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Original Article

A mutation of beta-tropomyosin gene in a Chinese family with distal arthrogryposis type I

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Abstract: Background: Distal arthrogryposis (DA) is the most common congenital limb malformation secondary to the functional defects of joints and muscles. DA1 is one of the most commonly described forms of DA. The characteristics of DA1 include bilateral and symmetric clenched fist, overlapping fingers, camptodactyly, ulnar deviation of fingers, and positional foot deformities such as talipes equinovarus. Previous studies demonstrate that mutations of *TPM2*, *TNNI2*, *TNNT3*, *MYH3* and *MYBPC1* may contribute to DA1. Materials and methods: The present study investigated 8 DA1 families/patients and 1 DA2B patient, determined sequences of *TPM2*, *TNNI2*, *TNNT3*, *MYH3* and *MYBPC1* and detected the mutation by multiple sequence alignments and bioinformatic prediction of mutation. Results: We identified a novel missense mutation of *TPM2* (c.463G>A; p.A155T) in a DA1 family without genetic mutant of *TNNI2*, *TNNT3*, *MYH3* and *MYBPC1*. Conclusion: The mutation of *TPM2* (c.463G>A; p.A155T) led to DA1 of the family. The identification of the mutation expands the spectrum of known *TPM2* mutations, and it may contribute to novel approaches to genetic diagnosis and counseling of families with DA1.

Keywords: *TPM2*, mutation, DA1, DA2

Introduction

Distal arthrogryposis (DA) occurring in 1/20000 human live births, is classified as arthrogryposis multiplex congenita (AMC), and is the most common congenital limb malformation secondary to the functional defects of joints and muscles [1-3]. The typical phenotype of DA mainly is congenital joint contractures of the hands and feet. Hall et al. (1982) described DA as arthrogryposis with mainly hand and foot involvement, which was classified into 5 subtypes (type I and type IIA-IID) [4]. Then, Banshad et al. (1996) revised and extended the classification [5]. And now, DAs have been recognized and classified into 10 different forms (DA1-DA10) (**Table 1**) [6-8].

The most commonly described forms of DA are DA1 (OMIM 108120) and DA2 [9]. The characteristics of DA1 are bilateral and symmetric clenched fist, overlapping fingers, camptodactyly, ulnar deviation of fingers, and positional foot deformities such as talipes equinovarus [10-12]. Besides aforementioned features, DA2A (OMIM 193700) also has facial phenotypes including a very small orifice, H-shaped dimpling of the chin, prominent nasolabial folds, increased philtrum length, small nose, blepharophimosis, deep-sunken eyes with hypertelorism [5, 13, 14]. And DA2B (OMIM 601680) has features intermediate between DA1 and DA2A [12, 15].

9 genes encoding muscle proteins, ion channels and converting enzymes are recognized as causative genes of DA [7]. Mutations of *TPM2*, *TNNI2*, *TNNT3* and *MYH3* were identified in DA1 and DA2B, and *MYBPC1* in DA1 [6, 7, 10, 16-18]. Of these genes, *TPM2* (9p13.2-p13.1) is a significant disease-causing gene of DA1. *TPM2* encodes bate-tropomyosin, which is an isoform of tropomyosins [19]. The tropomyosins exist as coiled-coil homo- or heterodimers forming head-to-tail polymers, running along the length of the actin filament [20]. There are 7 alternating actin bonding sites in every tropo-

Table 1. Classification of distal arthrogryposis

Classification	OMIM number	Key features	Causative genes
DA1	108120	Joint contractures of the hands and feet	*TPM2*, *TNNI2*, *TNNT3*, *MYH3*, *MYBPC1*
DA2A	193700	DA with facial contractures, small pursed mouth	*MYH3*
DA2B	601680	Intermediate between DA1 and DA2A	*TPM2*, *TNNI2*, *TNNT3*, *MYH3*,
DA3	114300	DA with cleft palate, short stature	*PIEZO2*
DA4	609128	DA with scoliosis	Unknown
DA5	108145	DA with ptosis, limited ocular mobility	*ECEL1*, *PIEZO2*
DA6	108200	DA with sensorineural hearing loss	Unknown
DA7	158300	DA with trismus, facultative finger contractures	*MYH8*
DA8	178110	DA with multiple pterygium	Unknown
DA9	121050	DA with ear deformity, long fingers	*FBN2*
DA10	187370	DA with plantar flexion contractures	Unknown

DA, distal arthrogryposis; unknown, causative genes have been recognized.

myosin [19]. It stabilizes the actin filament of the sarcomere and mediates the interactions between the troponin complex and actin to regulate muscle contraction in the striated muscle [21].

In this study, we collected 9 cases including 8 DA1 families/patients and 1 DA2B patient and investigated the potential causative gene of them by sequence analysis of *TPM2*, *TNNI2*, *TNNT3*, *MYH3* and *MYBPC1*.

Materials and methods

Patients

We collected 8 DA1 families/patients and 1 DA2B patient who went to Xiangya Hospital in 2016. The DA1 family of 8 living members, which was indentified mutations of diseasing-cause gene, is from Hunan province, China. Except 3 adults absent for working in other cities, 5 of these members across three generations participated in this study (**Figure 1A**). The proband, family member 3 from the 3rd generation (III: 3), I: 2 and III: 1 were diagnosed with DA1. The remaining 2 members (II: 4 and III: 2) were phenotypically normal. The proband (3-year-old) had congenital ulnar bilateral and symmetric arthrogryposes of 2-5 fingers without other malformation, I: 2 had arthrogryposes of 5th fingers, and III: 1 had arthrogryposes of 4/5 fingers (**Figure 1C**). In addition, it is said that II: 2 and II: 3 had DA, but I: 1 and II: 1 was normal. The Review Board of Xiangya Hospital of the Central South University (Hunan, China) approved this research and all family members involved gave written informed consent.

DNA extraction

Genomic DNA was extracted from the peripheral blood of the patient and the other family members using a DNeasy Blood & Tissue kit (Qiagen, Inc., Valencia, CA, USA) on the QIAcube automated DNA extraction robot (Qiagen, Inc.).

Mutation sequencing

The entire coding regions, including the flanking intronic sequences of *TPM2* [Refseq (https://www.ncbi.nlm.nih.gov/refseq/), NM_213674], *TNNI2* (NM_001145829), *TNNT3* (NM_001297646), *MYH3* (NM_002470) and *MYBPC1* (NM_002465) were amplified by polymerase chain reaction (PCR; primer sequences will be provided upon request). PCR product sequences were determined using the ABI 3100 Genetic Analyzer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) as previously described [22].

Multiple sequence alignments and bioinformatic prediction of mutation

The multiple TPM2 protein sequences across mammals were aligned using the multiple sequence comparison by log-expectation program (version 3.6; an online program at http://www.ncbi.nlm.nih.gov) [23].

Results

The present study investigated 8 DA1 families/patients and 1 DA2B patient and confirmed a novel mutation in 1 DA1 family from Hunan province, China. There are 8 living members in this family, and 5 of them across three genera-

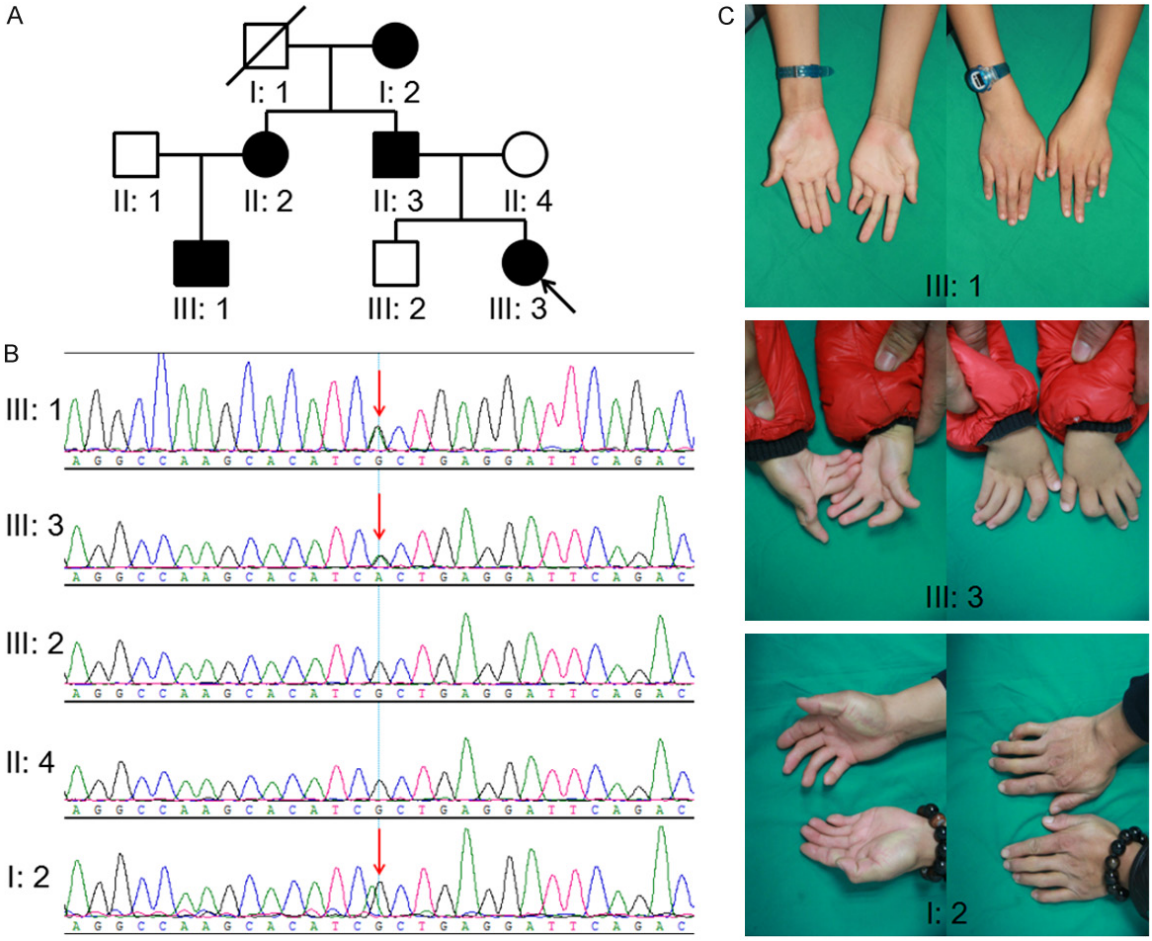

Figure 1. A. Pedigree of the family affected with AD1. Family members are identified by generations and number. Squares indicate male family members; circles, female members; a closed symbol, the affected member; open symbols, unaffected members; arrow, proband. B. Sequencing result of the TPM2 mutation. Sequence chromatogram indicates a heterozygous mutation (c.463G>A; p.A155T). C. Phenotypes of the part patients. III: 1 had arthrogryposes of 4/5 fingers, III: 3 had arthrogryposes of 2-5 fingers, I: 2 had arthrogryposes of 5th fingers.

tions participated in this study. The proband (III: 4), I: 2 and III: 1 were diagnosed with DA1 characterized by congenital ulnar bilateral and symmetric arthrogryposes of fingers. These arthrogryposes involved 2-5 fingers, 5th fingers and 4/5 fingers in III: 4, I: 2 and III: 1, respectively. Furthermore, II: 2 and II: 3, absent from this research, were narrated having ulnar arthrogryposes of fingers. All potential causative genes among all families/patients including *TPM2, TNNI2, TNNT3, MYH3* and *MTBPC1*, are investigated and only a mutation is identified in the 8-member family emphasized above. It is a novel heterozygous missense mutation (c.463G>A; p.A155T) in exon 4 of *TPM2*, and co-segregated with the affected family members (**Figure 1B**). No further relevant mutations were identified by direct sequencing of the genes for *TNNI2*, *TNNT3*, *MYH3* and *MYBPC1*. This newly discovered mutation of *TPM2* (c.463G>A; p.A155T) was not identified in the 200 control cohorts that our group studied previously. In addition, this mutation was not present in the dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/) or Exome Variant Server databases (http://evs.gs.washington.edu/EVS).

Discussion

Minttu et al. (2014) summarized the genotype-phenotype correlations of mutations in *TPM2* [20]. He identified the mutation (A155T, our identified mutation) in 2 “congenital myopathy patients lacking clinical details” from different families in a supplemental table, but he didn’t provide information of these families and se-

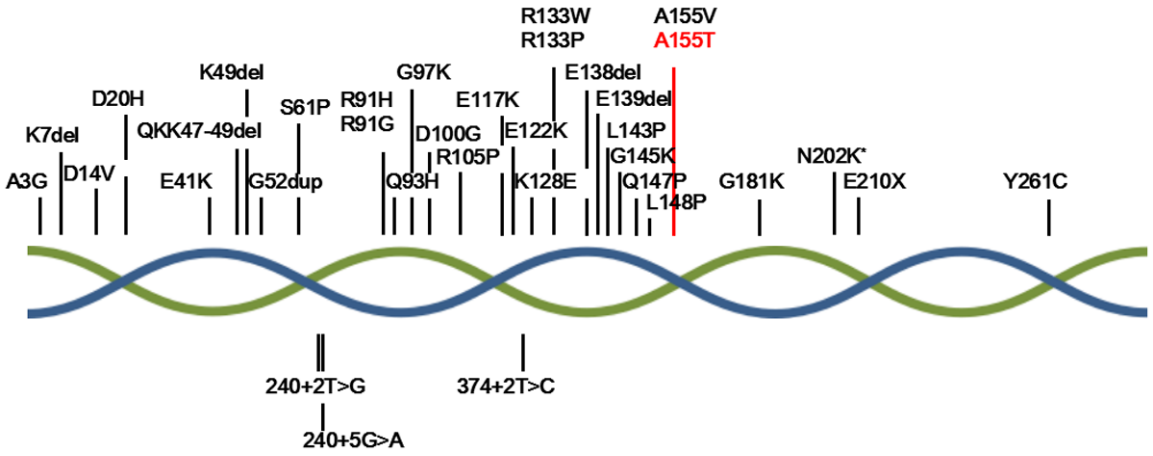

Figure 2. TPM2 disease mutations in congenital myopathy patients. The double helix represents the tropomyosin consisted by 2 bate-tropomyosin. The red word represents our mutation. *indicates the mutation caused by change of two different bases.

quence chromatograms. The present study identified the mutation in a DA1 family and cosegregated with the affected family members. Therefore, our study at least confirmed the existence and pathogenicity of the mutation.

TPM2 belongs to the family of tropomyosin genes (including *TPM1-4*), and encodes the skeletal muscle isoforms Tm2 [20, 24-27]. The isoform has 284 amino acid residues and a highly conserved N-terminal region [28]. TPM2 is a major causative gene of congenital myopathies and had been reported at least 35 mutations (**Figure 2**). The phenotypes resulting from these mutations are observably heterogeneous, mainly including DA1, DA2B, nemaline myopathy (NM), congenital fibre type disproportion (CFTD), cap disease and so on [12, 29, 30]. For example, the mutation (A3G) was reported causing NM, G97K causing DA1, and R105P causing DA2B [12, 20]. Moreover, the change of the same amino acid could lead to different diseases: E41K could cause NM, CFTD or cap disease [20, 31]. R133W could cause DA2B or NM, but R133P cause CFTD [9, 32]. A155V was reported to lead to CFTD by Nigel et al., but our identified mutation (A155T) resulted in symptoms of DA1 [33]. Although, phenotypes resulting from the change of 155th AA are different, the result of Nigel aspect demonstrated the pathogenicity of the mutation (A155T). It indicates that the genotype-phenotype correlations of mutations in *TPM2* need more clinical date and research to support.

TPM2, *TNNI2*, *TNNT3* and *MYH3* had been recognized as disease-causing genes of DA1 and DA2B, and mutations of *MYBPC1* were identified in DA1. In this study, of 8 DA1 families/patients and 1 DA2B patient, we only identified a mutation of *TPM2* in a DA1 family by sequence analysis of aforementioned genes. All forms of arthrogryposis are associated with decreased fetal akinesia [34]. Possible etiology and potential cause of fetal akinesia including: 1) myopathic processes, 2) neuropathic processes, 3) neuromuscular endplate abnormalities, 4) abnormalities of connective tissue, 5) the restriction of movement in utero, 6) maternal illness or exposures, 7) intrauterine vascular compromise, and 8) metabolic disturbances [35, 36]. The pathopoiesis of 8 remaining cases need to be proved by further study.

In conclusion, the present study identified a novel heterozygous missense mutation (c.463G>A; p.A155T) of *TPM2* in a Chinese family with DA1. The present identification of the mutation expands the spectrum of known *TPM2* mutations, and it may contribute to novel approaches to genetic diagnosis and counseling of families with DA1.

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Disclosure of conflict of interest

None.

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