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Genes on syndromic and idiopathic CTEV: A systematic review

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ABSTRACT

Background: Congenital talipes equinovarus (CTEV), also known as clubfoot, is a common but understudied developmental disease of the lower limb. The cause of congenital clubfoot is unclear, and the role of environmental and genetic factors remains unknown. Idiopathic CTEV and syndromic CTEV have rather different clinical features, proposed etiopathogenetic mechanisms, and treatment options. This study aimed to provide an update on the genes involved in idiopathic and syndromic CTEV.

Methods: We conducted this systematic review according to the guideline of the Preferred Reporting Items for Systematic Reviews and MetaAnalyses (PRISMA) 2020 Statement, only papers that have been published from the year 2019 until the search date was included. Three medical electronic databases (PubMed, Science Direct, and European PMC) were searched by a single author on October 24th, 2021. The titles and abstracts were screened where studies of any level of evidence reporting clinical or preclinical results published from 2019 onwards, mentioned gene(s) involved in cases presenting with CTEV (idiopathic and syndromic) were included. Data were synthesized with use of the Microsoft Excel (Microsoft, Redmond, WA).

Results: Fifty-three studies were included and analyzed in this paper, which met all inclusion criteria (11 articles that discussed genes involved in the presence of isolated CTEV and 42 articles for syndromes with CTEV phenotypes). The top three individual genes mentioned were PITX1, MTHFR, and ZC4H2 for the idiopathic, also HOX D13, SLC 26A2, and TBX4 for the syndromic. The top three family genes related to CTEV were HOX family, CASP family, and COL family. According to the results, the most often involved in idiopathic CTEV is HOX gene. Including studies of any level of evidence reporting clinical or preclinical results that mentioned gene(s) involved in cases presenting with CTEV carries a greater risk of being due to multiple biases. High heterogeneity and the paucity of high-profile studies on the etiology of CTEV also sets a major limitation for this study.

Conclusions: Genetic play a significant role in the etiopathogenesis of idiopathic and syndromic CTEV. PITX1 and MTHFR gene are the most frequently mentioned individual gene for idiopathic CTEV, whereas ZC4H2 gene being the most mentioned for syndromic CTEV. The HOX family genes were also found to be associated with both phenotypes.

1. Introduction

Congenital talipes equinovarus (CTEV), also known as clubfoot, is a common but understudied developmental disease of the lower limb. It is described as foot fixation in adduction, supination, and varus, that is, tilted inwards, axially rotated outwards, and pointed downwards [1]. In low- and middle-income countries, the birth prevalence of CTEV varies between 0,5 - 2 in every 1000 live births [2], approximately half of the cases have bilateral deformity whilst unilateral cases are mostly right-sided [3]. Congenital talipes equinovarus can occur alone as isolated congenital abnormalities with an "idiopathic" cause, or it can occur in conjunction with other traits as part of a genetic disease and be referred to as "syndromic". [1]. About 80% of clubfoot cases idiopathic [4] and the remaining 20% are caused by related malformations, chromosomal abnormalities, and genetic diseases such distal arthrogryposis (DA) or myleomeningocele [5].

The cause of congenital clubfoot is unclear, and the role of environmental and genetic factors remains unknown. There are compelling evidences for a genetic component to the etiology of idiopathic CTEV. Around 24,4% of all isolated cases report a family history of idiopathic

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CTEV [6]. A twin study found a higher concordance in monozygotic (33%) than dizygotic (3%) twins [7], and more recent study puts the heritability of isolated clubfoot at roughly 30% [8]. Although research on populations, families, and twins suggests a genetic component, pedigree analysis and the unusual sex ratio (2.0–2.5 : 1 male: female) suggests that the inheritance mode does not follow a typical Mendelian inheritance pattern [1].

Although syndromic CTEV's clinical presentation may resemble that of the idiopathic form, syndromic CTEV appears to derive from neurological/neuromuscular disorders [9] and fetal abnormalities [10]. As a result, idiopathic CTEV and syndromic CTEV have rather different clinical features, proposed etiopathogenetic mechanisms, and treatment options. This study aims to provide an update on the genes involved in idiopathic and syndromic CTEV.

2. Materials and METHODS

We conducted this systematic review according to the guideline of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 Statement. PRISMA flow diagram was utilized to present study search and selection process (Fig. 1) and the PRISMA 2020 checklist was used to ensure adherence to the guideline (Appendix 1) [45]. Three medical electronic databases (PubMed, Science Direct, and European PMC) were searched by a single author on October 24th, 2021. The search string used was ("club foot" OR "club feet" OR "club-footed" OR "congenital talipes equinovarus" OR "ctev") AND ("genetic*" OR "etiology*" OR "pathology*" OR "pathophysiology*" OR "physiology*" OR "embryology*" OR "etiopathogenesis*"). The wildcard term (*) was applied to increase the sensitivity of the search strategy. As we will include the results from a previous systematic review conducted by Pavone et al. [11], only papers that have been published from the year 2019 until the search date was included.

The titles and abstracts were screened using the following inclusion criteria: studies of any level of evidence reporting clinical or preclinical results published from 2019 onwards, mentioned gene(s) involved in cases presenting with CTEV (idiopathic and syndromic). As this review topic mainly focuses on genetic analysis, we expected non-randomised studies to be included although we would consider any randomized clinical trial (RCTs) if found. We excluded all duplicates, articles dealing with other topics, and articles that do not mention any genetic pathway leading to CTEV. The study protocol is available upon request.

Eligible studies were reviewed, and the following data were extracted: author's name, year of publication, subjects, pathway/molecule involved, results, and additional notes. The researchers performed analyses using descriptive statistics of the data and summarized the findings from these studies. using Microsoft Excel (Microsoft, Redmond, WA). Quality and risk of bias assessments for included studies were done using Newcastle-Ottawa Quality Assessment Scale for non-randomized studies where appropriate. The overall quality assessment of this systematic review was self-evaluated using the AMSTAR (Assessing the Methodological Quality of Systematic Reviews) Checklist as critical appraisal tool for systematic reviews with score of moderate quality review (Appendix 2) [46]. This review has been registered to the

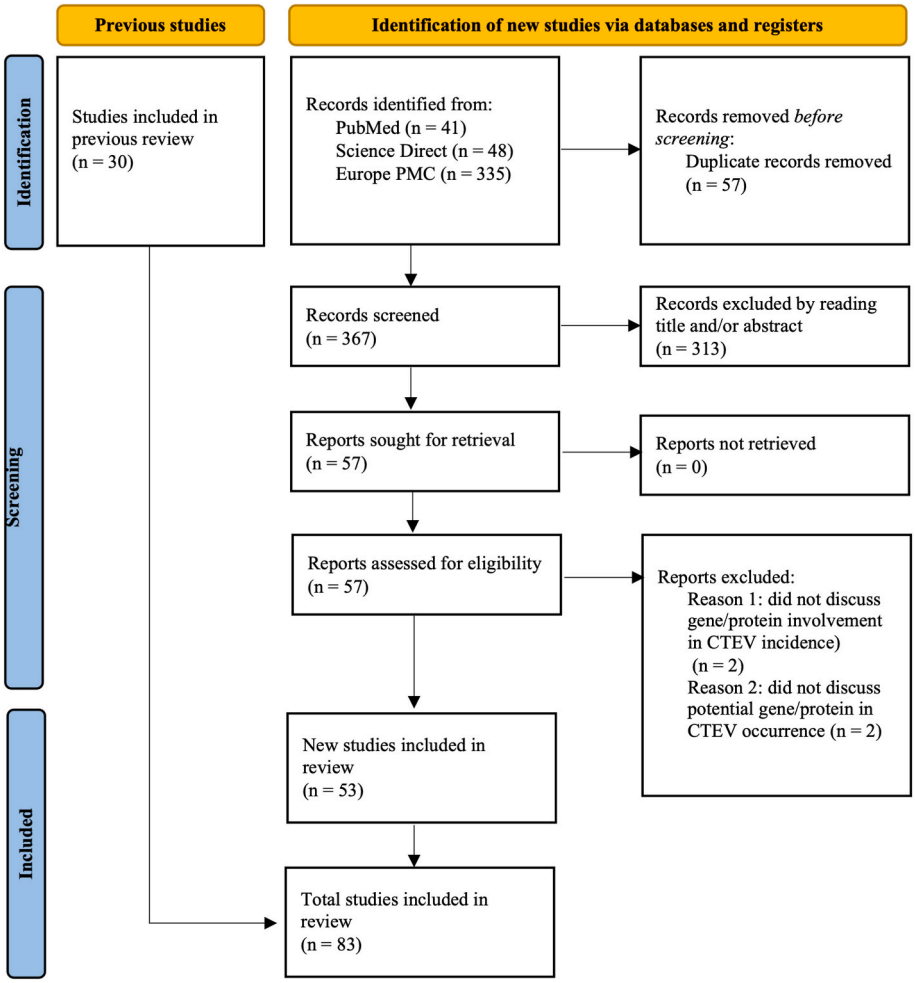

Fig. 1. Study search and selection process based on PRISMA flow diagram.

Research Registry with Unique Identifying Number (UIN) reviewregistry1445.

3. RESULTS

The literature search and selection process is illustrated in Fig. 1. The initial search across 3 medical databases resulted in a total of 424 articles before duplicates were removed. A total of 367 potentially relevant articles were assessed by reading the title and/or abstract after the exclusion of duplicated 57 studies. The full text of 57 articles was assessed for eligibility criteria. Four studies were excluded because the gene/protein discussed had no involvement in CTEV incidence or no genes/protein discussed in CTEV occurrence. Finally, 53 studies were included and analyzed in this paper, which met all inclusion criteria (11 articles that discussed genes involved in the presence of isolated CTEV and 42 articles for syndromes with CTEV phenotypes). Pavone et al. [11] included 30 articles that mainly focus on genetic research. From this previous study, only genes involved in idiopathic CTEV were mentioned whereas our search included genetic etiologies for both idiopathic and syndromic CTEV. The results from the previous study and our search results were summarized in tabular format in Supplementary Material 1, 2, and 3.

3.1. Top individual genes

The frequency of genes mentioned from both search strings was tallied, regardless of the phenotype they had (idiopathic or syndromic). The top three individual genes mentioned were PITX1 (n = 5), MTHFR and ZC4H2 (n = 4) and HOX D13, SLC 26A2 and TBX4 (n = 3). The most frequently mentioned individual gene is the PITX1 gene, which contribute to the idiopathic CTEV phenotype, that was mentioned in 5 different articles [12–16]. Gurnett et al. [12] conducted a large multi-generational family study and found that a single missense mutation (E130K) was identified in PITX1, a Bicoid-related homeodomain transcription factor located within the chromosome 17 region, through Genome-wide linkage analysis (Table 1).

The MTHFR and ZC4H2 genes are the second most mentioned individual genes. The MTHFR gene was included in articles with ICTEV cases[17–20], whereas the ZC4H2 gene was mentioned twice in cases of Wieacker-Wolff syndrome (WRWF) [21,22] and twice in ZC4H2-related Arthrogryposis Multiplex Congenital [23,24]. The HOX D13 [25–27] and TBX4 [28–30] were mentioned in three different articles, both genes were discussed being the genetic etiology of ICTEV. SLC 26A2 gene is also tied with HOX D13 and TBX4 genes, being mentioned in patients with Diastrophic Dysplasia (DTD) [31,32] and Atelosteogenesis type 2 syndromes [33].

3.2. Top family genes

The most frequently mentioned family gene involved in the CTEV phenotype was the HOX family which included HOXA, HOXA9, HOX C, HOX C13, HOX D, HOX D10, HOX D12, and HOX D13. Most articles that included HOX genes were those with idiopathic CTEV, except the HOX D10 gene that were mentioned in a syndromic congenital vertical talus (CVT) of Charcot Marie Tooth (CMT) deformity[34,35]. HOXD12 and HOXD13 single nucleotide polymorphisms (SNPs) were identified to be connected to idiopathic CTEV [27]. Interactions between HOXA and HOXD variants and apoptotic genes were discovered. Perturbation of HOX and apoptotic genes affects muscle and limb development, which may result in limb rotation failure into a plantar grade position [36]. Recent study stated that 5' HOXC microdeletions are associated with lower extremity malformation, including CTEV and vertical talus [37].

The CASP family gene comes in second with CASP3, CASP8, CASP9, and CASP 10 individual genes specifically reported in cases with idiopathic CTEV phenotypes. COL family gene (COL1A1, COL9A1, and COL1A2) was mentioned five times in different papers, reported in both idiopathic and syndromic cases. The COL1A1 gene mutation was found in an aborted fetus with a CTEV phenotype among other features and the differential diagnoses were metaphyseal chondroplasia, hyperparathyroidism, and spondylometaphyseal dysplasia, while the rest of the genes mentioned in the COL family gene represented idiopathic clubfoot. Although the frequency of TPM family gene report tied with the COL family gene (n = 5), the TPM2 gene specifically was reported to have no association with the incidence of clubfoot in two articles whilst one of the five articles suggested the functional role of both TPM1 and TPM2 gene but in an animal study.

The MYH family was reported in relation to idiopathic CTEV incidence, whereas the CHST and SLC gene families were in relation to syndromic clubfoot, all being reported four times. Syndromes related to the CHST gene family were Larsen syndrome or spondyloepiphyseal dysplasia (CHST3), Ehler Dahnlos Syndrome (EDS) musculocontractural type 1 (CHST14), and adducted thumb-clubfoot syndrome (CHST14). The SLC gene family reports comprised of SLC26A2 gene involvement in syndromes as discussed before with the addition of SLC12A5 gene in syndromic clubfeet of Tuberous Sclerosis Complex.

Last, the CHD and FOX gene families were mentioned three times each in relation to syndromic clubfoot of CHARGE syndrome/hypogonadotropic hypogonadism (CHD7), 15q26 deletion phenotype (CHD2), and 10q26 subtelomeric microdeletion syndrome (FOX12). CHD1 and FOXN3 genes contributed to these families' frequencies as they were associated with ICTEV cases (Table 2).

Table 1
Most frequently reported individual genes associated with clubfoot.

No	Genes	Frequency (*n* =)	CTEV Phenotype	Syndrome
1.	PITX1	5	ICTEV	–
2.	MTHFR	4	ICTEV	–
	ZC4H2	4	Syndromic	WRWF, ZARD
3.	HOXD13	3	ICTEV	–
	SLC26A2	3	Syndromic	DTD, atelosteogenesis type 2
	TBX4	3	ICTEV	–

*WRWF = Wieacker-Wolff syndrome.
*ZARD = ZC4H2-Related Diseases.
*DTD = Diastrophic Dysplasia.

Table 2
Most frequently reported gene families associated with clubfoot.

No	Family	Frequency (*n* =)	CTEV Phenotype	Syndrome
1.	HOX	13	Both	CMT
2.	CASP	6	ICTEV	
3.	COL	5	Both	metaphyseal chondroplasia ddx hyperparathyroidism ddx spondylometaphyseal dysplasia
	TPM	5	Both	
4.	CHST	4	syn	DTD, Larsen syndrome, EDS musculocontractural type 1, adductus thumb syndrome
	MYH	4	ICTEV	
	SLC	4	syn	DTD, atelosteogenesis type 2, TSC
5.	CHD	3	Both	CHARGE syndrome, hypogonadotropic hypogonadism, 15q26 deletion phenotype
	FOX	3	Both	10q26 subtelomeric microdeletion syndrome

*CMT = Charcot Marie Tooth.
*EDS = Ehler Dahnlos Syndrome.
*TSC = Tuberous Sclerosis Complex.
*CHARGE syndrome = Coloboma, heart defects, choanal atresia, growth retardation, genital abnormalities, and ear abnormalities.

4. Discussion

Genetics play a major role in occurrence of CTEV. CTEV can occurs idiopathically or together with a syndrome. Recently, several genes have been studied in context of their relationship with CTEV incidence.

According to the results, the most often involved in CTEV is HOX gene, in which HOX D10 that was highly related in syndromic CVT on CMT deformities. HOX genes encode a family of transcription factors of fundamental importance for body patterning during embryonic development. Humans have 39 HOX genes which is organized into four clusters, with major roles in the development of the central nervous system, axial skeleton, gastrointestinal and urogenital tracts, external genitalia, and limbs.

The first two limb malformations shown to be caused by mutations in the human Homeobox (HOX) genes were synpolydactyly and hand-foot-genital syndrome, which result from mutations in HOXD13 and HOXA13, respectively. Study done by Alvarado et al. stated that congenital vertical talus has been associated with 5' HOXC microdeletions. and A deletion of HOXC13 was also identified previously in one family with familial clubfoot [38].

Another gene, CASP also play a major role in occurrence of CTEV. Cysteine-dependent aspartate-directed proteases (caspases) are part of a family of cysteine proteases that play essential roles in apoptosis, necrosis, and inflammation processes. Firstly, identified in 2005 to plays a role in causing CTEV. However, CASP8, CASP10 and CFLAR after 40 SNPs genotyping were done, has no significant correlation. COL family is gene that regulates ECM. The ECM provides structural support for organs, tissues and cell membranes. They also play a role in cell differentiation, proliferation survival and migration. Extracellular matrix binding helps to regulate TGF-β signalling. Recently, COL3A1 are known causing vascular type of Ehler-Danloss syndrome, and regulations are estimated to be influence by use of minoxidil. The specific involvement of the foot explained with the PITX1 gene being predominantly expressed in the hindlimb rather than forelimb [39]. In general, MYH are known as main contractile components that plays role in the development of muscle. However, function of each isoform are remain unknown. CHD and FOX are highly correlated with formation of skeletal muscle, the role of this gene in causing CTEV is further to be studied.

ZC4H2-Associated Rare Diseases (ZARD), formerly known as Wieacker-Wolff syndrome (WRWF), is the most mentioned syndrome associated with syndromic CTEV. ZC4H2, located on the X chromosome, is a gene that encodes zinc finger C4H2-type containing protein essential for normal development. Multiple arthrogryposis, CTEV, achilles tendon contracture, distal muscle weakness, camptodactyly, knee flexion contracture, elbow flexion contracture, and hip subluxation were the most prevalent musculoskeletal symptoms [40].

Environmental factors also play an important role in the pathogenesis of CTEV. The hypothesis that maternal smoking is linked to a higher chance of a baby being born with idiopathic CTEV is supported by numbers of research [41–43]. Moreover, the combination of family history of CTEV and smoking increases the risk twenty-fold (Odds Ratio (OR) = 20,3 95% CI: 7.90–52.17) [44].

In contrast to earlier studies that primarily focused on the etiology of idiopathic CTEV, this study examined the genes involved in both idiopathic and syndromic CTEV. In specific, as this study also includes systematic review result by Pavone et al. (2018), it is important to point out the difference in population of interest may limit the information regarding genes involved in syndromic CTEV prior to 2019[11]. Relevant studies were identified using an extensive search strategy and a large number of bibliographic sources. We also opted to include studies of any level of evidence reporting clinical or preclinical results that mentioned gene(s) involved in cases presenting with CTEV, as every study and article could provide valuable information about the etiology of CTEV. Conversely, those type of studies carries a greater risk of being due to multiple biases. High heterogeneity and the paucity of high-profile studies on the etiology of CTEV also sets a major limitation for this study. Recently, there's no single gene that decided to be responsible causing CTEV. With the aid of a new animal model research, next-generation studies may have the potential to identify genes underlying the phenotype and elucidate the inheritance pattern and penetrance of the disorder.

5. CONCLUSION

Genetic factors play a significant role in the etiopathogenesis of idiopathic and syndromic CTEV. PITX1 and MTHFR gene are the most frequently mentioned individual gene that contribute to the development of idiopathic CTEV, whereas ZC4H2 gene being the most mentioned in the syndromic phenotype. The HOX family genes were also found to be associated with both phenotypes. Even though numerous studies have examined the genetic basis of the disease, there is still a lack of consensus on one or multiple gene targets. Recent data indicates a significant contribution from both genetic and environmental factors. In order to fully understand the causes of CTEV, which are likely complex and linked to numerous gene mutations, extensive multi-center studies are needed. More advanced studies with next-generation genome sequencing, genetic variant analysis, gene expression analysis, and metabolomics analysis are strongly encouraged. Understanding these pathways in CTEV will aid in diagnosis, counseling, and giving a roadmap for the most appropriate and effective treatment interventions, as well as broadening our comprehension of the illness process in general.

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Ethical approval

The ethical approval was waived for this study by The Ethics Committee of Faculty of Medicine, Gadjah Mada University because this study does not directly involve human nor animal subjects.

Consent

Not applicable for this type of study.

Author contribution

Hilmi Muhammad: Conceptualization, Methodology, Data curation, Writing-Original draft preparation, Validation. Sofia Mubarika Haryana: Conceptualization, Data curation, Methodology, Writing-Original draft preparation, Validation. Rahadyan Magetsari: Conceptualization, Methodology, Writing-Review and Editing, Validation. Shannen Karsten: Conceptualization, Methodology, Writing-Review and Editing, Validation. Paramita Ayu Saraswati: Conceptualization, Methodology, Writing-Review and Editing, Validation.

Registration of research studies

This study has been registered in the Research Registry with Unique Identifying Number reviewregistry1445.

(https://www.researchregistry.com/browse-the-registry#registryofsystematicreviewsmeta-analyses/registryofsystematicreviewsmeta-analysesdetails/631551c1ff15c70023aa2e00).

Guarantor

All of the authors.

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Informed consent (Patient/Guardian)

Consent to publish the study was not applicable for this type of study.

Institutional ethical committee approval

The ethical approval was waived for this study by The Ethics Committee of Faculty of Medicine, Gadjah Mada University.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijso.2022.100547.

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