International Journal of Scientific Engineering and Science
Volume 9, Issue 8, pp. 6-10, 2025. ISSN (Online): 2456-7361

From Genes to Environment: TGF-Mediated Mechanisms in Cleft Formation

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Abstract— *Orofacial clefts, including cleft lip and palate, result from the complex interaction of genetic and environmental factors affecting craniofacial development. Transforming growth factor-beta (TGF-β), particularly TGF-β3, is essential for palatal fusion by regulating the removal of the epithelial seam through Smad-dependent and independent pathways. Genetic mutations, such as those in YOD1, impair TGF-β3 signaling, reducing developmental robustness. Environmental toxins like 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) activate the aryl hydrocarbon receptor (AhR), suppressing BMP-2/TGF-β/Smad signaling and disrupting the osteogenic differentiation necessary for palate formation. These findings support a "two-hit" model where genetic vulnerability combined with environmental inhibition of TGF-β pathways crosses a critical threshold, causing cleft palate. Understanding how genetic factors and environmental exposures converge on TGF-β signaling pathways offers new insights for prevention and treatment. Future research should focus on clarifying pathway specific sensitivities and developing targeted therapies such as ligand supplementation, Smad stabilization, AhR inhibition, and nutritional support to protect high-risk pregnancies and reduce cleft incidence.*

Keywords— *Orofacial cleft; TGF-β; Genetic; Environmental factors.*

I. Introduction

One of the most common birth abnormalities affecting the craniofacial area is orofacial clefts, which include cleft lip and/or palate[1]. Globally, an estimated 700 infants are born each day with this condition[1,2] Orofacial clefts arise from incomplete fusion of the lip or palate during embryogenesis, a process influenced by multiple etiological factors, including both genetic and environmental determinants (Cawson & Odell, 2008; Rajendran R and B Sivapathasudharam, 2012; Sharma et al., 2021) Management of orofacial clefts is inherently complex and requires a multidisciplinary approach involving surgical, orthodontic, speech therapy, and other specialized interventions[1]. The primary objectives of treatment are the functional restoration of the lip and palate—structures that play a vital role in speech production, swallowing, and respiration (Cawson & Odell, 2008; Rajendran R and B Sivapathasudharam, 2012). Secondary objectives include improving facial aesthetics and enhancing the patient's quality of life[1]. Clinically, cleft lip is characterized by incomplete formation of the lip, with severity ranging from minor notching to extensive defects that may extend toward the nasal cavity (Cawson & Odell, 2008). Cleft palate results from failure of the palatal shelves to achieve complete fusion, creating an oronasal communication that may extend into the nasal cavity (Rajendran R and B Sivapathasudharam, 2012) The fissure might be unilateral or bilateral and may extend from the front hard palate to the back soft palate (Rajendran R and B Sivapathasudharam, 2012). The pathogenesis of orofacial clefts (OFC) is highly complex, involving intricate interactions between genetic predisposition and environmental influences during the early stages of embryonic development. In general, OFC results from the failure of fusion of facial prominences, which arise from embryonic layers, leading to clefts in the orofacial structures. This anomaly not only impairs essential functions such as feeding and speech but also imposes significant psychosocial challenges on affected individuals.

Recent studies have demonstrated that genetic factors, including mutations in specific genes, as well as exposure to environmental risk factors—such as maternal smoking or folate deficiency during pregnancy can substantially increase the risk of OFC. Consequently, a comprehensive understanding of OFC pathophysiology is crucial for effective prevention, accurate diagnosis, and holistic management of affected individuals. The etiology of cleft lip and/or palate (CL/P) is multifactorial, encompassing both genetic and environmental determinants. A few genes, such as MSX1, IRF6, and MTHFR, have been discovered to be closely linked to CL/P. Environmental contributors include maternal medication use, nutritional status, and exposure to teratogenic agents such as tobacco and alcohol.[4] Additionally, lower socioeconomic status has been frequently observed among patients with CL/P, suggesting the influence of broader social determinants of health. During early embryogenesis, the lip begins to form within a critical developmental window spanning approximately the fourth to the seventh week of gestation. Disruption or incomplete fusion of the embryonic tissues that contribute to the upper lip during this period may result in a congenital defect known as cleft lip. The morphological presentation can range from a subtle indentation to an extensive separation that continues upward into the nasal cavity. A cleft lip may be unilateral, bilateral, or, in exceptional circumstances, along the midline. This condition is frequently accompanied by cleft palate in the same individual. Palatal development follows shortly

International Journal of Scientific Engineering and Science
Volume 9, Issue 8, pp. 6-10, 2025. ISSN (Online): 2456-7361

thereafter, occurring between the sixth and ninth week of gestation. A cleft palate results when the palatal shelves fail to achieve complete fusion, leading to an opening in the roof of the oral cavity. The defect may involve both the hard (anterior) and soft (posterior) palate, or be confined to one specific region, depending on the severity and timing of the developmental disruption. (Khamila et al. 2021; Cox TC, 2004; Ma et al. 2016)

Non-syndromic orofacial clefts (OFCs) represent the majority of cases, comprising roughly 77% of the total, which is nearly triple the proportion of syndromic OFCs (23%). This distribution emphasizes the need for routine anticipation and screening for congenital anomalies, particularly in patients with non-syndromic presentations. A marked male predominance is observed, with males accounting for approximately 62% of non-syndromic cases, compared with 38% among females (Alhammad et al. 2021; Huang et al. 2019). Epidemiological patterns reveal distinct variations across populations: individuals of Asian and Native American descent exhibit the highest birth prevalence of non-syndromic orofacial clefts (NSOFC), populations of European ancestry show intermediate rates, and those of African ancestry demonstrate the lowest incidence. Across both sexes, cleft lip with palate (CLP) emerges as the predominant subtype (55%), and unilateral clefts (65%) occur almost twice as frequently as bilateral forms (35%) (Sundoro et al. 2023).

In the Indonesian context, cleft lip and palate constitute the second most prevalent congenital anomaly. National Basic Health Research (RISKESDAS) data indicate an increase in prevalence from 0.08% in 2013 to 0.12% in 2018. Furthermore, the National Health Service Guidelines on cleft management report a prevalence of 0.2%, with approximately 7,500 new cases diagnosed annually. [10] A regional analysis by Sjamsudin and Maifara (2017), drawing on data from the Cleft Lip and Palate Foundation and hospital records in West Java between 2011 and 2015, identified 1,596 cases of cleft lip with or without cleft palate. The most common presentation was combined cleft lip and palate (50.53%), followed by isolated cleft palate (25.05%) and isolated cleft lip (24.42%). Male patients slightly outnumbered females (55.95% vs. 44.05%), and left-sided clefts were observed more frequently than right-sided defects.[11] Numerous studies have linked the role of transforming growth factors (TGFs) to the occurrence of cleft formation due to their involvement in embryogenesis. A strong relationship between the risk of orofacial clefts, the infant's TGFa genotype, and the mother's cigarette usage during the periconceptional period has been established by prior studies. Given that cigarette smoking may reduce serum folate levels, and maternal periconceptional intake of folic acid–containing multivitamins has been correlated with a decreased risk of clefting, investigators have examined whether an interaction exists between infant TGFa genotype, maternal multivitamin supplementation, and the likelihood of developing specific orofacial cleft phenotypes. Therefore, this literature review aims to examine “From Genes to Environment: TGF-Mediated Mechanisms in Cleft Formation”.

II. Method

The PubMed database was used in this research to do a literature review and article search. The search utilized specific keywords [TGF signaling, genetic factors, environmental factors, and cleft formation]. The article selection process followed the guidelines set by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol. The process involved removing duplicate articles and further refining the selection to include studies published between 2015 and 2025, and those published in English. Book sections, studies involving animals, review articles, and conference proceedings were excluded. Data extraction encompassed a range of variables such as author names, article titles, publication years, study designs

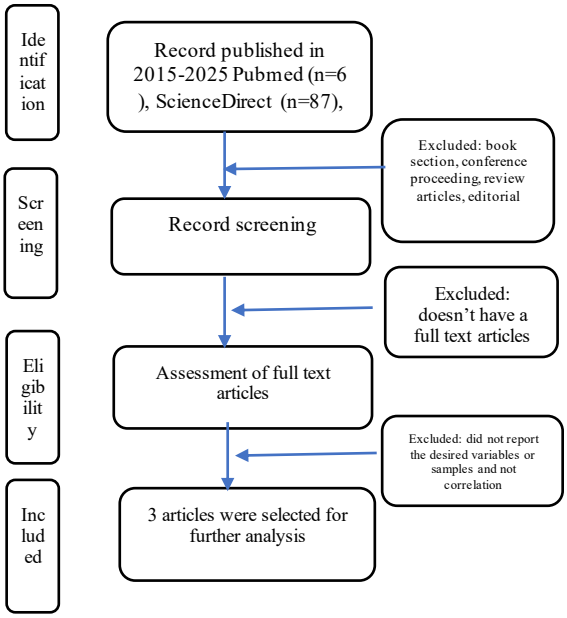

Figure 1. The article selection process flow diagram

III. Result and Discussion

The article selection process is outlined in Figure 1. a total of 93 articles were initially identified through the designated keywords in PubMed and Science Direct from 2015-2025. Thirteen articles satisfied the study's eligibility requirements after careful application of the inclusion and exclusion criteria and elimination of duplicate ones. Table 1 provides a summary of the extracted data from the selected studies.

IV. Discussion

The majority of orofacial clefts are multifactorial, and a number of environmental variables may be at play. Numerous environmental factors influence the occurrence of orofacial clefts, including maternal smoking during pregnancy, drug use, folic acid deficiency, alcohol consumption, maternal age, deficiencies in vitamin A and vitamin B, anorexia, stress, and consanguineous marriage. Transforming growth factor-beta (TGF-β) signaling is essential for normal palatal development, particularly in mediating the removal of the medial edge epithelium (MEE) during palatal shelf fusion. The current findings reveal that TGF-β3 signaling in the epithelium

operates through both canonical (Smad-dependent) and non-canonical (Smad-independent) pathways, with TAK1 and TRIM33 functioning as distinct mediators. (Iwata et al., 2013; Xu et al., 2008). TGF-β3 signaling regulates key downstream effectors such as Mmp13, which is rapidly induced in the MEE to facilitate extracellular matrix remodeling during seam disintegration (Blavier et al., 2001; Xu et al., 2006).

No	Authors/ year	Title	Methods	Result
1	Lane et al	Tak1, Smad4 and Trim33 redundantly mediate TGF-β3 signaling during palate development	The study used genetically modified Tgfb3F mice and crossbreeding with other floxed mouse models to produce mutants for palate development research. Gene expression of Tgfb3 was analyzed using in situ hybridization and RT-qPCR, normalized to β-actin via the 2ΔΔCt method	The study demonstrated that exposure to TCDD impairs osteogenic differentiation in human fetal palate mesenchymal cells (hFPMCs) by downregulating the BMP-2/TGF-β1/Smad signaling pathway. TCDD reduced TGF-β1 expression, its receptors (TβR1–3), and downstream Smad2/3 activation, leading to decreased osteoblast activity and mineralization. This inhibitory effect was mediated through aryl hydrocarbon receptor (AhR) activation, and co-treatment with TGF-β1 restored osteogenesis, indicating that TGF-β1 plays a key protective role against TCDD-induced disruption of palate bone formation
2	Qiang Ju et al/ 2018	Overexpression of Yod1 Promotes the Migration of Human Oral Keratinocytes by Enhancing TGF-B3 Signalin	HOKs were transfected with the plasmid pEGFP-N3-YOD1 containing YOD1. The mRNA levels of YOD1 and TGF-β were determined by qPCR. The protein expressions of YOD1, TGF-β, Smad2/3, Smad4, and phospho-Smad2/3 were determined by western blotting. Cell proliferation and migration were evaluated by Cell Counting Kit-8 assay and wound healing assay, respectively.	YOD1 overexpression in human oral keratinocytes (HOKs) promotes cell migration by upregulating TGF-β3 mRNA and protein expression, without affecting TGF-β1 or TGF-β2. It also enhances Smad2/3 phosphorylation, indicating TGF-β3 pathway activation, which may contribute to lip and palate formation. Conversely, YOD1 mutations may impair TGF-β3 signaling, hinder cell migration, and play a role in non-syndromic cleft lip and palate (NSCLP)
3	Liu et al/ 2020	TCDD inhibited the osteogenic differentiation of human fetal palatal mesenchymal cells through AHR and bmp-2/TGF-B/SMAD signaling	The study cultured osteoblasts from human fetal palate mesenchymal cells to assess TCDD's role in cleft palate formation, revealing that TCDD activates AhR signaling and inhibits the BMP-2/TGF-β1/Smad pathway. This suppression reduced osteogenic differentiation, while TGF-β1 co-treatment restored bone-forming activity.	TCDD exposure impaired osteogenic differentiation in human fetal palate mesenchymal cells by activating AhR signaling and suppressing the BMP-2/TGF-β1/Smad pathway, reducing p-Smad2/3, p-Smad1/5/8, and Smad4. Co-treatment with TGF-β1 restored pathway activity and osteogenesis, highlighting its role in protecting against TCDD-induced cleft palate mechanisms.

Additionally, cell cycle regulators such as p21, p16, and p57 were variably affected in pathway-specific mutants, linking TGF-β–mediated growth arrest to effective epithelial seam removal (Cui et al., 2003; Nawshad, 2008). Importantly, environmental toxicants such as **2**,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have been shown to disrupt osteogenic differentiation in human fetal palate mesenchymal cells (hFPMCs) by downregulating the BMP-2/TGF-β1/Smad axis, impairing mineralization, and ultimately compromising craniofacial development (Liu et al., 2024). The AhR-dependent suppression of TGF-β1 observed in TCDD exposure models parallels the genetic attenuation of TGF-β3 signaling seen in our mutant mice, suggesting a convergent pathogenic mechanism. This raises the possibility that environmental pollutants may exacerbate genetically predisposed cleft palate risk through interference with multiple TGF-β–mediated pathways.

Given that TGF-β3 is expressed in both MEE and peridermal cells (Lane et al., 2014), disruption at either site whether by genetic mutations or environmental exposures could impede periderm removal, palatal shelf adhesion, and fusion. The observation that Tgfb1 can rescue Tgfb3 loss in periderm but not MEE cells (Yang and Kaartinen, 2007) further emphasizes the cell-type specificity of TGF-β ligand function, a point of relevance when considering environmental agents that selectively alter ligand or receptor expression in certain tissues. That is overview that integrate molecular redundancy in TGF-β signaling with environmental vulnerability, suggesting that cleft palate pathogenesis may result from the cumulative effects of genetic pathway impairment and environmental suppression of TGF-β activity.

Study by Qiang Ju et al. demonstrates that YOD1 overexpression in human oral keratinocytes (HOKs) promotes cell migration by upregulating both mRNA and protein levels of TGF-β3 and increasing Smad2/3 phosphorylation. These findings suggest that YOD1 enhances TGF-β3 signaling, which is essential for lip and palate fusion during development. Given that YOD1 mutations are associated with non-syndromic cleft lip and palate (NSCLP), reduced YOD1 activity could impair TGF-β3 signaling, limit cell migration, and disrupt craniofacial morphogenesis. The TGF-β superfamily, which includes TGF-β1, TGF-β2, and TGF-β3 in mammals, is a crucial component of the mechanism of NSCLP 505 and plays a significant role in controlling cell proliferation, migration, and differentiation. The moderate association between TGF-3 and cleft palate has been established. By controlling gene transcription essential for epithelial seam removal and palatal fusion through the Smad2/3 pathway, TGF-3 regulates the development of the cleft palate. Genetic mechanism linking YOD1 to TGF-β3 signaling in cleft pathogenesis, further work using embryonic palatal mesenchymal cells and environmental exposure models is needed to clarify how gene environment interactions influence TGF-β mediated craniofacial development. Zhou et al (2018) reported that several modes of interaction explain how environment and genes may synergize: (1) By functionally resembling genetic loss-of-function in the TGF- axis, environmental ligands of AhR either reduce the

production of TGF-β ligands and receptors or lessen Smad activation; (2) gene variants that reduce baseline TGF-β3 signaling (e.g., *YOD1* mutations that impair TGF-β3 regulation) lower the threshold for environmental insults to produce phenotype; and (3) ubiquitin-dependent control of Smad stability (e.g., de-ubiquitinating enzymes such as YOD1 or OTUB1) can modulate amplitude/duration of TGF-β responses, making signaling more vulnerable to perturbation. Empirical support includes reports that YOD1 overexpression enhances TGF-β3 and phospho-Smad2/3 and promotes oral keratinocyte migration—while YOD1 disruption is associated with NSCLP—linking ubiquitin-regulated TGF-β control to cleft risk

Osteogenic differentiation in the developing palate is essential for normal craniofacial formation, and disruption can result in cleft palate. The environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a potent aryl hydrocarbon receptor (AhR) ligand, is known to cause craniofacial malformations, including cleft palate, in mammals. TCDD significantly decreased proliferation, alkaline phosphatase activity, extracellular matrix mineralization, and expression of key osteogenic markers (RUNX2, SP7, Sox9, Msx2, TGF-1, BMP-2) in human fetal palate mesenchymal cells (hFPMCs). Mechanistically, TCDD decreased TGF-β receptor 1 and 2 expression and suppressed BMP-2/TGF-β/Smad signaling, lowering p-Smad2/3, p-Smad1/5/8, and Smad4 protein levels. Activation of this pathway by recombinant TGF-β1, BMP-2, or Smad4 overexpression rescued osteogenic defects, while AhR deletion or knockdown prevented TCDD-induced Smad suppression. These findings indicate that TCDD impairs palate osteogenesis via AhR–TGF-β/Smad pathway crosstalk, providing a mechanistic link between environmental dioxin exposure and cleft palate pathogenesis. Taken together, the convergent evidence supports a model in which cleft palate arises when genetic impairments in TGF-β signaling (polymorphisms, loss-of-function alleles, or dysregulated ubiquitin modifiers) combine with environmental suppressors (TCDD and other AhR ligands, maternal smoking, folate deficiency) to fall below a critical signaling threshold required for MEE removal, palatal adhesion, osteogenesis, and fusion. This framework highlights several actionable research directions: (a) mechanistic studies comparing canonical versus non-canonical TGF-β pathway vulnerability to environmental ligands; (b) genotype-stratified toxicology to identify susceptible alleles; and (c) exploration of therapeutic rescue (ligand replacement, Smad stabilization, AhR antagonism, nutritional interventions) to mitigate risk in exposed or genetically at-risk pregnancies.

V. Conclusion

Cleft palate arises from the interplay between genetic control of TGF-β signaling and environmental factors. TGF-β3 is essential for palatal fusion, orchestrating epithelial seam removal through Smad-dependent and -independent pathways. Mutations such as YOD1 weaken this signaling, lowering developmental resilience. Environmental toxins like TCDD, a potent AhR ligand, mimic genetic defects by suppressing BMP-2/TGF-β1/Smad signaling, impairing osteogenesis, and disrupting fusion. This supports a “two-hit” model, where genetic predisposition and environmental inhibition together surpass a critical threshold for cleft formation. Future work should clarify pathway-specific pollutant sensitivity, link genotype to environmental risk, and explore interventions such as ligand supplementation, Smad stabilization, AhR blockade, and targeted nutrition to safeguard high-risk pregnancies.

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