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The Role of Transforming Growth Factor-Beta, Fibroblast Growth Factor, Platelet-derived Growth Factor, Epidermal Growth Factor, Insulin-like Growth Factor, Vascular Endothelial Growth Factor, and Sonic Hedgehog in the Non-syndromic Cleft Lip With or Without Cleft Palate Development: A Scoping Review

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Abstract: Non-syndromic cleft lip with or without cleft palate (NSCLP) birth defect, it imposes an enormous stress on society and requires nutrition, dental, speech, behavioural, and surgical therapies. The NSCLP multifactorial aetiology, including the environment and genetic factors. The environment and genetic factors affect the cellular mechanism, cell proliferation, cell differentiation, and cell migration and signalling pathways. Genetic growth factors including Transforming Growth Factor-Beta (TGF-β), Fibroblast Growth factors (FGFs), Platelet-derived Growth factors (PDGFs), Epidermal Growth factor (EGF), Insulin-like growth factors (IGF), Vascular Endothelial Growth factor (VEGF), Sonic Hedgehog (SHH). The study aims to understand the role of the growth factors "TGF-β, FGFs, PDGFs, EGF, IGF, VEGF, and SHH" in NSCLP development. Preferential Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) standards were followed when performing this scoping review. The 942 articles were extracted, and the following inclusion and exclusive criteria 43 articles were eligible for review. Twenty-seven studies identify 26 genes and 25 single-nucleotide polymorphisms (SNPs)/variants of the growth factors that are a significant risk for NSCLP development. In conclusion, the analysis of diverse populations and growth factors including TGF-β, FGFs, PDGFs, EGF, IGF, VEGF, and SHH were associated with NSCLP. The growth factors were involved in the cellular mechanism, cell proliferation, cell differentiation cell migration and signalling pathways that lead to the pathogenesis of NSCLP.

Keywords: Non-syndromic cleft lip with or without cleft palate, TGF-β, FGFs, PDGFs, EGF, IGF, VEGF, SHH.

***Abstrak*:** *Cacat lahir bibir sumbing non-sindrom dengan atau tanpa celah langit-langit (NSCLP), hal ini menimbulkan tekanan yang sangat besar pada masyarakat dan memerlukan terapi nutrisi, perawatan gigi, bicara, perilaku, dan bedah. Etiologi NSCLP multifaktorial, termasuk lingkungan dan faktor genetik. Lingkungan dan faktor genetik mempengaruhi mekanisme seluler, proliferasi sel, diferensiasi sel, serta migrasi sel dan jalur sinyal. Faktor pertumbuhan genetik termasuk Transforming Growth Factor-Beta (TGF-β), Fibroblast Growth Factors (FGFs), Platelet-derived Growth Factors (PDGFs), Epidermal Growth Factor (EGF), Insulin-like Growth Factors (IGF), Vascular Endothelial Growth faktor (VEGF), Sonic Hedgehog (SHH). Penelitian ini bertujuan untuk memahami peran faktor pertumbuhan "TGF-β, FGFs, PDGFs, EGF, IGF, VEGF, dan SHH" dalam pengembangan NSCLP. Standar Item Pelaporan Preferensial untuk Tinjauan Sistematis dan Meta-analisis (PRISMA) diikuti saat melakukan tinjauan pelingkupan ini. 942 artikel diekstraksi,*

dan kriteria inklusi dan eksklusif berikut 43 artikel memenuhi syarat untuk ditinjau. Dua puluh tujuh penelitian mengidentifikasi 26 gen dan 25 single-nucleotide polymorphisms (SNP)/varian faktor pertumbuhan yang merupakan risiko signifikan terhadap perkembangan NSCLP. Kesimpulannya, analisis beragam populasi dan faktor pertumbuhan termasuk TGF-β, FGFs, PDGFs, EGF, IGF, VEGF, dan SHH dikaitkan dengan NSCLP. Faktor pertumbuhan terlibat dalam mekanisme seluler, proliferasi sel, diferensiasi sel, migrasi sel, dan jalur sinyal yang mengarah pada patogenesis NSCLP.

Kata kunci: *Non-syndromic cleft lip with or without cleft palate, TGF-β, FGFs, PDGFs, EGF, IGF, VEGF, SHH.*

List of abbreviation: BMP4: Bone Morphogenetic Protein, CL: Cleft Lip; CNC: Cranial Neural Crest; CP: Clept Plate; CTGF or CCN2: Connective Tissue Growth Factor; EGF: Epidermal Growth Factor, FGF: Fibroblast Growth Factors, FGFR: Fibroblast Growth Factors Receptor; GLI3: GLI Family Zinc Finger; GWAS: Genome-Wide Association Study; HEPM: Human Embryonic Palatal Mesenchyme; IGF: Insulin-like Growth Factors; IGF1R: IGF1-Receptor; MTHFR: Methylenetetrahydrofolate Reductase; NS: Not Significant; NSCLO: Non Syndromic Cleft Lip Only; NSCLP: Non Syndromic Cleft Lip And Palate; NSCPO: Non Syndromic Cleft Palate Only; PDGFRA: Platelet-Derived Growth Factors Receptor-Alpha; PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; PRISMA: Preferential Reporting Items for Systematic Reviews and Meta-analyse; PICO: Population, Intervention, Comparison, and Outcomes; SNP: Single Nucleotide Polymorphisms; SHH: Sonic Hedgehog; SCLP: Syndromic Cleft Lip And Palate; TGF-β: Transforming Growth Factor-Beta; VEGF: Vascular Endothelial Growth Factor; VEGFA: Vascular Endothelial Growth Factor-A; WES: Whole-Exome Sequencing.

INTRODUCTION

Non-syndromic cleft lip with or without a cleft palate are the birth defect, incidence range is 1/700 worldwide (Shi *et al*. 2021). The non-syndromic cases are 70% and the syndromic cases are 30%. The NSCLP are multifactorial aetiology, environmental and genetic factors (Li *et al*. 2016[a]). Additionally, genetic factors are a potential risk for the development of NSCLP pathogenesis. According to the genome-wide association study (GWAS) of NSCLP, approximately 40 genes and loci are involved in the NSCLP pathogenesis (Gaczkowska *et al.* 2018). China GWAS identified 14 new loci that are significantly associated with NSCLP development (Yu *et al*. 2017).

Several candidate growth factors and genes in the NSCLP development provide significant evidence including TGF-β (Avasthi *et al*. 2022; Smane & Pilmane 2018; Živicová *et al*. 2017), FGFs (Rafiqdoost *et al*. 2014; Wang *et al*. 2013), PDGFs (Choi *et al*. 2009; Li *et al*. 2020; Raju *et al*. 2020), EGF (Avasthi *et al*. 2022; Tufekci *et al*. 2018), IGF (Sidhom & Pilmane 2017), VEGF (Sun *et al*. 2021), SHH (Dąbrowska *et al*. 2022; Vaivads *et al*. 2021). Growth factors are involved in the cellular mechanism, cell proliferation, cell differentiation, and cell migration. Regulate and stimulate the multi-signalling pathways, SNPs and variants disrupt the signalling pathways (Eswarakumar *et al*. 2005; Li *et al*. 2020; Sidhom & Pilmane 2017).

Northern Indian studies results show that the SNP of *MTHFR, BMP4* and *TGFA*, are at risk for NSCLP pathogenesis (Avasthi *et al*. 2022). According to (Wang *et al*. 2013) research finding FGF and FGFR gene family associated with NSCLP (Wang *et al*. 2013). *SHH* gene variation risk for NSCLP pathogenesis in Polish and Latvia populations (Dąbrowska *et al*. 2022; Vaivads *et al*. 2021). The various population studies of the *PDGF-C* gene SNP (rs28999109) were significantly associated with NSCLP in China, the United States, Spain, Turkey, Colombia and India (Choi *et al*. 2009). (Sun *et al*. 2021) identify the *VGFA* mutation effect on the cell function, cell migration and palate development.

The objective of the review understands the intricate roles that growth factors TGF-β, FGFs, PDGFs, EGF, IGF, VEGF, and SHH play in triggering the development of NSCLP. It seeks to clarify the individual and collective role growth factors paly in the cellular mechanisms, cell proliferation, cell differentiation cell migration and signalling pathways underpinning the pathogenesis of NSCLP. The current scoping review provides an insightful view of the growth factors, genetics variation and signalling pathways related to NSCLP pathogenesis. Twenty-seven studies give evidence of the genetic polymorphism significance in the cellular mechanism, signalling pathways and cell development, which has a fundamental role in the formation of the NSCLP and scoping review gives meaningful information about the diverse population understanding of the genetic issue.

MATERIAL AND METHODS

Review Design

A comprehensive examination of existing research was conducted according to the guidelines outlined by the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) framework (PRISMA; framework provides the checklist for the author-included and excluded

studies, to enhance the transparency of the study) (Faldini *et al*. 2022; Ramadhani *et al*. 2022).

Inclusion criteria considered papers describing genetic studies including Growth Factors TGF-β, FGFs, PDGFs, EGF, IGF, VEGF, and SHH associated with NSCLP development published in English peer-reviewed journals and full-text articles. Exclusive literature reviews, animal studies, not related to NSCLP, syndromic cleft lip and palate (SCLP), other genetic studies not assocated with NSCLP, abstract only, books and books chapters, systemic review and meta-analyses. The included article fulfils the PICO (Population, Intervention, Comparison, and Outcomes) criteria for the scoping review (PICO; the framework used for the clinical research questions and guide the development of research studies). The included studies are case-control, case-parent tries, cross-sectional, carries-free, cohort, surgical and molecular experimental study, tissue reconstructive molecular study, reproducible experimental model study, GWAS, histopathological molecular study, genetic analysis of case report, cellular and molecular experimental study, physical anatomical/ bioinformatics & molecular analysis. Respectively prospective research and retrospective research were used in these investigations.

Search strategy

Scopus, PubMed and Springer were searched over the years 2013-2022 to identify eligible studies in the English literature describing the genetic and growth factors associated with NSCLP development. The online literature search was conducted in August 2023 by three reviewers (IU, ES and AMM). The authors stated the following research question: "*What is the current state of research on the role of growth factors in the development of the NSCLP?*" This research question matched all four PICO concepts. Subsequently, the following key concepts were formulated "**:** non-syndromic cleft lip and palate", "non-syndromic cleft lip only", "non-syndromic cleft palate", and "growth factor" and various alternative terms were considered for each key concept to include the maximum number of articles available in the literature about the research question. Details on the search strategy are in the Supplementary Table S1.

The search was conducted using a combination of the following search criteria: "non-syndromic cleft" OR "cleft" OR "cleft lip" OR "cleft palate" OR "cleft lip and palate" OR "NSCLP" OR "NSCLO" OR "NSCPO" AND "growth factor" OR "TGF-β" OR "FGF" OR "PDGF" OR "EGF" OR "IGF" OR "VEGF" OR "Sonic Hedgehog" OR "SHH".

Study Selection

Identified citations were uploaded to the Mendeley-Desktop-1.19.8-win32 (Elsevier; Amsterdan, Netherland). Studies screened by titles and abstracts, we acquired and examined the full-text articles. Additionally, we conducted a manual examination of the reference lists in each of the pertinent articles to uncover any potentially overlooked eligible papers and duplicate articles were removed. The process of selecting studies adhered to the PRISMA flowchart, as depicted in Figure 1.

Data Extraction

Data extraction was carried out by two reviewers (IU, ES) using a standardized data collection form. Subsequently, the accuracy of the data was independently verified by three reviewers (IU, ES, AMM), with any inconsistencies in the results being subject to analysis and discussion. The study design's extraction of data, type of study design, age and gender, ethnicity (population), investigated growth factor, associated genes and SNPs, risk and significance, tools and techniques, and type of biological sample. All data is summarised in Table 1.

RESULT AND DISCUSSION

Included studies

In the initial search from 2013 to 2022, 10 years' duration of the literature total of 942 articles were identified, the 152 depulicate were remove the remaning 790 articles. The removal of the duplicate 152 and 700 other studies was excluded the final eligible 90 for full text review. After the full-text studies, the 29 abstracts only, 8 animal studies, 2 review articles, 7 not related to growth factor and one syndromic study were excluded. The literature flow of selection is shown on the PRISMA flow chart Figure 1. Eligible 43 articles were summarized in Table 1.

The eligible 43 studies, fulfil the inclusion criteria for the review. These studies include the case-control (Antunes *et al*. 2013; de Aquino *et al*. 2013; de Araujo *et al*. 2016; Avasthi *et al*. 2022; Bagheri *et al*. 2017; Blanco *et al*. 2017; Falagan-Lotsch *et al*. 2015; Junaid *et al*. 2018; Küchler *et al*. 2014; Kumari *et al*. 2019; Li *et al*. 2016[b]; Nasroen *et al*. 2016; Paranaíba *et al*. 2013; Rafiqdoost *et al*. 2014; Tufekci *et al*. 2018; Wang *et al*. 2017; Yan *et al*. 2017) , Case-parent trios (Kim *et al*. 2015; Mossey *et al*. 2017; Suazo *et al*. 2018; Wang *et al*. 2013; Yu *et al*. 2015) , case-control & case-parent study (Xu *et al*. 2014), Cross-sectional (Ghazali *et al*. 2014), Cohort & carries-free (Antunes *et al*. 2015), Cohort (Conte e*t al*. 2016), bioinformatics & molecular analysis (Wang *et al*. 2016), histopathological & surgical molecular experimental studies (Pilmane *et al*. 2021[a]; Smane & Pilmane 2018; Pilmane *et al*. 2021[b]; Sidhom & Pilmane 2017; Sun *et al*. 2021; Vaivads *et al*. 2021; Živicová *et al*. 2017), reproducible experimental model study (François *et al*. 2017), GWAS & case-control (Li *et al*. 2018), GWAS & case-parent trios , whole exome sequencing analysis (Li *et al*. 2020), genetic analysis

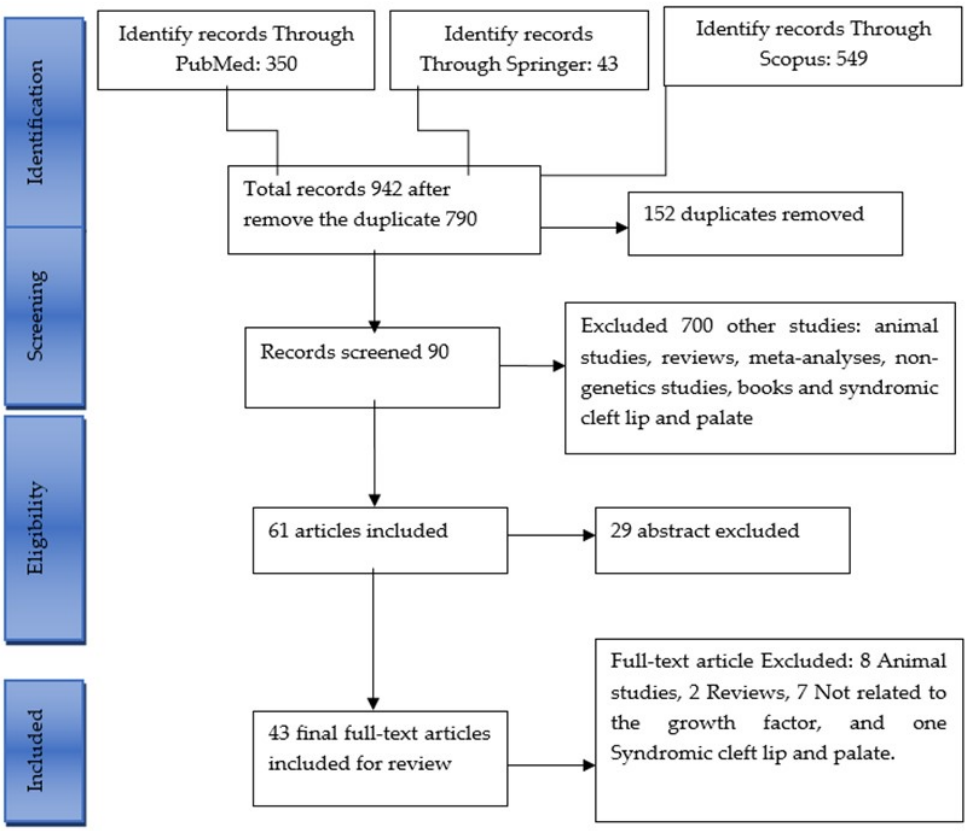

Figure: 1 Flow chart of PRISMA

of case report (Sufiawati *et al*. 2020), cellular and molecular experimental study (Sun *et al*. 2021) and GWAS & cohort study (Dąbrowska *et al*. 2022).

The studies analysed the sample size were inculeded one sample until 803 sample size, also included bioinformatics and cell lines analysis. Sample were isolated from the oral cells, peripheral blood, oral mucosa, buccal epithelial cells, lip mucosa tissue, skin sample (ear, nose, throat), whole blood, saliva, mouthwash sample, cell lines, human embryonic palatal mesenchyme (HEPM) cells, human embryonic kidney, buccal swabs, and bioinformatics analysis database data Table 1. The review were discuss growth factor, TGF-β, FGFs, PDGFs, EGF, IGF, VEGF, and SHH, in the NSCLP pathogenesis.

The eligible studies were conducted in various populations including Brazilian (Antunes *et al*. 2013; Antunes *et al*. 2015; de Araujo *et al*. 2016; Falagan-Lotsch *et al*. 2015; Küchler *et al*. 2014; Machado *et al*. 2016; Paranaíba *et al*. 2013) Maryland, Taiwan, Singapore, Korean (Kim *et al*. 2015; Wang *et al*. 2013), Malaysian (Ghazali *et al*. 2014), Chinese (Li *et al*. 2018; Li *et al*. 2019; Sun *et al*. 2021; Yan *et al*. 2017; Yu *et al*. 2015), Iranian (Bagheri *et al*. 2017; Rafiqdoost *et al*. 2014) , Turkish (Oner & Tastan 2016; Tufekci *et al*. 2018), European (Mossey *et al* 2017), Czech (Živicová *et al*. 2017), France (François *et al*. 2017), Indonesian (Nasroen *et al*. 2017), Chilean (Blanco *et al*. 2017; Suazo *et al*. 2018), Polish (Dąbrowska *et al*. 2022) and Indian (Avasthi *et al*. 2022; Junaid *et al*. 2018; Kumari *et al*. 2019). The diverse population studies collaborate in order to provide meaningful information that enhances our understanding of the subject matter at issue.

Characteristics of included Growth Factors

The included studies 6 genes realted to TGF-β, and 25 SNPs discussed in the 16 studies in Table 1. The TGF-β, genes included namely *BMP4, TGFB-1, TGFB3, BMP2/4, TGFB2, and BMP7. BMP4* (rs17563) (Antunes et al. 2013), *BMP7* (rs6127973) (Li *et al*. 2016[b]), *TGFB1 (Živicová et al. 2017), TGFB2* (Li *et al*. 2016[b]), *TGFB3, BMP2/4* (Smane & Pilmane 2018) were significant in the NSCLP patients. Furthermore, FGFs growth factor related 9 studies discussed *FGF2, FGFR1, FGF19, FGF12, TGFA, FGF3, FGF1, FGF5, FGF9, FGF8, FGF10, FGFR2* genes and 36 SNPs Table 1. The FGFs growth factor in the NSCLP pathogenesis significant genes and SNPs were include namely *FGFR1* (rs6987534, rs6474354, rs10958700), FGF19 (rs3737463, rs948992, rs1307968, rs1320706,

Table: 1 Growth Factor associated with NSCLP, (NS= not significant)

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
1	Case-control 367/413	Not available	Brazilian	TGF-β	*TGFβ3*	rs34019007, rs4252315	need further studies	PCR-RFLP	Oral Mucosa cells /Genomic DNA	(Paranaíba *et al.*)	2013
2	Case-control 383/450	Mean age± SD (17.09±11.4) Male 204(53.3) female 179(46.7)	Brazilian	TGF-β	*BMP4*	rs17563	Significant	Real-time PCR/TaqMan Method	Oral cells/Genomic DNA	(Antunes *et al.*)	2013
					TGFβ3	rs2268626	NS	Real-time PCR/TaqMan Method	Oral cells/Genomic DNA		
3	Case-parent trios /297	Male 185 female 112	Maryland 96, Taiwan 146, Singapore 35, Korea 40	FGF	*FGF2*	rs308395	NS	Illumina's assay	Peripheral blood /Genomic DNA	(Wang *et al.*)	2013
					FGFR1	rs6987534, rs6474354, rs10958700	significant	Illumina's assay	Peripheral blood /Genomic DNA		
					FGF19	rs3737463, rs948992, rs1307968, rs1320706, rs1789364	Significant	Illumina's assay	Peripheral blood /Genomic DNA		

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
4	Case-control 300/385	Male 52.2% Female 44.8%	Brazilian	FGF	*FGFi12*	rs11717284, rs6790664, rs1464942, rs12106855, rs 1875735	NS	TaqMan 5' -exonuclease allelic discrimination assay	Oral Mucosa cells /Genomic DNA	(de Aquino *et al*.,)	2013
5	Case-control 166/150 and case-parent 271	Not available	Chinese	FGF	*TGFA*	c.3851C rs11466285, c.3822A rs3771523	Significant	Microarray, PCR, Hybridization	Peripheral blood /Genomic DNA	(Xu *et al*.)	2014
6	Case-control 497/823	mean age 15.04 years (1 month to 60 years), not to mention gender distribution	Brazilian	FGF	*FGF v 3*	rs4980700	significant	RT-PCR, TaqMan method	oral cells/ Genomic DNA	(Küchler *et al*.)	2014
			Brazilian		*FGF3* interact with *PAX9* gene	rs2073242	Significant	RT-PCR, TaqMan method	oral cells/ Genomic DNA		
7	Case-control 100/100	Average age 12.12 (range: 1-54 years), Male 61 Female 39	Iranian	FGF	*FGF1*	rs34010	significant	TaqMan, tetra-ARMS-PCR	Peripheral blood /Genomic DNA	(Rafiqdoost *et al*.)	2014

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
					FGFR1	rs13317	significant	TaqMan, tetra-ARMS-PCR	Peripheral blood /Genomic DNA		
8	Cross-sectional study 96/96	Not available	Malay	TGF-β	*TGFβ3*	g.15812, g.15966	NS	PCR,	Peripheral blood /Genomic DNA	(Ghazali *et al*.)	2014
9	Case-parent trios /221	Male 80, Female 141	Chinese	TGF-β	*BMP7*	rs12438, rs6099486, rs6127973, rs230188, rs6025469	rs6127973 are Significant	Protein precipitation method, PCR, sequencing	Peripheral blood /Genomic DNA	(Yu *et al*.)	2015
10	Case-control 218/253	Mean age 17.2±10.8 years), Male 114 (52.3), Female 104 (47.7)	Brazilian	EGF	*EGF +61*	EGF+61 A>G	NS	PCR-RFLP	Oral Mucosa cells /Genomic DNA	(Falagan-Lotsch *et al*.)	2015
11	Cohort, 187 (38.5%) carries-free, 229(61.5%) caries	Male 197, Female 170	Brazilian	TGF-β	*TGFβ3*	rs2268626	NS	RT-PCR, TaqMan method	Buccal epithelial cells/Genomic DNA	(Antunes *et al*.)	2014
				TGF-β	*BMP4*	rs17563	NS	RT-PCR, TaqMan method	Buccal epithelial cells/Genomic DNA		

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
12	Case parent tries/ 218/119	Male 90, Female 52	Korean	EGF	*TGFA*	rs3821272, rs930655, rs3732247, rs765871, rs3771498, rs3771497, rs3755377, rs3771485, rs11466212, rs3771475	Not concluded in the Korean population	Illumina's assay	Peripheral blood /Genomic DNA	(Kim *et al*.)	2015
13	Cohort	Not available		FGF	*FGF2*		Significant	Bioinformatics tools / Linux BED tools	database data used for bioinformatics analyses	(Conte *et al*.)	2016
14	Case-control 602/605	Not available	Chinese	FGF	*FGF2*	rs1048201	Significant	Conventional phenol-chloroform method, Quantitative PCR, cell culture, transient co-transfection and dual-luciferase reporter assay	Peripheral blood /Genomic DNA	(Li *et al*.)	2016[b]
				FGF	*FGF5*	rs3733336	Significant		Peripheral blood /Genomic DNA		
					FGF9	rs546782	Significant		Peripheral blood /Genomic DNA		

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
15	Case-control 80/125	0-10 years	Turkish	TGF-β	*TGFβ1*	Pro10Leu	NS	PCR, Automated sequencer	Peripheral blood /Genomic DNA	(Oner & Tastan)	2016
						Arg25Pro	NS		Peripheral blood /Genomic DNA		
16	Bioinformatics and molecular analysis studies			TGF-β	*TGFβ2*		Significant (upregulate in the NSCLP)	Bioinformatics analysis, Cytoscape	Gene Expression Omnibus Database/ sven dental pulp stem cell samples	(Wang *et al.*)	2016
					TGFβ3		Significant (upregulate in the NSCLP				
17	Case-control/Individual ancestry proportion/ 803/599	not mention	Brazilian	FGF	*FGFR1*	rs7829058	NS	RT-PCR, TaqMan method	Buccal mucosa cells/ Genomic DNA	(Machado *et al*.)	2016
18	Case-control 182/355	Male 99, Female 83	Brazilian	TGF-β	*BMP4*		Significant	TaqMan Open Array system	Peripheral blood leukocytes /Genomic DNA	(de Araujo *et al*.)	2016

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
				SHH	*SHH*		Significant				
19	Surgical molecular experimental study	Male 5, Female 2, age 2 to 6 months	Latvia	TGF-β	*TGFB1*		NS	Immunohisto-chemistry	Lip Mucosa tissue	(Sidhom & Pilmane)	2017
				IGF	*IGF-1*		Significant				
				IGF	*IGF-1R*		Significant				
20	Case-parent triads/ 1020	Not available	European	EGF	*TGFA*	rs1348813	NS	PCR, ABI sequencer	Peripheral blood / buccal cell sample	(Mossey *et al*.)	2017
21	Tissue reconstructive molecular study	Neonates 24 sample age (1-6 days), older children 8 sample age (3 -6 months), control 8 sample adult age (23-77 years)	Czech	TGF-β	*TGFβ1*		The signalling of *TGFβ1* has a significant role in the NSCLP development.	cell culture, western blot analysis, ELISA, Microarray analysis,	Skin samples from the Ear, Nose, and Throat	(Živicová *et al*.)	2017

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
22	Case-control 113/ 209	Not available	Iranian	EGF	*TGFA*	rs731236	NS	PCR,	Peripheral blood samples/ Genomic Data	(Bagheri *et al*.)	2017
23	Case-control 285/315	Not available	Chinese	EGF	*EGF61*	rs4444903	Significant	mini sequencing (SNaPshot)	Peripheral blood/ genomic DNA	(Yan *et al*.)	2017
24	Case-control 504/455	Not available	Chinese	SHH	*GLI3*	rs3801161, rs7785287	rs3801161, significant	MassARAY platform	Peripheral blood/ genomic DNA	(Wang *et al*.)	2017
25	Reproducible experimental model study	Age average 44 (13-77) days	France	TGF-β	*TGFB3*		upregulate (the significance in the NSCLP not concluded	Histologically/ qPCR	tissue skin and muscle	(François *et al*.)	2017
					TGFB2		upregulate (the significance in the NSCLP not concluded				

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
					TGFB1		Downregulate (the significance in the NSCLP not concluded				
				VEGF	*VEGF*		Downregulate (the significance in the NSCLP not concluded				
					VGFA		Downregulate (the significance in the NSCLP not concluded				
					VGFR2		Downregulate (the significance in the NSCLP not concluded				
				FGFs	*FGF2*		Downregulate (the significance in the NSCLP not concluded				

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
				PDGF	*PDGFRA*		Downregulate (the significance in the NSCLP not concluded				
					PDGFC		Downregulate (the significance in the NSCLP not concluded				
26	Case-control 152/164	Female 38% Male 62%	Chilean	TGF-β	*BMP4*	rs2855532, rs762642, rs1957860	haplotype-based *BMP4* and *IRF6* significant in the NSCLP	PCR-RFLP			
					TGFB3	rs3917201, rs2268625, rs2268626	NS		Not mention		
27	Case-control 31/35	Not available	Indonesian	TGF-β	*TGFB3*	SfaN1	NS	PCR-RFLP	Peripheral blood samples/ Genomic Data	(Nasroen *et al*.)	2017

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
28	GWAS, case-control 504/455	Not available	Chinese	EGF	*EGFR*		EGFR interact with NHTN1, AP2B1 and NTRK1 gene, induce the develop of craniofacial	Affymetrix Axiom Genome-Wide CHB1 & CHB2 Array Plates	not mention	(Li *et al*.)	2018
29	Case-parent trios 152	38% Female, 69% no family history	Chilean	TGF-β	*BMP4*	rs2855532, rs762642, rs1957860	NS	PCR,	not mention	(Suazo *et al*.)	2018
					TGFB3	rs2268625, rs3917201, rs2268626	rs2268625 interact with MSX1 SNP rs6446693				
30	Case-control, 46/46	0-24 months	Indian	EGF	*TGFA*		not concluded	PCR-RFLP	Peripheral blood samples/ Genomic Data	(Junaid *et al*.)	2018
31	Case-control 70/85	Male: 42, Female 43	Turkish	EGF	*TGF-alpha/HinfI*		significant	PCR	Whole blood samples/ Genomic Data	(Tufekci *et al*.)	2018
32	Histopathological / Molecular studies	Male: 16, Female: 6	Latvia	TGF-β	*BMP2/4*		significant	Histological staining/ Immunohistochemistry	Tissue sample	(Smane & Pilmane)	2018
					TGFB3		significant				

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
33	GWAS/ Case-Parent Trios / 896	Not available	Chinese	FGF	*FGF8*		significant	Cordell's method	Whole blood, saliva, mouthwash sample/ genomic DNA	(Li *et al*. 2019)	
					FGF10	rs2330542, rs4866891, rs1011814, rs2121875, rs11750846	significant		Whole blood, saliva, mouthwash sample/ genomic DNA		
					FGFR1	rs7012413,	significant		Whole blood, saliva, mouthwash sample/ genomic DNA		
					FGFR2	rs2420946, rs100706, rs1047111, rs11199916	NS		Whole blood, saliva, mouthwash sample/ genomic DNA		
34	Case-control association study 245/201	Age: 3 months to 9 years	Indian	TGF-β	*TGFB3*	rs117462711	not concluded	Sequencing, SSCP, PCR-RELP	Peripheral blood samples/ Genomic Data	(Kumari *et al*. 2019)	

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
35	Whole-exome sequencing		Chinese study	PDGF	*PDGFC*		Significant inhibits the cell migration of the mutated PDGFC	Cell culture, siRNA sequence, siRNA transfection, qRT-PCR, Cell viability test kit- 8 (CCK-8) assay, Wound healing assay	HOK cell lines	(Li *et al.* 2020)	
36	Genetic analysis of Case report	Age, mother; 29 years, baby; newborn	Indonesian	EGF	*TGFA*	BamHI - rs11466297, RsaI- rs3732248	NS	PCR-RFLP, sequencing	Whole blood/ genomic DNA	(Sufiawati *et al.*)	2020
37	Histopathological / Molecular studies	Newborns	Latvia	TGF-β	*TGFB-1*		Significant interaction with cytokines	ELISA, RIPA	lip tissue sample	(Pilmane *et al.*)	2021[b]
38	Surgical molecular experimental study, 10 patients / 5 controls	Not available	Latvia	SHH	*SHH*		NS, However, the SHH correlate with the development of the cleft lip	immunohistochemical method	Mucosa tissue	(Vaivads *et al.*)	2021
39	Surgical molecular experimental study, 14 patients	Female 2, male 10,	Latvia	FGF	*FGFR1*		Significant role in the cellular proliferation	immunohistochemical method	Lip Mucosa tissue	(Pilmane *et al.*)	2021[a]

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
					bFGF or FGF2		NS				
					FGFR2		Significant role in the cellular proliferation				
40	Cellular and Molecular Experimental Study		Chinese study	vascular endothelial growth factor	*VEGFA*	C.733 T>C	significant	Cell culture, plasmid construction, transient transfection, Western-bolt, ELISA, proliferation assay and iCELLigence real-time cell analysis system (RTCA), Wound-healing assay, cell cycle&apoptosis experiments, RNA-sequencing, RNA isolation, RT-PCR, ALP-analysis, Quantitative alizarin red s staining	Human embryonic palatal mesenchyme (HEPM) cells, Human embryonic kidney 293 (HEK-293), MC3T3 cell line	(Sun *et al.*)	2021
41	Two-generation family study physical, anatomical and molecular study/ bioinformatic analysis	Not available	Chinese study	vascular endothelial growth factor	*VEGFA*	C.733 T>C	significantly pathogenic in two-generation family	Whole-exome sequencing (WES)	peripheral blood from the case and buccal swabs from control/ Genomic DNA	(Sun *et al.*)	2020

No	Study design	Age (Mean/Range) Gender	Ethnicity	Growth factors	Genes analysed	SNPs	Result	Technique	Biological sample	Authors	Year
42	GWAS/ cohort 135	Male; 83, Females 52, age (1-15 years)	Polish	FGF	*FGF4*	c.460G>A	significant	Next-generation sequencing	Peripheral blood/ genomic DNA	(Dąbrowska *et al*.)	2022
					FGF8	c.599A>G	significant				
					FGF12	c.FGF12	significant				
				SHH	*SHH*	c.787C>G	significant				
43	Case-control 200/200	Age: 5 to 7 years	Indian	TGF-β	*BMP4*	rs17563	significant	RELP, Sanger sequencing,	Peripheral blood/ genomic DNA	(Avasthi *et al*.)	2022
				EGF	*TGFA*	rs11466297	significant				

rs1789364), FGF3 (rs4980700), FGF1 (rs34010 C/A), FGFR1 (rs13317 A/G), FGF2 (rs1048201), FGF5 (rs3733336), FGF9 (rs54682), FGF8 (c.599A>G), FGF10, FGFR2, FGF4 (c.460G>A), and FGF12 (c.FGF12) Table 2.

The PDGFs two studies discussed the PDGFC and PDGFRA, one study not conclude the role of PDGFC and PDGFRA in the NSCLP pathogenesis (François *et al*. 2017), and another study identified the significance of the PDGFC gene in NSCLP pathogenesis (Li *et al*. 2020). Ten studies analysed 4 genes and 22 SNPs of the EGF the details on the Table 2. The significant were EGF61 (rs4444903), TGF-alpha/HinfI, TGFA (c.3851C, rs11466285, c.3822A rs3771523, rs11466297), and EGFR role in the NSCLP development. One study discussed the IGFs one gene IGF1 and IGF-1 receptor (IGF-1R) both are significant in the cleft lip development (Sidhom & Pilmane 2017). VEGF discuss 3 studies VEGFA (c.733T>C), VGFR2 and VGFA. VEGFA (c.733T>C) two studies identify the significance for the NSCLP pathogenesis (Sun *et al.* 2020; Sun *et al*. 2021). The SHH growth factor were analyse 4 studies two genes and three SNP. The SHH (c.787C>G) and GLI3 (rs380116) were significantly involved in the NSCLP pathogenesis Table 2.

The role of the Growth Factor in the NSCLP development

TGF-β

TGF-β further investigation related genes and SNPs. The *Bone Morphogenetic protein-4 (BMP4)* gene investigated in the Indian, Chilean, Brazilian. The role of the *BMP4* gene involve in the *BMP4* signalling pathway. The result shows the significant role the development of the craniofacial formation stages, the palatogenesis, facial structures and fusion. The mutation in the *BMP4* gene significantly disrupt the *BMP4* signalling pathway the risk for the development of NSCLP pathogenesis. Brazilian, Indian studies identify the *BMP4* SNP (rs17563), (P=0.00009, P=0.005), significance in the NSCLP pathogenesis (Antunes *et al*. 2013). However, in the Chilean population *BMP4* SNP (rs285532, rs762642, rs1957860) were no significance association with NSCLP (Suazo *et al*. 2018).

Chilean population haplotype-based study of the *BMP4* SNP (rs285532, rs762642, rs1957860) and *IRF6* interaction P=0.023 have significant role in the NSCLP development (Blanco *et al*. 2017). The *BMP2/4* and *BMP7* are also the BMP family gene, potential role in cartilage formation and craniofacial development. China researchers analyse five SNP of the *BMP7* gene rs6127973 P=0.0061 significantly involve in the NSCLP development (Yu *et al*. 2015). *BMP2/4* regulate the normal tissue function. Moreover, Latvia researcher Pilmane identified *BMP2/4* P=<0.001 significantly involve in the cleft lip and palate development (Smane & Pilmane 2018).

TGFB-1, 2,3 genes are involved in the TGF-B signalling pathway. TGF-B family gene regulate the cell growth, embryonic development and craniofacial formation. The variation in these genes induce the risk of NSCLP development. Czech and Latvia researcher identify the role of *TGFB-1* gene interacting with cytokines and variation risk for the NSCLP development (Pilmane *et al*. 2021[a]; Živicová *et al*. 2017). However, the Franch study not concluded the role of *TGFB-1* (François *et al*. 2017). Moreover, Turkish research Oner & Tastan analyze the *TGFB-1* (Pro10Leu, Arg25Pro) were not associated with NSCLP (Oner & Tastan 2016). Bioinformatics and molecular analysis of *TGFB-2* and *TGFB3* gene expression are significantly upregulated in NSCLP (Wang *et al*. 2016). Chilean population study was identified gene-gene (GxG) interaction between *TGFB3* (rs2268625) and *MSX1* (rs6446693) P=0.038 were significant risk for NSCLP development (Suazo *et al*. 2018). Latvia researcher Pilmane histopathological analysis *TGFB3* P=<0.001 significant for the NSCLP (Smane & Pilmane 2018). The study of Brazilian *TGFB3* (rs34019007, rs4252315) (Paranaíba *et al*. 2013), Franch *TGFB3,* (François *et al*. 2017). Indian *TGFB3* (rs117462711) (Kumari *et al*. 2019), were not concluded and Brazilian *TGFB3* (rs2268626) (Antunes *et al*. 2013), Chilean *TGFB3* (rs3917201, rs2268625, rs2268626) (Blanco *et al*. 2017), and Indonesian *TGFB3* (sfaN1) (Nasroen *et al*. 2017) were not significant risk for the NSCLP pathogenesis.

FGF

FGFs involve in the cell proliferation, differentiation, tissue repair and embryonic development depend on the FGF signalling. Dysregulation of the FGFs signalling has been implicated in the pathogenesis NSCLP. *FGF receptor-1 (FGFR1)* SNPs (rs6987534, rs6474354, rs10958700) P=0.04 and OR (95% CI) 0.51 (0.31-0.82) significantly risk for the Maryland, Taiwan, Singapore and Korean Population (Wang *et al*. 2013). Furthermore, Iranian researcher identify the risk of *FGFR1* (rs13317 A/G) P=0.004, OR (100% CI) 0.226 (0.082-0.621) (Rafiqdoost *et al*. 2014) and Chinese researcher (Li *et al*. 2019) *FGFR1* P=<0.001 and Latvia researcher identify *FGFR1* P=0.018, and *FGFR2* P=<0.01 (Jain & Pilmane 2021), the p-value were less than 0.050 were significant risk for the NSCLP development.

FGFR1 (rs7829058), investigate in the Brazilian population were not associate with NSCLP (Machado *et al*. 2016) and *FGFR2* (rs2420946, rs100706, rs1047111, rs11199916) and NSCLP were not associate in the Chinese Population (Li *et al*. 2019). *Fibroblast Growth Factors (FGF-19, 1,2,3, 4,5,8, 9,10, 12)* are members of the FGF family growth

Table 2. Significant growth factors and genes associated with NSCLP pathogenesis, (NS=non-significant, OR= odds ratio, CL=cleft lip, CL±P= cleft lip with or with palate, CL/P= cleft lip with palate, CP= cleft palate, NSOC=Non-syndromic orofacial cleft, CPO=cleft palate only)

No	Growth factors	Associated genes	SNPs	Molecular pathway	Sensitivity/ Specificity	Cleft type	OR (95% CI)	P-Value	Main findings or Genetic associations	Reference	Year
1	TGF-β	*BMP4*	rs17563	Bmp signalling pathway	Cell proliferation, differentiation, apoptosis, chondrogenesis	CL		0.00009	The variations of amino acid in *BMP4* associated with CL and unilateral clefts	(Antunes *et al*.)	2013
2	FGF	*FGFR1*	rs6987534	FGF signalling pathway	The FGF family gene and FGF receptor interacts with environmental factor, e.g. smoking, nutrition	NSCLP	0.51 (0.31-0.82)	0.04	The FGF family gene interaction has a significant role in the NSCLP pathogenesis	(Wang *et al*.)	2013
		FGFR1	rs6474354			NSCLP	0.51 (0.31-0.82)	0.04			
		FGFR1	rs10958700			NSCLP	0.51 (0.31-0.82)	0.04			
		FGF19	rs3737463			NSCLP	1.45 (1.07-1.92)	<.05			
		FGF19	rs948992			NSCLP	1.45 (1.07-1.92)	<.05			

No	Growth factors	Associated genes	SNPs	Molecular pathway	Sensitivity/ Specificity	Cleft type	OR (95% CI)	P-Value	Main findings or Genetic associations	Reference	Year
		FGF19	rs1307968			NSCLP	1.45 (1.07-1.92)	<.05			
		FGF19	rs1320706			NSCLP	1.45 (1.07-1.92)	<.05			
		FGF19	rs1789364			NSCLP	1.45 (1.07-1.92)	<.05			
3	EGF	*TGFA*	rs11466285, c.3851C	EGF signalling pathway	The FGF family gene and FGF receptor interacts with environmental factor, e.g. smoking, nutrition	(CLP)		0.028	The microarray analysis is suitable for SNP analysis in the NSCLP, and *TGFA* SNP is significant for the non-syndromic cleft lip with or without palate but not significant for cleft palate only	(Xu *et al*.)	2014
		TGFA	rs3771523, c.3822A			(CL±P)		0.025			
		TGFA	rs11466285, c.3851C			CP		0.335 *(NS)			
		TGFA	rs3771523, c.3822A			CP		0.133 *(NS)			

No	Growth factors	Associated genes	SNPs	Molecular pathway	Sensitivity/ Specificity	Cleft type	OR (95% CI)	P-Value	Main findings or Genetic associations	Reference	Year
		TGFA	rs11466285, c.3851C			CL/P		0.006			
		TGFA	rs3771523, c.3822A			CL/P		0.019			
4	FGF	*FGF3*	rs4980700	FGF3 interact with PAX9 gene	Fundamental role in cancer and craniofacial	Oral cleft	0.43 (0.27-070)	0.0002	The interaction between the *PAX9* gene and the *FGF3* gene is significant for the oral clefts.	(Küchler *et al*.)	2014
5		*FGF1*	rs34010 C/A	FGF signalling pathway	These are involved in the development stages and cranial neural crest (CNC) cell induction	NSCLP	0.226 (0.082-0.621)	0.004	*FGF1* rs34010 C/A and *FGFR1* rs13317 A/G are significant for the pathogenesis of NSCLP in the Iranian population.	(Rafiqdoost *et al*.)	2014
		FGFR1	rs13317A/G			NSCLP	0.226 (0.082-0.621)	0.004			
6	TGF-β	*BMP7*	rs6127973	Bmp signalling pathway	Palate development	NSOCs		0.0061	The paternal and maternal have no significant difference, however, the rs6127973 G allele was over-transmitted paternally.	(Yu *et al*.)	2015

No	Growth factors	Associated genes	SNPs	Molecular pathway	Sensitivity/ Specificity	Cleft type	OR (95% CI)	P-Value	Main findings or Genetic associations	Reference	Year
7	FGF	*FGF2*	rs1048201	miRNA interacts with the FGF signalling pathway	Craniofacial development	NSOCs	0.83 (0.71-0.98)	0.026	*FGF2, FGF5* and *FGF9* interact with miRNA-FGF to increase the risk for NSOCs.	(Li *et al*.)	2016[b]
		FGF5	rs3733336		cell proliferation and neural crest formation	NSOCs	0.73 (0.60-0.88)	0.001			
		FGF9	rs546782		regulate cell proliferation	NSOCs	0.57 (0.60-0.98)	0.04			
8	TGF-β	*TGFβ2*		TGF-β signalling pathway	involve in the cell cycle	NSCLP			TGF-β signalling pathway understanding better for the NSCLP pathogenesis	(Wang *et al*.)	2016
		TGFβ3				NSCLP					
9	IGF	*IGF1*		IGF interact with multiple signalling pathways	Morpho-pathogenesis, collagenous connective tissue development, postnatal facial formation,	CL			*IGF-1R* and *IGF-1* have significant role in the cleft morphopathogenesis	(Sidhom & Pilmane)	2017

No	Growth factors	Associated genes	SNPs	Molecular pathway	Sensitivity/ Specificity	Cleft type	OR (95% CI)	P-Value	Main findings or Genetic associations	Reference	Year
		IGF-1R				CL					
10	TGF-β	*TGFβ1*		TGF-β signalling	regulate the signalling pathway of TGFB	CL			Cytokines and TGF*β1* regulate the TGFB signalling pathway the downregulation of the *TGFB1* effect on the signalling pathway risk for cleft lip	(Živicová *et al*.)	2017
11	EGF	*EGF61*	rs4444903	EGF signalling pathway	Promoting growth, for the mesenchymal cells, epidermal cells, and tumour cells has a mitogenic factor role, inhibiter of apoptosis, and significant role in the cell differentiation and proliferation		0.59 (0.42-0.84)		The *EGF61* G allele associated with NSCLP development	(Yan *et al*.)	2017
		EGF61	rs4444903			NSCLP		0.012			
		EGF61	rs4444903			CLP		0.008			
		EGF61	rs4444903			CPO		0.33			

No	Growth factors	Associated genes	SNPs	Molecular pathway	Sensitivity/ Specificity	Cleft type	OR (95% CI)	P-Value	Main findings or Genetic associations	Reference	Year
12	SHH	*GLI3*	rs3801161,	SHH signalling pathway	Sonic Hedgehog pathway has role in the NSCLP development	NSCLP	1.56 (1.15-2.11)	0.004	The *GLI3* is a significant factor for the NSCLP pathogenesis	(Wang *et al*.)	2017
13	TGF-β	*BMP4*		Bmp signalling pathway	Morphogenesis, craniofacial development	NSCLP		0.023	*BMP9* and *IRF6* indication has a risk for NSCLP pathogenesis	(Blanco *et al*.)	2017
14	EGF	*EGFR*		RTKs, and EGFR/MAPK signalling pathway	Migration of neural crest cells, development of the embryonic stage,	NSCLP			This study provide evidence the of significance of RTKs, and EGFR/MAPK signalling pathway in the development of the NSCLP pathogenesis.	(Li *et al*.)	2018
15	TGF-β	*TGFB3*	rs2268625 interact with MSX1 SNP rs6446693/ (GxG)	TGF-β signalling	cell proliferation, craniofacial development	NSCLP		0.038	The interaction between *MSX1* and *TGFB3* risk for the NSCLP in the Chilean population	(Suazo *et al*.)	2018
16	EGF	*TGF-alpha/HinfI*			mesenchymal cell migration	NSCLP		0.029	The study finds evidence of the role of *TGF-alpha/HinfI* in the pathogenesis of NSCLP	(Tufekci *et al*.)	2018

No	Growth factors	Associated genes	SNPs	Molecular pathway	Sensitivity/ Specificity	Cleft type	OR (95% CI)	P-Value	Main findings or Genetic associations	Reference	Year
17	TGF-β	*BMP2/4*			Regulate the normal tissue function	CLP		<0.001	*BMP2/4* and *TGFB3* have significant role in the bone remodelling	(Smane & Pilmane)	2018
		TGFB3			regulate osteoblast differentiation, bone remodelling	CLP		<0.001			
18	FGF			*SNP and SNP interaction between TBX and FGF		NSCLP			The *FGF* and *TBX* gene molecular mechanisms involved in the NSCLP pathogenesis	(Li *et al*.)	2019
		FGF8				NSCLP		<0.001			
		FGF10				NSCLP		<0.001			
		FGFR1				NSCLP		<0.001			
19	PDGF	*PDGFC*		PDGF signalling pathway	The low expression of the PDGFC affects cell migration	NSCLP		0.024	The mutated *PDGFC* inhibit cell proliferation, and cell migration, this risk for the pathogenesis of NSCLP	(Li *et al*.)	2020
20	TGF-β	*TGFB-1*		TGF-β signalling pathway	TGFB1 regulate the many cell events, including cell growth	CLP			*TGFB1* low expression or mutation has a potential risk for	(Pilmane *et al*.)	2021[b]

No	Growth factors	Associated genes	SNPs	Molecular pathway	Sensitivity/ Specificity	Cleft type	OR (95% CI)	P-Value	Main findings or Genetic associations	Reference	Year
									orofacial clefting		
21	SHH	*SHH*		SHH signalling pathway	facial development and cranial crest cell development	Unilateral-CL		0.019	SHH signalling pathway is involved in the development of cleft lip	(Vaivads *et al*.)	2021
22	FGF	*FGFR1*		FGF/FGFR signalling	cell proliferation, apoptosis, angiogenesis	NSOC		0.018	*FGF/FGFR* low expression effect on cell proliferation, this is a significant risk for the orofacial cleft	(Pilmane *et al*.)	2021[a]
		FGFR2				NSOC		<0.01			
23	VEGF	*VEGFA*	C.733 T>C		Palatogenesis, Osteogenesis, bone development	NSCLP			*VEGFA* polymorphism inhibits cell migration and proliferation, which induces the pathogenesis of the NSCLP	(Sun *et al*.)	2021
24	VEGF	*VEGFA*	C.733 T>C			NSCLP			The study found the missense mutation in the *VEGFA* gene this mutation is pathogenic for NSCLP, in the position of NM_001025366.2 c.773T>C p.Val258Ala	(Sun *et al*.)	2020

No	Growth factors	Associated genes	SNPs	Molecular pathway	Sensitivity/ Specificity	Cleft type	OR (95% CI)	P-Value	Main findings or Genetic associations	Reference	Year
	FGF	*FGF4*	c.460G>A	FGF and SHH signalling pathways		NSCLP			GWAS identifies the 17 genes including the *FGF4, FGF8, FGF12* and *SHH*, the polymorphism on these genes is are risk for the NSCLP pathogenesis	(Dąbrowska *et al*.)	2022
		FGF8	c.599A>G			NSCLP					
		FGF12	c.FGF12			NSCLP					
	SHH	*SHH*	c.787C>G	SHH signalling pathways		NSCLP					
26	TGF-β	*BMP4*	rs17563			NSCLP	1.85 (1.19-2.89)	0.005	The finding of the research is the *BMP4* and *TGFA* SNPs interact with *MTHFR* and *IRF6* gene SNPs which induces risk for NSCLP pathogenesis.	(Avasthi *et al*.)	2022
27	EGF	*TGFA*	rs11466297			NSCLP	1.69 (1.01-2.82)	0.036		(Avasthi *et al*.)	2022

factors. *FGF1* (rs34010 C/A), OR (100% CI) 0.22(0.82-0.621) P=0.004, *FGF2* (rs1048201) OR(100%CI) 0.83 (0.71-0.98), P=0.026, *FGF3* (rs4980700), OR(100% CI) 0.43 (0.27-0.070), P=0.0002, *FGF4* (c.460G>A), *FGF5* (rs3733336) OR (100% CI) 0.73 (0.60-0.88), P=0.001, *FGF8* (c.599A>G) P=<0.001, *FGF9* (rs546782), OR(100%CI) 0.57(0.60-0.98) P=0.04, *FGF10* P=<0.001, *FGF12* (c.FGF12) and *FGF19* (rs3737463, rs948992, rs1307968, rs1320706, rs1789364) OR(100% CI) 1.45(1.07-1.92) P=<0.05, these gene and receptor have association with NSCLP development in various population, the details are summarize in the Table 2.3.

EGF

EGF, have key role in the tissue repair, wound healing, facial tissue regeneration and craniofacial development. The variation in the EGF family genes effect on the EGF signalling pathway. Studies identify EGF family gene mutation has risk for the NSCLP development. TGFA, EGF61, EGFR, TGF-alpha/HinfI, are the EGF family gene, Transforming growth factor-A (TGFA), (c.3851C, rs11466285, c.3822A rs3771523) significant (P=0.25, to P=0.006) risk in the cleft lip with or without palate except cleft palate (P=0.335, 0.133) only non-significant (Xu *et al*. 2014). Turkish papulation TGF-alpha/HinfI (Tufekci *et al*. 2018) P=0.029 and TGFA SNP rs11466207 OR(100% CI) 1.69(1.01-2.82) P=0.036 were risk for the Indian population (Avasthi *et al.* 2022).

However, one Indian (Junaid *et al*. 2018) and Korean (Kim *et al.* 2015) study not concluded the role of TGFA and European TGFA (rs1348813) (Mossey *et al*. 2017), Iranian TGFA (rs731236), (Bagheri *et al*. 2017) and Indonesian population TGFA (BamHI-rs11466297, Rasl-rs3732248) (Sufiawati *et al*. 2020) were non-significant in the NSCLP. EGF61 rs4444903 significant associate with NSCLP P=0.012, OR(100%CI), 0.59(0.42-0.84) and non-significantly with NSCLO P=0.33 in the Chinese population (Yan *et al.* 2017). However, in the Brazilian study the variant EGF+61 A>G has no significant association. (Falagan-Lotsch *et al*. 2015) The Chinese GWAS study of the epidermal growth factor receptor, significantly interact with the NHTN1, AP2B1 and NTRK1 gene, regulate the neural crest cell migration and development by the RTKs, and EGFR/MAPK signalling pathway, the variation the EGFR induce the pathogenesis of NSCLP (Li *et al*. 2018).

VEGF

The VEGF growth factor fundemntal role in the signalling in the tissue nourishment and the formation of the palate. Variation and polymorphism in the VEGF the consequence the disruption of the signalling pathway and NSCLP pathogenesis. Vascular endothelial growth factor-A (VEGFA) are the VEGF family gene researcher (Sun *et al*. 2021), analyse the variant c.733 T>C, in were inhibits the cell migration and cell proliferation, and induce the pathogenesis of NSCLP (Sun *et al*. 2020; Sun *et al*. 2021). However, the French researcher not concluded the VEGF and VEGF-Receptor (VEGFR) in the France population (François *et al*. 2017).

IGF

IGFs, involve in the development of the tissue, epithelial, the gene expression and the signalling pathway. Sidhom & Pilmane identify in the Latvia population *IGF1* and *IGF1-receptor (IGF1R)* were involve in the CL development (Sidhom & Pilmane 2017).

PDGF

The PDGF family protein and genes involve in the palatogenesis, tissue repair, cell proliferation and craniofacial development. The Franch researcher analyse *PDGF-C* gene and *PDGF-receptor (PDGFR)* (François *et al*. 2017) expression were downregulate in the Cleft lip and Whole-exome sequencing (WES) analysis the mutated *PDGF-C* gene cell lines were significantly inhibit the cell migration (Li *et al*. 2020)

SHH

The SHH signalling molecule regulate and plays a crucial role in the embryonic and craniofacial development. In the polish and Latvia population respectively *SHH* (c.787C>G) (Dąbrowska *et al*. 2022) in NSCLP and *SHH* P=0.019 were involve in the unilateral cleft lip (Vaivads *et al*. 2021). In the Chinese population G*LI3* (rs3801161) OR(100%CI) 1.56(1.15-2.11) P=0.004, were associated with NSCLP pathogenesis, Conversely, the SNP rs7785287 no association with NSCLP, in the same population (Wang *et al*. 2017).

DISCUSSION

The review highlights the prospective importance and consequences of TGF-β, FGFs, PDGFs, EGF, IGF, VEGF, and SHH growth factors and genetic variation/SNP in the development of NSCLP. Significant difference in sample size, sample types, and statistical methodologies between studies. While summarised in Table 1 various study designs, sample sizes, populations, methods and techniques were used in the research. The scoping review's main finding is the out of 43 articles reviewed, 26 genes and 25 SNP/variants were a fundamental role in cellular mechanisms and various signalling pathways as a consequence of the polymorphism risk for the NSCLP pathogenesis, in the diverse population.

In this scoping review, we evaluate the Seven growth factors in the NSCLP, patients, the NSCLP is a common birth defect in the worldwide population

the 1/700 children affected (Nasroen *et al*. 2022). NSCLP complex aetiology, involve the environmental and genetic factor including the growth factors, additionally the genetic more influence in the NSCLP pathogenesis. According to the GWAS of NSCLP, approximately 40 gene and loci are involve in the NSCLP pathogenesis (Gaczkowska *et al*. 2018). The review of the growth factor to explore the biological pathway and SNP/variation of genes' role and association with NSCLP development.

TGF-β (Premaraj & Moursi 2013) were studies in different population, TGF-β associated genes were significantly involved in the development of the craniofacial, palatogenesis, facial structures and fusion. BMP4 (Blanco *et al*. 2017) BMP2/4 (Krivicka *et al*. 2013), BMP7 (Yu *et al*. 2015), TGFB1, TGFB2, and TGFB3 (Paranaíba *et al*. 2013) genes and SNP/variants involve in the TGF-β signalling, BMP signalling pathways (Krivicka *et al*. 2013). The studies evidence were identified that the SNP/variants disrupted the signalling pathways and induced development NSCLP pathogenesis. The mice study of gene expression profiling of the TGF-B signalling fundamental role in the signalling pathways, cholesterol metabolism, extracellular matrix including palatogenesis (Pelikan *et al*. 2013). FGFs regulate the FGF signalling cell proliferation, embryonic development, and cell differentiation. However, the disruption of the FGF signalling and FGF gene SNP/variants induce the pathogenesis of the NSCLP development. FGF family genes polymorphism in the Chinese, Maryland, Taiwan, Iranian, Brazilin, Singapore and Korean population were significantly involving pathogenesis of NSCLP development (Li *et al*. 2019; Machado *et al*. 2022; Wang *et al*. 2013). FGFR bioinformatics analyses researcher identify that the FGFR lead and stimulates the intracellular signalling pathways, cellular mechanism and cell proliferation (Eswarakumar *et al*. 2005).

Tarr *et al*. 2018 studies result show that the connective tissue growth factor (CTGF or CCN2) interact with the multi signalling molecules including FGF, EGF TGF-B, BMPs signalling molecule significant role in the palatogenesis (Tarr *et al*. 2018). EGF Family gene and signalling pathway regulate the facial tissue regeneration, tissue repair and craniofacial development. The current review studies shows TGFA, EGF61, EGFR, and TGF-alpha/HinfI genes evidence that SNPs/variants develop the NSCLP pathogenesis (Avasthi *et al*. 2022; Tufekci *et al*. 2018; Xu *et al*. 2014) Table 2. According to the China GWAS EGF interact with multiple signalling pathway and regulated different cellular mechanism and development (Li *et al*. 2018). VEGF, and genes role the results proof that new insight for the palatogenesis and osteogenesis, the mutation consequences lead the NSCLP (Sun *et al*. 2021). The histopathological study of the IGF (IGF-1, IGF1R), the finding that IGF are potential role in the morphopathogenesis of the CLP. (Sidhom & Pilmane 2017). Certain studies of PDGF family gene and SNP are associated with NSCLP development (Choi *et al*. 2009; Raju *et al*. 2020). This Scoping review two included studies provide the evidence that the mutated PDGF-C and PDGFA significantly inhibit the cell migration and proliferation (François *et al*. 2017; Li *et al*. 2020). Zhou *et al*. 2013 identify that the SHH signalling molecule interact with Pax9, BMP4 and FGF10 and Osr2 signalling pathway and regulate the morphogenesis and palatal shelf development (Zhou *et al*. 2013). The review included studies also support the that the SHH signalling molecule and associated gene SNP/variants disrupt the SHH signalling as a consequence the development of the NSCLP pathogenesis (Dąbrowska *et al*. 2022; Vaivads *et al*. 2021; Wang *et al*. 2017)

Scoping Review Limitations

There are some significant limitations to this scoping review. First off, hardly all of the genetic studies on the subject are covered by the included publications; instead, they mostly concentrate on growth factors. This more restricted approach may lead to the possible removal of important genetic findings that could further our understanding of the topic. Second, the period that can be included in this review is limited to ten years, from 2013 to 2022. This period permits a modern examination of the literature, but it can omit earlier research that offers historical background or adds crucial basic knowledge. Furthermore, the reference to "some database" PubMed, Springer, and Scoups, suggests that the study search approach was restricted to particular databases. This can create bias since pertinent research that is available in other databases might go unnoticed, which could result in an incomplete picture of the body of knowledge on the subject.

CONCLUSION

In conclusion, this scoping review focused on TGF-β, FGFs, PDGFs, EGF, IGF, VEGF, and SHH. The included growth factor 26 genes and 25 SNP/variants were significantly involved in the pathogenesis of NSCLP development. The 10-years results show that NSCLP associated growth factors, played a significant role in the cellular mechanism, cell proliferation, cell differentiation, cell migration and various signalling pathways that's lead the craniofacial development, facial morphogenesis and palatogenesis.

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Supplementary -1

Table S1 Database search strategy

S. No	Database	Search strategy / 2013-2022 ten years	Specifically,	Extracted article
	PubMed	**#1 "non-syndromic cleft" [Title/Abstract] OR "cleft" [Title/Abstract] OR "cleft lip" [Title/Abstract] OR "cleft palate" [Title/Abstract] OR "cleft lip and palate" [Title/Abstract] OR "NSCLP" [Title/Abstract] OR "NSCLO" [Title/Abstract] OR "NSCPO" [Title/Abstract]** **#2** "Growth factor" **[Title/Abstract]** OR "TGF-β" **[Title/Abstract]** OR "FGF" **[Title/Abstract]** OR "PDGF" **[Title/Abstract]** OR "EGF" **[Title/Abstract]** OR "IGF" **[Title/Abstract]** OR "VEGF" **[Title/Abstract]** OR "Sonic Hedgehog" **[Title/Abstract]** OR "SHH" **[Title/Abstract]** **#1 AND #2**	Not specify	350
2	Scopus	"non-syndromic cleft" OR "cleft" OR "cleft lip" OR "cleft palate" OR "cleft lip and palate" OR "NSCLP" OR "NSCLO" OR "NSCPO" AND "Growth factor" OR "TGF-β" OR "FGF" OR "PDGF" OR "EGF" OR "IGF" OR "VEGF" OR "Sonic Hedgehog" OR "SHH"	subject area Medicine, Biochemistry, Genetics and Molecular Biology, Dentistry, Pharmacology, toxicology and pharmaceutics English, final article, Source type: Journal	549
3	Springer	(("non-syndromic cleft" OR "cleft" OR "cleft lip" OR "cleft palate" OR "cleft lip and palate" OR "NSCLP" OR "NSCLO" OR "NSCPO") AND ("growth factor" OR "TGF-β" OR "FGF" OR "PDGF" OR "EGF" OR "IGF" OR "VEGF" OR "Sonic Hedgehog" OR "SHH"))	Article, English	43
	Total			942