Universidad de Cuenca

Facultad de Odontología

Especialización en Ortodoncia I Cohorte

"ASSOCIATION OF TGFB3 AND FGFs GENE POLYMORPHISMS WITH CLEFT LIP WITH OR WITHOUT CLEFT PALATE. A SYSTEMATIC REVIEW"

Trabajo de titulación previo a la obtención del título de Especialista en Ortodoncia

Autor:

Andrea Soledad Quizhpi Quito

Director:

Diego Mauricio Bravo Calderón

ORCID: 0000-0003-1813-8051

Cuenca, Ecuador

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Resumen

Objetivo: Realizar una revisión sistemática de la posible asociación entre los polimorfismos de los genes del factor de crecimiento transformante B3 (TGFB3) y de los factores de crecimiento de fibroblastos (FGFs) y la fisura labial no sindrómica con o sin paladar hendido (FLNS/P). **Métodos:** Dos revisores seleccionaron de forma independiente los estudios examinando todos los títulos y resúmenes. Los estudios se incluyeron si cumplían los siguientes criterios: el resultado de interés era FLNS/P; los polimorfismos estudiados eran TGFB3 y FGF; presentaban datos suficientes, es decir, frecuencia alélica/genotípica entre casos y controles; o su Odds Ratio (OR) con intervalo de confianza (IC) del 95%. La calidad de los estudios se evaluó de forma independiente mediante una valoración del riesgo de sesgo para los estudios de asociación genética. **Resultados:** Basándonos en los criterios de inclusión hemos seleccionado un total de 6 artículos (4 para TGFB y 2 para FGF). Particularmente para el gen TGFB hemos encontrado resultados significativos en el exón 4 en la variante g.15812T>G, y en el SNP rs2300607 A/T, en la distribución entre casos y controles. Por otro lado, para el gen FGF hemos observado un estadísticamente significativo en el genotipo rs34010 CA. **Conclusiones:** Ninguna de las variaciones genéticas que muestran asociación está verificada en diferentes poblaciones, por lo tanto, no existe suficiente validación científica respecto a la asociación entre el polimorfismo de TGFB y FGF y FLNS/P. Los hallazgos de los diferentes estudios sugieren la necesidad de nuevas investigaciones con muestras compuestas por un mayor número de individuos en diferentes poblaciones, las cuales deben ser realizadas con todos los estándares para estudios genéticos, permitiendo así comprender las bases moleculares de la enfermedad.

Palabras clave: gen TGFB3, genes FGFs, polimorfismo, fisura labial no sindrómica con o sin paladar

Abstract

Objective: To conduct a systematic review of the possible association between transforming growth factor B3 (TGFB3) and fibroblast growth factors (FGFs) gene polymorphisms and non-syndromic cleft lip with or without cleft palate (NSCL/P). Methods: Two reviewers independently screened studies by examining all titles and abstracts. Studies were included if they met the following criteria: the outcome of interest was NSCL/P; the polymorphisms studied were TGFB3 and FGF; they presented sufficient data, i.e., allele/genotype frequency between cases and controls; or their odds ratio (OR) with 95% confidence interval (CI). Study quality was independently assessed by a risk of bias assessment for genetic association studies. **Results:** Based on the inclusion criteria we have selected a total of 6 articles (4 for TGFB and 2 for FGF). Particularly for the TGFB gene we have found significant results in exon 4 in the variant g.15812T>G, and in the SNP rs2300607 A/T, in the distribution between cases and controls. On the other hand, for the FGF gene we observed a statistically significant in the genotype rs34010 CA. **Conclusion:** None of the genetic variations that show association is verified in different populations, therefore, there is not enough scientific validation regarding the association between TGFB and FGF polymorphism and NSCL/P. The findings of the different studies suggest the need for further investigations with samples composed of a larger number of individuals in different populations, which should be performed with all the standards for genetic studies, thus allowing an understanding of the molecular basis of the disease.

Keywords: TGFB3 gene, FGFs Genes, Polymorphism, Nonsyndromic cleft lip with or without cleft palate

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Introduction

Clefts of the lip and/or palate are among the most common congenital defects worldwide, characterized by their high genetic component and affect 1 in every 700 live births, [1] of which 70% of the cases are non-syndromic and 30% are syndromic. [2-3-4] Syndromic forms are usually caused by chromosomal aberrations or monogenic diseases, while non-syndromic forms derive from the interaction between genetic and environmental factors. [2-3]

The upper lip and palate are formed by the fusion of components of the frontonasal process and the maxillary processes; and failures in these events during development cause the appearance of the cleft lip with or without cleft palate. [5-6] Thus, murine and knock-out mouse models have allowed the identification of several genes and even different chromosomal loci as involved in cleft formation. [7-8]

For example, the Transforming Growth Factor-B3 Gene (TGFB3, 14q24.3) is expressed in the epithelial cells of the medial border of the palatal processes and participates in events including: membrane degradation, metalloproteinase-mediated remodeling of the extracellular matrix and epithelial-mesenchymal transformation; which together generate the confluence of processes during palate formation. [9] Interestingly, studies in ethnically contrasting populations have verified positive findings between TGFB3 gene alterations and nonsyndromic cleft lip with or without cleft palate (NSCL/P). [10-11] Most of these studies have been performed in white and Asian populations; thus, reflecting the heterogeneity of the cleft phenotype as well as variability in environmental risk factors. [10-11-12]

Similarly, it has been verified that the Fibroblast Growth Factors (FGFs) and its receptors play a crucial role through the regulation of cell proliferation, differentiation and motility which are necessary for the development of the palate and upper lip. [13] Mutations in the genes coding these molecules have been shown to contribute significantly to the development of syndromic orofacial clefts, such as Apert syndrome. [14] In addition, case-control studies have shown evidence of association of single nucleotide polymorphisms (SNPs) in FGFs genes with susceptibility or decreased risk of NSCL/P, depending on the polymorphism and the gene analyzed. [15]

However, no single candidate gene has been consistently identified in all studies. [10-11-12-15] Therefore, the present study aims to perform a systematic review of the possible association between polymorphisms in TGFB3 and FGFs genes and NSCL/P.

Materials and Methods

Based on the guidelines for preferred reporting elements for systematic reviews in the RevMan manual, [16] the specific PICO question [17] "What is the association of polymorphisms in TGFB3 and FGFs genes with nonsyndromic cleft lip with or without cleft palate?", was developed. being:

(P) Participants: Patients with cleft lip and palate.

(I) Interventions of interest: TGFB3 and FGFs genes.

(C) Controls: groups of patients without cleft lip and palate.

(O) Outcome measures: would be the association of polymorphism with cleft lip and palate.

The relevant studies for the present systematic review were identified in the digital databases of PubMed, Scielo, BVS, AJODO, Google Scholar, and the last search in all databases was performed on January 31, 2022. The search terms used were the following: "gene polymorphism and cleft lip", "gene polymorphism and cleft palate", "gene polymorphism and cleft lip and cleft palate", "genetic and cleft lip", "genetic and cleft palate".

Two reviewers (ASQ-Q, DMB-C) independently selected studies by examining all titles and abstracts. Any association study in humans, and meeting the following criteria were included: the outcome of interest was NSCL/P, the polymorphisms studied were TGFB3 and FGFs, presenting sufficient data, i.e., allele/genotype frequency between cases and controls; or their odds ratio (OR) with 95% confidence interval (CI). Reference lists of selected articles were also reviewed to identify additional relevant publications. On the other hand, animal studies, family-based studies, case reports, publications with insufficient information such as letters from the author were excluded and, specifically for the TGFB3 gene, studies prior to December 2013 were not considered because the most recent systematic review on the subject was published on the aforementioned date. [18] In sequence, the lists of articles were compared, validated and disagreements were resolved by consensus among the reviewers. Figure 1 summarizes the literature search strategy according to PRISMA guidelines. The pattern of the present systematic review was customized to summarize mainly relevant data.

Data Extraction:

A data extraction sheet was developed in Microsoft Excel, from the selected studies, the information was extracted from each study including: title, author, year of publication,

database, type of gene, SNPs, type of study, and being genetic in nature, it was checked if they performed Hardy-Weinberg Equilibrium (HWE).

Risk of Bias Assessment

Study quality was independently assessed using a risk of bias assessment for genetic association studies. [19-20] Briefly, the following domains were analyzed: selection bias, information bias, and Hardy-Weinberg equilibrium (HWE) assessment, whereby, each item was scored as 2; 1 or 0; corresponding to low risk of bias, high risk of bias, or unclear/insufficient information, respectively. Total scores ranged from 0 (worst) to 13 (best).

Results

The search in the databases PubMed, Scielo, BVS, AJODO, Google Scholar allowed us to locate a total of 373 studies, being that, after the exclusion of 33 duplicate records, 340 remained for further review. Of these 340 articles, 334 studies were discarded because after reading the abstracts, these articles clearly did not meet the inclusion criteria (Figure 1).

The reasons for ineligibility were mainly because of non-relevant genes or populations and studies published in years prior to the range established in the present review. Thus, there were finally 6 publications that were included in the systematic review, 4 for the TGFB3 gene and 2 for the FGFs genes. The PRISMA flow chart provides an overview of the selection process of the polymorphisms studied and the number of studies selected (Figure 1).

Figure 1. Flow chart for item selection

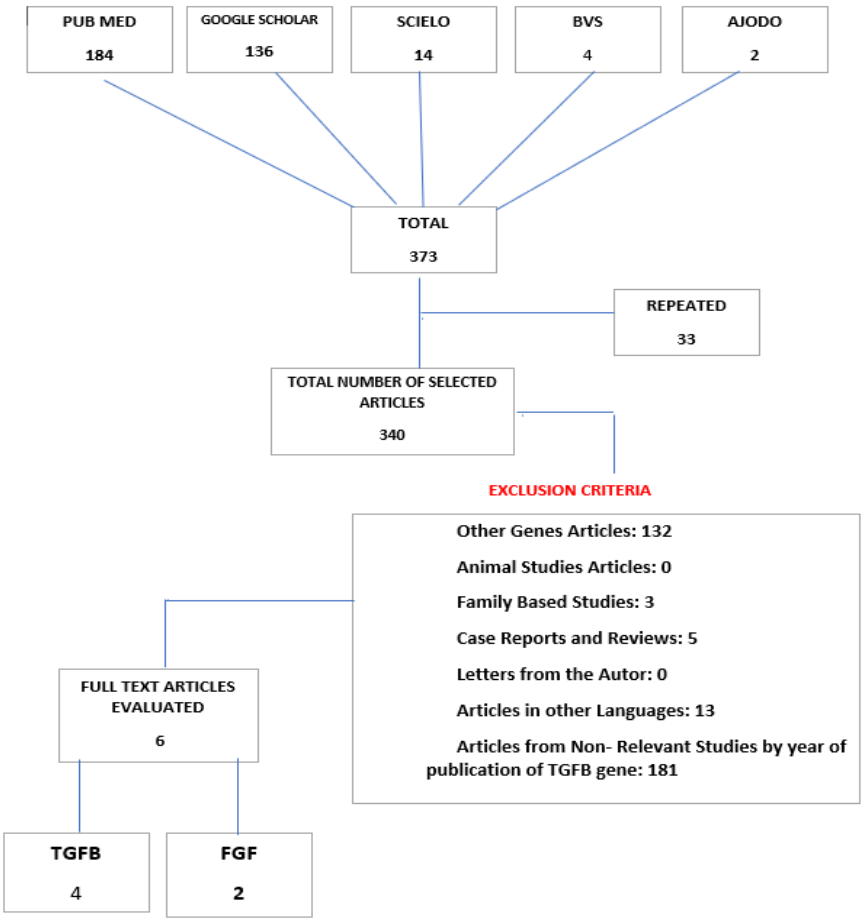

Table 1 describes the general characteristics of the selected studies. Among the selected articles, five had a case-control design, and 1 was of cross-sectional type. Regarding ethnicity, two studies were conducted in Asian, two in European and two in Latino populations. Particularly for the TGFB3 gene, genetic variations located in exons 1 to 7 [10-21] and the SNPs: rs2300607, rs2268625, [11] and rs369477964 rs375973742, rs117462711, [22] were studied.

In the study conducted by Ghazali.2014 exons 1 to 7 were analyzed, finding significant results for exon 4, indicating that individuals harbored the g.15812T.G. variant with an OR of 0.313 and 95% CI: 0.082 - 1.191, P=0.089; while, for the g.15966A.G variant with an OR of 0.356 and 95% CI: 0.092- 1.382. p=0.315. (Table 1).

On the other hand, the study by Deniz Aslar Oner.2017 revealed 2 polymorphisms in exon 1: Pro10Leu and Arg25Pro, analyzing a total of 80 case and 125 control patients. The differences in frequencies of CT and TT genotypes in the case group were not statistically significant compared to controls, OR=2.18; 95% CI= 0.94 - 5.6; P= 0.06 and OR=1.46; 95% CI= 0.67 -

3.19; P=0.315, respectively. For the Arg25Pro polymorphism there were 74 subjects out of the group of 80 cases. The frequency of the GC genotype was higher compared to the control group OR=1.31 95% CI:0.48-3.64, P=0.79, but was not statistically significant. The frequency of C alleles was higher in cases than in controls OR = 0.29, 95% CI:0.48-3.52, p=0.8, but the differences were not significant. (Table 1)

SNP rs2300607 A/T, from the study by Aljabeiti 2017, had a total of 237 cases and 168 controls, the $\chi 2$ test revealed a statistically significant difference in the distribution of genotypes between cases and controls with the following p-values=0.0206; $\chi 2$=0.7626. (Table 1). Likewise for SNP rs2268625 C/T, 237 cases and 98 controls were analyzed, indicating that the distribution of rs2268625 C/T SNP genotypes differed significantly between cases and controls with a value of p=0.041. (Table 1).

In the study by Kumari 2018, the SNP rs3917219, was genotyped in 162 cases and 108 controls but revealed no association with FLAPNS, The SNP rs369477964 and rs375973742, were present in 2 cases and 2 controls, of the 162 cases and 108 controls analyzed, and finally the SNP rs117462711 registered only 2 cases, obtaining a value of P<0.001 (Table 1).

Table 1. General characteristics of the included studies about TGFB genes.

Title	Author	Country	Ethnia	Study Design	Number of Cases	Number of Controls	Snp/ Exon	Locus	Variant/ Allele	OR	95 % IC	p-value
Screening of Transforming Growth Factor Beta 3 and Jagged2 Genes in the Malay Population with Nonsyndromic Cleft Lip with or Without Cleft Palate	Ghazali et al. 2014	Malasia	European	Cross-Sectional	96	96	EXON 1-7	TGFB3	g.15812T.G	0.313	[0,082-1,191]	p=0,089
									g.15966A.G	0.356	[0,92-1.382]	p=0,315
Association Between the Transforming Growth Factor Beta 1 Gene Polymorphisms and Turkish Patients with Nonsyndromic Cleft Lip With/Without Cleft Palate	Oner et al. 2016	Turqui	European	Case Control	80	125	EXON 1	TGFB3	Pro10Leu	CT 2,18 TT 1,46	CT [0,94-5,06] TT [6,67-3,19]	CT p=0,06 TT p=0,66
									Arg25Pro	GC 1,31 CC 0,29	GC [0,48-3,64] C [0.48–3.52]	GC p=0,79 C p=0,80

Association of TGFβ3 variants with Nonsyndromic Cleft Lip and Palate in Guatemalan Population	Aljabeiti et al.2017	Guatemala	American	Case Control	237	266	rs2300607 rs2268625	TGFB3	rs2300607 A/T			p=0.020
									rs2268625 C/T			p=0,041
TGFB3, MSX1, and MMP3 as Candidates for NSCL+P in an Indian Population	Kumari et at.2018	India	Asia	Case Control	162	108	rs3917219 rs369477964 rs375973742 rs117462711	TGFB3	rs3917219			p=0,008
									rs369477964 rs375973742 rs117462711			p<0,001

While, for FGFs the following SNPs were studied: rs6790664, rs11717284, rs1464942, rs12106855, rs1875735, [24] rs34010 and rs13317. [15] Regarding the FGF SNPs, of the genotypic frequencies observed in the study conducted by Sibele Nascimento de Aquino, with 300 cases and 365 controls, none showed an association with NSCL/P, likewise the controls did not show significant differences. In the SNPs studied by Zahra Rafiqdoost, a statistically significant difference was observed between cases and controls in the rs34010 CA genotype with the following OR values = 0.29, 95% CI = 0.16-0.55, p= 0.001. With respect to the FGF rs13317 AG variant, no statistically significant values were found between the genotypic frequencies of patients and controls in the models analyzed (Table 2).

Table 2. General characteristics of the included studies about FGF genes.

Title	Author	Country	Ethnia	Study Design	Number of Cases	Number of Controls	Snp/ Exon	Locus	Variant/ Allele	OR	95 % IC	p-value
Polymorphisms in FGF12, VCL, CX43 and VAX1 in Brazilian patients with nonsyndromic cleft lip with or without cleft palate	Aquino et al.2013	Brasil	American	Case Control	300	385	rs6790664 rs11717284 rs1464942 rs12106855 rs1875735	FGF	rs6790664	1,11	[0,87-1,41]	p=0,56
									rs11717284	0,74	[0,58-0,95]	p=0,91
									rs1464942	0,94	[0,72-1,24]	p=0,5
									rs12106855	1,12	[0,87-1,43]	p=0,53
									rs1875735	0,93	[0,72-1,18]	p=0,76
Investigation of FGF1 and FGFR gene polymorphisms in a group of Iranian patients with nonsyndromic cleft lip with or without cleft palate	Rafiqdoost et al.2014	Irani	Asia	Case Control	100	100	rs34010	FGF1	rs34010 C/A	0,29	[0,16-0,55]	p=0,001
							rs13317		rs13317 A/G	0,84	[0,46-1,56]	p=0,588

As shown in Table 3, the quality analysis verified a score that varied between 6 and 10 points for the studies on the TGFB gene, and as for HWE only 50% performed it.

Table 3. Bias Risk Analysis of included studies about TGFB Genes.

PARAMETERS	ARTICLES ON TGFB GENE POLYMORPHISMS											
	Ghazali et al. 2014			Oner et al. 2016			Aljabeiti et al. 2017			Kumari et al. 2018		
	2	1	0	2	1	0	2	1	0	2	1	0
REPRESENTATIVENESS OF THE CASES												
Consecutives/randomly selected from the population of cases with a clearly defined sampling frame												
Consecutive/randomly selected from the case population without a clearly defined sampling frame or broad inclusion/exclusion criteria.		x			x			x			x	
No selection method is described												
REPRESENTATIVENESS OF THE CONTROLS												
Controls were drawn consecutively/randomly from the same sampling frame (neighborhood/community) as cases.												
Controls were drawn consecutively/randomly from a different sampling frame as cases.		x			x			x			x	
Not described												
DETERMINATION OF FLAPS												
Clearly described objective criteria for the diagnosis of NSCL/P	x			x			x			x		
Diagnosis of NSCL/P by patient self-report or patient history												
Not described												
VERIFICATION OF CONTROLS												
Controls were tested to rule out NSCL/P.	x			x						x		
Controls were subjects who did not report NSCL/P; no objective tests								x				
Not described												
EVALUATION OF THE PARTNERSHIP												
Evaluate the association between genotypes and NSCL/P with appropriate statistics and adjustment for confounding factors.	x									x		
To evaluate the association between genotypes and NSCL/P with appropriate statistics without adjustment for confounding factors.					x			x				
Inappropriate statistics used												
GENOTYPING TEST*												
Genotyping performed under the "blind" condition without knowledge of the sample group												
Not blinded or not mentioned			x			x			x			x
HARDY-WEINBERG EQUILIBRIUM												
Hardy-Weinberg equilibrium in the control group				x						x		
Hardy-Weinberg disequilibrium in the control group												
Without testing Hardy-Weinberg equilibrium			x						x			
TOTAL, RISK OF BIAS	8			9			6			10		

On the other hand, the value ranged between 9 and 10 points for the investigations on FGFs genes, and all FGFs polymorphisms measured HWE (Table 4).

Table 4. Bias Risk Analysis of included studies about FGF Genes.

PARAMETERS	ARTICLES ON FGF GENE POLYMORPHISMS					
	Aquino et al. 2013			Rafiqdoost et al. 2014		
	2	1	0	2	1	0
REPRESENTATIVENESS OF THE CASES						
Consecutives/randomly selected from the population of cases with a clearly defined sampling frame						
Consecutive/randomly selected from the case population without a clearly defined sampling frame or broad inclusion/exclusion criteria.		x			X	
No selection method is described						
REPRESENTATIVENESS OF THE CONTROLS						
Controls were drawn consecutively/randomly from the same sampling frame (neighborhood/community) as cases.						
Controls were drawn consecutively/randomly from a different sampling frame as cases.		x			X	
Not described						
DETERMINATION OF FLAPS						
Clearly described objective criteria for the diagnosis of NSCL/P	x			x		
Diagnosis of NSCL/P by patient self-report or patient history						
Not described						
VERIFICATION OF CONTROLS						
Controls were tested to rule out NSCL/P.	x			x		
Controls were subjects who did not report NSCL/P; no objective tests						
Not described						
EVALUATION OF THE PARTNERSHIP						
Evaluate the association between genotypes and NSCL/P with appropriate statistics and adjustment for confounding factors.						
To evaluate the association between genotypes and NSCL/P with appropriate statistics without adjustment for confounding factors.		x			X	
Inappropriate statistics used						
GENOTYPING TEST*						
Genotyping performed under the "blind" condition without knowledge of the sample group		x				
Not blinded or not mentioned						x
HARDY-WEINBERG EQUILIBRIUM						
Hardy-Weinberg equilibrium in the control group	x			x		
Hardy-Weinberg disequilibrium in the control group						
Without testing Hardy-Weinberg equilibrium						
TOTAL, RISK OF BIAS	10			9		

DISCUSSION

The findings found in each study analyzed are detailed below. Once the articles have been selected, it should be noted that, for the TGFB gene, two papers, despite not performing the HWE balance, we consider that they have a high score in the quality analysis; this is because they meet other methodological parameters but emphasize the need to perform the HWE analysis in genetic studies.[23]

Although nonsyndromic cleft lip with or without cleft palate is among the most common congenital defects, the exact genetic and environmental events associated with its pathogenesis are still unknown. [11-24] Identification of the genetic alterations causing NSCL/P could lead to a better understanding of the molecular basis of the disease, and of craniofacial development. [22-24]

The present systematic review focuses on 2 genes, TGFB3 and FGF, which are involved in facial development, and some of their variants have been shown to be risk factors for NSCL/P, although to different degrees. [10-22]

Particularly for the TGFB3 gene, Gazhali et al.2014, considered the g.15812T>G variant as a polymorphism because it was present in more than 1% of the study population, and also indicates that the side most affected by clefting was the left side, which was affected 2.8 times more frequently than the right, possibly because blood is more abundant on the right side than on the left [10].

On the other hand, Oner et al. 2016, indicates that no statistically significant differences were found in the Pro10Leu and Arg25Pro variants in exon 1. [21] However, it is demonstrated that genetic evidence of TGFB is functionally required for secondary palate formation. [10-21]

Likewise, in the study conducted by Aljabeiti et al.2017, in the population of Guatemala the results showed, for the first time, the association between TGFB3 rs2268625 C/T polymorphism and NSCL/P, which coincide with the results found in the study Ichikawa et al in a Japanese population in the rs2300607 A/T polymorphism. [25] In this study, comparisons of allele frequencies for both mutations were made, finding a higher proportion of mutated alleles in cases compared to controls. [11] Therefore, it is suggested that both mutations of the TGFB3 gene are involved in the etiology of NSCL/P. [11]

In relation to FGFs genes, in the study conducted by Aquino et al. 2013, in a Brazilian population, no significant association of FGF12 with NSCL/P was found, the author indicates

that this is due to the sample size used in the study; therefore, it is possible that associations of polymorphisms and cleft risk were overlooked, indicating that their study was based on samples from European studies, [24] and also performed additional analyses on the dominant and recessive genetic models which also revealed no differences in the distribution between groups.[24]

In the SNPs studied by Zahra Rafiqdoost et al.2014, a statistically significant difference was observed between NSCL/P patients and the control group in SNP rs34010. [15] The FGF1 rs34010 CA genotype had a markedly higher frequency in controls compared to NSCL/P; and in the CA + AA genotype it was more frequent in the control group. [15] With respect to the FGF1 rs13317 AG variant, no statistically significant values were found between genotypic frequencies. [15] Highlighting the protective role of the FGF1 rs34010 C/A polymorphism, therefore, the FGFs signaling pathway has constant functions in lip and palate morphogenesis, and the perturbation of its expression patterns sometimes leads to cleft pathogenesis. [15]

In summary, understanding the etiology of nonsyndromic CL/P patients may help to predict and prevent its occurrence in the future. Specifically, the present review did not verify significant associations between gene polymorphisms TGFB3 and FGF with non-syndromic CL/P, and these results may be based on the limited number of studies exploring these relationships and the ethnic variability of the populations among the investigations analyzed.

Moreover, facial development and its alterations, such as non-syndromic CL/P, are not only determined by genes, but also by their interaction with environmental factors such as habits, nutrition and trauma. [26]

On the other hand, it should be noted that in this review age or gender was not considered as a determining factor because they are birth defects and measurements of genetic expressions are taken among affected individuals.

CONCLUSION

None of the genetic variations that show association is verified in different populations, therefore, there is not enough scientific validation regarding the association between TGFB and FGF polymorphism and NSCL/P. Therefore, the findings of the different studies suggest the need for new research with samples composed of a larger number of individuals in different populations, which should be performed with all the standards for genetic studies, thus

allowing to have certified information that would allow a better understanding of the molecular basis of the disease.

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