Journal of Clinical Orthopaedics and Trauma 35 (2022) 102064

Contents lists available at ScienceDirect

Journal of Clinical Orthopaedics and Trauma

journal homepage: www.elsevier.com/locate/jcot

Assessment of the COL9A1 Single Nucleotide Polymorphism with Severity of clubfoot in a paediatric population along with their biological mothers

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ARTICLE INFO

Article history:
Received 3 August 2022
Received in revised form
6 October 2022
Accepted 26 October 2022
Available online 29 October 2022

ABSTRACT

Background: A frequent birth abnormality known as congenital talipes equinovarus (CTEV) results in severe abnormalities of one or both feet. The risk of CTEV has been shown to be significantly influenced by genetics. It is a typical congenital malformation. Diseases involving the articular cartilage and COL9A1 polymorphisms are linked.

Materials and method: This case parent dyad research was conducted in a premier care medical and educational facility by the Departments of Paediatric Orthopedics and Biochemistry. Children who had been diagnosed with clubfoot as well as their biological mothers took part in the study. 125 kids were able to join in the research when all the screening, inclusion, and exclusion criteria were met. Baseline demographic information was collected, including the child's age and sex, the kind of clubfoot, any family members who have the condition, and whether or not the mothers smoke or drink. Pirani score is used to clinically evaluate every case. Only one peripheral blood sample was taken from each patient, including their Biological Mothers.

Results: out of 125 children enrolled with biological mothers, Col9A1 SNP rs1135056 is substantially related.

Conclusion: Additionally, patients with the GG genotype for rs592121 have a higher chance of developing CTEV than those with other genotypes. In this investigation, we found possible associations between COL9A1 gene polymorphisms in the mother and offspring with the risk of CTEV. Our research may help us comprehend the genetic makeup of CTEV better and lay the groundwork for creative intervention strategies.

1. Introduction

Idiopathic clubfoot, typically referred to as congenital talipes equinovarus, is the most common congenital abnormality in orthopaedics. Club foot, which is bilateral in around 50% of cases and affects one in every 1000 live births, is common.[1] Its origin may be influenced by a number of variables, including genetic predisposition, gestational abnormalities, and a range of histological abnormalities. But the exact cause is still a mystery.[2] This is a three-dimensional malformation that is readily noticeable at birth. The tibia is internally rotated, the ankle is equinus, the midfoot is cavus, the forefoot is adducted and supinated, and the heel is varus and internally rotated. There are a few hereditary abnormalities linked to CTEV that are linked to congenital malformations. It is necessary to conduct more research to determine the exact aetiology of isolated CTEV.[3] Genetic research is shedding light on key aspects of clubfoot's pathophysiology. A positive family history of clubfoot is present in around 25% of all individuals with isolated clubfoot, indicating that the disorder may have a genetic origin.[4] Recent research has revealed that variations in bone marrow hypoplasia can result from mutations in the collagen IX genes. These mutations may also be the genetic foundation for disorders connected to

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https://doi.org/10.1016/j.jcot.2022.102064
0976-5662/

articular cartilage, the risk of which has been linked to COL9A1 expression in earlier research. This gene's expression may change as a result of polymorphisms, which may then affect how the encoded protein functions. According to reports, multiple epiphyseal dysplasia, lumbar disc disease, and osteoarthritis can all be brought on by COL9A1 sequence variations. So far, only one study has looked at the relationship between the COL9A1 rs1135056 and rs35470562 polymorphisms and the risk of congenital talipes equinovarus. The severity of Pirani was connected with the gene polymorphism in the current study, which assessed the genetic polymorphism in children and their biological mothers in SNPs of COL9A1 variants rs135056 and rs59212.[5]

2. Methods

All children under the age of five who presented with clubfoot for the first time and their mothers were included in the study, which was conducted from March 2020 to December 2021 in the department of Paediatric Orthopedics at King George Medical University Lucknow in collaboration with the Department of Biochemistry. For the purpose of documentation, the baseline and sociodemographic information were recorded. After receiving their mothers' agreement, the research participants were enrolled before being asked to provide 2 ml of blood. Blood samples were obtained in simple vials and sent for COL9A1 gene SNP (Single Nucleotide Polymorphism) analysis. Pirani Scoring was used to assess the severity of clubfoot, and the findings were retrospectively analyzed.

2.1. Inclusion/exclusion criteria

All patients, regardless of sex or age group, who sought treatment for CTEV along with their natural mothers were counted as cases. Excluded instances included those that had undergone surgery, developed CTEV, had various congenital defects or syndromes connected with them, and those whose biological mothers were not accessible for inclusion.

2.2. Genomic DNA isolation and genotyping

Using the QI amp DNA kit (quigen, C A), DNA was extracted from the blood according to the manufacturer's instructions. The extracted DNA was measured, and then CARY 300 bio - UV/visible spectrophotometry was used to further verify for purity. By electrophoresis on a 0.8% agarose gel, the integrity of this DNA was examined. Col9A1(rs1135056 & rs592121) gene polymorphism was genotyped using polymerase chain reaction (PCR) and enzyme digestion (restriction fragment length polymorphism). Products of the polymerase chain reaction were first digested with ***HinfI*** and then separated by electrophoresis on 3% agarose gel. Ethidium bromide staining and UV transillumination were then used to see them. A large variety of ds-DNA fragments were sized and roughly quantified on the gel using a 100bp DNA ladder.

2.3. Statistical analysis

Categorical variables were shown as percentages and numbers (%). The presentation of continuous variables was as mean ± SD. Calculated odds ratios with 95% confidence intervals for certain factors and their importance. When the data sets were not normally distributed, the Unpaired *t*-test/Mann-Whitney Test was used to compare quantitative variables between two groups. For non-parametric data, the Anova/Kruskal Wallis test was used to compare quantitative variables between three groups. The appropriate Chi-Square test or Fisher's exact test was used to compare qualitative variables. A 0.05 p-value was regarded as statistically significant. The Statistical Package for Social Sciences (SPSS) version 22.0 was used to conduct the analysis after the data were input into an MS EXCEL spreadsheet.

3. Results

In this prospective case control mother child dyad study, the patients with clubfoot those are below 5 years of age were taken as control and the biological mothers of these children were taken as control. The aim of the study was to examine the COL9A1 gene polymorphism in mother and child to observe the genetic association of the gene, based on this the following observations were collected is as follows. In patients with CTEV afflicted newborns and their biological mothers, this study intends to look at the expression of the COL9A1 gene and the distribution of the gene's Single Nucleotide Polymorphism (SNP). The study analyzed the genomic SNP and frequency of SNP of COL9A1 gene and its expression in both mother and child the study also correlated the severity of Pirani scoring with that of col9a1 gene polymorphism. The mean age of patient was 70.84[SD:63.08] days and mean age of the mother age was 26.12[SD:5.09] Out of 125 Children enrolled 63 (50.40%) were boy children and 62(49.60%) were girl children. The mean age of boys was 76.25(SD-62.12) in days with mean pirani of 1.89(SD-1.17), while the mean age of girls were 1.95(SD-0.99) in days and the mean pirani was 1.95(SD-0.99). Most of the case were having bilateral deformity were 105(84%) while the 20(16%) case were of unilateral deformity. The family history of clubfoot was noticed in 52(41.6%) of the cases, and the addiction used by the mothers was seen in 8(6.4%) of children. The genotyping distribution of COL9A1 gene polymorphism of SNP rs113056 is AA 78(62.40%), AG 32(25.60%) and GG 15(12%) and rs592121 AA 54(43.2%), AG 57(45.6%) and GG 14(11.2%) in Children, the genetic distribution in Mothers was for rs1135056 AA 55(44%), AG 54(43.2%), GG 16(12.8%) and for rs592121 it is AA 55(44%), AG 59(47.2%) and GG 11(8.8%). The Minor allele frequency of the SNPs was greater in G allele 70.4% in cases for rs1135056 as compared to control group which was 64%(χ2-1.16; p-value:0.17) while the frequency of rs592121 was higher in A allele 65.6% as compared to control 62.4%(χ2-0.27; p = 0.34) however the there was no significant difference found among alleles of mother and child (Table 1).

The genotype distributions of *COL9A1* rs1135056 and rs592121 were presented in Table 2 By chi-square test, there was significant difference in the genotype frequencies of AA, AG and GG in *COL9A1* rs1135056 between patients with congenital talipes equinovarus and control subjects (χ2-9.63, *P*-0.0008), but no significant difference was found in the genotype distributions of *COL9A1* rs592121 (χ2-0.40, *P*-0.86).(Table 2).

Using independent sample *t*-test, SNP rs1135056 cases with AA genotype has compared AG genotype had a score of (0.26; p = 0.80), when compared with AG and GG score was(t-0.81; p-0.42), and AA and GG was (t-0.80; p-0.42), SNP rs592121 cases with AA genotype compared to AG had score of (t-0.24; p-0.80), when

Table 1
Minor allele frequency.

SNPs	Allele	Cases (n = 125)		Control (n = 125)		
		No.of carrier	%	No.of Carrier	%	P-Value[a]
rs1135056	A	37	29.6%	45	36%	
	G	88	70.4%	80	64%	0.17
rs592121	A	82	65.6%	78	62.4%	
	G	43	34.4%	47	37.6%	0.36

[a] Chi-Square.

Table 2
Genotype distribution of COL9A1 rs1135056 and rs592121 in study population.

SNP	Cases	%	Control	%	Chi	P value
rs1135060					9.63	0.008*
AA	78	62.40	55	44		
AG	32	25.60	54	43.20		
GG	15	12	16	12.80		
rs592121						
AA	54	43.20	55	44	0.40	0.82
AG	57	45.60	59	47.20		
GG	14	11.2	11	8.8		

compared with AG and GG(t-1.26; p-0.09) which was weakly or borderline significant, and AA and GG (t-1.27; p-0.20). The mean and SD for the SNPs is given in (Table 3).

Using the multinomial regression GG genotype of rs592121 was observed to be at increased risk of CTEV, however it didn't show any significant difference(OR-1.36, 95%CI-0.54-3.10; P-0.56), when compared to other genotypes, other SNPs, and other genotypes doesn't found to be at increase risk of CTEV (Table 4).

4. Discussion

In this Mother-Child dyad study, the role of two important polymorphisms (rs592121 and rs1135056) was evaluated. The results of this study evaluated that there is risk of developing CTEV in patients with rs592121 gene polymorphism. Isolated club foot's aetiology hasn't been well researched and is still unknown.

Previous studies have indicated that both genetic and environmental factors contribute to the risk of congenital talipes equinovarus (CTEV). The *COL9A1* gene encodes one of the three alpha chains of type IX collagen, which is a key collagen component of hyaline cartilage. A study by Jianwu Zhao et al., indicated that there was a combined effect between genetic and environmental factors in CTEV and suggested that there should be a genetic counselling of families who are at risk of developing it.[6]

Multiple lines of evidence imply that environmental triggers are at work in a genetically susceptible person.[7–10] The genetic and environmental influences have not been fully understood. Mutations in COL9A1 have been linked to multiple epiphyseal dysplasia in a Finnish population, according to Czarny-Ratajczak et al.[9]

This collagen is quantitatively a small component that is in charge of covalently cross-linking to the surface of type II collagen fibrils, and it is encoded by the COL9A1, COL9A2, and COL9A3 genes.[11] Previous experimental research has shown that polymorphisms in the collagen IX gene in people and animals are associated to osteochondropathy and are correlated with the functional lifetime of joint cartilages.[12,13] According to reports, COL9A1 is linked to cartilage tissue and contributes to the integrity of the articular cartilage's internal environment.[14]

In a research conducted in a Chinese population, Liu et al. featured that patients with club foot had significantly elevated

Table 3
Mean of pirani score in COL9A1 gene polymorphism.

SNP	Cases	Pirani Score	
rs1135056		Mean	SD
AA	78	1.94	1.04
AG	32	1.99	1.18
GG	15	1.70	1.14
rs592121			
AA	54	1.99	1.20
AG	57	1.94	1.94
GG	14	1.57	1.57

Table 4
Association between COL9A1 rs1135056, and rs592121 Polymorphisms and risk of Congenital Talipes Equinovarus.

SNP	PATIENT	CONTROL	OR	CI	P VALUE
rs1135056					
AA	78	55	1.0	0.64–1.86	0.73
AG	32	54	1.1	0.50–2.40	0.77
GG	15	16	0.89	0.41–1.96	0.77
rs592121					
AA	54	55	0.98	0.58–1.67	0.95
AG	57	59	1.0	0.60–1.70	0.96
GG	14	11	1.3	0.54–3.10	0.56

levels of COL9A1 mRNA expression than healthy individuals.[15] 25 children with Club Foot and 5 healthy controls participated in a study by Liu et al., and they discovered that the COL9A1 rs1135056 polymorphism was connected to the aetiology of this condition.[16] In co-dominant, dominant, and recessive models, Huibin et al. (2016) discovered that the COL9A1 rs1135056 polymorphism was linked to an elevated risk of club foot, whereas the rs592121 polymorphism was not.[20]

Numerous environmental and lifestyle variables, including prenatal growth retardation, maternal smoking, soft tissue contracture, vascular abnormalities, skeletal dysplasia of bone, and neuromuscular illness, among others, contribute to the development of club feet.[17] Kimura et al. discovered the COL9A1 in 1989, and Warman et al. determined its location to be 6q12-q13. There are two transcripts for this gene, and their respective complete lengths of cDNA are 2985 and 3704 base pairs, or 38 and 32 exons, respectively.[18] The articular cartilage is protected and stabilised by COL9A1 by combining matrix metalloproteinases, limiting growth factors, and blocking the receptor on the surface of the cartilage cell membrane[19]

In a case-control research, Huibin et al. (2016) investigated the relationship between COL9A1 polymorphism and the onset of club foot. They found that the COL9A1 rs1135056 polymorphism was linked to a higher risk of this condition in co-dominant, dominant, and recessive models[20]

Numerous illnesses, including primary osteoarthritis, hip osteoarthritis, degenerative lumbar spinal stenosis, and various epiphyseal dysplasia mutations, have been linked to the COL9A1 rs1135056 polymorphism, according to earlier research.[21–23] In a study of a Finnish population, Noponen-Hietala et al. discovered that COL9A1 mutation had a significant role in the aetiology of lumbar spinal stenosis.[24] On a research by Alizadeh et al. in a population from the Netherlands, it was discovered that the COL9A1 gene was closely associated with hip osteoarthritis susceptibility. [25]

A Case control study conducted by Fang Li et al., in 2016, to evaluate the relationship between COL9A1 rs1135056 and rs35470562 and susceptibility to congenital talipes equinovarus they observed that the GG genotype of COL9A1 rs1135056 was associated with an increased risk of congenital talipes equinovarus, their study suggested that the GG genotype and G allele of COL9A1 rs1135056 polymorphism could influence the development of congenital talipes equinovarus in a Chinese population.[26]

According to Zhengdong Wang et al., in 2013, stated that the cause of the high expression levels of COL9A1 in muscle samples of ICTEV patients remains unclear. Specific in vitro and in vivo studies have revealed that the SRY (sex-determining region Y)- box 9 (SOX9) transcription factor binds to consensus sequence pairs in the upstream region of the COL2A1 and COL9A1 genes and regulates expression of COL9A1 and was found to be regulated by SOX9 and thus found the correlation between the two.[27]

5. Conclusion

According to our findings, COL9A1 gene polymorphism in Mother and Children affects both the risk of congenital talipes and the severity of the condition. The etiopathogenesis of clubfoot is heavily influenced by genetic factors. Although various research has looked at the genetic basis of CTEV, there is still no agreement on one or more target genes. This is because there are numerous genes that usually contribute to CTEV development. Recent research has mostly concentrated on genetic and environmental variables. Future research should be done on the link between the two parameters to validate the candidate genes for improved findings and clinical outcomes, since our study only focused on COL9A1 gene polymorphism. Our findings show how the COL9A1 gene variation affects clubfoot in both mothers and their offspring. Despite improvements in screening and therapy, a genetic approach to this illness might help physicians better understand the condition and develop more effective treatment regimens. We now know a lot more about the genetic makeup of the Indian population. More research with a bigger sample size is necessary to confirm our findings.

Author contribution

In the conceptualization and design of the study, Ajai Singh, Archana Raikwar, and Abbas Ali Mahdi were engaged. Data gathering was done by Manish Yadav and Archana Raikwar. Data analysis and interpretation were the responsibilities of Archana Raikwar, Manish Yadav, and Ajai Singh. The text was written with the assistance of Ajai Singh, Archana Raikwar, Manish Yadav, and Abbas Ali Mahdi after conducting a literature study. Critical manuscript changes were performed by Ajai Singh and Abbas Ali Mahdi.

Funding

The research received the grant from Uttar Pradesh Council of Science & Technology(UP-CST).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Archana Raikwar reports financial support was provided by State Council of Science and Technology Uttar Pradesh.

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