Concise Review

# Advancements in Pathogenic Genes and Biomarkers for Non-syndromic Cleft Lip With or Without Cleft Palate Via Multiomics

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ABSTRACT

Non-syndromic cleft lip with or without cleft palate (nsCL/P) is a common congenital malformation influenced by a combination of environmental and genetic factors. nsCL/P is usually diagnosed using fetal ultrasound during the late second trimester; however, these results are often affected by factors such as instruments, fetal position, and maternal obesity. Moreover, by this time, structural anomalies in the fetuses are already formed and missed optimal time for intervention. Therefore, identifying more efficient and non-invasive biomarkers before fetal ultrasound is essential. In recent years, rapidly evolving omics technologies, including genomics, transcriptomics, proteomics, lipidomics, epigenomics, and single-cell omics, have been used to identify several nsCL/P-associated risk genes. Additionally, omics technologies have proven invaluable for investigating non-invasive biomarkers for prenatal diagnosis of nsCL/P. Therefore, this article reviews the current applications of multi-omics technologies in nsCL/P research, focusing on their use to identify pathogenic genes and the research advances in prenatal diagnosis. We highlighted the technological landscape and applications of multi-omics in nsCL/P, and explored the potential opportunities and challenges for future clinical practice.



## Introduction

Oral and maxillofacial clefts are the most common congenital craniofacial anomalies and are categorized into non-syndromic and syndromic types, depending on the presence or absence of other congenital anomalies. Approximately 70% of cleft lip with or without cleft palate cases occur as non-syndromic and the remaining 30% are a part of syndromic phenotypes.[1] The incidence of non-syndromic cleft lip with or without cleft palate (nsCL/P) is higher and the causes are more complex. nsCL/P encompasses non-syndromic cleft lip (nsCL), nonsyndromic cleft palate (nsCP), and non-syndromic cleft lip with palate (nsCLP). The global prevalence of nsCL/P among live births ranges from 1 in 500 to 1 in 2,500, varying slightly among different regions.[2] The development of nsCL/P is influenced by a combination of genetic and environmental factors.[3] Maternal nutritional status, smoking, alcohol consumption, passive smoking, air pollution, and heavy metal exposure are some of the environmental factors that may contribute to the development of nsCL/P.[4-9] Children with nsCL/P have facial malformations that affect their appearance and result in varying degrees of impaired swallowing, breathing, and speech functions. These children often require multiple surgeries, which poses serious psychological and financial burdens for patients as well as their families.

As palate morphological and genetic development in mice closely resembles that of humans, mice are considered the most suitable animal model for studying cleft palate. Common teratogens used in cleft palate models include all-trans

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retinoic acid (atRA) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). atRA is the main metabolically active product of vitamin A. Though, it plays major roles in embryonic organ development and is involved in cell proliferation, differentiation, and migration.[10] Clinically, atRA is commonly used to treat skin diseases and cancers such as acute promyelocytic leukemia. However, excessive exposure to atRA induces cleft palate. TCDD, on the other hand, is a common environmental pollutant, primarily produced as a byproduct of various industrial activities such as metal smelting and waste incineration. TCDD can be absorbed into the human body through the gastrointestinal tract, respiratory system, and skin, inducing various physiological and toxicological responses and leading to endocrine disruption, reproductive and developmental defects, immunosuppression, and carcinogenesis.[11-13] Previous studies have widely used human specimens and mouse models of cleft palate to explore pathogenic genes and the associated mechanisms. In humans, palatogenesis is initiated in the early 6$^{th}$ week of gestation and palatal fusion is completed by the 12$^{th}$ week of gestation.[14] The secondary palate develops in three stages. In mice, the palate grows from the lateral aspects of the maxillary process on embryonic day E11.5 (corresponding to the 6$^{th}$ week of gestation in humans). During E12.5-13.5 (gestation weeks 6-7 in humans), the palate grows vertically downward along the sides of the tongue and then ascends to a horizontal position on E14.0. Finally, on E14.5-E16.5 (gestation weeks 9-12 in humans), the bilateral palatine processes begin to converge along the midline and merge to form a complete palate.[15] Disruptions at any point in this entire process can lead to the formation of cleft palate. Presently, multi-omics technologies have been performed on human and mice tissues.

Currently, ultrasound imaging and blood tests are the most common screening methods for detecting fetal developmental defects. Currently, nsCL/P is primarily diagnosed using fetal ultrasound during the late second trimester. However, factors such as instruments used, maternal obesity, fetal position, and physician skills may interfere with the accuracy of the diagnostic results. Ultrasound detection rates vary widely between different nations and regions, with second-trimester detection rates for nsCL/P ranging from 28% to 88%.[16] Moreover, structural anomalies are already formed in the late second trimester, crossing the most appropriate time window for clinical intervention. Owing to its non-invasive nature, peripheral blood testing of pregnant women is considered a better method of prenatal diagnosis than amniocentesis or cord blood sampling. Moreover, peripheral blood can be easily collected and can be readily examined in primary hospitals to provide guidance for further clinical examinations. However, there are no clinical biomarkers for the diagnosis of nsCL/P.

In recent years, the rapid development in multi-omics technologies, including genomics, transcriptomics, proteomics, lipidomics, epigenomics and single-cell omics, have significantly contributed to the identification of genes responsible for congenital malformations. Genetic screening played an important role in the detection of diseases. Clinical data have revealed that the presence of 2 or 3 oral features was commonly seen in PTEN Hamartoma Tumour Syndrome (PHTS) patients.[17] PTEN inhibitor could partially rescue the mouse embryonic CP induced by atRA.[18] Dental practitioners are crucial for the early detection of PHTS by identifying oral features, such as high palate and cleft palate, which allows for timely genetic diagnosis and the initiation of preventative cancer care.[17,19] These technologies are now innovatively utilized in early screening for common congenital malformations, such as neural tube defects and congenital heart diseases.[20,21] Deng *et al.* identified a panel of six potential prenatal diagnostic genes for neural tube defects through integrating bulk- and scRNA-seq transcriptome.[22] Multi-omics technologies are constantly emerging that have enabled researchers to access multi-layer information from the genome, transcriptome, proteome, metabolome, and more. Presently, there is no review summarized recent advances in biomarkers for the prenatal diagnosis of fetal nsCL/P or pathogenic genes based on multi-omics technologies. To delve deeper into this aspect, in this review, we aimed to discuss and summarize the studies that have applied multi-omics technologies to discover pathogenic genes associated with nsCL/P, as well as its prenatal biomarkers. The search strategy is presented in file S1. Here is the figure abstract of this study (Figure 1).

## Genomics

Various genomics research methods have been applied to screen for candidate causative genes of nsCL/P, including genome-wide association studies (GWASs), whole exome sequencing (WES), whole genome sequencing (WGS) and so on. GWAS is a conventional method for discovering mutation sites known as single-nucleotide polymorphisms (SNPs) in disease-associated risk genes. The first GWAS on nsCL/P identified the presence of a susceptibility locus on chromosome 8q24.[23] This finding was subsequently confirmed by other GWASs in several independent populations; in addition, these GWASs studies identified some additional susceptibility loci on chromosome 17q22, 10q25, and 9q22.[24-27] Bian *et al.* identified 14 susceptibility loci associated with nsCLP and confirmed 12 previously reported loci in the Chinese population.[28] A GWAS study identified 14 susceptibility loci for nsCL/P and suggested different subtypes harbor different genetic etiologies.[29] Many other nsCL/P-associated genes and SNPs within these genes, such as *IRF6, MAFB, SHTN1, PAX7, FGFR2* and *NOG* have been identified through GWASs.[30-32] Although GWASs have been highly successful in identifying susceptibility genes for nsCL/P, the genes or SNPs identified to date represent only a small fraction of the total genes involved in this condition.[33]

Exons constitute only 1% of the human genome; however, 85% of disease-causing mutations in humans are located in these coding regions.[34] WES, also known as targeted exome capture, is a genomic analysis method that utilizes sequence-capturing techniques and exon DNA sequence microarrays to capture and enrich DNA from exons throughout the genome for high-throughput sequencing. WES has been performed in various populations to identify pathogenic SNPs and small insertions/deletions (indels) associated with nsCL/P.[35] Several candidate causative genes and loci associated with nsCL/P have been identified using WES, including *CHD7, PDGFC,*

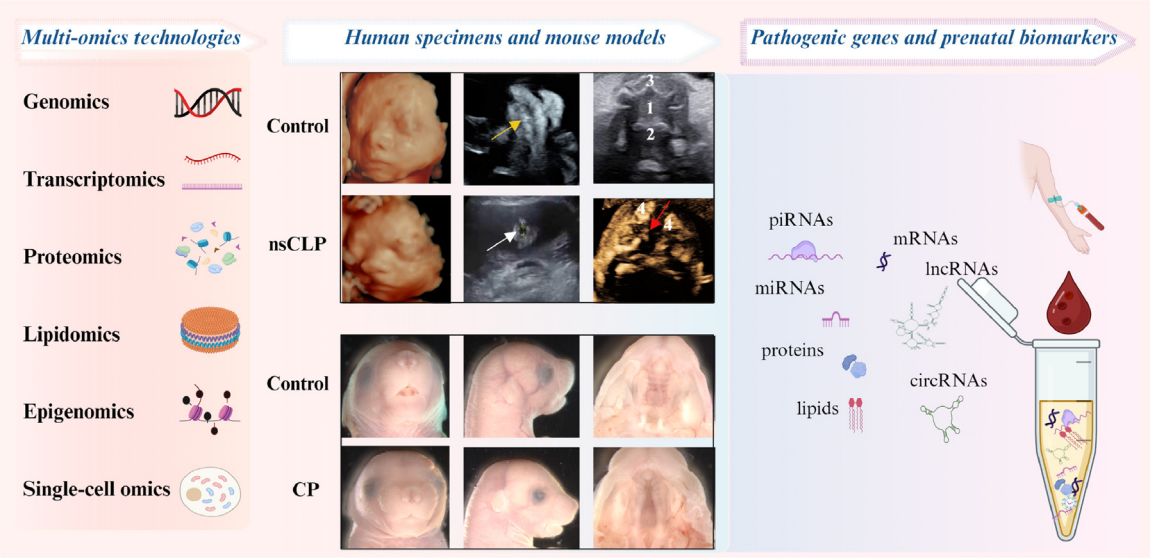


**Fig. 1 – The technological landscape and applications of multi-omics technologies in nsCL/P. Multi-omics techniques were performed in human and mouse specimens to search for pathogenic genes and prenatal biomarkers of nsCL/P. yellow arrow, intact lip; 1, hard palate; 2, soft palate; 3, intact maxilla; white arrow, cleft lip; 4, hemimaxilla; red arrow, cleft palate.**

*ACSS2*, *PHYH*, *LRP6*, *MTR*, *ARHGAP29*, *GLI2*, *TP63*, *IRF6*, *NRP1*, *RPL27A*, *FZD6*, and *PTCH1*.[36-45] With an increase in the discovery of candidate causative genes and mutation loci, studies investigating their functions are gradually progressing. A mutation locus in the *LAMA5* gene has been detected in nsCL/P patients from eastern China and subsequently found to be involved in the temporal and spatial development of the palate in mouse models of cleft palate.[46] WGS, unlike WES, can be used to detect SNPs and indels in non-coding regions. WGS analysis of case-patients with orofacial anomalies from European and Colombian revealed a new disease locus on chromosome 21.[47] Loss-of-function de novo mutations in *IRF6*, *TFAP2A*, and *ZFHX4* have been identified in the tissues of patients with oral and maxillofacial anomalies.[48] The characteristics of the studies mentioned above are presented in Table 1. These findings imply that genomics technologies are a reliable methodological approach to identify the causative genes of nsCL/P with enhanced accuracy and efficiency, thereby significantly contributing to genetic counseling, prenatal diagnosis, and prevention. Circulating cell-free DNA (cfDNA) in maternal bodily fluids carry information concerning the fetal origin. Over the past decade, non-invasive prenatal test employing cfDNA to screen for fetal aneuploidy has been used in clinical prenatal care. As we look ahead, future studies may focus on the potential clinical applications of cfDNA in nsCL/P.

## Transcriptomics

Figure 2. presented the applications of transcriptomics to exploring the pathogenic genes and biomarkers of nsCL/P. Cai *et al*. performed transcriptomics analysis of the upper lip and primary palate from C57BL/6J mouse embryos at three stages (E10.5, E11.5 and E12.5) and revealed a series of key genes involved during development.[49] Following the completion of the Human Genome Project, researchers discovered that over 80% of disease-causing mutations occur in the non-coding regions of genes.[50] Non-coding RNAs include long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), microRNAs (miRNAs), and PIWI-interacting RNAs (piRNAs), among others. lncRNAs are non-coding RNAs longer than 200 nucleotides in length, and their function is closely associated with subcellular localization. In the cytoplasm, lncRNAs form sponge complexes with miRNAs and competitively exert the mechanism of competitive endogenous RNAs. In the nucleus, lncRNAs interact with various molecules, including DNA, RNA, and proteins, to maintain chromosome structure and function, regulate gene transcription in cis or trans, and influence mRNA splicing, stabilization, and translation.[51-53] Many studies have reported the involvement of lncRNAs in nsCL/P development.[54-56] Wang *et al*. systematically investigated lncRNAs with these functions in nsCL/P and characterized the *lncRNA USP17L6P/miR-449c-5p/c-Myc* regulatory network.[57] Liu *et al*. found that the *lncRNA Meg3/TGF-β/Smad* regulatory axis affects the proliferation and apoptosis of mouse embryonic palatal mesenchymal (MEPM) cells.[58] Li *et al*. found that *lncRNA MIR31HG* is present in the susceptibility locus of nsCL/P and affects the proliferation and migration of human embryonic palatal mesenchymal (HEPM) cells and human oral keratinocyte (HOK) cells.[59] Additionally, the *lncRNA NONMMUT100923.1/miR-200a-3p/Cdsn* regulatory axis contributes to the failure of palate fusion by impeding the epithelial-mesenchymal transition of cells in the midpalatal suture.[60] Similarly, circRNAs form sponge complexes with miRNAs and affect the post-transcriptional level regulation of miRNA target genes. Shu *et al*. proposed and validated the *circRNA 0954/miRNA-881-3p/RKAR1α* regulatory axis through omics data of mouse cleft palate tissues.[61] RNA sequencing (RNA-seq) was used on palatal tissues at four stages (E10.5, E13.5, E15, and E17), three lncRNAs (*H19*, *Malat1*, and *Miat*) and four mRNAs (*Cdh1*, *Irf6*, *Grhl3*, and *Efnb1*) were validated as hub genes

**Table 1 – Characteristics of the genomics studies in nsCL/P.**

| Technologies | Subtypes of orofacial clefts | Study participants | Areas or races | Genetic susceptibility factors | References |
|---|---|---|---|---|---|
| GWAS | nsCL/P | 224 cases, 383 controls | Central European | chr. 8q24 | Birnbaum, S. *et al.*,[23] |
| | nsCL/P | 111 cases, 5951 controls | Greater Philadelphia | chr. 8q24 | Grant, S F. *et al.*,[24] |
| | nsCL/P | 401 cases, 1323 controls | Central European | chr. 17q22 and 10q25.3 | Mangold, E. *et al.*,[25] |
| | nsCL/P | 149 cases, 303 controls | Mayan Mesoamerican populations | IRF6, chr.10q25, and 8q24 | Rojas-Martinez A, *et al.*,[26] |
| | nsCL/P | 515 cases, 151 controls | Honduran and Colombian populations | chr. 8q24, 9q22, 10q25, and 17q22 | Lennon C J, *et al.*,[27] |
| | nsCLP | 7404 cases, 16059 controls | Chinese populations | chr. 2p25.1, 4p16.2, 4q28.1,5p12, 6p24.3, 8p11.23, 8q22.1, 9q22.32, 12q13.13, 12q13.2, 12q21.1, 14q22.1, 14q32.13, and 17q21.32 | Yu Y, *et al.*,[28] |
| | nsCL/P | 6986 cases, 10165 controls | Chinese populations | chr. 3p22.1, 4p16.3, 11q14.2, 12q21.31, 13q32.3, 14q13.3, 14q32.2, 16q24.2, 19p13.11, 16q24.1, 1q32.2, 2p24.2, 10q25.3, and 20q12 | Huang, L, *et al.*,[29] |
| | nsCL/P | 1863 case–parent trios | European and Asian populations | chr. 8q24, IRF6, MAFB, and ABCA4 | Beaty, T. H, *et al.*,[30] |
| | nsCL/P | 1931 cases, 2258 controls | Chinese populations | chr. 10q25.3 and SHTIN1 | Wang Y, *et al.*,[31] |
| | nsCL/P | 1409 cases, 1409 controls | Asian and European populations | NTN1, PAX7, FGFR2, and NOG | Leslie EJ, *et al.*,[32] |
| WES | nsCL/P, syndromic CL/P | 107 singleton pregnancies and their fetuses | Chinese populations | CHD7 | Yan S, *et al.*,[36] |
| | nsCLP | 52 case–parent trios | Honduran populations | ACSS2 and PHYH | Aylward A, *et al.*, [37] |
| | nsCP | 30 cases, 30 controls | Brazilian populations | LRP6 and MTR | Machado RA, *et al.*,[38] |
| | nsCL/P | a four-generation family | Chinese populations | ARHGAP29 | Tang JX, *et al.*,[39] |
| | nsCL/P | a three-generation family | Chinese populations | GLI2 | Meng P, *et al.*,[40] |
| | nsCL/P | a three-generation family | Chinese populations | TP63 | Xu T, *et al.*,[41] |
| | nsCL/P | a three-generation family | Chinese populations | IRF6 | Wang Y, *et al.*,[42] |
| | nsCL/P | a four-generation family | Saudi populations | NRP1 and RPL27A | Al Mahdi HB, *et al.*,[43] |
| | nsCL/P | a three-generation family | Chinese populations | FZD6 | Zhang J, *et al.*,[44] |
| | nsCL/P | a three-generation family | Chinese populations | PTCH1 | Zhong W, *et al.*,[45] |
| | nsCL/P | 30 cases | Chinese populations | LAMA5 | Fu, *et al.*,[46] |
| WGS | nsCL/P | 580 case–parent trios | European and Colombian populations | chr. 21q | Mukhopadhyay, *et al.*,[47] |
| | nsCL/P | 756 case–parent trios | European, Colombian, and Taiwanese | IRF6, TFAP2A, and ZFHX4 | Bishop MR, *et al.*,[48] |

during palatogenesis.[62] miRNAs are small non-coding RNAs of 18-25 nucleotides whose primary function in diseases is to inhibit target genes at the post-transcriptional level.[63] miRNAs have been demonstrated to be involved in spatiotemporal developmental processes in orofacial and palatal sites in mice and to participate in the regulatory network of causative genes of cleft palate.[64,65] Many similar studies have been reported to date. For example, *miR-124-3p* and *miR-340-5p* have been shown to inhibit the proliferation of MEPM cells and the neural crest stem cell line O9-1 by regulating the expression of target genes such as *Sox5h*, *Trp53*, and *Tgfbr1*; further, a cocktail of *miR-124-3p* and *miR-340-5p* inhibitors can reduce the malformation rate of cleft palate in mice.[66] *miRNA-470-5p* inhibits epithelial-mesenchymal transition in MEPM cells by regulating the expression of its target gene *Fgfr1*, which in turn results in failure of palate fusion.[67] *miR-27b*, *miR-133b*, and *miR-205* inhibit the proliferation of lip mesenchymal cells in humans and mice, leading to the development of cleft lip.[68] The *Let-7c-5p/PIGA* and *miR-193a-3p/TGFB2* regulatory axes decrease the proliferation and increase the apoptosis of HEPM cells and oral epithelial cells, which are involved in nsCL/P.[69] Tang *et al.* performed RNA-seq on plasma from nsCL/P patients, and found three differentially expressed miRNAs (*miR-212-3p*, *miR-200b-3p* and *miR-130b-3p*) and their related ceRNA network.[70] All of these studies suggest that miRNAs are involved in the process of lip and palate

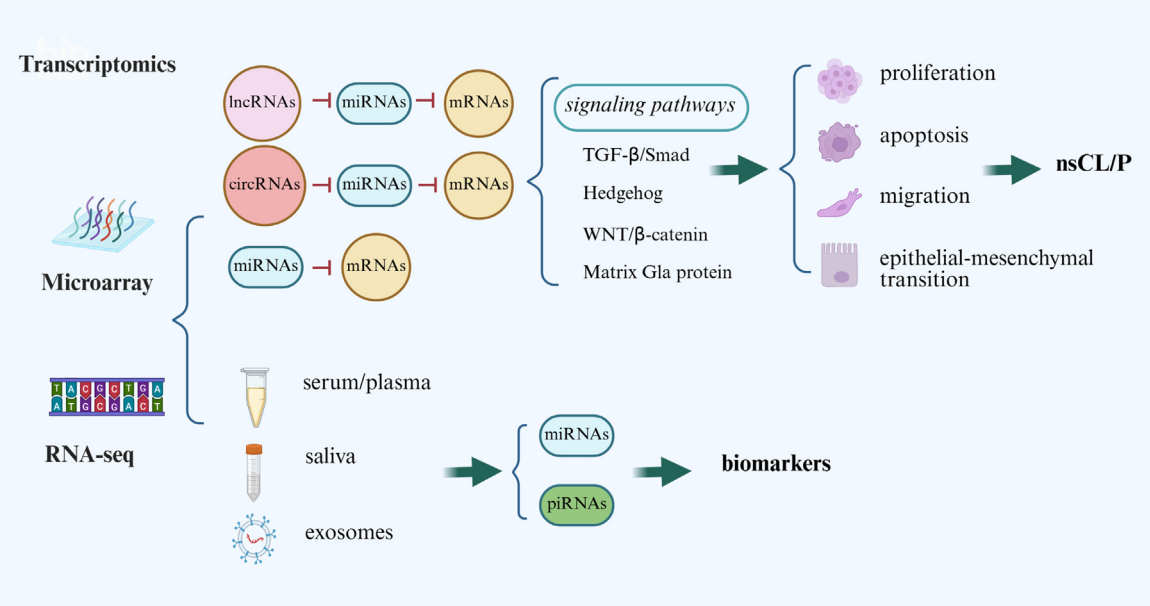


**Figure 2 – Application of transcriptomics to exploring the pathogenic genes and biomarkers of nsCL/P. Transcriptomics includes microarray and RNA-seq analysis. LncRNA, circRNAs, miRNAs and mRNAs are found as pathogenic genes and participate in the regulatory networks of nsCL/P. miRNAs and piRNAs from serum/plasma, saliva and exosomes are as potential biomarkers for nsCL/P.**

development. Large quantities of free RNA can be found in the peripheral blood of pregnant women and can be characterized using transcriptomics or microarray techniques. Exosomes, which are a class of lipid bilayer vesicles rich in miRNAs and resistant to enzyme degradation in body fluids, are present in the peripheral blood.[71] Exosomes participate in embryonic development by facilitating cellular and cell-cell transport and communication. In a previous study, we highlighted the clinical applicability of the Let-7 family, either from maternal plasma or plasma-derived exosomes, in predicting nsCLP. We found that *EN2* regulates *LIN28A/hsa-let-7a-3p*-suppressed hedgehog signaling pathway, inhibiting HOK cell proliferation by suppressing *GLI2* activity.[72] The genes involved in this regulatory axis were screened using RNA-seq analysis of lip tissues from nsCLP fetuses and further validated using additional lip specimens from nsCLP and control fetuses. Specifically, we identified eight miRNAs from the Let-7 family (*hsa-let-7a-3p, hsa-let-7a-5p, hsa-let-7c-5p, hsa-let-7d-3p, hsa-let-7d-5p, hsa-let-7e-5p, hsa-let-7f-5p*, and *hsa-miR-98-5p*) through small RNA sequencing of the plasma and exosomes derived from the plasma of pregnant women carrying nsCLP fetuses. Our findings indicate that these miRNAs hold promise as potential diagnostic biomarkers of nsCLP. The area under the receiver operating characteristic curve (AUC) of these eight miRNAs in the plasma was 0.893, with sensitivity and specificity values of 84.40% and 80.00%, respectively. When combined with diagnosis using plasma-derived exosomes, the AUC was 0.992 and the sensitivity and specificity were 100.00% and 93.30%, respectively. In a previous study, a miRNA microarray conducted on saliva collected from 12 children with cleft lip and palate and 12 healthy children revealed significant differences in *miR-141, miR-223*, and *miR-324-3p* expression levels.[73] Another study performed miRNA microarray analysis using plasma specimens from three children with cleft lip and three healthy children and subsequently conducted real-time quantitative reverse transcription polymerase chain reactions to validate the findings on three miRNAs (*miR-16-2-3p, miR-365a-3p*, and *miR-877-5p*) with high AUC values.[74] Li *et al*. included serum specimens from three children with cleft lip, three with cleft lip and palate, and three healthy children for miRNA microarray studies. Further sample validation revealed significant differences in the expression of six miRNAs (*miR-340-5p, miR-877-5p, miR-3648, miR-1260a, miR-494-3p*, and *miR-1304-3p*) in the patients with CL/P.[75] piRNAs, ranging from approximately 26-31 nucleotides in length, along with miRNAs, are a major class of small non-coding RNAs. These RNAs bind to PIWI proteins and play important roles in embryonic stem cell differentiation and early embryonic development.[76] In our previous study, we identified three piRNAs (*hsa-piR-009228, hsa-piR-016659*, and *hsa-piR-020496*) derived from the plasma exosomes of pregnant women as potential diagnostic biomarkers for the early diagnosis of nsCLP in the early second trimester (gestation weeks 15-19).[77] The characteristics of the studies mentioned above are presented in Table 2.

Compared with DNA, RNA is less stable, has more stringent storage requirements, and necessitates more complicated experimental handling. Thus, uniform, rigorous, and standardized systems should be used to ensure the reproducibility of the experimental results from such studies.

## Proteomics

Being downstream of the genomics and transcriptomics analysis, the proteomics can reflect the combined effects of

**Table 2 – Characteristics of the transcriptomics studies in nsCL/P.**

| Subtypes of orofacial clefts | Samples | Species | RNA names | References |
|---|---|---|---|---|
| nsCL/P | peripheral blood samples | human | lncRNA USP17L6P, hsa-miR-449c-5p, and MYC | Wang X, *et al.*,[57] |
| nsCP | palatal shelf tissues | 57BL/6N mice | lncRNA Meg3, TGF-$\beta$, and Smad | Liu X, *et al.*,[58] |
| nsCL/P | lip tissues | human | lncRNA MIR31HG | Li X, *et al.*,[59] |
| nsCP | palatal shelf tissues | C57BL/6J mice | lncRNA NONMMUT100923.1, miR-200a-3p, and Cdsn | Zhang M, *et al.*,[60] |
| nsCP | palatal shelf tissues | C57BL/6J mice | circRNA_0954, miRNA-881-3p, and PRKAR1$\alpha$ | Shu X, *et al.*,[61] |
| nsCP | palatal shelf tissues | ICR mouse | lncRNAs H19, lncRNAs Malat1, lncRNAs Miat, Cdh1, Irf6, Grhl3, and Efnb1 | Huang W, *et al.*,[62] |
| nsCP | palatal shelf tissues | C57BL/6J mice | miR-129-5p, miR-340-5p, Sox5, Trp53, Chd7, Fign, and Tgfbr1 | Yoshioka H, *et al.*,[66] |
| nsCP | palatal shelf tissues | C57BL/6J mice | miRNA-470-5p and Fgfr1 | Zhou J, *et al.*,[67] |
| nsCL | lip tissues | human, mice | miR-27b, miR-133b, and miR-205 | Yoshioka H, *et al.*,[68] |
| nsCL/P | palatal shelf tissues, lip tissues | human, mice | Let-7c-5p, miR-193a-3p, PIGA, and TGFB2 | Fu C, *et al.*,[69] |
| nsCL/P | plasma | human | miR-212-3p, miR-200b-3p, and miR-130b-3p | Tang J, *et al.*,[70] |
| nsCLP | plasma and plasma-derived exosomes | human | hsa-let-7a-3p, hsa-let-7a-5p, hsa-let-7c-5p, hsa-let-7d-3p, hsa-let-7d-5p, hsa-let-7e-5p, hsa-let-7f-5p, and hsa-miR-98-5p | Jia S, *et al.*,[72] |
| nsCLP | saliva | human | miR-141, miR-223, and miR-324-3p | Grassia V, *et al.*,[73] |
| nsCL | plasma | human | miR-16-2-3p, miR-365a-3p, and miR-877-5p | Zou J, *et al.*,[74] |
| nsCL/P | serum | human | miR-340-5p, miR-877-5p, miR-3648, miR-1260a, miR-494-3p, and miR-1304-3p | Li J, *et al.*,[75] |
| nsCLP | plasma-derived exosomes | human | hsa-piR-009228, hsa-piR-016659, and hsa-piR-020496 | Jia S, *et al.*,[77] |

multiple upstream factors more accurately and comprehensively, helping us to understand the pathogenesis of the disease. The development of proteomics relies on two-dimensional gel electrophoresis (2-DE) and mass spectrometry. One conventional assay method in proteomics is 2-DE with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). Compared with 2-DE, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has high detection throughput, accuracy, reproducibility, and can be used for comparative and quantitative proteomics analyses. The isobaric tags for relative and absolute quantification (iTRAQ) technique, in which amino acid residues in a protein sample are labeled by isobaric tags to produce specific mass signals in mass spectrometry analysis, enables the quantitative analysis of proteins. Shotgun proteomics is another LC-MS-based technique used in proteomics analysis. Proteins are first enzymatically digested, and peptides of appropriate size are detected by mass spectrometry. Next, the proteins corresponding to the peptides are determined through alignment with database entries. Finally, qualitative and quantitative analyses are performed.[78] Tandem mass tagging (TMT) is an in vitro peptide labeling technique whose principle is similar to that of iTRAQ. Peptide fragments are labeled by linking them to specific amino acid sites, and then tandem mass spectrometry is performed to monitor the tagged fragments and quantify the peptides, thus achieving higher throughput compared with iTRAQ. These common proteomics technologies have been used to study pathogenic proteins associated with nsCL/P and in prenatal diagnosis. The changes in protein profiles using mouse models of cleft palate have been previously investigated. For example, Yuan *et al.* constructed a mouse model of cleft palate using TCDD and performed 2-DE in conjunction with MALDI-TOF-MS on palate tissues. They found that peroxidase-1 is involved in energy metabolism, cell migration, and apoptosis in palate development in mice.[79] Huang *et al.* identified 10 hub proteins including motor proteins and extracellular matrix components in pathogenesis using iTRAQ proteomic analysis on mice model of cleft palate[80]. Wang *et al.* constructed a mouse model of cleft palate using TCDD and atRA separately and extracted palate tissues for iTRAQ proteomic analysis with further validation through western blot, revealing significant enrichment of 14-3-3 sigma and annexin A1 protein expression in the cleft palate group.[81] Moreover, Wang *et al.* investigated prenatal diagnostic proteins as biomarkers for cleft palate by constructing animal models. They used the teratogens atRA and TCDD for the construction of mouse models, extracted amniotic fluid for antibody array to screen for differentially expressed proteins, confirmed these results through enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry, and revealed that receptor of advanced glycosylation end products (RAGE) and epiregulin were significantly decreased in amniotic fluid.[82] Subsequently, they found that interleukin (IL)-12p40 and soluble

RAGE were differentially expressed in the serum of mice with cleft palate, indicating that these differentially expressed proteins can be used as diagnostic biomarkers for cleft palate in mice.[83] Following initial investigations in mouse models, subsequent studies have shifted their focus on humans. Several differentially expressed secreted proteins, including actin, salivary cystatin, and keratin, were screened from saliva collected from children with cleft palate and healthy children using MALDI-TOF-MS.[84] Zhang *et al.* collected serum from children with nsCLP and healthy children and applied shotgun proteomics to analyze differentially expressed proteins and found that retinol-binding protein 4 and vitamin A were significantly decreased in the serum of children with nsCL/P.[85] This finding provides a theoretical basis for vitamin A supplementation during pregnancy to prevent the development of nsCL/P. Hou *et al.* conducted TMT-based quantitative proteomics analysis on serum from children with non-syndromic orofacial cleft compared with healthy children, followed by quantitative validation using parallel reaction monitoring techniques and found significant differences in the expression levels of four proteins (sex hormone-binding globulin, periostin, osteomodulin, and aggrecan core protein) in the non-syndromic orofacial cleft group.[86] Wang *et al.* categorized pregnant women in nsCLP, nsCL, and healthy control groups, collected serum samples from the women at 16-28 weeks of gestation for iTRAQ proteomic analysis, and validated them using MRM and ELISA methods.[87] They found that Haptoglobin was significantly downregulated in the nsCLP and nsCL groups, whereas levels of Apolipoprotein(a) and C-reactive protein were significantly increased in the nsCLP group. The combined diagnostic efficiency of these three proteins was better in the nsCLP group, with an AUC of 0.820 (95% confidence interval: 0.701-0.906). This finding suggests a practical avenue for investigating protein biomarkers for prenatal diagnosis of fetal cleft lip and palate. In contrast to traditional data acquisition, data-independent acquisition (DIA) as a sensitive proteomic method enables in-depth protein profiling with low sample requirements in an unbiased manner. Future studies may use DIA proteomics to explore more promising protein biomarkers for nsCL/P. The characteristics of the studies mentioned above are presented in Table 3.

## Lipidomics

Lipidomics is a relatively new high-throughput methodology compared with conventional omics technologies such as genomics, transcriptomics, and proteomics. Lipidomics is a major branch of metabolomics that has benefited from the development of mass spectrometry technology. It uses qualitative and quantitative analysis and involves the identification of lipid metabolites to reveal relationships between lipid synthesis, lipid metabolism, and physiological and pathological processes, thus enabling researchers to predict the development, progression, and outcomes of diseases. Lipidomics is categorized into untargeted Lipidomics (which is characterized by broad coverage) and targeted Lipidomics (which is characterized by high accuracy).[88,89] Lipids act as major components of cell membranes, energy stores, signaling

**Table 3 – Characteristics of the proteomics studies in nsCL/P.**

| Technologies | Subtypes of orofacial clefts | Study participants | Tissues | Protein names | References |
|---|---|---|---|---|---|
| 2-DE with MALDI-TOF-MS | nsCP | C57BL/6J mice | palates | Peroxiredoxin-1 | Yuan X, *et al.*,[79] |
| iTRAQ | nsCP | Kun Ming mice | palates | Tnnc2, Myl1, Myh8, Ckm, Myh3, Rps16, Col2a1, Col9a1, Matn3, and Acan | Huang Z, *et al.*,[80] |
| iTRAQ | nsCP | C57BL/6J mice | palates | 14-3-3 sigma and annexin A1 | Wang C, *et al.*,[81] |
| label-based mouse antibody array | nsCP | C57BL/6J mice | amniotic fluid | receptor for advanced glycation end products (RAGE) and epiregulin | Wang X, *et al.*,[82] |
| label-based mouse antibody array | nsCP | C57BL/6J mice | serum | interleukin (IL)-12p40 and soluble RAGE | Wang X, *et al.*,[83] |
| MALDI-TOF-MS | nsCLP | 31 patients and 20 controls | saliva | actin, salivary cystatin, keratin, TGF-b3, and dermokine | Szabo GT, *et al.*,[84] |
| shotgun proteomics | nsCLP | 13 patients and 10 controls | serum | Retinol binding protein 4 and vitamin A | Zhang J, *et al.*,[85] |
| TMT | nsCLP | 5 patients and 5 controls | serum | sex hormone-binding globulin, periostin, osteomodulin, and aggrecan core protein | Hou Y, *et al.*,[86] |
| iTRAQ | nsCLP, nsCL | 20 pregnant women carrying nsCLP/nsCL fetuses and 20 pregnant women with healthy ones | serum | Haptoglobin, Apolipoprotein(a), and C-reactive | Wang X, *et al.*,[87] |

molecules, and critical players in embryonic development. Zhang *et al.* found that unsaturated triglycerides play a crucial role in palate formation through non-targeted lipidomic analysis of palate tissues with atRA-treated cleft palate compared with control embryos.[90] Abnormal lipid metabolism also reportedly affects the proliferation and migration of MEPM cells. For example, intracellular lipid droplets were found to accumulate in a Tgfbr2 mutant mouse model of cleft palate, in which MEPM cells proliferation was impaired by abnormal lipid metabolism.[91] Similarly, another study found decreased expression of the adipose triglyceride lipase Pnpla2 (a major enzyme involved in lipolysis process) in palate tissues from an atRA-induced mouse cleft palate model, led to impaired proliferation and migration of MEPM cells.[92] Combining lipidomics and machine learning, we explored a panel of 3 lipid biomarkers showed great potential for nsCLP diagnosis.[93] Lipid-related studies are particularly important for investigating prenatal screening and the pathogenic mechanisms of nsCL/P (Figure 3A).

## Epigenomics

Epigenomics is a comprehensive analysis of a range of epigenetics, including but not limited to, DNA methylation and histone modification. Epigenetics is a critical mediator of the interplay between genes and the environment, and influences phenotypic outcomes. As mentioned, nsCL/P is influenced by a combination of environmental and genetic factors. DNA methylation is well-recognized as one of the mechanisms of epigenetics and involved in the development of orofacial clefts. Aberrant DNA methylation levels result in craniofacial malformations, including orofacial clefts.[94-96] DNA hypomethylation or hypermethylation depends on the tissue- and timing-dependent in orofacial development.[97] Different orofacial cleft subtypes have distinct methylation profiles.[98] Up till now, DNA methylation has been examined in patients with nsCL/P from multiple specimens, including lip, palate, whole blood, and saliva, as well as experimental studies in mice. DNMT1, an active DNA methyltransferase enzyme, regulates cranial neural crest cell (cNCC) proliferation and differentiation, and required for orofacial morphogenesis.[94] Zhang *et al.* illustrated the DNA methylation profile of lip tissues from nsCLP patients by whole-genome bisulfite sequencing (WGBS) and found the DNA hypomethylation of GLI2, the well- known susceptibility gene of nsCL/P.[99] Our previous study detected numerous aberrantly methylated loci in fetal lip tissues of nsCL fetus via Infinium HumanMethylation450 BeadChi array.[100] Two individual CpGs and multiple differentially methylated regions were identified in blood DNA

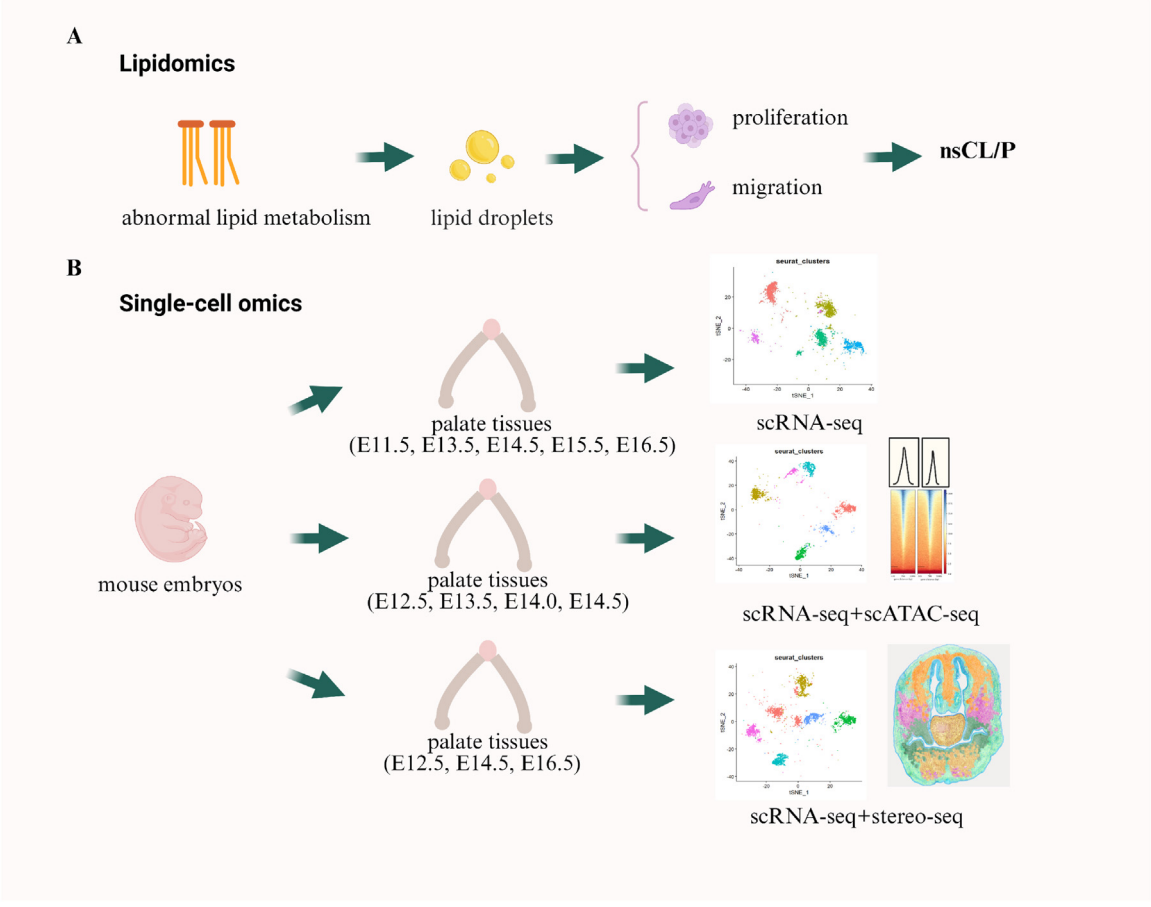


**Figure 3 – Application of Lipidomics and single-cell omics to exploring the pathogenic genes of nsCL/P. Lipidomics and single-cell omics techniques were performed in nsCL/P studies. A: Lipidomics studies in nsCL/P. B: Single-cell omics studies in nsCL/P.**

between newborn babies with and without nsCL/P.[101] Other studies have confirmed that saliva and blood samples between monozygotic twins discordant for nsCL/P present significant differences in DNA methylation.[102,103] Histone acetylation is involved in the critical palate fusion during E13.5-E15.5 in TCDD induced cleft palate formation.[104] Wilderman, A. *et al.* profiled human embryonic craniofacial samples from each of four distinct Carnegie stages (CSs) (CS13, CS14, CS15, and CS17) encompassing 4-5 post conception weeks (pcw) to 6 pcw and found multiple histone modifications via chromatin immunoprecipitation sequencing (CHIP-seq).[105] The characteristics of the studies mentioned above are presented in Table 4. Understanding epigenomics in orofacial clefts will contribute to the development of new genetic tools for early detection, the enrichment of prognostic information, and ultimately, the prevention of orofacial clefts.

## Single-cell omics

Conventional approaches involve the use of small RNA microarray or bulk transcriptomic analysis, which fail to provide cell type-specific gene expression profiles owing to the inherent heterogeneity within tissue blocks. Under these circumstances, single-cell RNA sequencing (scRNA-seq) offers an accurate and efficient way to analyze the interactions between different cells involved in craniofacial development (Figure 3B). scRNA-seq has been performed on mouse facial tissues to identify and analyze gene expression profiles of ectodermal, mesenchymal, and endothelial cell populations involved in the development of lip and palate tissues.[106] An scRNA-seq study of palate tissue in mouse embryos at day E13.5, E14.5 and E15.5 revealed complex cellular heterogeneity during craniofacial development, describing the complex regulatory role of cells derived from cNCC in the development of the craniofacial musculoskeletal system.[107] Another scRNA analysis of the anterior palate in mouse embryos at day E13.5 revealed the heterogeneity of mesenchymal cells.[108] scRNA-seq analysis of the palate tissues of mice with atRA-induced cleft palate and normal mice on day E16.5 revealed different subcellular patterns of gene expression, such as the increased levels of M1-type macrophages and monocytes, as well as activation of the IL-17 signaling pathway in mice with cleft palate.[109] In another recent study, gene expression and chromatin accessibility in the same cells at different developmental periods (E12.5, E13.5, E14.0, and E14.5) were analyzed using single-cell multi-omics technologies, including scRNA-seq and single-cell assay for transposase-accessible chromatin sequencing (scATAC-seq), to elucidate the linkage between spatial and temporal gene expression in palatal cells at the transcriptomic and genomic levels.[110] A recent study generated genome-wide maps of the enhancer-associated histone mark H3K27ac, accessible chromatin, and gene expression via multi-omics techniques (CHIP-seq, ATAC-seq and RNA-seq) from embryonic face tissue for CS 18, 19, 22, and 23, which are within the critical developmental window for palate formation.[111] Meanwhile, they combined the considerable temporal dynamics of human craniofacial enhancers with single-cell-resolved data of mouse embryonic face to define the regulatory landscape of craniofacial development at both bulk and single-cell resolution. The combination of conventional RNA sequencing methods with scRNA-seq analysis can help explore the role of known nsCL/P-related pathogenic genes during development.[112] Combining scRNA-seq and spatially enhanced resolution omics-sequencing (stereo-seq) at three critical stages of palate formation (E12.5, E14.5, and E16.5) establishes a transcriptomic regulatory landscape across single-cell and spatial scales during the development of palate in mice.[113] Single-cell omics technologies can offer insights into gene expression at single-cell resolutions, allowing the study of cellular heterogeneity and the effective selection of cell types associated with lip and palate development.

**Table 4 – Characteristics of the epigenomics studies in nsCL/P.**

| Technologies | Subtypes of orofacial clefts | Study participants | Areas or races | Tissues | References |
|---|---|---|---|---|---|
| Infinium Human Methylation 450 K Bead-Array | nsCL/P | 238 patients and 246 controls | Brazil | whole-blood and lip | Alvizi, L. *et al.*,[95] |
| Illumina Infinium HumanMethylation450 BeadChip | CL/P | 150 patients | United Kingdom | whole-blood, lip, and palate | Sharp, GC, *et al.*,[98] |
| WGBS | nsCLP | 8 nsCLP patients | Chinese populations | lip | Zhang, *et al.*,[99] |
| Illumina Infinium HumanMethylation450 BeadChip | nsCL | 3 nsCL fetuses and 3 control fetuses | Chinese populations | lip | Xu, *et al.*,[100] |
| Illumina Infinium HumanMethylation450 BeadChip | nsCL/P | 308 patients and 436 controls | Norway | whole-blood | Xu, *et al.*,[101] |
| WGBS | nsCLP | 6 monozygotic twin pairs | Non-Hispanic White and Hispanic populations | saliva | Young, JI., *et al.*,[102] |
| MethylationEPIC BeadChip | nsCL, nsCLP | 6 monozygotic twin pairs | Chinese populations | whole-blood | Shi, *et al.*,[103] |

## Conclusion

Omics technologies are commonly employed in nsCL/P research to understand its causation and development and improve prenatal diagnosis. Utilizing multi-omics technologies enables a multifaceted search for the relevant causative genes of nsCL/P, providing a theoretical basis for further investigation of their molecular mechanisms. This article provides a comprehensive and timely review of multi-omics technologies in nsCL/P, effectively synthesizing their applications in identifying pathogenic genes and promising non-invasive prenatal biomarkers. These findings provide valuable clues for further understanding the underlying mechanisms and offer insights for the future development of prenatal diagnostics. Integrating multi-omics studies has contributed to a better knowledge of aetiology of nsCL/P. Nine novel candidate genes involved in nsCL/P were revealed by the integrative multi-omics analysis.[114] Recently, a multi-omics approach was employed to provide genetic variants contributing to nsCL/P.[115] Despite these advances, several shortcomings remain in the current body of research. Distinct genetic backgrounds across ethnic populations introduce significant genetic heterogeneity. This can lead to the omission of population-specific biomarkers and the generation of biased biological models, thereby compromising the generalizability and clinical applicability of multi-omics findings. The samples included in the research articles on prenatal diagnosis of nsCL/P presented in this review were primarily from single centers and included a small number of samples, which may have led to a diagnostic bias in biomarkers. In addition, detection techniques differed between studies, indicating a lack of uniformity and standardization. Therefore, future multicenter, large-sample studies with improved designs can enable timely translation of prenatal diagnosis research to the clinic. Data integration, standardization, and the substantial cost barriers that currently limit the widespread clinical implementation of multi-omics approaches. Further technological developments and cost reductions may eventually allow genomics, transcriptomics, proteomics, lipidomics, epigenomics, and single-cell multi-omics technologies to become routine clinical tests. Non-invasive prenatal diagnostic techniques based on omics technologies are likely to be key to early and accurate diagnosis of nsCL/P and will thus play a crucial role in prenatal medicine. Although multi-omics studies on nsCL/P have been carried out, integrated multi-omics analysis has rarely been reported. While multi-omics analysis provides a powerful tool for nsCL/P, it still faces multiple challenges, including data heterogeneity, computational complexity, difficulties in biological interpretation, and high demands for interdisciplinary collaboration. Artificial intelligence (AI) is particularly well-suited for the complexities of heterogeneous and unstructured data, excelling at discovering the non-linear, high-dimensional correlations present in multi-modal information.[116] AI-based multi-omics analysis has great promise for prenatal diagnosis and mechanism research. Moreover, establishing an open-source database for nsCL/P multi-omics data would significantly advance research in this field.

## Author contributions

CQ. Y., SS. J., and ZW. Y. wrote the main manuscript text. CQ. Y. and L.D., prepared the figures and tables. YZ. D., Y. W., and SY. C. edited the manuscript. All authors reviewed the manuscript.

## Conflicts of interests

None disclosed.

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## Supplementary materials

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## REFERENCES

1. Jugessur A, Farlie PG, Kilpatrick N. The genetics of isolated orofacial clefts: from genotypes to subphenotypes. Oral Dis 2009;15(7):437–53.
2. Wang M, Yuan Y, Wang Z, et al. Prevalence of orofacial clefts among live births in China: a systematic review and meta-analysis. Birth Defects Res 2017;109(13):1011–9.
3. Dixon MJ, Marazita ML, Beaty TH, et al. Cleft lip and palate: understanding genetic and environmental influences. Nat Rev Genet 2011;12(3):167–78.
4. Krapels IP, Van Rooij IA, Ocke MC, et al. Maternal nutritional status and the risk for orofacial cleft offspring in humans. J Nutr 2004;134(11):3106–13.
5. Honein MA, Rasmussen SA, Reefhuis J, et al. Maternal smoking and environmental tobacco smoke exposure and the risk of orofacial clefts. Epidemiology 2007;18(2):226–33.
6. Boyles AL, Deroo LA, Lie RT, et al. Maternal alcohol consumption, alcohol metabolism genes, and the risk of oral clefts: a population-based case-control study in Norway, 1996-2001. Am J Epidemiol 2010;172(8):924–31.
7. Hwang BF, Jaakkola JJ. Ozone and other air pollutants and the risk of oral clefts. Environ Health Perspect 2008;116(10):1411–5.
8. Ni W, Yang W, Yu J, et al. Association between selected essential trace element concentrations in umbilical cord and risk for cleft lip with or without cleft palate: a case-control study. Sci Total Environ 2019;661:196–202.

9. Guo Y, Liu L, Ni W, et al. Uranium concentration in umbilical cord may increase the risk for orofacial clefts. Environ Res 2020;182:109103.
10. Brown G. Retinoic acid receptor regulation of decision-making for cell differentiation. Front Cell Develop Biol 2023; 11:1182204.
11. Behnisch PA, Hosoe K, Sakai S. Combinatorial bio/chemical analysis of dioxin and dioxin-like compounds in waste recycling, feed/food, humans/wildlife and the environment. Environ Int 2001;27(6):495–519.
12. Vorderstrasse BA, Fenton SE, Bohn AA, et al. A novel effect of dioxin: exposure during pregnancy severely impairs mammary gland differentiation. Toxicol Sci: 2004;78(2):248–57.
13. Chopra M, Schrenk D. Dioxin toxicity, aryl hydrocarbon receptor signaling, and apoptosis-persistent pollutants affect programmed cell death. Crit Rev Toxicol 2011;41(4): 292–320.
14. Bush JO, Jiang R. Palatogenesis: morphogenetic and molecular mechanisms of secondary palate development. Development 2012;139(2):231–43.
15. Gritli-Linde A. Molecular control of secondary palate development. Dev Biol 2007;301(2):309–26.
16. Sander FH, Jorgensen DS, Jakobsen LP, et al. Prenatal detection of orofacial clefts in Denmark from 2009 to 2018. Ultrasound Obstet Gynecol 2024;63(4):507–13.
17. Schei-Andersen AJ, van Oirschot B, Drissen M, et al. Exploring the prevalence of oral features for early detection of PTEN hamartoma tumour syndrome. Int Dent J 2024;74 (6):1424–31.
18. Wang Y, Peng X, Wang X, et al. Glycolysis regulates palatal mesenchyme proliferation through Pten-Glut1 axis via Pten classical and non-classical pathways. Cell Biol Toxicol 2025;41(1):53.
19. Nestler U, Grafe D, Strehlow V, et al. A newborn with cleft palate associated with PTEN hamartoma tumor syndrome. Clin Pract 2025;15(1).
20. Huang W, Gu H, Yuan Z. Identifying biomarkers for prenatal diagnosis of neural tube defects based on "omics". Clin Genet 2022;101(4):381–9.
21. Chen L, Guan J, Wei Q, et al. Potential role of "omics" technique in prenatal diagnosis of congenital heart defects. Clin Chim Acta 2018;482:185–90.
22. Wang W, Ji Y, Dong Z, et al. Characterizing neuroinflammation and identifying prenatal diagnostic markers for neural tube defects through integrated multi-omics analysis. J Transl Med 2024;22(1):257.
23. Birnbaum S, Ludwig KU, Reutter H, et al. Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. Nat Genet 2009;41(4):473–7.
24. Grant SF, Wang K, Zhang H, et al. A genome-wide association study identifies a locus for nonsyndromic cleft lip with or without cleft palate on 8q24. J Pediatr 2009;155(6):909–13.
25. Mangold E, Ludwig KU, Birnbaum S, et al. Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. Nat Genet 2010;42(1):24–6.
26. Rojas-Martinez A, Reutter H, Chacon-Camacho O, et al. Genetic risk factors for nonsyndromic cleft lip with or without cleft palate in a Mesoamerican population: Evidence for IRF6 and variants at 8q24 and 10q25. Birth Defects Res A Clin Mol Teratol 2010;88(7):535–7.
27. Lennon C J, Birkeland A C, Nunez J A, et al. Association of candidate genes with nonsyndromic clefts in Honduran and Colombian populations. The Laryngoscope 2012;122(9): 2082–7.
28. Yu Y, Zuo X, He M, et al. Genome-wide analyses of non-syndromic cleft lip with palate identify 14 novel loci and genetic heterogeneity. Nat Commun 2017;8(14364).
29. Huang L, Jia Z, Shi Y, et al. Genetic factors define CPO and CLO subtypes of nonsyndromicorofacial cleft. PLoS Genet 2019;15(10):e1008357.
30. Beaty TH, Murray JC, Marazita ML, et al. A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. Nat Genet 2010;42(6):525–9.
31. Wang Y, Sun Y, Huang Y, et al. Validation of a genome-wide association study implied that SHTIN1 may involve in the pathogenesis of NSCL/P in Chinese population. Sci Rep 2016;6:38872.
32. Leslie EJ, Taub MA, Liu H, et al. Identification of functional variants for cleft lip with or without cleft palate in or near PAX7, FGFR2, and NOG by targeted sequencing of GWAS loci. Am J Hum Genet 2015;96(3):397–411.
33. Leslie E J, Carlson J C, Shaffer J R, et al. Genome-wide meta-analyses of nonsyndromic orofacial clefts identify novel associations between FOXE1 and all orofacial clefts, and TP63 and cleft lip with or without cleft palate. Human genetics 2017;136(3):275–86.
34. Teer JK, Mullikin JC. Exome sequencing: the sweet spot before whole genomes. Hum Mol Genet 2010;19(R2):R145–51.
35. Manojlovic Z, Auslander A, Jin Y, et al. Genome analysis using whole-exome sequencing of non-syndromic cleft lip and/or palate from Malagasy trios identifies variants associated with cilium-related pathways and asian genetic ancestry. Genes (Basel) 2023;14(3):665.
36. Yan S, Fu F, Li R, et al. Exome sequencing improves genetic diagnosis of congenital orofacial clefts. Front Genet 2023; 14:1252823.
37. Aylward A, Cai Y, Lee A, et al. Using whole exome sequencing to identify candidate genes with rare variants in nonsyndromic cleft lip and palate. Genet Epidemiol 2016;40(5): 432–41.
38. Machado RA, Martelli-Junior H, Reis SRA, et al. Identification of novel variants in cleft palate-associated genes in Brazilian patients with non-syndromic cleft palate only. Front Cell Develop Biol 2021;9:638522.
39. Tang JX, Xiao XS, Wang K, et al. Identification of a novel variant of ARHGAP29 in a Chinese family with nonsyndromic cleft lip and palate. Biomed Res Int 2020:8790531 2020.
40. Meng P, Zhao H, Huang W, et al. Three GLI2 mutations combined potentially underlie non-syndromic cleft lip with or without cleft palate in a Chinese pedigree. Mol Genet Genomic Med 2019;7(9):e714.
41. Xu T, Du M, Bu X, et al. Identification of a novel TP63 mutation causing nonsyndromic cleft lip with or without cleft palate. BMC Med Genomics 2021;14(1):53.
42. Wang Y, Ma C, Jiang C, et al. A Novel IRF6 variant detected in a family with nonsyndromic cleft lip and palate by whole exome sequencing. J Craniofac Surg 2021;32(1):265–9.
43. Al Mahdi HB, Edris S, Bahieldin A, et al. Identification of causative variants contributing to nonsyndromic orofacial clefts using whole-exome sequencing in a saudi family. Genet Test Mol Biomarkers 2020;24(11):723–31.
44. Zhang J, Zhao H, Huang W, et al. A novel FZD6 mutation revealed the cause of cleft lip and/or palate in a Chinese family. Genes Dis 2020;7(3):440–7.
45. Zhong W, Zhao H, Huang W, et al. Identification of rare PTCH1 nonsense variant causing orofacial cleft in a Chinese family and an up-to-date genotype-phenotype analysis. Genes Dis 2021;8(5):689–97.
46. Fu Z, Yue J, Xue L, et al. Using whole exome sequencing to identify susceptibility genes associated with nonsyndromic cleft lip with or without cleft palate. Mol Genet Genomics 2023;298(1):107–18.
47. Mukhopadhyay Bishop M, Mortillo M, et al. Whole genome sequencing of orofacial cleft trios from the Gabriella Miller

Kids First Pediatric Research Consortium identifies a new locus on chromosome 21. Human genetics 2020;139(2):215–26.
48. Bishop M R, Diaz Perez K K, Sun M, et al. Genome-wide enrichment of De novo coding mutations in orofacial cleft trios. Am J Hum Genet 2020;107(1):124–36.
49. Cai S, Si N, Wang Y, et al. Transcriptomic analysis of the upper lip and primary palate development in mice. Front Genet 2022;13:1039850.
50. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. Nature 2009;461(7265):747–53.
51. Salmena L, Poliseno L, Tay Y, et al. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 2011;146(3):353–8.
52. Jiang S, Zhang Q, Li J, et al. New sights into long non-coding RNA LINC01133 in Cancer. Front Oncol 2022;12:908162.
53. Geisler S, Coller J. RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol 2013;14(11):699–712.
54. Wu C, Liu H, Zhan Z, et al. Unveiling dysregulated lncRNAs and networks in non-syndromic cleft lip with or without cleft palate pathogenesis. Sci Rep 2024;14(1):1047.
55. Shi C, Jiao P, Chen Z, et al. Exploring the roles of noncoding RNAs in craniofacial abnormalities: a systematic review. Dev Biol 2024;505:75–84.
56. Wang E, Guo Y, Gao S, et al. Long non-coding RNAs MALAT1 and NEAT1 in non-syndromic orofacial clefts. Oral Dis 2023;29(4):1668–79.
57. Wang X, Guo S, Zhou X, et al. Exploring the molecular mechanism of lncRNA-miRNA-mRNA networks in non-syndromic cleft lip with or without cleft palate. Int J Gen Med 2021;14:9931–43.
58. Liu X, Song S, Wang G, et al. Upregulated LncRNA-Meg3 modulates the proliferation and survival of MEPM cells via interacting with Smad signaling in TCDD-induced cleft palate. Food Chem Toxicol 2023;185:114410.
59. Li X, Xu X, Liu L, et al. lncRNA MIR31HG Regulates Proliferation and Migration by Targeting Matrix Gla Protein in Non-syndromic Cleft Lip With or Without Cleft Palate. DNA Cell Biol 2023;42(7):390–8.
60. Zhang M, Zhou J, Ji Y, et al. LncRNA-NONMMUT100923.1 regulates mouse embryonic palatal shelf adhesion by sponging miR-200a-3p to modulate medial epithelial cell desmosome junction during palatogenesis. Heliyon 2023;9(5):e16329.
61. Shu X, Cheng L, Dong Z, et al. Identification of circular RNA-associated competing endogenous RNA network in the development of cleft palate. J Cell Biochem 2019;120(9):16062–74.
62. Huang W, Zhong W, He Q, et al. Time-series expression profiles of mRNAs and lncRNAs during mammalian palatogenesis. Oral Dis 2023;29(5):2163–76.
63. Huang HY, Lin YC, Li J, et al. miRTarBase 2020: updates to the experimentally validated microRNA-target interaction database. Nucleic Acids Res 2020;48(D1):D148–D54.
64. Yan F, Simon LM, Suzuki A, et al. Spatiotemporal MicroRNA-Gene Expression Network Related to Orofacial Clefts. J Dent Res 2022;101(11):1398–407.
65. Iwaya C, Suzuki A, Iwata J. MicroRNAs and Gene Regulatory Networks Related to Cleft Lip and Palate. Int J Mol Sci 2023;24(4):3552.
66. Yoshioka H, Suzuki A, Iwaya C, et al. Suppression of microRNA 124-3p and microRNA 340-5p ameliorates retinoic acid-induced cleft palate in mice. Development 2022;149(9):dev200476.
67. Zhou J, Zhang M, Zhang M, et al. MiRNA-470-5p suppresses epithelial-mesenchymal transition of embryonic palatal shelf epithelial cells by targeting Fgfr1 during palatogenesis. Exp Biol Med (Maywood) 2023;248(13):1124–33.
68. Yoshioka H, Li A, Suzuki A, et al. Identification of microRNAs and gene regulatory networks in cleft lip common in humans and mice. Hum Mol Genet 2021;30(19):1881–93.
69. Fu C, Lou S, Zhu G, et al. Identification of New miRNA-mRNA Networks in the Development of Non-syndromic Cleft Lip With or Without Cleft Palate. Frontiers in cell and developmental biology 2021;9:631057.
70. Tang J, Lian SB, Bai Y, et al. Comprehensive analysis of plasma miRNA and related ceRNA network in non-syndromic cleft lip and/or palate. Int J Pediatr Otorhinolaryngol 2022;162:111306.
71. Kalluri R, Lebleu VS. The biology, function, and biomedical applications of exosomes. Science 2020;367(6478):eaau6977.
72. Jia S, Zhang Q, Wang Y, et al. Identification by RNA-Seq of let-7 clusters as prenatal biomarkers for nonsyndromic cleft lip with palate. Ann N Y Acad Sci 2022;1516(1):234–46.
73. Grassia V, Lombardi A, Kawasaki H, et al. Salivary microRNAs as new molecular markers in cleft lip and palate: a new frontier in molecular medicine. Oncotarget 2018;9(27):18929–38.
74. Zou J, Li J, Li J, et al. Expression profile of plasma microRNAs in nonsyndromic cleft lip and their clinical significance as biomarkers. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 2016;82:459–66.
75. Li J, Zou J, Li Q, et al. Assessment of differentially expressed plasma microRNAs in nonsyndromic cleft palate and non-syndromic cleft lip with cleft palate. Oncotarget 2016;7(52):86266–79.
76. Rojas-Rios P, Simonelig M. piRNAs and PIWI proteins: regulators of gene expression in development and stem cells. Development 2018;145(17):dev161786.
77. Jia S, Zhang Q, Wang Y, et al. PIWI-interacting RNA sequencing profiles in maternal plasma-derived exosomes reveal novel non-invasive prenatal biomarkers for the early diagnosis of nonsyndromic cleft lip and palate. EBioMedicine 2021;65:103253.
78. Shteynberg D, Deutsch EW, Lam H, et al. iProphet: multilevel integrative analysis of shotgun proteomic data improves peptide and protein identification rates and error estimates. Mol Cell Proteomics 2011;10(12) M111 007690.
79. Yuan X, Liu L, Pu Y, et al. 2,3,7,8-Tetrachlorodibenzo-p-dioxin induces a proteomic pattern that defines cleft palate formation in mice. Food Chem Toxicol 2012;50(7):2270–4.
80. Huang Z, Zhang C, Sun M, et al. Proteomic analysis illustrates the potential involvement of motor proteins in cleft palate development. Sci Rep 2024;14(1):21868.
81. Wang C, Zhai SN, Yuan XG, et al. Common differentially expressed proteins were found in mouse cleft palate models induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin and retinoic acid. Environment Toxicol Pharmacol 2019;72:103270.
82. Wang X, Zhu J, Fang Y, et al. Lower concentrations of receptor for advanced glycation end products and epiregulin in amniotic fluid correlate to chemically induced cleft palate in mice. Environment Toxicol Pharmacol 2017;51:45–50.
83. Wang XH, Peng Y, Meng LY. IL-12p40 and sRAGE in serum correlate with chemically induced cleft palate in mice. Human Experiment Toxicol 2020;39(12):1661–70.
84. Szabo GT, Tihanyi R, Csulak F, et al. Comparative salivary proteomics of cleft palate patients. Cleft Palate Craniofac J 2012;49(5):519–23.
85. Zhang J, Zhou S, Zhang Q, et al. Proteomic Analysis of RBP4/Vitamin A in Children with Cleft Lip and/or Palate. J Dent Res 2014;93(6):547–52.
86. Hou Y, Xu Y, Fu D, et al. Quantitative proteomic analysis of nonsyndromic orofacial cleft patient serum. Oral Dis 2023;29(1):206–10.

87. Wang X, Yang X, Huang P, et al. Identification of maternal serum biomarkers for prenatal diagnosis of nonsyndromic orofacial clefts. Ann N Y Acad Sci 2022;1510(1):167–79.
88. Chen H, Peng L, Zhao C, et al. Protective Mechanism of Polygonum perfoliatum L. Extract on Chronic Alcoholic Liver Injury Based on UHPLC-QExactive Plus Mass Spectrometry Lipidomics and MALDI-TOF/TOF. Mass Spectrometry Imaging. Foods 2022;11(11):1583.
89. Castane H, Baiges-Gaya G, Hernandez-Aguilera A, et al. Coupling machine learning and lipidomics as a tool to investigate metabolic dysfunction-associated fatty liver disease. Gen Overview. Biomolecules 2021;11(3):473.
90. Zhang W, Zhao H, Chen J, et al. A LCMS-based untargeted lipidomics analysis of cleft palate in mouse. Mech Dev 2020;162:103609.
91. Iwata J, Suzuki A, Pelikan RC, et al. Modulation of lipid metabolic defects rescues cleft palate in Tgfbr2 mutant mice. Hum Mol Genet 2014;23(1):182–93.
92. Ye QN, Zheng K. Expression and function of patatin-like phospholipase domain-containing 2 in cleft palate induced by retinoic acid. Br J Oral Maxillofac Surg 2023;61(3):215–20.
93. Jia S, Xie W, Yang C, et al. Combining lipidomics and machine learning to identify lipid biomarkers for nonsyndromic cleft lip with palate. JCI insight 2025;10(9):e186629.
94. Ulschmid CM, Sun MR, Jabbarpour CR, et al. Disruption of DNA methylation-mediated cranial neural crest proliferation and differentiation causes orofacial clefts in mice. Proceed Nat Acad Sci USA 2024;121(3):e2317668121.
95. Alvizi L, Ke X, Brito LA, et al. Differential methylation is associated with non-syndromic cleft lip and palate and contributes to penetrance effects. Sci Rep 2017;7(1):2441.
96. Enkhmandakh B, Joshi P, Robson P, et al. Single-cell transcriptome landscape of DNA methylome regulators associated with orofacial clefts in the mouse dental pulp. Cleft Palate Craniofac J 2023:10556656231172296.
97. Charoenvicha C, Sirimaharaj W, Khwanngern K, et al. alterations in DNA methylation in orofacial clefts. Int J Mol Sci 2022;23(21):12727.
98. Sharp GC, Ho K, Davies A, et al. Distinct DNA methylation profiles in subtypes of orofacial cleft. Clin Epigenetics 2017;9(63).
99. Zhang B, Zhang Y, Wu S, et al. DNA methylation profile of lip tissue from congenital nonsyndromic cleft lip and palate patients by whole-genome bisulfite sequencing. Birth Defects Res 2023;115(2):205–17.
100. Xu XY, Wei XW, Ma W, et al. Genome-wide screening of aberrant methylation loci for nonsyndromic cleft lip. Chin Med J (Engl) 2018;131(17):2055–62.
101. Xu Z, Lie RT, Wilcox AJ, et al. A comparison of DNA methylation in newborn blood samples from infants with and without orofacial clefts. Clin Epigenetics 2019;11(1):40.
102. Young JI, Slifer S, Hecht JT, et al. DNA Methylation Variation Is Identified in Monozygotic Twins Discordant for Non-syndromic Cleft Lip and Palate. Frontiers in cell and developmental biology 2021;9(656865).
103. Shi X, Wang Q, Sun C, et al. Study on the role of methylation in nonsyndromic cleft lip with or without cleft palate using a monozygotic twin model. Int J Pediatr Otorhinolaryngol 2021;143(110659).
104. Yuan X, Qiu L, Pu Y, et al. Histone acetylation is involved in TCDDinduced cleft palate formation in fetal mice. Mol Med Rep 2016;14(2):1139–45.
105. Wilderman A, Vanoudenhove J, Kron J, et al. High-resolution epigenomic atlas of human embryonic craniofacial development. Cell reports 2018;23(5):1581–97.
106. Li H, Jones KL, Hooper JE, et al. The molecular anatomy of mammalian upper lip and primary palate fusion at single cell resolution. Development 2019;146(12):dev174888.
107. Han X, Feng J, Guo T, et al. Runx2-Twist1 interaction coordinates cranial neural crest guidance of soft palate myogenesis. eLife 2021;10:e62387.
108. Ozekin YH, O'rourke R, Bates EA. Single cell sequencing of the mouse anterior palate reveals mesenchymal heterogeneity. Development Dynamics 2023;252(6):713–27.
109. Wang B, Xu M, Zhao J, et al. Single-cell Transcriptomics Reveals Activation of Macrophages in All-trans Retinoic Acid (atRA)-induced Cleft Palate. J Craniofac Surg 2024;35(1):177–84.
110. Rajderkar S S, Paraiso K, Amaral M L, et al. Dynamic enhancer landscapes in human craniofacial development. Nat Commun 2024;15(1):2030.
111. Yan F, Suzuki A, Iwaya C, et al. Single-cell multiomics decodes regulatory programs for mouse secondary palate development. Nat Commun 2024;15(1):821.
112. Cui X, Zhu G, Han M, et al. Genetic variants in BCL-2 family genes influence the risk of non-syndromic cleft lip with or without cleft palate. Birth Defects Res 2024;116(1):e2288.
113. Wang B, Zhang Z, Zhao J, et al. Spatiotemporal Evolution of Developing Palate in Mice. J Dent Res 2024;103(5):546–54.
114. Yan F, Dai Y, Iwata J, et al. An integrative, genomic, transcriptomic and network-assisted study to identify genes associated with human cleft lip with or without cleft palate. BMC Med Genomics 2020;13(Suppl 5):39.
115. Lou S, Yang J, Zhu G, et al. Integrative multi-omics analysis identifies genetic variants contributing to non-syndromic cleft lip with or without cleft palate. Chin J Dent Res 2024;27(1):65–73.
116. He X, Liu X, Zuo F, et al. Artificial intelligence-based multi-omics analysis fuels cancer precision medicine. Semin Cancer Biol 2023:187–200.