Whole-exome sequencing study identifies two novel rare variations associated with congenital talipes equinovarus

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Abstract. Congenital talipes equinovarus (CTEV) is a common birth defect with an unclear genetic pathogenesis that results from both genetic and environmental factors. The present study aimed to identify novel variants in patients with CTEV using whole-exome sequencing (WES) and to investigate the genetic factors responsible for the development of CTEV.A cohort of nine neonates/infants with suspected CTEV was recruited. Subsequently, sequential tests, including chromosome karyotyping and WES, were performed for each of the participants. Familial validation was performed using Sanger sequencing and low-coverage copy-number variation (CNV) sequencing. A novel CNV containing the mediator complex subunit 13L gene at 12q24.21-q24.23 was detected by WES and further investigated by CNVseq. Additionally, a novel *de novo* missense variation, transforming growth factor-β receptor 2: c.1280T>C, was identified by WES and further investigated by Sanger sequencing. The two identified variations were hypothesized to be causative genetic factors for the development of CTEV in the two cases the variations were identified in. In the present study, two pathogenic variations (one CNV and one single-base variation) were detected in two Chinese families with CTEV. The results of the present study may aid in investigating the molecular basis of CTEV; however, further investigation is required.

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Introduction

Congenital talipes equinovarus (CTEV), also known as clubfoot, is one of the most common inborn musculoskeletal abnormalities, with a worldwide incidence of 1 in 1,000 livebirths (1). CTEV is identified by four clinical foot characteristics: Forefoot adduction, hindfoot varus, midfoot cavus and hindfoot equinus (2).A total of ~80% of CTEV cases are idiopathic, namely ICTEV (3), with the remaining 20% being characterized as secondary or syndromic CTEV (4). ICTEV affects males more than females (5), with a male-to-female ratio of 2:1 across different ethnic groups (1,6).

Although a number of studies on familial characteristics, especially twins, have strongly suggested that genetic factors play an important role in the pathogenesis of ICTEV (4,7-9), the particular locality of the etiologyis still unclear compared with syndromic CTEV (5).

Previous studies on the association between ICTEV and genetic factors have identified several causative gene families and genes, including Hox (10,11), caspase (2,11), paired like homeodomain 1 (7-9,12), T-box transcription factors and GLI family zinc finger 3 (10,13) genes. However, a major candidate gene remains to be identified (5). Previous studies have focused on the interaction between genetic and environmental factors, displaying the multifactorial identity of the disease (4,5,14,15). At present, multifactorial identity remains the most validated theory (5). Additionally, numerous other phenotypes of syndromic clubfoot in newborns are not recognizable and can be easily misdiagnosed as ICTEV (16,17). Accordingly, these patients require the development of improved molecular methods for accurate differential diagnosis.

In the present study, whole-exome sequencing (WES) was used to investigate nine neonates/infants with CTEV, among which seven displayed isolated CTEV and two displayed CTEV combined with other abnormalities.

Materials and methods

Subjects. The present study was approved by the Ethics Committee of Shijiazhuang Obstetrics and Gynecology Hospital (approval no. 20180015) and written informed

consent was obtained from the parents of all patients. Patients with suspected CTEV, identified by routine clinical and ultrasound diagnostic criteria (2) without clear genetic diagnosis, were recruited for the present study. Patients with isolated (both bilateral and unilateral) and combined CTEV were included in the present study. The exclusion criteria were as follows: i) Clear prenatal genetic diagnosis; ii) mechanical injury during labor. A total of nine neonates or infants with CTEV were recruited from the Center of Prenatal Diagnosis in Shijiazhuang Obstetrics and Gynecology Hospital and the Department of Pediatric Orthopedic in The Third Hospital of Hebei Medical University between January 2016 and December 2018. Patient characteristics are shown in Table I. Peripheral blood (5 ml) was collected from each subject for genetic testing.

Chromosome karyotyping. Conventional chromosome karyotyping by G-banding was performed on the peripheral blood samples to detect overall chromosomal anomalies, as previously described (18). The karyotype results were analyzed in accordance with the International System for Human Cytogenomic Nomenclature (2016 edition) (19).

DNA extraction. Total genomic DNA (1 μg) was extracted from 200 μl peripheral blood using the DNA Blood Midi/Mini kit (Qiagen GmbH), according to the manufacturer's protocol.

WES analysis. DNA library construction, quality testing and WES experiments were performed as previously described (18); however, the 'proband only' analysis strategy was adopted for the identification of causative variants. WES analysis was performed using the Novaseq6000 platform (Illumina). Sequencing reads were mapped to the human reference genome (hg19/GRCh37) and underwent standard quality control screening. The Verita Trekker® Variants Detection system (Berry Genomics, Inc.) was used to identify single-nucleotide polymorphisms, insertion and deletions, copy number variants (CNVs), mitochondrial gene variants and runs of homozygosity. Subsequently, the Enliven® Variants Annotation Interpretation (Berry Genomics, Inc.) system was used to fulfill the annotation and interpretation progress referring to multiple databases. The overall workflow is presented in Fig. 1. After the variation filtering process, several disease-associated variants were identified and selected for familial validation using Sanger or CNV sequencing as previously described (20).

To identify the novel missense variation, homological analysis among species was performed using the NCBI blast online software (blast.ncbi.nlm.nih.gov/Blast.cgi; 2019/Oct/29). *In silico* analysis was performed using Sorting Intolerant from Tolerant (SIFT; sift.bii.a-star.edu.sg; 2019/Oct/29) and Polymorphism Phenotyping (PolyPhen; version 2; genetics.bwh.harvard.edu/pph2) software to calculate the respective pathogenicity indices. Biophysical analysis was conducted using Modeller software (version 9.21; salilab.org/modeller).

Results

Clinical data. The main clinical data are presented in Table I. All the subjects were patients with ICTEV with no family history of the disease (Table I). The parents had no adverse habits, for example, smoking. Representative clinical and ultrasonic illustrations are presented in Fig. 2.

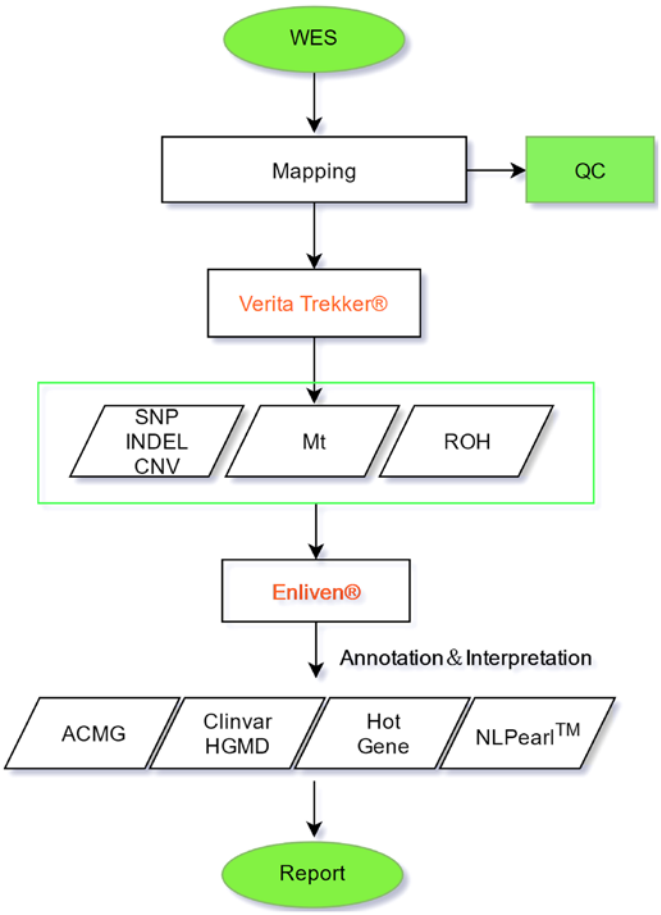

Figure 1. Data analysis and variation identification work flow. The green block represented the variation calling process. SNP, single nucleotide polymorphism; INDEL, insertion/deletion; CNV, copy number variation; Mt, mitochondrial variation; ROH, runs of homozygosity; WES, whole-exome sequencing; Red words represent patented software of Berry Genomics; QC, quality control; ACMG, American College of Medical Genetics and Genomics; HGMD, Human Gene Mutation Database; NLpearl™, Natural Language Pearl processing and analysis system by Berry Genoimcs, Inc.

Genetic analysis. The karyotyping results were normal for each of the subjects. However, two potentially causative variations were identified by WES and were further investigated by familial validation. The two potential causative variations identified were: seq[GRCh37]del(12)(q24.21-q24.23) g.116399065-120555197 encompassing the mediator complex subunit 13L (MED13L) gene in case 1 and transforming growth factor-β receptor 2 (TGFBR2):NM_001024847.2:exon5:c.1280T>C:p.L427P in case 3 (Fig. 3A and B). Several suspected variations were detected in other cases, but did not match the inheritance pattern of the corresponding disease-causing genes (data not shown).

It was indicated that the novel *de novo* missense variation, TGFBR2: p.L427P, was highly conserved among multiple species (Fig. 3C). Furthermore, *in silico* analysis results obtained using the SIFT (with the index of '0') and PolyPhenV2 (with the index of '0.996') softwares identified the variation as 'deleterious/probably damaging'. Additionally, biophysical

Table I. Clinical data of the recruited subjects.

Subject	Sex	Sampling time (after birth)	Main manifestations	Family history
Case 1	Male	2 months	Bilateral CTEV; VSD and patent ductus arteriosusat 4 month follow-up	G1P1, no family history of CTEV
Case 2	Female	1 month	Bilateral CTEV	G2P1, onemiscarriage at early pregnancy (without genetic testing), no family history of CTEV
Case 3	Female	1 month	Bilateral CTEV	G1P1, no family history of CTEV
Case 4	Male	5 days	Unilateral (left) CTEV	G1P1, no family history of CTEV
Case 5	Female	15 days	Unilateral (left) CTEV	G2P2, one 3-year-old healthy son, no family history of CTEV
Case 6	Male	17 days	Unilateral (right) CTEV; arthrogryposis at 3 month follow-up	G1P1, no family history of CTEV
Case 7	Male	1 month	Bilateral CTEV	G2P2, one 4-year-old healthy daughter, no family history of CTEV
Case 8	Female	25 days	Unilateral (right) CTEV	G1P1, no family history of CTEV
Case 9	Male	1 month	Unilateral (right) CTEV	G1P1, no family history of CTEV

CTEV, congenital talipes equinovarus;VSD, ventricular septal defect; G, gravida; P, para.

analysis suggested that the helix in the kinase domain was damaged by the missense variant, the hydrogen bond force was reduced and the hydrophobic pocket was affected to some extent (Fig. 3D and E).

According to the 2019 American College of Medical Genetics and Genomics (ACMG) & Clinical Genome Resource guideline for CNV interpretation (21), the CNV detected, seq[GRCh37]del(12)(q24.21-q24.23)g.116399065-120555197, was interpreted as pathogenic with a score >1 (variant classification, 2A). Similarly, in accordance with the 2015ACMG guideline for sequence variant interpretation (22), the TGFB R2:NM_001024847.2:exon5:c.1280T>C variant was deemed to be likely pathogenic (variant classification evidence, PS2+PM2+PP2+PP3).

Additionally, two pathogenic variations not associated with CTEV were detected, which were a paternal MYBPC3:NM_000256.3:exon6: c.769C>T: p.H257Y variation in case 4 and a maternal BRCA1:NM_007300.3:exon14:c.4497G>T: p.E1499D variation in case 5 (data not shown).

Discussion

CTEV seriously affects the aesthetics and functions of patients. The incidence and pathogenesis of the condition varies among different ethnicities, suggesting that genetic factors play an important role (23). ICTEV only follows the Mendelian inheritance pattern in minorities, including Polynesians (24), and therefore further investigation into the multifactorial pathogenesis of ICTEV in other ethnicities is required. Previous studies identified certain potential pathogenic genes primarily by single-nucleotide polymorphism typing combined with statistical analysis (2,11,25). Subsequently, further studies performed chromosomal microarray analysis and other methods to detect clinically significant CNVs containing important genes (7,26). However, few attempts have been made to apply next generation sequencing methods, including WES, to the identification of CTEV genetic variations (16,27). A study conducted by Yang *et al* (28) identified variants in the filamin B gene, which is consistent with the findings of the present study (17). In the present study, the detection rate of variants was low for the majority of ICTEV cases, indicating that the pathogenesis of ICTEV in the Chinese population is relatively complicated and further suggesting multifactorial inheritance. Therefore, to explore the pathogenesis of ICTEV by detecting rare variants, an effective method would be to increase the sample size and subsequently perform gene ontology enrichment analysis (29).

The MED13L gene on chromosome 12q24 encodes a subunit of the large mediator complex that functions with DNA-binding transcription factors and RNA polymerase II for gene activation or repression (30). The gene is part of the evolutionarily conserved Thyroid Hormone Receptor Associated Protein gene family, which encode proteins that regulate embryonic development (31). Several studies have indicated that heterozygous loss of function variations resulting in haploin sufficiency could cause Mental Retardation and Distinctive Facial Features with or without Cardiac Defects [MRFACD; Mendelian Inheritance in Man (MIM), #616789] (32-35), and clubfoot was a notable phenotype in these cases. In the present study, it was hypothesized that the microdeletion of 12q in case 1 would result in MRFACD, particularly on the basis of the aforementioned evidence. Therefore, the mental and cardiovascular development of this infant should be closely monitored.

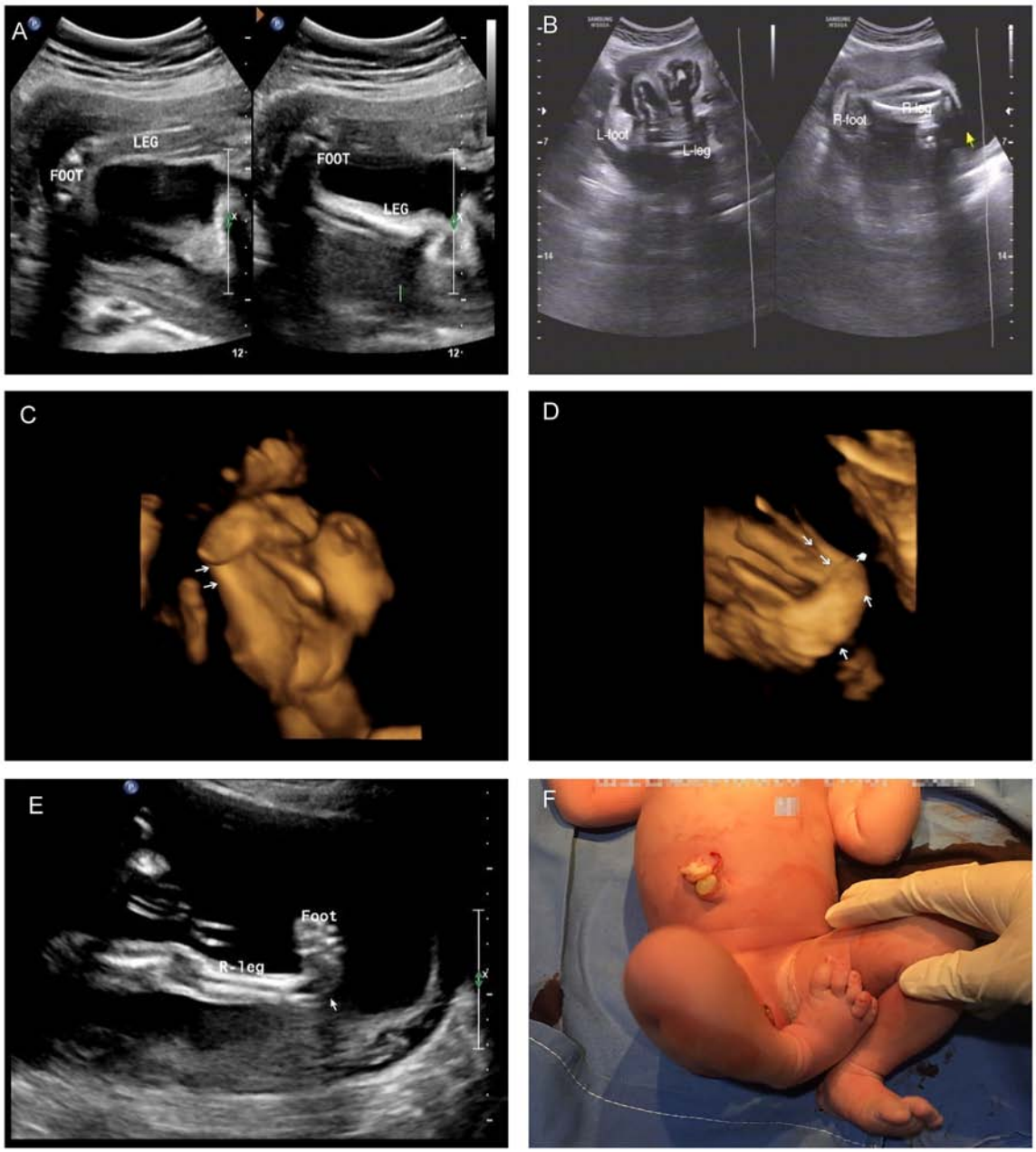

Figure 2. Representative clinical and ultrasonic images of the recruited subjects. (A) Case 1: Bilateral CTEV at 22 gestational weeks. (B) Case 2: Bilateral CTEV at 20 weeks. View (C) 1 and (D) 2 of case 3: Bilateral CTEV at 21 weeks. (E) Case 8: Unilateral (right) CTEVat 23 weeks. (F) Case 9: Unilateral (right) CTEV at birth. Arrows indicate the affected parts. CTEV, congenital talipes equinovarus.

The TGFBR2(MIM, *190182) gene on chromosome 3p24 belongs to the serine-threonine kinase family (36).The activities of TGF-β1 (TGFB1; MIM,*190180) in regulating cell proliferation, differentiation and extracellular matrix production are mediated via these receptors. In the present study, the variant in case 3 was located in the protein kinase domain of TGFBR2. Previous studies have reported that missense variants occurring in this domain can cause Loeys-Dietz syndrome type II (37,38). Therefore, based on the results of genetic analysis, the child incase3 should receive long-term follow-up in order to determine whether they display other phenotypes, as well as to provide further counseling and guidance for the subsequent pregnancies within the family. Moreover, although biophysical analysis predicted how the variant may alter the structure of TGFBR2, further studies are required to determine how the interaction between TGFBR2 and its receptor maybe affected.

According to the present study, the use of WES as a first-line method for the detection of ICTEV in children may not be efficient. However, WES provided ample sensitivity for the detection of rare syndromic CTEVs and consequently may be beneficial for the accurate differential diagnosis. The main limitations of the present study were the small sample size and the heterogeneity of CTEV. To validate the results of the present study, studies including larger and more well defined patient cohorts without congenital co-morbidities are required. Additionally, reporting the detection of pathogenic variants unrelated to proposed phenotypes presents an ethical challenge, which must be well communicated with patients and clearly reflected in informed consent.

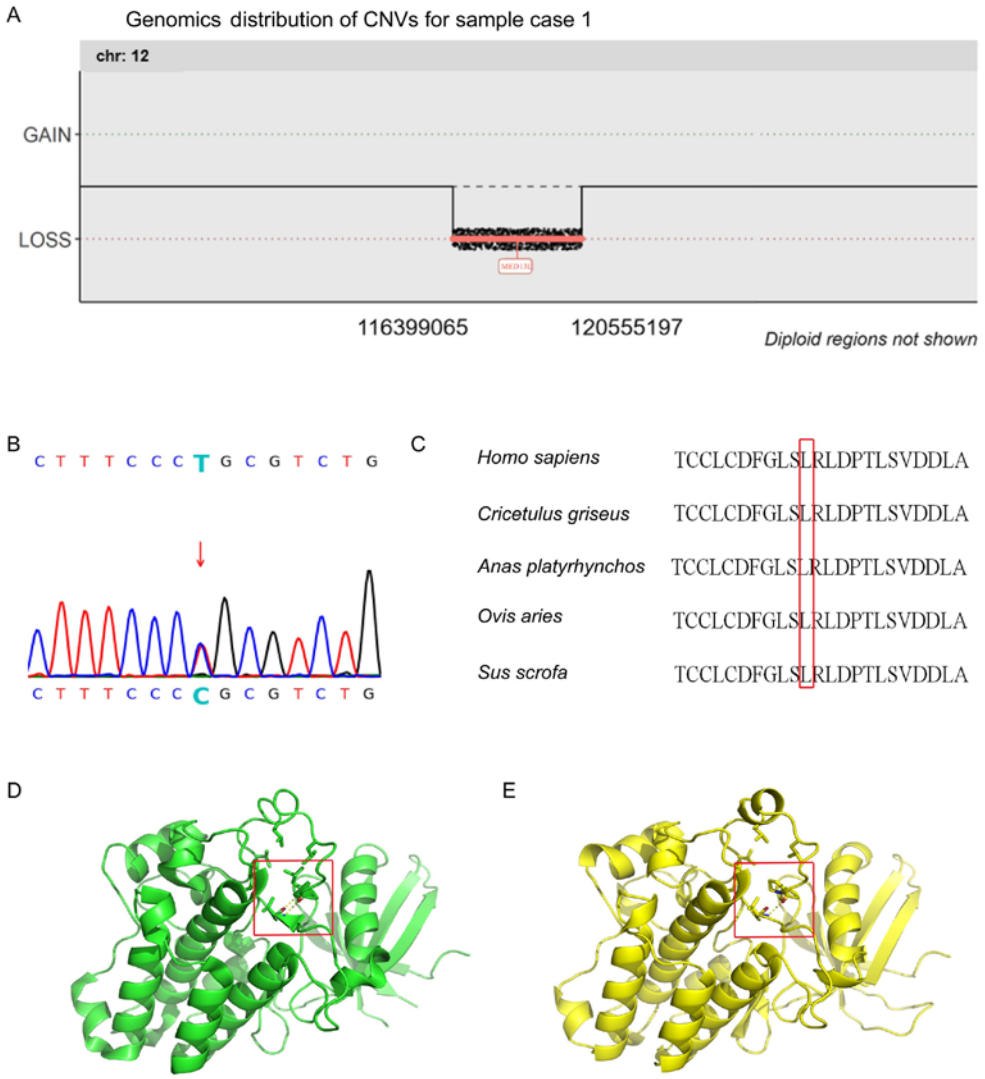

Figure 3. Variations detected in two cases and the results of the biophysical analysis. (A) The microdeletion at 12q in case 1. (B) TGFBR2: c.1280T>C in case 3. (C) The conservation of TGFBR2: p.427L (red box) among species. (D) The wild-type structure of the kinase domain of TGFBR2 encompassing p.427L (indicated by the red box). (E) The mutant structure of kinase domain of TGFBR2 encompassing p.427P (indicated by the red box). TGFBR2, transforming growth factor-β receptor 2; CNV, copy number variation.

In conclusion, the present study suggested that WES may serve as a comprehensive method for the detection of rare variants in coding sequences. In particular, WES displayed the advantage of detecting syndromic CTEV, which has a phenotype that is not easy to determine.

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Availability of data and materials

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

Authors' contributions

JZ and YL designed the current study. JZ and SL analyzed the data and drafted the manuscript. SM and YLiu recruited the case studies and performed the experiments. XW performed the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Shijiazhuang Obstetrics and Gynecology Hospital (approval no. 20180015) and written informed consent was obtained from the parents of all patients.

Patient consent for publication

This study has followed the principles of anonymity; no direct or indirect identifiers of our participants were used for publication. Written informed consent was obtained for publication.

Competing interests

The authors declare that they have no competing interests.

References

1. Wynne-Davies R: Genetic and environmental factors in the etiology of talipes equinovarus. Clin Orthop Relat Res 84: 9-13, 1972.
2. Ester AR, Tyerman G, Wise CA, Blanton SH and Hecht JT: Apoptotic gene analysis in idiopathic talipes equinovarus (clubfoot). Clin Orthop Relat Res 462: 32-37, 2007.
3. Wynne-Davies R: Family studies and the cause of congenital club foot. Talipes Equinovarus, Talipes Calcaneo-Valgus and Metatarsus Varus. J Bone Joint Surg Br 46: 445-463, 1964.
4. Pavone V, Chisari E, Vescio A, Lucenti L, Sessa G and Testas G: The etiology of idiopathic congenital talipes equinovarus: A systematic review. J Orthop Surg Res 13: 206, 2018.
5. Basit S and Khoshhal KI: Genetics of clubfoot; recent progress and future perspectives. Eur J Med Genet 61: 107-113, 2018.
6. Ching GH, Chung CS and Nemechek RW: Genetic and epidemiological studies of clubfoot in Hawaii: Ascertainment and incidence. Am J Hum Genet 21: 566-580, 1969.
7. Alvarado DM, Buchan JG, Frick SL, Herzenberg JE, Dobbs MB and Gurnett CA: Copy number analysis of 413 isolated talipes equinovarus patients suggests role for transcriptional regulators of early limb development. Eur J Hum Genet 21: 373-380, 2013.
8. Alvarado DM, Aferol H, McCall K, Huang JB, Techy M, Buchan J, Cady J, Gonzales PR, Dobbs MB and Gurnett CA: Familial isolated clubfoot is associated with recurrent chromosome 17q23.1q23.2 microduplications containing TBX4. Am J Hum Genet 87: 154-160, 2010.
9. Alvarado DM, McCall K, Aferol H, Silva MJ, Garbow JR, Spees WM, Patel T, Siegel M, Dobbs MB and Gurnett CA: Pitx1 haploinsufficiency causes clubfoot in humans and a clubfoot-like phenotype in mice. Hum Mol Genet 20: 3943-3952, 2011.
10. Cao D, Jin C, Ren M, Lin C, Zhang X and Zhao N: The expression of Gli3, regulated by HOXD13, may play a role in idiopathic congenital talipes equinovarus. BMC Musculoskelet Disord 10: 142, 2009.
11. Ester AR, Weymouth KS, Burt A, Wise CA, Scott A, Gurnett CA, Dobbs MB, Blanton SH and Hecht JT: Altered transmission of HOX and apoptotic SNPs identify a potential common pathway for clubfoot. Am J Med Genet A 149A: 2745-2752, 2009.
12. Gurnett CA, Alaee F, Kruse LM, Desruisseau DM, Hecht JT, Wise CA, Bowcock AM and Dobbs MB: Asymmetric lower-limb malformations in individuals with homeobox PITX1 gene mutation. Am J Hum Genet 83: 616-622, 2008.
13. Wang Y, Sun Y, Huang Y, Pan Y, Shi B, Ma J, Ma L, Lan F, Zhou Y, Shi J, *et al*: The association study of nonsyndromic cleft lip with or without cleft palate identified risk variants of the GLI3 gene in a Chinese population. J Genet 96: 687-693, 2017.
14. Dickinson KC, Meyer RE and Kotch J: Maternal smoking and the risk for clubfoot in infants. Birth Defects Res A Clin Mol Teratol 82: 86-91, 2008.
15. Parker SE, Mai CT, Strickland MJ, Olney RS, Rickard R, Marengo L, Wang Y, Hashmi SS and Meyer RE; National Birth Defects Prevention Network: Multistate study of the epidemiology of clubfoot. Birth Defects Res A Clin Mol Teratol 85: 897-904, 2009.
16. Zhang Z, Kong Z, Zhu M, Lu W, Ni L, Bai Y and Lou Y: Whole genome sequencing identifies ANXA3 and MTHFR mutations in a large family with an unknown equinus deformity associated genetic disorder. Mol Biol Rep 43: 1147-1155, 2016.
17. Yang K, Shen M, Yan Y, Tan Y, Zhang J, Wu J, Yang G, Li S, Wang J, Ren Z, *et al*: Genetic analysis in fetal skeletal dysplasias by trio whole-exome sequencing. BioMed Res Int 2019: 2492590, 2019.
18. Arsham MS, Barch MJ and Lawce HJ: The AGT Cytogenetics Laboratory Manual. John Wiley & Sons Inc., Hoboken, NJ, 2017.
19. McGowan-Jordan J, Simons A and Schmid M (eds): An International System for Human Cytogenomic Nomenclature. Karger, Basel, Switzerland, 2016.
20. Chen Y, Bartanus J, Liang D, Zhu H, Breman AM, Smith JL, Wang H, Ren Z, Patel A, Stankiewicz P, *et al*: Characterization of chromosomal abnormalities in pregnancy losses reveals critical genes and loci for human early development. Hum Mutat 38: 669-677, 2017.
21. Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, Raca G, Ritter DI, South ST, Thorland EC, *et al*: Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med 22: 245-257, 2019.
22. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, *et al*: ACMG Laboratory Quality Assurance Committee: Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17: 405-424, 2015.
23. Wang JH, Palmer RM and Chung CS: The role of major gene in clubfoot. Am J Hum Genet 42: 772-776, 1988.
24. Chapman C, Stott NS, Port RV and Nicol RO: Genetics of club foot in Maori and Pacific people. J Med Genet 37: 680-683, 2000.
25. Zhao XL, Wang YJ, Wu YL and Han WH: Role of COL9A1 genetic polymorphisms in development of congenital talipes equinovarus in a Chinese population. Genet Mol Res: Nov 3, 2016 (Epub ahead of print). doi: 10.4238/gmr15048773.
26. Lu W, Bacino CA, Richards BS, Alvarez C, VanderMeer JE, Vella M, Ahituv N, Sikka N, Dietz FR, Blanton SH, *et al*: Studies of TBX4 and chromosome 17q23.1q23.2: An uncommon cause of nonsyndromic clubfoot. Am J Med Genet A 158A: 1620-1627, 2012.
27. Alvarado DM, Buchan JG, Gurnett CA and Dobbs MB: Exome sequencing identifies an MYH3 mutation in a family with distal arthrogryposis type 1. J Bone Joint Surg Am 93: 1045-1050, 2011.
28. Yang H, Zheng Z, Cai H, Li H, Ye X, Zhang X, Wang Z and Fu Q: Three novel missense mutations in the filamin B gene are associated with isolated congenital talipes equinovarus. Hum Genet 135: 1181-1189, 2016.
29. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL, Jagodnik KM, Lachmann A, *et al*: Enrichr: A comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res 44: W90-W97, 2016.
30. Utami KH, Winata CL, Hillmer AM, Aksoy I, Long HT, Liany H, Chew EG, Mathavan S, Tay SK, Korzh V, *et al*: Impaired development of neural-crest cell-derived organs and intellectual disability caused by MED13L haploinsufficiency. Hum Mutat 35: 1311-1320, 2014.
31. Muncke N, Jung C, Rüdiger H, Ulmer H, Roeth R, Hubert A, Goldmuntz E, Driscoll D, Goodship J, Schön K, *et al*: Missense mutations and gene interruption in PROSIT240, a novel TRAP240-like gene, in patients with congenital heart defect (transposition of the great arteries). Circulation 108: 2843-2850, 2003.
32. Asadollahi R, Oneda B, Sheth F, Azzarello-Burri S, Baldinger R, Joset P, Latal B, Knirsch W, Desai S, Baumer A, *et al*: Dosage changes of MED13L further delineate its role in congenital heart defects and intellectual disability. Eur J Hum Genet 21: 1100-1104, 2013.
33. Hamdan FF, Srour M, Capo-Chichi JM, Daoud H, Nassif C, Patry L, Massicotte C, Ambalavanan A, Spiegelman D, Diallo O, *et al*: De novo mutations in moderate or severe intellectual disability. PLoS Genet 10: e1004772, 2014.
34. van Haelst MM, Monroe GR, Duran K, van Binsbergen E, Breur JM, Giltay JC and van Haaften G: Further confirmation of the MED13L haploinsufficiency syndrome. Eur J Hum Genet 23: 135-138, 2015.
35. Adegbola A, Musante L, Callewaert B, Maciel P, Hu H, Isidor B, Picker-Minh S, Le Caignec C, Delle Chiaie B, Vanakker O, *et al*: Redefining the MED13L syndrome. Eur J Hum Genet 23: 1308-1317, 2015.
36. Lin HY, Wang XF, Ng-Eaton E, Weinberg RA and Lodish HF: Expression cloning of the TGF-β type II receptor, a functional transmembrane serine/threonine kinase. Cell 68: 775-785, 1992.
37. Mizuguchi T, Collod-Beroud G, Akiyama T, Abifadel M, Harada N, Morisaki T, Allard D, Varret M, Claustres M, Morisaki H, *et al*: Heterozygous TGFBR2 mutations in Marfan syndrome. Nat Genet 36: 855-860, 2004.
38. Loeys BL, Chen J, Neptune ER, Judge DP, Podowski M, Holm T, Meyers J, Leitch CC, Katsanis N, Sharifi N, *et al*: A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. Nat Genet 37: 275-281, 2005.