Research Article

A Novel Missense Variant of TP63 Heterozygously Present in Split-Hand/Foot Malformation

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1. Introduction

Split-hand/foot malformation (SHFM) is a severe congenital abnormality mainly characterized by the absence or hypoplasia of the central ray of the hand/foot, which can be isolated or syndromic [1]. The reported incidence of SHFM ranges from 1/6000 to 1/20000, worldwide. The incidence in China could be higher, underlying higher disabilities in infants [2, 3]. Genetic and environmental factors have been proven to contribute significantly to the occurrence of congenital malformations. Several candidate genes have been reported to be associated with SHFM, including TP63 (OMIM 603273), DLX5 (OMIM 600028), DLX6 (OMIM 600030), FGFR1 (OMIM 136350), WNT10B (OMIM 601906), and BHLHA9 (OMIM 615416). The majority of SHFM cases display autosomal dominant inheritance, but other modes of inheritance have also been described [4, 5]. In addition, environmental exposure to medication and chemicals also increases the risk of limb malformations [6, 7].

In the present study, we investigated an isolated Chinese family with no history of exposure to environmental risk factors. In this family, the proband and his son suffered from SHFM. Whole-exome sequencing (WES) was used to detect possible genetic lesions, and a novel missense variant (NM_003722.4:c.948G>A; p.Met316Ile) of TP63 in SHFM was thus identified, which may enlarge the spectrum of known TP63 variants and also provide new approaches for genetic counselling of families with SHFM.
bilateral split-foot malformations, and his son suffered from cleft hand and foot deformities. No other abnormalities were found in the proband or his son. The clinical and imaging features of the affected individuals are shown in Figure 2. Notably, in this family, the proband’s father (I-1) died before seeking genetic counselling; thus, the clinical features were not recorded. However, based on descriptions given by his family members, he did not show any clinical signs of limb malformations.

Using WES, we identified a novel heterozygous variant (NM_003722.4:c.948G>A; p.Met316Ile) of TP63 in the proband and his son (Figure 1). This new variant is not found in the gnomAD, 1000G, and ExAC databases (Table 1). An amino acid sequence alignment suggests that the 316th amino acid in TP63 protein is highly conserved among different species (Figure 3(a)). This novel variant was predicted to be disease-causing/probably damaging by MutationTaster and PolyPhen-2 (Table 1). Subsequently, we constructed a partial model of TP63 protein using Swiss-model; the mutated one exhibits an altered three-dimensional structure of TP63 (Figure 3(b)). Finally, Sanger sequencing found this new variant in affected family members but not in healthy individuals, conforming to the cosegregation principle.

4. Discussion

SHFM is a severe congenital heterogeneous limb abnormality that mainly affects the development of the central rays in the hand/foot. It may occur in an isolated or syndromic manner. The clinical phenotypes of SHFM are highly variable, ranging from hypoplasia in a single phalanx or syndactyly to aplasia in one or more central limbs [9]. The development of limbs is a very complex process that begins with the formation of limb buds. The apical ectodermal ridge (AER), located at the distal edge of the developing limb bud, acts as the main signal centre regulating growth along the proximal/distal axis. Disruption of the AER may contribute to SHFM [4].

Recently, it has been reported that genetic factors play a crucial role in the occurrence of SHFM. Several chromosomal loci have been identified that associate with the occurrence of SHFM. Chromosomal rearrangements in 7q21 lead to SHFM1; DLX5 and DLX6 located in this area are involved in the development of limb malformation [10, 11]. SHFM2 is caused by mutations in Xq26 [12]. Duplications involving BTRC and FBXW4 in 10q24 contribute to the occurrence of SHFM3 [13, 14]. SHFM4-associated mutations mapping to 3q28 have been found to be in TP63 [15–17]. Dysregulation of the HOXD gene cluster located in 2q31 plays a key role in SHFM5 [18]. WNT10B mutations in 12q13 are involved in the development of SHFM6 [19, 20]. In addition, there exists a specific SHFM with fibula deficiency called SHFMD. BHLHA9-associated duplications in 17p13 display significant association with SHFMD [21]. SHFM1, 3, 4, and 5 mainly exhibit an autosomal dominant inheritance pattern, while SHFM2 and 6 display X-linked and autosomal recessive models of inheritance, respectively.

Heterozygous expression of mutant TP63 could underlie the occurrence of SHFM4 [4, 5]. Hence, it is essential to
provide families with histories of SHFM with molecular genetic testing and counselling. In the present study, we identified a novel heterozygous variant of TP63 in an isolated SHFM family. Based on clinical features and WES results, this type was diagnosed as SHFM4, probably inherited in an autosomal dominant inheritance pattern. However, the proband’s father died before molecular testing; although he did not show any clinical signs of limb malformations, we cannot exclude paternal inheritance.

TP63 is a protein-coding gene comprising 17 exons, 2 promoters, and some variable splice sites. The TP63 isoforms encoded by this gene can be divided into two categories (TAp63 and ΔNp63) whose expression is driven by different promoters. TAp63 isoforms own an N-terminal transactivation (TA) domain, which is absent in ΔNp63 isoforms. Both the TAp63 and ΔNp63 isoforms can be further divided into TAp63α, β, and γ variants after undergoing mRNA alternative splicing. TAp63α is the longest isoform, containing a TA domain, a central DNA-binding domain

**Table 1**: TP63 variant (NM_003722.4:c.948G>A; p.Met316Ile) in a Chinese family with SHFM.

| Gene   | TP63                               |
|--------|------------------------------------|
| DNA change | NM_003722.4:c.948G>A (heterozygous) |
| Amino acid alteration | p.Met316Ile |
| Variant type | Missense |
| **Allele frequency** |                      |
| 1KGP    | 0                                  |
| ExAC_all | 0                                  |
| gnomAD  | 0                                  |
| **Function prediction** |                      |
| MutationTaster | Disease causing (1,000) |
| PolyPhen-2   | Probably damaging (0.937)        |
| SIFT     | Tolerated (0.074)                 |

Abbreviations: SHFM: split-hand/foot malformation; 1KGP: 1000 Genomes Project; ExAC_all: all the data of Exome Aggregation Consortium; gnomAD: the Genome Aggregation Database.
As a member of the p53 family of transcription factors, TP63 plays a key role in the formation and differentiation of the AER and is crucial to limb development [4]. The newly discovered amino acid substitution (p.Met316Ile) confirmed in this study occurred at a mutational hotspot in DBD, which is responsible for DNA binding. According to the Alamut Visual software and the ACMG 2015 guidelines, this variant is regarded as a class 3-unknown pathogenicity. However, this site in TP63 is evolutionarily highly conserved among different species. Despite there was small physicochemical difference between Met and Ile according to Grantham scores, bioinformatics software (MutationTaster and PolyPhen-2) predicted that this new variant would be disease-causing/probably damaging. Importantly, Swiss-model software also suggested that this novel variant may change the TP63 partial structure in its DNA-binding domain, which may affect the formation and differentiation of the AER, probably leading to limb malformation.

In conclusion, a novel heterozygous missense variant (NM_003722.4:c.948G>A; p.Met316Ile) of TP63 was detected in a Chinese family by whole-exome sequencing. It must be included in genetic diagnoses and counselling discussions of families with SHFM.

**Data Availability**

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

**Ethical Approval**

This study was approved by the Ethics Review Board of the First Affiliated Hospital of Anhui Medical University.

**Consent**

Written informed consent was obtained from all patients.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

XH and ZZ designed the study. HG, CX, and DT collected the data. HG and DT analyzed the data. HG and DT wrote the paper. All authors have read and approved the final manuscript. Hao Geng and Dongdong Tang contributed equally to this work.
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References

[1] A. Sowińska-Seidler, M. Socha, and A. Jamsheer, "Split-hand/-foot malformation-molecular cause and implications in genetic counseling," Journal of Applied Genetics, vol. 55, no. 1, pp. 105–115, 2014.

[2] A. M. Elliott, M. H. Reed, A. E. Chudley, B. N. Chodirker, and J. A. Evans, "Clinical and epidemiological findings in patients with central ray deficiency: split hand foot malformation (SHFM) in Manitoba, Canada," American Journal of Medical Genetics Part A, vol. 140, pp. 1428–1439, 2006.

[3] L. Dai, Y. H. Li, Y. Deng et al., "Prevalence of congenital split hand/split foot malformation in Chinese population," Journal of Sichuan University, vol. 41, pp. 320–323, 2010.

[4] P. N. Kantaputra and B. M. Carlson, "Genetic regulatory pathways of split-hand/foot malformation," Clinical Genetics, vol. 95, pp. 132–139, 2018.

[5] F. Gurrieri and D. B. Everman, "Clinical, genetic, and molecular aspects of split-hand/foot malformation: an update," American Journal of Medical Genetics Part A, vol. 161, no. 11, pp. 2860–2872, 2013.

[6] A. M. Al-Jobair and A. I. Al-Saleem, "Possible association between acetzalamide administration during pregnancy and multiple congenital malformations," Drug Design Development & Therapy, vol. 10, pp. 1471–1476, 2016.

[7] H. Kang, C. Magee, C. Mahan et al., "Pregnancy outcomes among U.S. Gulf War veterans: a population-based survey of 30,000 veterans," Annals of Epidemiology, vol. 11, no. 7, pp. 504–511, 2001.

[8] C.-W. Lam, K.-S. Wong, H.-W. Leung, and C.-Y. Law, "Limb girdle myasthenia with digenic RAPSIN and a novel disease gene AK9 mutations," European Journal of Human Genetics, vol. 25, pp. 192–199, 2017.

[9] A. Jamsheer, "Genetic background of isolated forms of congenital malformations of the hand," Medycyna Wieku Rozwojowego, vol. 12, no. 3, pp. 729–737, 2008.

[10] H. E. Shamseldin, M. A. Faden, W. Alashram, and F. S. Alkuraya, "Identification of a novel DLX5 mutation in a family with autosomal recessive split hand and foot malformation," Journal of Medical Genetics, vol. 49, pp. 16–20, 2012.

[11] A. Ullah, M. F. Ullah, Z. M. Khalid, and W. Ahmad, "Novel heterozygous frameshift mutation indistal-less homeobox 5underlies isolated split hand/foot malformation type 1," Pediatries International, vol. 58, no. 12, pp. 1348–1350, 2016.

[12] M. Faiyaz-Ul-Haque, S. H. E. Zaidi, L. M. King et al., "Fine mapping of the X-linked split-hand/split-foot malformation (SHFM2) locus to a 5.1-Mb region on Xq26.3 and analysis of candidate genes," Clinical Genetics, vol. 67, pp. 93–97, 2005.

[13] R. Lyle, U. Radhakrishna, J.-L. Blouin et al., "Split-hand/split-foot malformation 3 (SHFM3) at 10q24, development of rapid diagnostic methods and gene expression from the region," American Journal of Medical Genetics Part A, vol. 140A, no. 13, pp. 1384–1395, 2006.

[14] S. Sifakis, D. Basel, P. Ianakiev, M. W. Kilpatrick, and P. Tsipouras, "Distal limb malformations: underling mechanisms and clinical associations," Clinical Genetics, vol. 60, no. 3, pp. 165–172, 2001.

[15] H. van Bokhoven, B. C. J. Hamel, M. Bamshad et al., "p63 gene mutations in EEC syndrome, limb-mammary syndrome, and isolated split hand-split foot malformation suggest a genotype-phenotype correlation," American Journal of Human Genetics, vol. 69, no. 3, pp. 481–492, 2001.

[16] J.-Y. Jin, L. Zeng, K. Li et al., "A novel mutation (c.1010G>T; p.R337L) in TP63as a cause of split-hand/foot malformation with hypodontia," The Journal of Gene Medicine, vol. 21, no. 10, article e3312, 2019.

[17] L. U. Alves, E. Pardon, P. A. Otto, and R. C. Mingroni Netto, "A novel c.1037C>G (p.Ala346Gly) mutation in TP63 as cause of the ectrodactyly-ectodermal dysplasia and cleft lip/palate (EEC) syndrome," Genetics and Molecular Biology, vol. 38, no. 1, pp. 37–41, 2015.

[18] B. Długaszewska, A. Silahtaroglu, C. Menzel et al., "Breakpoints around the HOXD cluster result in various limb malformations," Journal of Medical Genetics, vol. 43, no. 2, pp. 111–118, 2006.

[19] A. Ullah, A. Gul, M. Umair et al., "Homozgyous sequence variants in the WNT10B gene underlie split hand/foot malformation," Genetics & Molecular Biology, vol. 41, no. 1, pp. 1–8, 2018.

[20] S. A. Ugur and A. Tolun, "Homozygous WNT10B mutation and complex inheritance in split-hand/foot malformation," Human Molecular Genetics, vol. 17, no. 17, pp. 2644–2653, 2008.

[21] S. Malik, F. E. Percin, D. Bornholdt et al., "Mutations affecting the BHLHA9 DNA-binding domain cause MSSD, mesoaxial synostotic syndactyly with phalangeal reduction, Malik-Percin type," American Journal of Human Genetics, vol. 95, no. 6, pp. 649–659, 2014.

[22] J. Celli, P. Duijf, B. C. J. Hamel et al., "Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome," Cell, vol. 99, no. 2, pp. 143–153, 1999.

[23] J. A. McGrath, P. H. Duijf, V. Doetsch et al., "Hay-Wells syndrome is caused by heterozygous missense mutations in the SAM domain of p63," Human Molecular Genetics, vol. 10, no. 3, pp. 221–229, 2001.

[24] T. Rinne, S. E. Clements, E. Lammme et al., "A novel translation re-initiation mechanism for the p63 gene revealed by amino-terminal truncating mutations in Rapp-Hodgkin/Hay-Wells-like syndromes," Human Molecular Genetics, vol. 17, no. 13, pp. 1688–1777, 2008.

[25] P. Ghioni, F. Bolognese, P. H. G. Duijf, H. van Bokhoven, R. Mantovani, and L. Guerrini, "Complex transcriptional effects of p63 isoforms: identification of novel activation and repression domains," Molecular and Cellular Biology, vol. 22, no. 24, pp. 8659–8668, 2002.