered, leading to interaction impairment with the light chain or actin, as well as dysfunction of ATPase activity (9,15,21).

The present study detected the mutation c.2506A>G (p.K836E) in exon 22 of *MYH3*, where the short neck of embryonic

MYH was affected, according to the protein structure modeling. This mutation may impair the interaction between the embryonic MYH and RLC, damaging the regulatory mechanism and skeletal muscle activity (14,21). In addition, the SNP c.5263A>G (p.K1755E) was located in the myosin tail domain, where the change was rarely relevant for binding ATP or other proteins (20). Therefore, the SNP c.5263A>G (p.K1755E) appeared not to be critical for *MYH3* functioning. Additionally, the proband's father (II<sub>1</sub>) exhibited a normal appearance, which also provided further evidence to confirm that the disease-causing mutation was c.2506A>G (p.K836E) in the present study.

Thus far, 28 mutations in the *MYH3* gene have been reported to be associated with DA1, DA2A, DA2B or DA8 (9,15-17,19-20,27). It is of note that there may be an association between the position of the mutations in *MYH3* and the type of disease. For instance, all mutations resulting in DA1 are located in the globular motor domain (16,19), and the majority of pathogenic mutations for DA2A and DA2B are situated within the head and neck of embryonic MYH (9,15-17). In DA8, one small deletion has been reported in the globular motor domain of *MYH3*; however, the remaining two mutations associated with DA8 are located in the tail of the embryonic MYH near the hinge (20). In the spatial structure, the mutations causing DA2A are mainly located in the groove lying between two large domains in the head, which are involved in the binding



and hydrolyzing of ATP (9,28). When mutations occur in this area, the ATP metabolic process is disrupted. Conversely, almost all DA2B mutations are on the surface of the embryonic MYH directly exposed to the outside, leading to dysfunction in the interactions made with other proteins such as myosin light chain or actin (9,14-16,28). Consistent with this, the novel pathogenic mutation p.K836E identified in the present study is located in the neck of embryonic MYH corresponding to the surface of the spatial model, which is also adjacent to a mutation site (p.K838E) reported by Toydemir *et al* (9) in 2006. However, the underlying mechanism contributing to this phenomenon is still unclear.

In conclusion, the present study identified a novel pathogenic missense mutation of the *MYH3* gene in a Chinese family with DA2B. To the best of our knowledge, this was the first report of an *MYH3* gene mutation leading to DA2B in the Asian population. Furthermore, based on the construction and analysis of the protein structure model, the mechanism underlying the DA formation induced by *MYH3* mutation was also assessed. The present study expanded the mutation spectrum of *MYH3* and supported the results of previous studies regarding the association between mutation locations and DA types; thereby, contributing to developments in the clinical and genetic diagnosis of DA.

### Acknowledgements

The authors would like to thank Professor Qinglin Kang (Department of Orthopedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, P.R. China) for his support and advice.

### Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 81572121).

### Availability of data and materials

The primary data generated or analyzed in this study are included in this published article. The additional data used and analyzed in the current study are available from the corresponding author on reasonable request.

### Authors' contributions

WW collected the clinical information and peripheral blood samples of all of the participants and contributed to the analysis of sequencing outcomes. LK primarily analyzed the outcomes of whole-exome sequencing and Sanger sequencing and was a major contributor in writing the manuscript. RZ contributed to the conducting of the whole-exome sequencing and Sanger sequencing for the participants. QK conceived and designed the study and experimental methods. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

Written informed consent was obtained from all of the participants. The present study was approved by the Ethics

Committees of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China).

### Patient consent for publication

Written informed consent was obtained from all of the participants approving this non-commercial research and the publication of any associated data/images.

### Competing interests

The authors declare that they have no competing interests.

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