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Exploring the genetic and pathobiological pathways of talipes equinovarus: a short narrative review

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Abstract. Talipes equinovarus (TEV), also known as club foot, is one of the common congenital foot deformities. TEV deformity includes the components of osteochondral tissues namely supination, or inversion of the subtalar joint (hind foot varus), forefoot adduction deformity (metatarsus adductus), exaggerated midfoot arch (cavus) and limited ankle dorsiflexion (equinues). Its prevalence ranges from 0.5 to 2.0/1000 live births. It is hypothesized that TEV manifestation is due to molecular genetic defects that are influenced by environmental factors. The deformity had been recognized for centuries, but its main cause remains elusive. This narrative review summarizes the literature data that have accumulated over the past few decades on the environmental, molecular, and genetic factors along with the pathobiological mechanisms underlying TEV and analyze the role of these factors in the development of this disease. TEV segregates in families with both autosomal dominant and recessive mode of inheritance or autosomal dominant with incomplete penetrance. This supports the involvement of the genetic component underlying TEV. Genetic factor underlying TEV is further supported by the fact that a much higher concordance rate is seen in monozygotic (32%) rather than dizygotic (2.9%) twins. Various genetic studies including candidate gene association studies, copy number variation analysis, linkage analysis, whole exome sequencing and whole genome sequencing have shown the involvement of certain genes in the development of TEV. The research work done so far is still deficient for the exact genetic cause in families with TEV as most studies have focused on the sporadic cases and the genetic causes documented so far are still speculative. TEV is considered as a multifactorial congenital deformity where both genetic and environmental factors disrupt the normal mitotic division of the cytoskeleton in the lower limbs and ultimately leads to the formation of deformed foot. Hence, large multiscale, multicenter collaborative studies using genetic techniques like genome wide association studies (GWAS) with single nucleotide polymorphisms scan and linkage analysis in large families are required.

Keywords: talipes equinovarus, genetic, pathology.

INTRODUCTION

Skeletal disorders include a series of different conditions where the development and architecture of the chondro-osseous tissue is adversely affected leading to deformities of the musculoskeletal system. TEV is one of the most common congenital abnormalities affecting the limbs characterized by deformity of the foot with four components of osteochondral tissues, hind foot varus, forefoot (metatarsus) adducts, exaggerated midfoot arch (cavus) and equinus (1,2). The deformity is illustrated in Figure 1.

The birth prevalence of TEV varies from 0.5 to 2.0/1000 live births (3). In the low to middle income countries, it varies between 0.15 to 2.03/1000 live births (4). TEV incidence is high in the Māori population in New Zealand which is explained by the genetic load carried by closed societies (5). Around 80 % of all the reported cases of TEV are classified as isolated club foot also called non syndromic, while the other 20% are associated with other clinical abnormalities, thus called syndromic. Syndromic TEV is more commonly associated with myelomeningocele and distal arthrogryposis, multiple epiphyseal dysplasia, Larsen syndrome (LS), Lambert syndrome, and Ehlers-Danlos syndrome (EDS) (6,8) as shown in the Figure 2. The studies performed on the isolated TEV are carried out during their childhood, however no data is available for these cases in their adulthood to elaborate if they have delayed presentations of other features. Male to female ratio is 2:1 which is not variable in different societies (7). Kursi et al in 2008 explained the polygenic and multifactorial pattern of inheritance where females would also be more likely to transmit clubfoot to their children thus having

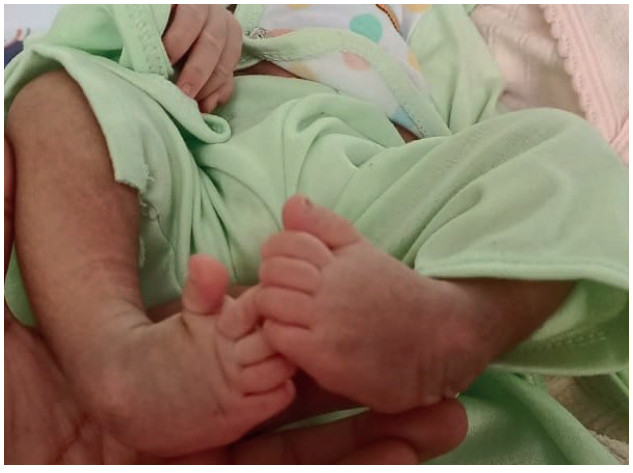

Figure 1. An infant with bilateral club foot (Courtesy of orthopedic unit, District headquarter hospital Rawalpindi Pakistan).

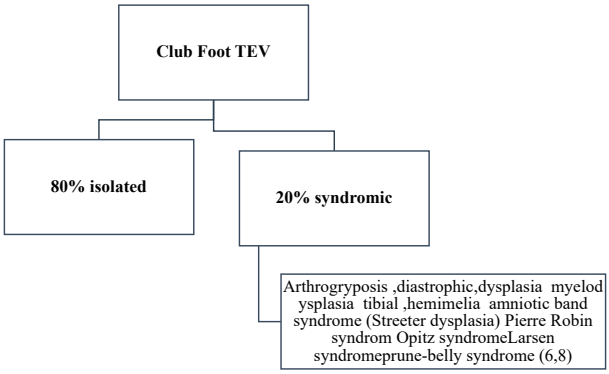

Figure 2. Showing the presentation of TEV.

the Carter affect. Club foot can be unilateral (30-40%) or bilateral (60-70%). TEV manifests a large variety of biomolecular genetic defects which are further influenced by environmental factors (9).

Historically, TEV was also described by Hippocrates during his era (10). Egyptian Pharaoh Tutankhamen in 1332 was also featured to be suffering from club foot (11). A 17th century painting of a dubbed *clubfoot child* by Jusepe de Ribera still hangs in the Musee du Lauvre (12). Since old times, a lot of work and advancements have been achieved in the management of TEV. But the main cause of this deformity remains evasive. In this review, our focus will be on the molecular genetic factors and pathobiological mechanisms underlying TEV.

ETIOLOGY

Despite multiple theories, the exact cause underlying this deformity is still unknown. It is multifactorial where both environmental and genetic factors are thought to play a significant role (13). Some of the environmental factors like maternal psychological distress during pregnancy, alcohol use, parental age, parental education, and birth season have been excluded due to lack of significant statistical association (14). Hippocrates postulated that, during the intrauterine life of a fetus, increased intrauterine pressure compressing the lower limbs plays a role in the development of this deformity. But later, studies showed that congested fetus with oligohydramnios, twins' pregnancy, large babies and primigravida fetuses are not affected by club foot. (15). Moreover, it zhas also been proved that TEV can be diagnosed even at the second trimester of pregnancy before the intrauterine pressure rises. (16) The most common and congruous environmental factor involved in etiology of this anomaly is exposure of the women

to the tobacco smoke during pregnancy (17). Previous studies showed that tobacco smoke is biotransformed in modulation with N-acetylation genes *NAT1*, *NAT2*. It is supported by the studies that variations in *NAT1, NAT2* genes are associated with TEV (18). Moreover, decreased acetylation activity of *NATE 2* gene polymorphism is also found to be associated with TEV (18,19). Additionally, genetic variation in smoking metabolism genes were also studied in relation to clubfoot where it is found that perturbation of *CYP1A1 gene results in* adverse effects on the development of lower limb. (20).

As TEV is segregating in families with both autosomal dominant and recessive mode of inheritance, concordance rate is more in monozygotic (32%) than in dizygotic twins (2.9%) (21,22,23). Various genetic technologies have been used to identify the genetic defects involved in the development of TEV. Candidate association studies, copy number variation analysis, linkage analysis, whole genome sequencing and whole exome sequencing have pointed out the involvement of certain genes in the development of TEV (13). But the genetic molecular regulatory network underlying the TEV is yet to be identified. Other studies showed that the TEV is a multifactorial deformity of the lower limb where genetic factors are predominant but modifiable by environmental factors (24,25).

GROSS PHENOTYPIC ABNORMALITIES OF THE TEV

In TEV the tibia and fibula bones are slightly shortened due to the incomplete developmental process. (26,27). Hence, distal tibial metaphyseal fractures due to diastasis in the tibiofibular joint have been reported in several cases while doing forced dorsiflexion and eversion of the ankle joint. (28,29,30). Relations of talus bone with other abnormal bones divided into different subgroups like medical subluxation of the navicular bone on the talar head and medially subluxated cuboid bone over calcaneal head, which ultimately leads to restriction of movements in the ankle joint. (28,29). However, some bone anomalies in TEV like small dome shaped deformation is associated with better range of movements in the ankle joint (31).

Congenital hypoplasia of the calf muscles even after the treatment of TEV was reported (32). The total number of the muscle fibers was normal, but they showed type 1 fiber atrophy, deficiency of type 2b and increased number of types 2c muscle fibers suggested abnormal development of these muscle fibers in TEV cases (33).

GENETICS OF TEV

Usually, mutations in genes coding for the contractile proteins of skeletal myofibrils like *MYBPH*, *TPM2*, *TNNT3*, *TPM1*, *MYH13* and *MYH3* are involved in the TEV (34,35). However, Gurnett et al. in his study of 31 patients out of whom 20 were suffering from familial clubfoot TEV patients could not detect mutations in TNNT3, MYH3, and TPM2 genes (35). Although he found several previously undescribed single-nucleotide polymorphisms of unknown importance.

GENES IMPLICATED IN TEV

PITX- gene

PITX1 gene participates in the development of the hind limb primarily the legs and feet. The first brachial arch, an embryonic structure located at the base of the brain, is also found to preferentially express *PITX1 (36,37).* A study of six proband of Ghanaian parents were reported with co-occurrences of orofacial clefts (congenital malformations of the face and palate) and club foot with variable genetic causes (38). The defect in bronchial arches explain the co-occurrence. Most studies show that genetic defects in *PITX1* can cause lower limb developmental deformities such as developmental hip dysplasia, patella hypoplasia, tibial hemimelia, preaxial polydactyly together with bilateral TEV (39,40,41,42). Moreover, copy number variations as well as haploinsufficiency of PITX1 have been shown to play a crucial role in the development of isolated clubfoot. (44,45).

TBX4- gene

T-box family, T-box factor 4 (*TBX4*) is a known transcriptional factor and its expression has been observed in multiple organs and tissues (46). Knock out studies revealed that *TBX4* gene has a significant role in the development of the lower limbs and the respiratory pathways (47). Hence, mutations in the *TBX4* lead to skeletal dysplasia and pulmonary hypertension due to developmental lung diseases (48). Specifically, mutations in *TBX4* gene have been reported with pulmonary developmental disorders and lower limb deformity (49). Moreover, microduplication of the 17q23.1q23.2 region which involves TBX4 has been identified in familial cases of TEV (50).

HOX (homeobox A, C, D) gene

HOX family participates in patterning (51). Genes in HOX cluster (*HOXA-D*) engage in controlling the lower limb axis development. Members of HOXD cluster, *HOXD12* and *HOXD13* were found to be involved in the development of TEV (52). Transmission defects in HOXA and HOXD apoptosis gene cluster were also found in TEV (53). Moreover, microdeletion of the *5'* region of *HOXC* is also found to be a contributing factor in the development of both vertical talus and TEV (54).

Filamin B (FLNB)

FLNB is a cytoplasmic protein that regulates intracellular communication and signaling by cross-linking the protein actin and thus allows direct communication between the cell membrane and cytoskeletal network, thereby controlling and guiding proper skeletal development. *FLNB* mutation may lead to boomerang dysplasia atelosteogenesis I and III and Larsen syndrome (55,56,57,58). Although mutation in filamin B can cause a complex syndrome but three novel missense mutations in this gene were established in the development of TEV (59). A deletion in *FLNB E1792del* is also documented in isolated club foot patients (60).

COL9A1

Type IX collagen proteins are produced by COL9A1 gene, which helps in strengthening the connective tissues, ligaments, tendon, cartilage, bone, and skin (61). A study on the Chinese population showed that COL9A1 rs35470562 variant may contribute to the development of the TEV (62). Liu et al demonstrated the high expression of a protein from COL9A1 gene located on the rs1135056 encoding region may increase the susceptibility to develop TEV (63).

Table 1. The most common genes involved in the development of TEV.

Genes	Associated with other phenotype	References
PITX- gene	Orofacial clefts Developmental hip dysplasia Patella hypoplasia, Tibial hemimelia, Preaxial polydactyly	39,40,41,42, 43,44,45
TBX4- gene	Skeletal dysplasia Pulmonary hypertension Coxodopattelar syndrome Amelia	46,47,48
HOX A	Severe limb and genital abnormalities.	52,53,54
FLNB	Dysplasia atelosteogenesis I and III and Larsen syndrome	55,56,57,58,60
COL9A1	Osteoarthritis Epiphyseal dysplasia, Stickler syndrome	61,62,63

DISCUSSION

Recently mutations in several candidate genes or SNPs have been hypothesized to be involved in the molecular genetic basis of the TEV, but the main mechanism remains elusive (10,64,65). TEV inheritance among heterogenic families shows that it is associated with multiple genetic factors. These genetic factors and their regulatory sequences are further influenced by a few environmental factors making their discovery more complicated. To know the common denominator a neurovascular hypothesis has been proposed. Spinal anomalies and spina bifida frequently seen in the TEV deformities are suggested to be associated with neurodegenerative disorders (66,67). A study on sciatic nerve defect in the chicken and mice model of TEV phenotype found that upregulation of *LIMK1* gene causes peroneal muscular atrophy (68). Vascular insufficiencies and occlusion of vascular tree at Sinus tarsi canal of the ankle and foot is found in limbs of patients affected by TEV (69,70). The finding of joint laxity in TEV patients supports the theory of connective tissue defect (68). Calcaneus and talus bone anomalies with reduced fetal movement also support the concept of physiological deformity of the foot (1,71). All these mechanisms for the development of TEV are related to the genes which are key players in the cytoskeleton formation. A healthy and genetically maintained cytoskeleton of feet during intrauterine life is important for the maturity of feet. Any disruption in the physical property or shape of the cytoskeleton of bones, muscles, ligaments, and soft tissues may lead to deformed foot.

Since its description by Hippocrates in 400BC, search for its possible causes was started. Data on segregation analysis suggested the "major genes" involved in the development of TEV. However, because of heterogeneity of the genetic causes the candidate gene approach has failed to reveal the exact number of causative variants in both isolated and syndromic TEV phenotypes. Moreover, Sadler et al classified the genetics of isolated and syndromic club foot and concluded that FLNB is involved in both types (72). As shown in table 2 genes involved in the club foot are also involved in the other

disease processes. Studies published on the management of club foot mentioned additional features appear even after doing surgical intervention in the clubfoot patients during their adulthood. (73). Case reported by Milenkovic. et al in a 56 year of age suggesting additional feature like degenerative changes in the hindfoot and thoracolumbar scoliosis with degenerative spondyloarthrosis in thoracolumbar spine segments (74). Sequencing analysis in one study failed to detect genetic variants in *TBX4, PITX1, HOXD12,* and *HOXD9* in two Pakistani families (75). They suggested that mutations may be present at the regulatory region of these genes. The literature review on a good number of studies proved that relapse of the disease process occurs even after corrective surgery because of tissue deposition indicating that pathobiological factors do persist and keep on causing the issues (76,77).

Recently more advanced techniques became available to get a promising result for the underlying cause of TEV. Among all the reported genetic studies using different techniques, the next generation sequencing (NGS) including whole genome sequencing and whole exome sequencing (WES) are getting popularity in common clinical diagnosis as well as in research on gene identification in inherited diseases. To get the most effective results it is useful to use the combination of these techniques with linkage analysis (78).

CONCLUSION

TEV is a multifactorial condition with genetic disruption of multiple molecules in the cytoskeleton effected by the exposure to adverse environmental factors as well as to genetic factors. The available literature shows deficit in high profile studies as many studies focusing on the genetic causes of this deformity lacked speculating harmony in proving exact genetic cause in both isolated and syndromic types of talipes equinovarus. Therefore, refinement of the existing genes of isolated clubfoot and clubfoot-associated phenotypes in early and late adulthood will lead to more effective genotype determination and it will improve the diagnostic capabilities and management options. Hence, large multiscale, multicenter collaborative studies using genetic techniques like GWAS with SNP scan for linkage analysis are required on families affected with TEV. Furthermore, inheritance pattern and penetrance will help to skim through novel genes involved in this disorder. Discovery of the genetic factors will revolutionize the management of the TEV and can eliminate the disability of the patients that often persists.

Nonetheless, we would like to put forward the hypothesis however that genes found to be implicated in club foot do not only cause club foot but will lead to multiple symptoms and the affected patients are suffering from syndromic club foot. So, we need to thoroughly investigate the affected individuals for the possibility of other syndromes. In other words, the classification of club foot into isolated and syndromic club foot needs to be revised.

AUTHOR CONTRIBUTIONS

Yasir Naseem khan wrote the initial draft of the article under the supervision of Mohammad Imad. Mohammad Imad has reviewed and suggested, and changes required for publications. Bothall authors contributed to and have approved the final article.

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