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A novel mutation (c.1010G>T; p.R337L) in *TP63* as a cause of split-hand/foot malformation with hypodontia

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Running title: A NOVEL MUTATION OF *TP63* CAUSING SHFM

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Abstract

Background: TP63-related disorders can be divided into at least 6 categories, including ectrodactyly-ectodermal dysplasia-cleft lip/palate syndrome 3 (EEC syndrome 3), ankyloblepharon-ectodermal defects-cleft lip/palate syndrome (AEC syndrome), acro-dermo-ungual-lacrimal-tooth syndrome (ADULT syndrome), limb-mammary syndrome (LMS), Rapp-Hodgkin syndrome (RHS) and split-hand/foot malformation 4 (SHFM4), and are caused by heterozygous mutations of *TP63*. The phenotypes of TP63-related disorders broadly involve ectodermal dysplasias, acromelic malformation and orofacial cleft. SHFM and hypodontia are prominent clinical manifestations of TP63-related disorders.

Methods: The present study investigated a family with SHFM and hypodontia; determined the sequences of *DLX5*, *WNT8B*, *WNT10B*, *BHLHA9*, *CDH3*, *DYNC1I1* and *FGFR1*; and performed SNP-array analysis. We detected the mutation by multiple sequence alignments and a bioinformatic prediction.

Results: We identified a novel missense mutation of *TP63* (c.1010G>T; R337L) in the family without mutations of *DLX5*, *WNT8B*, *WNT10B*, *BHLHA9*, *CDH3*, *DYNC1I1*, *FGFR1* and copy number variants (CNVs) causing SHFM.

Conclusion: A mutation of *TP63* (c.1010G>T; R337L) leads to SHFM with hypodontia. Identification of this mutation expands the spectrum of known *TP63* mutations, and it may contribute to novel approaches to the genetic diagnosis and counseling of families with TP63-related disorders.

Key words: *TP63*, TP63-related disorder, SHFM, hypodontia, ADULT syndrome

Introduction

Tumor protein p63 (TP63) is a member of p53 family, homolog to TP53 and TP73[1]. As a transcription factor, TP63 plays a crucial role in regulating epithelial, limb, and craniofacial development[2]. Correspondingly, the phenotypes of TP63-related disorders broadly involve ectodermal dysplasias, acromelic malformation and orofacial cleft, which include split-hand/foot malformation (SHFM) with/without syndactyly, tooth abnormalities, nail dysplasia, sparse hair, cleft lip/palate (CL/P), hypopigmentation, lacrimal duct obstruction, hypohidrosis and so on[3-5]. TP63-related disorders can be phenotypically divided into at least 6 overlapping categories, including ectrodactyly-ectodermal dysplasia-cleft lip/palate syndrome 3 (EEC syndrome 3; OMIM_604292), ankyloblepharon-ectodermal defects-cleft lip/palate syndrome (AEC syndrome; OMIM_106260), acro-dermo-ungual-lacrimal-tooth syndrome (ADULT syndrome; OMIM_103285), limb-mammary syndrome (LMS; OMIM_603543), Rapp-Hodgkin syndrome (RHS; OMIM_129400) and SHFM4 (OMIM_605289)[6-11].

TP63 is encoded by *TP63* locating on 3q28[12]. *TP63* has 2 promoters, 15 exons and some alternative splicing sites, to express 6 different isoforms[13]. Three of them include a transactivation domain, respectively named TAp63-α, -β, and -γ, whereas the other three do not, called delta-Np63-α, -β, and -γ[14, 15]. Heterozygous mutations of *TP63* could result in one of 6 diseases or some symptoms of them[16]. Our patient has SHFM with hypodontia conforming to the symptoms of TP63-related disorders. Other diseases can also have SHFM, for example SHFM1-6 (except SHFM4) and SHFLD caused by mutations of *DLX5*, *WNT8B*, *WNT10B*, *BHLHA9*, *CDH3*, *DYNC1I1* (15 and 17 exons), or *FGFR1*, or by copy number variants (CNVs) including 10q24, 7q21-22, 2q31 and Xq26[17, 18].

In the present study, we report a family with SHFM and hypodontia from Jiangxi province, China. We identified a novel missense mutation (c.1010G>T; R337L) of *TP63* in the proband and co-segregated with the affected family members. To the best of our knowledge, this mutation has not been reported in previous study or presented in our control cohorts and various single nucleotide polymorphism (SNP) databases.

Materials and methods

Patients

We identified a family with SHFM and hypodontia from Jiangxi province, China, by detailed clinical and X-ray examinations. Five members (I:1, II:1, II:2, II:3, III:1) across three generations from this family participated in the present study (Fig.1A). The proband (III:1), a 11-year-old boy, went to Xiangya Hospital for limb malformation. He has severe defects of all four limbs, absence of the third fingers/toe of the bilateral hands/left foot and the second and third toes of the right foot, and syndactyly of the 1/2 fingers/toes of the right hand/left foot and the 4/5 toes of the bilateral feet. Furthermore, we observed that the proband has hypodontia and mild nail dysplasia without other evident abnormities (Fig.1B-H). The mother (II:2) has the same symptoms. In addition, his grandmother (I:2) and uncle (II:3), were said to have also suffered from at least SHFM and hypodontia. Other family members are normal. The Review Board of Xiangya Hospital of the Central South University (Hunan, China) approved this research and all family members involved provided 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.).

SNP-array analysis

Genomic DNA samples of the patient and his parents were adjusted to a final concentration of 50 ng/μL. The HumanOmni1-Quad Chip (Illumina Inc., San Diego, USA) contains over 1.1 million loci across the human genome, including markers derived from the three HapMap, the 1000 Genomes Project and recently published studies. The Illumina BeadScan genotyping system (Beadstation Scanner) was employed to obtain the signal intensities of the SNP probes. The GenomeStudio V2011 software was used to analyze the genotypes (human genome build 37/Hg19 for analysis) and evaluate the experimental quality. The call rates of the samples

were greater than 99.0%.

Mutation sequencing

The entire coding regions, including the flanking intronic sequences of *TP63* [Refseq (https://www.ncbi.nlm.nih.gov/refseq/), NM_003722.4], *DLX5* (NM_005221.5), *WNT8B* (NM_003393.3), *WNT10B* (NM_003394.4), *CDH3*(NM_001793.6), *DYNC1I1* (NM_004411.4), *FGFR1* (NM_023110.2) and *BHLHA9* (NM_001164405.1) 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).

Multiple sequence alignments and bioinformatic prediction of mutation

Multiple TP63 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). The pathogenicity of variants was predicted by MutationTaster (http://www.mutationtaster.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://provean.jcvi.org/index.php). The 1000 Genomes Project (https://www.genome.gov/27528684/1000-genomes-project/) and the Exome Aggregation Consortium (http://exac.broadinstitute.org/) were used to predict the variant frequency in the population.

Result

This study identified a family with SHFM and hypodontia. The proband (III:1) has SHFM with syndactyly, hypodontia and mild nail dysplasia. We predict the disease is autosomal dominant according to the pedigree. We examined the possible causative genes and performed SNP-array analyses among all subjects, confirming a novel missense mutation (c.1010G>T; R337L) of *TP63* in the proband, his mother (II:2) and his uncle (II:3) (Fig.1A, 2A). And the mutation was not identified in his grandfather (I:1) and father (II:1). The mutation co-segregated with the affected family members. No further relevant mutations

were found by direct sequencing of the genes for *DLX5*, *WNT8B*, *WNT10B*, *CDH3*, *DYNC1I1*, *FGFR1*and *BHLHA9*, and CNVs referring to 10q24, 7q21-22, 2q31, 17p13.3 and Xq26 were not detected (Table S1). This newly identified mutation (c.1010G>T; R337L) of *TP63* was not found in our 200 control cohorts. The mutation was predicted to be disease-causing by bioinformatics programs and was not presented in the 1000G and ExAC databases (Table 1).

Discussion

TP63-related disorders can be broadly divided into 6 categories, including EEC syndrome 3, AEC syndrome, ADULT syndrome, LMS, RHS (orofacial cleft 8/nonsyndromic cleft lip with or without cleft palate 8 included) and SHFM4[19, 20]. SHFM, hypodontia and nail dysplasia are prominent clinical manifestations of TP63-related disorders in our patients (including EEC syndrome 3, AEC syndrome and ADULT syndrome) (Table 2). Given CL/P is reported in approximately 100% of patients with AEC syndrome but was not observed in our patients, we excluded the possibility of AEC syndrome[21]. Furthermore CL/P is a frequent phenotype of EEC syndrome 3 and RHS [22]. In addition, it was observed that the proband had sparse hair (although the proband's father firmly denied this conclusion and insisted it was an intended hair style) and a few scattered freckles. Therefore, except for the symptoms described above, our patients did not appear to show signs of ectodermal dysplasia, and thus we thought the disease of our patients approached to ADULT syndrome more. We analyzed the sequences of *TP63* and detected a novel mutation. To confirm, we excluded mutations in other genes (*DLX5*, *WNT8B*, *WNT10B*, *BHLHA9*, *CDH3*, *DYNC1I1* and *FGFR1*) and CNVs (10q24, 7q21-22, 2q31, 17p13.3 and Xq26) known to cause SHFM by sequencing and SNP-array analyses.

TP63 encodes a transcription factor acting as a sequence-specific DNA binding transcriptional activator or repressor to play a critical in the maintenance of progenitor-cell populations[23]. *TP63* expresses 6 isoforms transcribed from 2 promoters, named TAp63 and delta-Np63 which lacks a transactivation domain; combined with alternative splicing, the isotypes α, β and γ are produced[24]. All p63 isoforms share a DNA binding domain (DBD) and an oligomerization domain (OD). TAp63 isoforms have a N-terminal transactivation

domain (TA)[25]. The C-terminal full-length α-isoforms contain a sterile alpha motif (SAM) and a transactivation inhibitory domain (TI)[23]. The mutation in our patients (c.1010G>T; R337L) occurred in the DBD, possibly influencing the function of TP63, resulting in SHFM and hypodontia. Bioinformatics predicted this site to be highly conserved across species (Fig.2B).

There are at least 112 mutations occurring in TAp63-α (partial mutations from HGMD (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=TP63))[26-28] (Fig.3). EEC syndrome and AEC syndrome are the most frequent and are mainly caused by alterations in the DBD domain and SAM domain, respectively. RHS results from mutations in the SAM and TI domains. The other diseases show no preference. The mutation we reported (c.1010G>T; R337L) was situated in the DBD domain. Other substitutions of this site (R337Q and R337G) have been reported to result in ADULT syndrome[29, 30]. For these three amino acid alterations, glutamine (Q) and glycine (G) belong to polar amino acids, but leucine (L) is nonpolar, which may cause the different levels of functional damage to give rise to different symptoms.

In conclusion, the present study identified a novel heterozygous missense mutation (c.1010G>T; R337L) of *TP63* in a Chinese family with SHFM and hypodontia. The identification of the mutation expands the spectrum of known *TP63* mutations and may contribute to novel approaches to the genetic diagnosis and counseling of families with TP63-related disorders.

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Conflict of interest

None.

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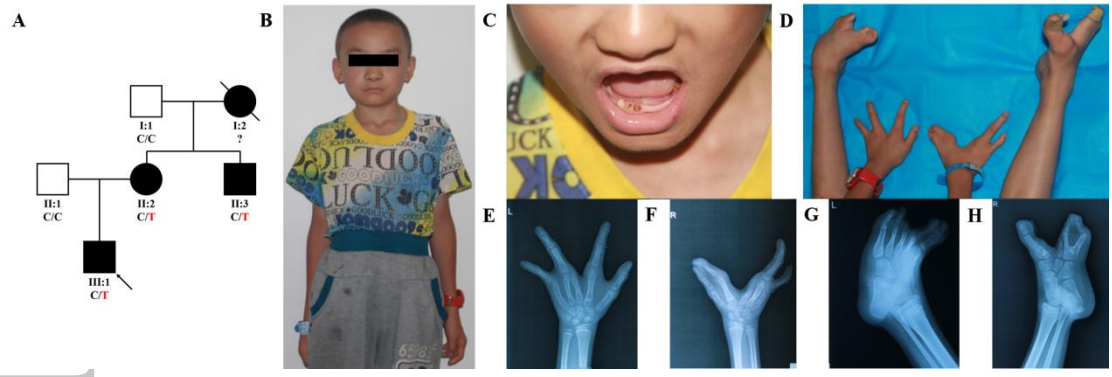

Figure 1. (A) Ancestry of the family affected with SHFM and hypodontia. Family members are identified by their generation (indicated by roman numerals) and a number. Squares represent male family members and circles, female members. The black symbols represent a member with SHFM and hypodontia, and the white symbols represent unaffected members. The arrow indicates the proband. Genotype are identified by letters and slash, red representing mutations. (B-H) Phenotypes of the proband. The proband has SHFM, hypodontia and nail dysplasia.

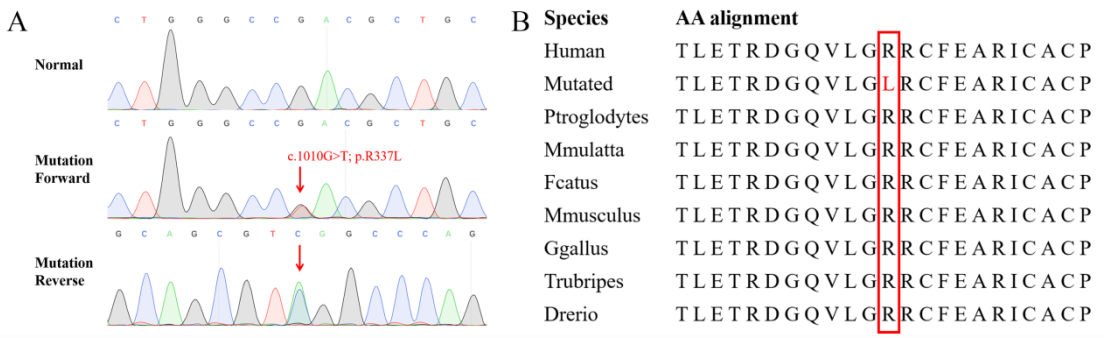

Figure 2. (A) Sequencing results of the *TP63* mutation. Sequence chromatograms indicate a heterozygous missense mutation (c.1010G>T; R337L) in the proband. (B) This site of TP63 is highly evolutionary conserved cross species. Red grapheme represents mutated amino acid, red box emphasizes these sites cross species to compare.

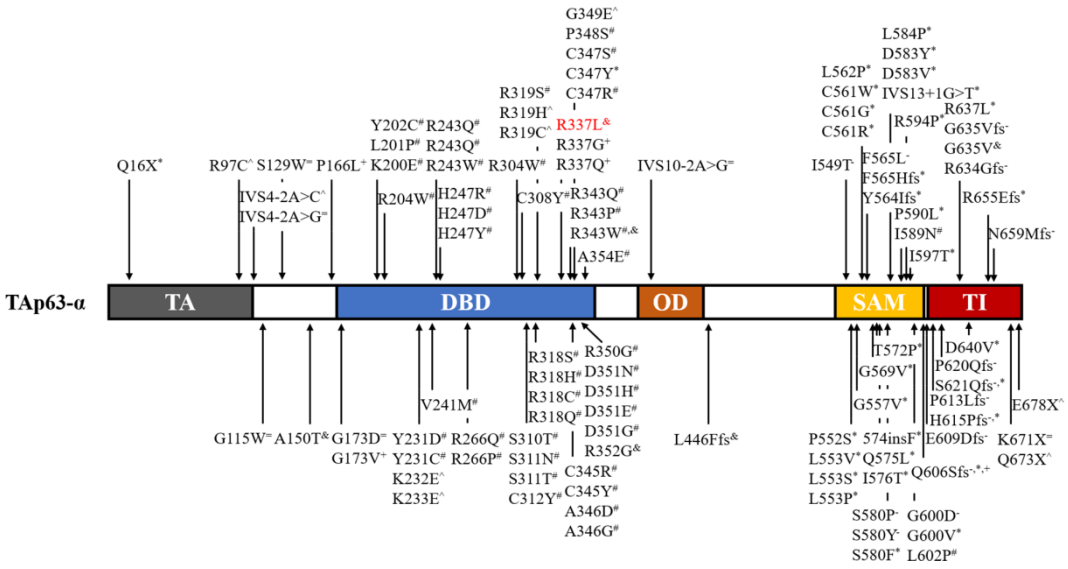

Figure 3. *TP63* mutations identified in Tap63-α. The rectangular box represents the Tap63-α protein with the N-terminus on the left and C-terminus on the right. Known functional domains include a transactivation domain (TA), a DNA binding domain (DBD), an oligomerization domain (OD), a sterile alpha motif (SAM) and a transactivation inhibitory domain (TI). Mutations are grouped based on the mutation site. The red word represents previous mutation. * represent AEC syndrome; ^ represent SHFM; = represent LMS; + represent ADULT syndrome; # represent EEC syndrome; - represent RHS; & represent other disorders.

Table 1. The TP63 mutation (c.1010G>T; p.R337L) in this family.

Gene	Variant	MutationTaster	PolyPhen-2	SIFT	1000G	ExAC
TP63	NM_003722.4: c.1010G>T; p.R337L	D (1.000)	D (0.993)	D (0.002)	Never	Never

D, disease causing.

Table 2. The clinical symptoms of 6 major TP63-related disorders.

Disorder	SHFM	CL/P	hypodontia	Nail dysplasia	Lacrimal-duct abnormalities	Sparse hair	Dry skin	Ankyloblepharon	Breast and/or nipple hypoplasia	Hypospadias
EEC3 (604292)	+	++	++	++	+	+	++	-	-	+
AEC (106260)	±	++	++	++	+	+	++	++	-	+
ADULT (103285)	+	-	+	+	+	+	+	-	+	-
LMS (603543)	+	+	±	±	+	-	±	-	++	-
RHS (129400)	-	++	+	+	+	+	+	-	-	+
SHFM4 (605289)	+	±	-	-	-	-	-	-	-	-
proband	+	-	+	+	-	+	-	-	-	-

EEC3: ectrodactyly- ectodermal dysplasia-cleft lip/palate syndrome; AEC: ankyloblepharon-ectodermal defects-cleft lip/palate syndrome; ADULT: acro-dermo-ungual-lacrimal-tooth syndrome; LMS: limb-mammary syndrome; RHS: Rapp-Hodgkin syndrome (orofacial cleft 8 included); SHFM: split-hand/foot malformation; CL/P: cleft lip/palate; ++: characteristic phenotype; +: regularly observed phenotype; ±: rare phenotype; -: never observed phenotype.