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REVIEW

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Research progress of proteomics in congenital craniofacial anomalies

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Abstract

Congenital craniofacial anomalies (CFAs) are among the most common birth defects, significantly affecting the appearance, oral function and mental health of patients. These anomalies are etiologically complex, involving genetics, environmental factors and gene-environment interactions. While genetic studies have identified numerous potential causal genes/risk loci for CFAs, the pathogenic mechanisms still largely remain elusive. Proteomics, the large-scale analysis of proteins, offers a comprehensive view of disease pathogenesis and their systemic effects. During the past two decades, the application of proteomics in CFA research has uncovered many biomarkers for early diagnosis and shed light on underlying mechanisms driving these anomalies. Here, we review the advancements and contributions of proteomics to congenital CFA research, outlining technological advances, novel findings from human body fluid proteomics, and integrative multi-omics approaches.

Keywords

Craniofacial anomaly; Proteomics; Mass spectrometry; Human body fluid proteomics; Research progress

1. Introduction

Congenital craniofacial anomalies (CFAs) account for over one-third of all congenital birth defects [1], causing considerable neonatal morbidity and mortality. Affecting the head and face of patients, CFAs impact not only the patient's physical appearance, but also their orofacial function and mental health, thereby raising the financial burden and reducing the quality of life for both patients and their families [2]. The etiologies of CFAs are complex, including genetics, environmental factors and gene-environment interactions [3].

Over the past two decades, omics technologies, such as genomics [4, 5], transcriptomics [6, 7] and proteomics [8], have enriched CFA research. While genome-wide association studies (GWAS) and whole-exome sequencing (WES) have spotlighted numerous potential causal genes/risk loci [9–12], much remains to be uncovered about CFAs, from its etiology to overall health impacts.

Proteins, as the primary effectors of genomic information, provide cells with their structure and drive most functions of cells [13]. Positioned downstream of the genome and transcriptome, the proteome can capture the cumulative effects of multiple upstream factors, offering a comprehensive view into disease pathogenesis [14]. Furthermore, proteomics could unveil systemic health impacts from diseases, such as the nutritional deficiencies related to CFAs [15]. Thus, delving into proteomics for CFAs not only elucidates their etiologies but also broadens our grasp on their overall health implications. In the present review, we discuss the pivotal roles and advancements of proteomics in congenital CFA research, touching on aspects like technological advances, novel findings from human body fluid proteomics, and multi-omics approaches (Fig. 1).

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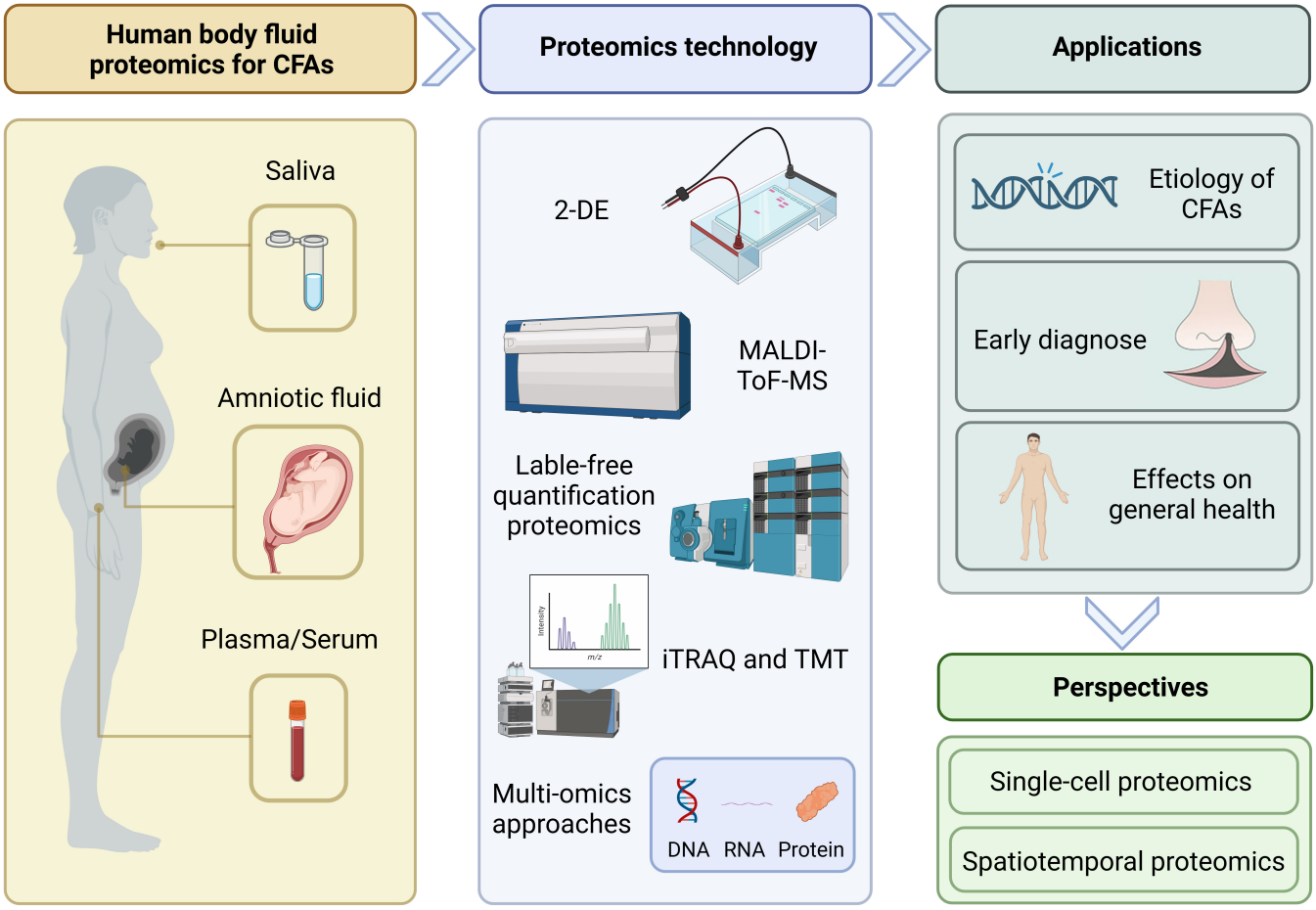

FIGURE 1. Advancements and contributions of proteomics in congenital craniofacial anomaly research. This figure illustrates recent advancements and contributions of proteomics to the field of congenital craniofacial anomaly (CFA) research. We focus on the novel findings gained from the proteomic analysis of human body fluids such as plasma/serum, saliva and amniotic fluid. The evolution of proteomics technology from 2-D gel electrophoresis to quantitative proteomics and multi-omics approaches has deepened our understanding of CFA etiology, facilitated more precise prenatal molecular diagnosis, and provided insights into CFA's effects on general health. In the future, the application of single-cell proteomics and spatiotemporal proteomics can promise to further revolutionize CFA research, offering more sophisticated tools for in-depth study. 2-DE: two-dimensional gel electrophoresis; MALDI-TOF-MS: matrix-assisted laser desorption-time of flight mass spectrometry; iTRAQ: isobaric tags for relative and absolute quantitation; TMT: tandem mass tags.

2. Overview of craniofacial anomalies: classifications, etiologies and omics research

CFAs can affect the head, face, or both, encompassing malformations like cranial, ocular, nasal and orofacial defects [16, 17]. These anomalies not only hinder vital functions like breathing and feeding, potentially leading to infant mortality, but also negatively impact speech, hearing, mental health and social integration [18, 19]. Moreover, certain CFAs may increase the risk of complications, such as obstructive sleep apnea syndrome (OSAS) from micrognathia, leading to long-term health issues [20].

CFAs can occur alone (non-syndromic CFAs) or alongside other congenital anomalies like neural, cardiac or skeletal defects (syndromic CFAs) [21]. Syndromic CFAs can be categorized into craniosynostoses and cleft syndromes [22]. Craniosynostosis involves the premature fusion of one or more cranial sutures, which can limit skull growth, alter its features, and eventually lead to skull base asymmetries [23]. As for cleft syndromes, prevalent ones include Van der Woude, Pierre-Robin, Treacher-Collins and Nager syndrome [24, 25]. Non-syndromic CFAs most commonly manifest as non-syndromic orofacial clefts (NSOFCs), which include cleft lip only, cleft palate only and cleft lip and palate [26]. NSOFCs are a diverse group of disorders that affect the lips and oral cavity. Their occurrence ranges from 1/700 to 1/1000 live births globally, varying by ethnicity and geography [27, 28]. Another notable CFA is craniofacial microsomia (also known as first and second branchial arch anomalies or lateral facial dysplasia). These malformations affect about 1 in 5500 to 26,000 newborns globally, and can lead to facial asymmetry due to deformities in the facial skeleton and soft tissue [29].

Syndromic CFAs are primarily Mendelian monogenic disorders that have been extensively studied over the past years. With genomic technology advancements, numerous genes and

variants causing syndromic CFAs have been identified, and confirmed using animal models [30, 31]. Non-syndromic CFAs often show phenotype variability and non-Mendelian inheritance patterns, which are thought to be complex diseases influenced by both genetic and environmental factors [32]. Techniques like GWAS and next-generation sequencing (NGS) have identified at least 45 risk loci [33, 34] and various candidate genes for NSOFCs [4, 12, 35]. Transcriptomics, meanwhile, is a popular tool in CFA research, helping to uncover mechanisms by analyzing transcriptomic shifts during normal and abnormal tissue development [6, 36].

3. Proteomics to study craniofacial anomalies

Proteomics is the comprehensive analysis of proteins on a large scale, significantly enhancing our understanding of physiological and pathological processes in the post-genomic era [37]. Through proteomics, researchers can identify biomarkers across various diseases at protein levels, delve into post-translational modifications, and investigate protein-protein interactions using mass spectrometry (MS) [38, 39]. Many studies have applied MS-based proteomics to congenital CFAs, shedding light on their etiology and underlying mechanisms.

3.1 Proteomics technology applications to CFAs: from 2-D gel electrophoresis to quantitative proteomics

Two-dimensional gel electrophoresis (2-DE) is a well-established method for protein separation, allowing simultaneous analysis of multiple samples [40]. Using 2-DE in combination with matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS), Xingang Yuan *et al.* [41] identified a potential link between peroxiredoxin1 (PRX1) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced cleft palate in mice. However, 2-DE faces challenges in accurately separating and quantifying proteins, sometimes leading to loss of certain proteins [42]. To address these limitations, shotgun proteomic technology was introduced to CFA research. This approach can identify hundreds of proteins at once, making it an invaluable tool for comparative proteomics due to its enhanced efficiency and sensitivity [43]. Sosa-Acosta *et al.* [44] utilized shotgun proteomics to uncover dysregulated extracellular matrix (ECM)-related proteins modulating the microcephalic phenotype. As technological advancements continue, various quantitative proteomic methods have emerged in CFA research. Broadly speaking, these can be classed into two categories: label-free quantification and mass tag [45, 46]. Mauricio Quiñones-Vega *et al.* [47] utilized label-free quantification proteomics to suggest that higher expression of integrins in microcephaly might be associated with high internalization of Zika virus (ZIKV). Turning to mass tag quantitative proteomics, isobaric tags for relative and absolute quantitation (iTRAQ) and tandem mass tags (TMT) are two prominent techniques at present. For instance, Chen Wang *et al.* [48] combined iTRAQ with liquid chromatography tandem-MS (LC-MS/MS) to uncover that 14-3-3σ and annexin A1 (ANXA1) are pivotal in cleft palate formation, while we [49] deployed TMT-labeled quantitative MS to discern unique protein profiles in NSOFC patient serum. In sum, the advancement of proteomics equips researchers with increasingly efficient, sensitive and precise techniques to investigate CFAs.

3.2 Human body fluid proteomics for craniofacial anomalies

3.2.1 Plasma/serum

Blood, an indispensable sample for clinical tests, holds great potential for detecting disease biomarkers. The plasma proteome, one of the most complex proteomes in the human body, comprises secreted proteins from various organs and tissues, positioning it as an ideal matrix for comprehensive protein biomarker discover [49]. For early disease surveillance of congenital CFAs, maternal blood is preferred over methods like amniocentesis or chorionic villus sampling due to its non-invasive nature [50].

Srinivasa R. Nagalla *et al.* [51] analyzed maternal serum samples from the first and second trimesters to discover potential serum biomarkers linked to Down's syndrome, one of the most prevalent gross chromosomal abnormalities in live births. They employed a suite of complementary proteomic techniques, including fluorescence 2-DE, 2-dimensional liquid chromatography-chromatofocusing (2D-CF), multidimensional protein identification technology, and MALDI-TOF-MS peptide profiling. Their research identified 19 proteins specific to the first trimester, 16 specific to the second, and 10 that were differentially present in both trimesters, paving the way for developing a novel Down's syndrome screening test.

The prenatal diagnosis of NSOFCs is challenging due to the ultrasonographic examination limitations: the reported detection rate of NSOFCs is only 65%, with over half of cleft palates going undetected [52]. Consequently, there's growing interest in using maternal serum for the prenatal diagnosis of NSOFCs. In a study by Xinhuan Wang *et al.* [53], iTRAQ-based MS were employed to investigate the differences in maternal serum protein profiles. They compared 20 pregnant women with NSOFC fetuses to 20 with healthy fetuses, later confirming their findings through multiple reaction monitoring-MS and enzyme linked immunosorbent assay (ELISA). This work suggested that apolipoproteins A (APOA), haptoglobin (HPT) and c-reactive protein (CRP) proteins as potential serum biomarkers for NSOFC prenatal diagnosis, introducing a novel proteomics-based prenatal diagnostic approach for NSOFCs.

In addition to the application in prenatal diagnosis of CFAs, serum proteomics can reflect the physiological state of patients, helping us understanding the etiology of CFAs and their impact on general health. In a previous study [15], we utilized the shotgun approach to examine the plasma proteome of 13 children with NSOFCs and 10 healthy controls. Our findings indicated that reduced levels of retinol-binding protein 4 (RBP4) and vitamin A were associated with newborns with NSOFCs, suggesting a potential need for early vitamin A supplementation. In another study of ours [49], we employed a TMT-labeled quantitative MS technique to study

the serum proteomics of NSOFC patients, indicating that the levels of sex hormone binding globulin (SHBG), periostin sapiens (POSTN), osteomodulin (OMD) and aggrecan core protein (ACAN) were significantly higher in the NSOFC group compared to the healthy control group. This work suggested that NSOFCs may influence the expression of collagen-related and bone-associated proteins. Table 1 provides a summary of recent studies on CFAs based on plasma/serum proteomics.

3.2.2 Saliva

Saliva, secreted by salivary glands, whose development is influenced by certain CFAs. Due to this connection with CFAs, saliva serves as an ideal source of biomarkers for various diseases, particularly oral-facial diseases [60]. Saliva collection is less invasive and faster than blood collection. Over the past 20 years, proteomics has extensively studied human saliva, especially with MS [61].

In 2012, Gyula Tamas Szabo *et al.* [56] employed a top-down strategy to identify potential biomarkers for elucidating the mechanism underlying cleft lip and palate at protein level. By analyzing proteins in the saliva collected from 31 patients with cleft lip and palate, and comparing them with 20 healthy volunteers using MALDI-TOF, they identified several biomarkers, including adaptor protein-3 (AP-3), dermokine (DMKN), nidogen 1 (NID1)-precursor, transforming growth factor-β3 (TGF-β3) and zinc finger ran-binding domain-containing protein 2 (ZRANB2). These proteins might play crucial roles in tissue regeneration and the molecular repair mechanisms in cleft lip and palate patients. Notably, this method not only showed significant disease-associated protein biomarkers, but also provided an accessible diagnostic platform that reduces patient discomfort. Table 1 provides a summary of recent studies on CFAs based on saliva proteomics.

3.2.3 Amniotic fluid

Amniotic fluid is derived from both maternal and fetal tissues, encompassing substances from the placenta, fetal lung secretions, skin, urine and gastric fluid [62]. As amniotic fluid directly reflects the internal environment of the fetus, analyzing it can provide valuable information about the condition of fetal structures [63]. Since the 1950s, when amniotic fluid was first utilized in prenatal diagnosis for fetal conditions [64], it has become a widely used tool.

Chan-Kyung J. Cho *et al.* [57] explored the proteome of amniotic fluid using 2-dimensional liquid chromatography-chromatofocusing (2D-CF) and MS/MS to analyze samples from both chromosomally normal and Down's syndrome-affected pregnancies, identifying amyloid precursor protein (APP) and tenascin (TNC) as potential biomarkers. Similarly, George Th. Tsangaris *et al.* [59] studied amniotic fluid from Down's syndrome and chromosomally normal fetuses at the 17th week of gestation using 2-DE, matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) and nano-electrospray ionization tandem mass spectrometry (nano-ESI-MS/MS), which indicated that SFRS4 as a unique marker present only in the amniotic fluid of Down's syndrome fetuses. Fetal Alcohol Syndrome (FAS) is characterized by craniofacial defects, mental retardation and stunted growth [65]. Aiming to discover protein biomarkers in amniotic fluid to facilitate early diagnosis of FAS, Susmita Datta *et al.* [58] administered alcohol to pregnant mice (the exposed group) and saline to a control group on the 8th day of gestation. On the 17th day, utilizing LC-MS/MS and multidimensional protein identification technology (MudPIT), they identified AFP as a potential biomarker for distinguishing FAS-positive from FAS-negative pregnancies.

ZIKV is associated with multiple birth defects such as microcephaly [66]. This virus can exhibit vertical transmission during pregnancy [55], providing a pathway to investigate the mechanisms underlying ZIKV-induced microcephaly through the analysis of amniotic fluid. Patricia Sosa-Acosta *et al.* [44] employed a shotgun proteomic approach to examine the amniotic fluid from pregnant women infected with ZIKV. They compared those carrying microcephalic fetuses to those with non-microcephalic fetuses and ZIKA-negative controls. This study suggested that the down-regulation of ECM-related proteins and impairment of innate immune system processes might modulate microcephalic phenotypes. We summarize recent studies on CFAs based on amniotic fluid proteomics in Table 1.

3.3 Multi-omics approaches to investigate craniofacial anomalies

Congenital CFAs are complex disorders. With the advancement of multi-omics, researchers now employ integrated multi-omics approaches to uncover the mechanisms of CFAs [36].

Huanhuan Pang *et al.* [67] conducted a multi-omics study on ZIKV-infected mouse brains, encompassing transcriptomics, proteomics, phosphoproteomics and metabolomics. They observed a dramatic alteration in NAD+-related metabolic pathways. Furthermore, they inferred a potential link between MAPK and cyclic GMP–protein kinase G signaling with ZIKV-induced microcephaly. In another study, Xin Chen *et al.* [7] employed RNA microarray and TMT-labeled quantitative proteomics to explore key genes of nonsyndromic microtia. Notably, this research, being the first to integrate transcriptomic and proteomic analyses for nonsyndromic microtia, identified several candidate genes, including laminin β2 (*LAMB2*), cartilage oligomeric matrix protein (*COMP*), *APOA2*, apolipoprotein C-II (*APOC2*), *APOC3* and alpha-2 macroglobulin (*A2M*). Similarly, Angèle Tingaud-Sequeira *et al.* [68] revealed the link between *EYA3* gene and the oculo-auriculo-vertebral spectrum (OAVS), which manifests as asymmetric ear anomalies, hemifacial microsomia and ocular and vertebral defects. Through WES, researchers identified variants in eyes absent 3 (*EYA3*) gene, and using proteomic analyses, unveiled four potential upstream regulators: peroxisome proliferator-activated receptor-gamma coactivator-1beta (PPARGC1B), yes-associated protein 1 (YAP1), nuclear factor erythroid 2 like 2 (NFE2L2) and myelocytomatosis viral oncogene homolog (MYC). We summarize recent studies on CFAs using multi-omics approaches in Table 2.

TABLE 1. Summary of recent studies on CFAs based on plasma/serum proteomics, saliva proteomics and amniotic fluid proteomics.

Study	Sample type	Participants	Objective	Method of proteomics	Results	Subsequent verification	Ref.
Hou *et al.* [49] (2023)	Supernatant serum	5 NSOFC patients and 5 healthy controls	Analyze the proteomic changes in postnatal patients with NSOFC	TMT-labeled quantitative MS	NSOFC patient serum exhibited distinct protein profiles relative to healthy controls, and NSOFCs might change the expression of collagen-related and bone-related proteins	PRM	PMID: 34170602
Wang *et al.* [53] (2022)	Maternal serum	20 pregnant women with NSOFC fetuses and 20 pregnant women with healthy fetuses	Identify specific biomarkers in maternal serum for predicting NSOFC prenatally	iTRAQ-based MS; MRM-MS	APOA, HPT and CRP proteins are potential serum biomarkers for prenatal diagnosis of NSOFC	ELISA	PMID: 34951699
Zhao *et al.* [4] (2022)	Maternal serum	10 non-pregnant women, 10 pregnant women with healthy fetuses, and 10 pregnant women with Down's syndrome fetuses	Identify Down's syndrome biomarkers in maternal serum	iTRAQ	11 proteins had significantly different serum levels between the Down's syndrome fetus group and healthy fetuses' group	NP	PMID: 31793358
Zhang *et al.* [15] (2014)	Serum	13 children with NSCLP and 10 control children, aged 2 to 3.5 years	Identify and evaluate the differential expression of serum protein profiles in NSCLP children and unaffected babies	Shotgun proteomics	Reduced levels of RBP4 and vitamin A were related to newborns with NSCLP	WB; HPLC	PMID: 24695672
Yu *et al.* [54] (2012)	Maternal serum	6 pregnancies with fetuses affected by Down's syndrome and 6 pregnancies with normal fetuses	Identify novel proteins for Down syndrome screening	2-DE; MS	29 proteins were identified in maternal serum obtained from pregnancies with fetuses affected by Down's syndrome	ELISA	PMID: 22678011
Nagalla *et al.* [51] (2007)	Maternal serum	56 women with Down's syndrome fetuses and 56 control women with non-Down's syndrome fetuses	Identify potential serum biomarkers to detect Down's syndrome	2D-DIGE; 2D-CF; MudPIT; MALDI-TOF-MS peptide profiling	28 and 26 proteins were differentially present in first- and second-trimester samples, respectively	NP	PMID: 17373838
Zuanazzi *et al.* [55] (2017)	Saliva	A 25-y-old woman clinically diagnosed with Zika fever in the first trimester and her dizygotic twins: a female with microcephaly and a male without microcephaly	Explored the potential biomarkers to diagnose Zika virus related microcephaly	LC-ESI-MS/MS	The virus may have entered the oral cavity through the salivary glands, leading to an infection that persists into the postnatal period	RT-PCR	PMID: 28825520

TABLE 1. Continued.

Study	Sample type	Participants	Objective	Method of proteomics	Results	Subsequent verification	Ref.
Szabo *et al.* [56] (2012)	Saliva	31 cleft lip and palate patients and a control group with 20 healthy volunteers	Evaluate protein composition differences between samples with cleft lip and palate and healthy controls	MALDI-TOF/TOF-MS	The presence of cleft lip and palate stimulated the expression of several proteins, included AP-3, DMKN, NID1-precursor, TGF-β3 and ZRANB2	NP	PMID: 21504360
Acosta *et al.* [44] (2022)	Amniotic fluid	2 pregnant women who were positive for ZIKV and bearing non-microcephalic fetuses, two pregnant women who were positive for ZIKV and bearing microcephalic fetuses	Identify and understand the biological processes affected by CZS development	Shotgun proteomic	The microcephalic phenotypes are modulated by a down-regulation of the ECM proteins and the impairment of the innate immune system processes	NP	PMID: 34676661
Cho *et al.* [57] (2010)	Amniotic fluid	10 chromosomal normal and 10 Down syndrome-affected pregnancies in the second trimester of pregnancy	Discover novel biomarkers for Down syndrome	2D-LC; MS/MS; LTQ-Orbitrap mass spectrometer, spectral counting	APP and TNC-C showed a 2-fold increase in Down Syndrome-amniotic fluid samples compared to controls	ELISA	PMID: 20459121
Datta *et al.* [58] (2008)	Amniotic fluid	C57BL/6J and C57BL/6N mice treated with alcohol (exposed) or saline (control) on day 8 of gestation	Screen amniotic fluid for biomarkers that could discriminate between FAS-positive and FAS-negative pregnancies	MALDI-TOF-MS; LC-MS/MS; MudPIT	AFP deficiency is a biomarker for FAS-positive	NP	PMID: 18240165
Tsangaris *et al.* [59] (2006)	Amniotic fluid	18 women in the 17th week of pregnancy: 12 pregnancies with normal fetuses and 6 pregnancies with fetuses affected by Down syndrome	Discover novel biomarkers for Down syndrome	2-DE, 3 MALDI-TOF-MS, nano-ESI-MS/MS	CO1A1, CO3A1, CO5A1 and PGBM are differentially expressed in all pregnancies with DS tested	WB	PMID: 16847874

Abbreviations: NP: not performed; CZS: congenital Zika syndrome; FAS: fetal alcohol syndrome; NSOFC: non-syndromic orofacial clefts; NSCLP: non-syndromic cleft of the lip and/or palate; PRM: parallel reaction monitoring; TMT: tandem mass tag; 2-DE: two-dimensional electrophoresis; MS: mass spectrometry; MALDI-TOF-MS: matrix-assisted laser desorption-time of flight mass spectrometry; LC-MS/MS: liquid chromatography tandem mass spectrometry; MudPIT: multidimensional protein identification technology; MS/MS: tandem mass spectrometry; 2D-LC: two-dimensional liquid chromatography; 2D-DIGE: fluorescence 2-dimensional gel electrophoresis; 2D-CF: 2-dimensional liquid chromatography-chromatofocusing; LC-ESI-MS/MS: liquid chromatography electrospray Ionization tandem mass spectrometry; HPLC: high-performance liquid chromatography; iTRAQ: isobaric tags for relative and absolute quantitation; MRM: multiple reaction monitoring; ELISA: enzyme linked immunosorbent assay; WB: western blot; ECM: extracellular matrix; AFP: alpha fetoprotein; TF: serotransferrin; A1BG: alpha-1b-glycoprotein; DES: desmin; SERPINA1: alpha-1-antitrypsin; CP: ceruloplasmin; APCS: serum amyloid P-component; APP: amyloid precursor protein; TNC-C: tenascin-C; APOA: apolipoprotein A; HPT: haptoglobin; CRP: c-reactive protein; CO1A1: collagen alpha 1 (I) chain; CO3A1: collagen alpha 1 (III) chain; CO5A1: collagen alpha 1 (V) chain d; PGBM: basement membrane-specific heparin sulfate proteoglycan core protein; RBP4: retinol-binding protein 4; AP-3: adaptor protein-3; DMKN: dermokine; NID1: nidogen 1; TGF-β3: transforming growth factor-β3; ZRANB2: zinc finger ran-binding domain-containing protein 2; LTQ: linear trap quadrupole.

TABLE 2. Summary of recent studies on CFAs using multi-omics approaches.

Study	Sample type	Participants	Objective	Method of multi-omics	Results	Subsequent verification	Ref.
Riedhammer *et al.* [69] (2022)	Fibroblasts; blood samples	6 individuals with Suleiman-El-Hattab syndrome; zebrafish	Describe the Suleiman-El-Hattab syndrome and explore the consequences of biallelic TASP1 loss-of-function variants	Genomics; proteomics	Possible downstream mechanisms of TASP1 deficiency include perturbed HOX gene expression and dysregulated TFIIA complex causing Suleiman-El-Hattab syndrome	WB	PMID: 35512351
Chen *et al.* [7] (2022)	Cartilage tissues	10 patients with third-degree nonsyndromic microtia and five control subjects	Explored its potential pathogenesis of nonsyndromic microtia	Transcriptomics; proteomics	The dysregulation of LAMB2, COMP, APOA2, APOC2, APOC3 and A2M, contributing to nonsyndromic microtia	PRM	PMID: 35647449
Sequeira *et al.* [68] (2021)	Blood samples	124 OAVS patients	Identify and characterize a new gene associated with OAVS	Genomics; proteomics	EYA3 is the gene identified to be associated with OAVS	Immunocytochemistry; WB; RT-qPCR	PMID: 33475861
Dong *et al.* [8] (2021)	Auricular cartilage samples	16 unilateral microtia patients who had undergone ear reconstruction surgery	Explore the pathogenesis of microtia	Proteomics; transcriptomics	47 genes as molecular markers of microtia progression	NP	PMID: 33840765
Pang *et al.* [67] (2021)	Mouse brain samples	ICR mice (8–10 weeks old) and their pups after birth	Explore the mechanisms of how ZIKV infection cause microcephaly in newborns	Transcriptomics; proteomics; phosphor-proteomics; metabolomics	MAPK and cGMP–protein kinase G signaling may be associated with ZIKV-induced microcephaly, and NAD+ metabolic reprogramming is a pathogenic driver	Immunohistochemistry; WB	PMID: 34385701
Kato *et al.* [70] (2021)	Blood leucocytes	A patient with global developmental delay, who presented macrocephal, characteristic facial features, and cutis marmorata	Explore the mechanisms of CUL3-related disorders	Genomics; proteomics	A de novo missense variant in *CUL3* was identified	NP	PMID: 33130828
Magini *et al.* [71] (2019)	Fibroblasts	Children from 12 unrelated families presented with developmental disorder characterized by microcephaly	Explore the function of SMPD4 in microcephaly	Genomics; proteomics	SMPD4 links homeostasis of membrane sphingolipids to cell fate by regulating the cross-talk between the ER and the outer nuclear envelope, while its loss reveals a pathogenic mechanism in microcephaly	Flow cytometry; immunofluorescence; WB	PMID: 31495489

Abbreviations: NP: not performed; OAVS: oculo-auriculo-vertebral spectrum; ZIKV: Zika virus; MS: mass spectrometry; PRM: Parallel Reaction Monitoring; TFIIA: transcription initiation factor IIA; ER: endoplasmic reticulum; cGMP: cyclic guanosine monophosphate; MAPK: mitogen-activated protein kinase; NAD+: nicotinamide adenine dinucleotide; GMP: guanosine monophosphate; TASP: taspase 1; HOX: homeobox; WB: western blot; LAMB: laminin beta; COMP: cartilage oligomeric matrix protein; APOA: apolipoprotein A; APOC: apolipoprotein C; A2M: alpha-2 macroglobulin; RT-qPCR: real-time quantitative polymerase chain reaction; ICR: institute of cancer research; CUL: cullin; SMPD4: sphingomyelin phosphodiesterase 4.

Luke Reilly *et al.* [72] provided a thorough proteo-genomic strategy to uncover potential biomarker candidates for human diseases. The strategy begins with the use of genome sequencing to screening variant sites in the sample. Subsequently, MS technology evaluates the expression levels of proteins, highlighting potential biomarkers/targets. Finally, by integrating both genomic and proteomic data, a deeper understanding of disease mechanisms can be revealed.

3.4 Limitations and suggestions for using proteomics in analyzing predictors of congenital craniofacial anomalies

While mass spectrometry technology has significantly advanced, it's important to recognize the limitations in studies using mass-spectrometry-based proteomics for understanding the mechanisms and identifying biomarkers of CFAs. Predominantly, these studies utilize a bottom-up approach, analyzing peptides through LC-MS/MS [73]. This method complicates protein identification and obscures the recognition of certain protein forms in the sample, as the connection between peptides and their original proteoforms is lost during digestion [74]. Consequently, reconstructing the proteome's complexity at the proteoform level from peptides becomes challenging. An alternative top-down approach, which starts with intact proteins, can circumvent some of these issues [75]. However, limitations related to protein abundance and molecular weight restrict its application.

Although this review concentrates on proteomics in CFAs, it's crucial to acknowledge that no single "omics" technique can entirely elucidate all molecular events leading to disease. Therefore, to understand CFAs comprehensively, it is essential to integrate multi-omics data, including genomics, transcriptomics and proteomics [36, 70]. Such integration allows for evaluating the effects of pathogenic genetic variants at multiple levels, clarifying the molecular and biochemical pathways underlying pathological phenotypes and predicting the occurrence of CFAs.

4. Future perspectives

Proteomics, particularly MS-based proteomics, facilitates large-scale protein analysis, shedding light on the molecular mechanisms and pathological processes of CFAs. However, there are still challenges to overcome, including handling minuscule-size samples, detecting low-abundance proteins with crucial functions, and investigating protein function in specific spatiotemporal contexts [76]. In light of these challenges, emerging proteomics technologies might soon be applied in this field.

Single-cell proteomics offers a detailed understanding into the composition and function of proteins in individual cells [77]. This method can exhibit the nuances of cell-to-cell variability and heterogeneity [78]. Unlike traditional proteomics which often requires larger sample sizes, even small samples suffice for robust analyses due to its sensitivity [79]. Beyond merely identifying proteins within cells, this approach may also be used to investigate protein functions and interactions between cells [80]. Thus, it greatly enriches our understanding of intercellular signaling communication and regulatory networks.

Imaging proteins *in situ* within whole organs or organisms has posed significant challenges for many years [81]. To address this issue, Harsharan Singh Bhatia *et al.* [82] introduced a breakthrough solution with their technology termed three-dimensional (3D) imaging of solvent-cleared organs profiled by mass spectrometry (DISCO-MS). This method integrates whole-organ/organism clearing and imaging, deep-learning-based image analysis, robotic tissue extraction and ultra-high-sensitivity MS. DISCO-MS allows researchers to obtain spatial-molecular profiles in various disease models by analyzing fluorescently labeled target regions. Unlike traditional proteomic studies, DISCO-MS preserves the sample's integrity, offering comprehensive information into the entire sample, including both the structure and function of tissues and organs.

5. Concluding remarks

Proteomics applications have significantly enhanced our understanding of the molecular mechanisms behind CFAs, deepened our comprehension of the overall health impacts from CFAs, and offered new possibilities for early diagnosis. In this review, we present recent advancements in mass-spectrometry-based proteomics for CFA research, emphasizing the importance of body fluid analysis in biomarker discovery. Such analysis holds immense potential for prenatal diagnosis and treatment of CFAs, with the prospect of translating this information into innovative clinical applications in precision medicine and personalized nutrition. We also discuss the importance of integrating multi-omics approaches and offers new perspectives for future research. Looking ahead, we believe that establishing proteomic databases or resources specific to CFAs, akin to the creation of transcriptomics databases for craniofacial development, is essential for the effective identification of biomarkers through proteomics. This approach promises to open new avenues for future research and clinical application in the field.

ABBREVIATIONS

NP, not performed; CZS, congenital Zika syndrome; FAS, fetal alcohol syndrome; NSOFC, non-syndromic orofacial clefts; NSCLP, non-syndromic cleft of the lip and/or palate; OAVS, oculo-auriculo-vertebral spectrum; ZIKV, Zika virus; MS, mass spectrometry; PRM, Parallel Reaction Monitoring; TMT, tandem mass tag; 2-DE, two-dimensional electrophoresis; MALDI-TOF-MS, matrix-assisted laser desorption-time of flight mass spectrometry; LC-MS/MS, liquid chromatography tandem mass spectrometry; MudPIT, multidimensional protein identification technology; MS/MS, tandem mass spectrometry; 2D-LC, two-dimensional liquid chromatography; 2D-DIGE, fluorescence 2-dimensional gel electrophoresis; 2D-CF, 2-dimensional liquid chromatography-chromatofocusing; LC-ESI-MS/MS, liquid chromatography electrospray Ionization tandem mass spectrometry; HPLC, high-performance liquid chromatography; iTRAQ, isobaric

tags for relative and absolute quantitation; MRM, multiple reaction monitoring; ELISA, enzyme linked immunosorbent assay; WB, western blot; TFIIA, transcription initiation factor IIA; ER, endoplasmic reticulum; cGMP, cyclic guanosine monophosphate; MAPK, mitogen-activated protein kinase; NAD+, nicotinamide adenine dinucleotide; GMP, guanosine monophosphate; BTB, broad-complex/tramtrack/bric-a-brac; ECM, extracellular matrix; AFP, alpha fetoprotein; TF, serotransferrin; A1BG, alpha-1b-glycoprotein; DES, desmin; SERPINA1, alpha-1-antitrypsin; CP, ceruloplasmin; APCS, serum amyloid P-component; APP, amyloid precursor protein; TNC-C, tenascin-C; APOA, apolipoproteins A; HPT, haptoglobin; CRP, c-reactive protein; CO1A1, collagen alpha 1 (I) chain; CO3A1, collagen alpha 1 (III) chain; CO5A1, collagen alpha 1 (V) chain d; PGBM, basement membrane-specific heparin sulfate proteoglycan core protein; PRX1, peroxiredoxin1; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; ANXA1, annexin A1; RBP4, retinol-binding protein 4; SHBG, sex hormone binding globulin; POSTN, periostin sapiens; OMD, osteomodulin; ACAN, aggrecan core protein; AP-3, adaptor protein-3; DMKN, dermokine; NID1, nidogen 1; TGF-β3, transforming growth factor-β3; ZRANB2, zinc finger ran-binding domain-containing protein 2; LTQ, linear trap quadrupole; APP, amyloid precursor protein; MALDI-MS, matrix-assisted laser desorption/ionization-mass spectrometry; LAMB2, laminin β2; COMP, cartilage oligomeric matrix protein; APOC2, apolipoprotein C-II; A2M, alpha-2 macroglobulin; EYA3, eyes absent 3; PPARGC1B, peroxisome proliferator-activated receptor-gamma coactivator-1beta; YAP1, yes-associated protein 1; NFE2L2, nuclear factor erythroid 2 like 2; MYC, myelocytomatosis viral oncogene homolog; TASP, taspase; HOX, homeobox; RT-qPCR, real-time quantitative polymerase chain reaction; ICR, institute of cancer research; CUL, cullin; SMPD4, sphingomyelin phosphodiesterase 4.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article.

AUTHOR CONTRIBUTIONS

HXZ, JNZ and HMH—designed this review. SJH—collected the data. SJH, JNZ and HXZ—analyzed the data. HXZ, SJH, XQL, YHJ, KYL and HML—wrote the original draft. HXZ, BXY, SJH, YD, JNZ and HMH—revised the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Twigg SR, Wilkie AO. New insights into craniofacial malformations. Human Molecular Genetics. 2015; 24: R50–R59.

[2] de Oliveira Júnior AG, Montagna E, Zaia V, Barbosa CP, Bianco B. Oral health-related quality of life in patients aged 8 to 19 years with cleft lip and palate: a systematic review and meta-analysis. BMC Oral Health. 2023; 23: 670.

[3] Alvizi L, Nani D, Brito LA, Kobayashi GS, Passos-Bueno MR, Mayor R. Neural crest E-cadherin loss drives cleft lip/palate by epigenetic modulation *via* pro-inflammatory gene-environment interaction. Nature Communications. 2023; 14: 2868.

[4] Zhao H, He Q, Wu X, Liang X, Jiao Y, Zhang Y, *et al*. Identification of rare loss-of-function variants in FAM3B associated with non-syndromic orofacial clefts. Genomics. 2023; 115: 110630.

[5] Bishop MR, Diaz Perez KK, Sun M, Ho S, Chopra P, Mukhopadhyay N, *et al*. Genome-wide enrichment of de novo coding mutations in orofacial cleft trios. The American Journal of Human Genetics. 2020; 107: 124–136.

[6] Huang W, Zhong W, He Q, Xu Y, Lin J, Ding Y, *et al*. Time-series expression profiles of mRNAs and lncRNAs during mammalian palatogenesis. Oral Diseases. 2023; 29: 2163–2176.

[7] Chen X, Xu Y, Li C, Lu X, Fu Y, Huang Q, *et al*. Key genes identified in nonsyndromic microtia by the analysis of transcriptomics and proteomics. ACS Omega. 2022; 7: 16917–16927.

[8] Dong W, Jiang H, He L, Pan B, Yang Q. Changes in the transcriptome and proteome of cartilage in microtia. Journal of Craniofacial Surgery. 2021; 32: 2301–2304.

[9] Lansdon LA, Dickinson A, Arlis S, Liu H, Hlas A, Hahn A, *et al*. Genome-wide analysis of copy-number variation in humans with cleft lip and/or cleft palate identifies COBLL1, RIC1, and ARHGEF38 as clefting genes. The American Journal of Human Genetics. 2023; 110: 71–91.

[10] Awotoye W, Mossey PA, Hetmanski JB, Gowans LJJ, Eshete MA, Adeyemo WL, *et al*. Whole-genome sequencing reveals de-novo mutations associated with nonsyndromic cleft lip/palate. Scientific Reports. 2022; 12: 11743.

[11] Ray D, Venkataraghavan S, Zhang W, Leslie EJ, Hetmanski JB, Weinberg SM, *et al*. Pleiotropy method reveals genetic overlap between orofacial clefts at multiple novel loci from GWAS of multi-ethnic trios. PLOS Genet. 2021; 17: e1009584.

[12] Zhao H, Zhong W, Leng C, Zhang J, Zhang M, Huang W, *et al*. A novel PTCH1 mutation underlies nonsyndromic cleft lip and/or palate in a Han Chinese family. Oral Diseases. 2018; 24: 1318–1325.

[13] Gao Y, Hong Y, Huang L, Zheng S, Zhang H, Wang S, *et al*. B2-microglobulin functions as an endogenous NMDAR antagonist to impair synaptic function. Cell. 2023; 186: 1026.e20–1038.e20.

[14] Jiang L, Wang M, Lin S, Jian R, Li X, Chan J, *et al*. A quantitative proteome map of the human body. Cell. 2020; 183: 269–283.

[15] Zhang J, Zhou S, Zhang Q, Feng S, Chen Y, Zheng H, *et al*. Proteomic Analysis of RBP4/vitamin a in children with cleft lip and/or palate. Journal of Dental Research. 2014; 93: 547–552.

[16] Heike CL, Wallace E, Speltz ML, Siebold B, Werler MM, Hing AV, *et al*. Characterizing facial features in individuals with craniofacial microsomia: a systematic approach for clinical research. Birth Defects

Research Part A: Clinical and Molecular Teratology. 2016; 106: 915–926.

[17] Alam MK, Alfawzan AA, Abutayyem H, Kanwal B, Alswairki HJ, Verma S, *et al*. Craniofacial characteristics in Crouzon's syndrome: a systematic review and meta-analysis. Science Progress. 2023; 106: 003685042311562.

[18] Deatherage J, Bourgeois T, O'Brien M, Baylis AL. Examining risk of speech-language disorders in children with cleft lip. Journal of Craniofacial Surgery. 2022; 33: 395–399.

[19] Collett BR, Wallace ER, Kapp-Simon KA, Johns AL, Drake AF, Heike CL, *et al*. Cognitive, motor, and language development of preschool children with craniofacial microsomia. The Cleft Palate-Craniofacial Journal. 2021; 58: 1169–1177.

[20] Chkadua TZ, Libin PV, Sufiomarov NS. Surgical treatment of a patient with mandibular micrognathia accompanied by obstructive sleep apnea syndrome using extended genioplasty. Stomatologiya. 2023; 102: 48.

[21] Whitaker LA, Pashayan H, Reichman J. A proposed new classification of craniofacial anomalies. The Cleft Palate Journal. 1981; 18: 161–176.

[22] Buchanan EP, Xue AS, Hollier LH. Craniofacial syndromes. Plastic and Reconstructive Surgery. 2014; 134: 128e–153e.

[23] Xue AS, Buchanan EP, Hollier LH. Update in management of craniosynostosis. Plastic & Reconstructive Surgery. 2022; 149: 1209e–1223e.

[24] Darjazini Nahas L, Hmadieh M, Audeh M, Yousfan A, Almasri IA, Martini N. Cleft lip and palate risk factors among otorhinolaryngology: head and neck surgery patients in two hospitals. Medicine. 2023; 102: e34419.

[25] Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Watanabe Y, *et al*. Mutations in IRF6 cause van der woude and popliteal pterygium syndromes. Nature Genetics. 2002; 32: 285–289.

[26] das Neves LT, de Carvalho IMM, Cobourne MT, Gomide MR. Dental anomalies in non-syndromic orofacial clefts: a clinical approach. Oral Diseases. 2022; 28: 1351–1368.

[27] Auslander A, McKean-Cowdin R, Feigelson D, Brindopke F, DiBona M, Magee K, *et al*. The international family study of nonsyndromic orofacial clefts: design and methods. The Cleft Palate-Craniofacial Journal. 2022; 59: S37–S47.

[28] Saleem K, Zaib T, Sun W, Fu S. Assessment of candidate genes and genetic heterogeneity in human non syndromic orofacial clefts specifically non syndromic cleft lip with or without palate. Heliyon. 2019; 5: e03019.

[29] Ronde EM, Nolte JW, Kruisinga FH, Maas SM, Lapid O, Ebbens FA, *et al*. Evaluating international diagnostic, screening, and monitoring practices for craniofacial microsomia and microtia: a survey study. The Cleft Palate Craniofacial Journal. 2023; 60: 1118–1127.

[30] Petrin AL, Zeng E, Thomas MA, Moretti-Ferreira D, Marazita ML, Xie XJ, *et al*. DNA methylation differences in monozygotic twins with Van der Woude syndrome. Frontiers in Dental Medicine. 2023; 4: 1120948.

[31] Lau MCC, Kwong EML, Lai KP, Li J, Ho JCH, Chan T, *et al*. Pathogenesis of POLR1C-dependent type 3 Treacher Collins syndrome revealed by a zebrafish model. Biochimica Et Biophysica Acta (BBA)—Molecular Basis of Disease. 2016; 1862: 1147–1158.

[32] Haaland ØA, Lie RT, Romanowska J, Gjerdevik M, Gjessing HK, Jugessur A. A genome—wide search for gene—environment effects in isolated cleft lip with or without cleft palate triads points to an interaction between maternal periconceptional vitamin use and variants in ESRRG. Frontiers in Genetics. 2018; 9: 60.

[33] Yu Y, Zhen Q, Chen W, Yu Y, Li Z, Wang Y, *et al*. Genome—wide meta—analyses identify five new risk loci for nonsyndromic orofacial clefts in the Chinese Han population. Molecular Genetics & Genomic Medicine. 2023; 11: e2226.

[34] Dąbrowska J, Biedziak B, Szponar-Żurowska A, Budner M, Jagodziński PP, Płoski R, *et al*. Identification of novel susceptibility genes for non-syndromic cleft lip with or without cleft palate using NGS-based multigene panel testing. Molecular Genetics and Genomics. 2022; 297: 1315–1327.

[35] Leslie E, Taub M, Liu H, Steinberg K, Koboldt D, Zhang Q, *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. The American Journal of Human Genetics. 2015; 96: 397–411.

[36] Yan F, Dai Y, Iwata J, Zhao Z, Jia P. An integrative, genomic, transcriptomic and network-assisted study to identify genes associated with human cleft lip with or without cleft palate. BMC Medical Genomics. 2020; 13: 39.

[37] Messner CB, Demichev V, Muenzner J, Aulakh SK, Barthel N, Röhl A, *et al*. The proteomic landscape of genome-wide genetic perturbations. Cell. 2023; 186: 2018e.21–2034.e21.

[38] Li Y, Ma C, Liao S, Qi S, Meng S, Cai W, *et al*. Combined proteomics and single cell RNA-sequencing analysis to identify biomarkers of disease diagnosis and disease exacerbation for systemic lupus erythematosus. Frontiers in Immunology. 2022; 13: 969509.

[39] Coscia F, Doll S, Bech JM, Schweizer L, Mund A, Lengyel E, *et al*. A streamlined mass spectrometry–based proteomics workflow for large-scale FFPE tissue analysis. The Journal of Pathology. 2020; 251: 100–112.

[40] Miltner N, Kalló G, Csősz É, Miczi M, Nagy T, Mahdi M, *et al*. Identification of SARS-CoV-2 main protease (Mpro) cleavage sites using two-dimensional electrophoresis and *in silico* cleavage site prediction. International Journal of Molecular Sciences. 2023; 24: 3236.

[41] Yuan X, Liu L, Pu Y, Zhang X, He X, Fu Y. 2,3,7,8-Tetrachlorodibenzo-p-dioxin induces a proteomic pattern that defines cleft palate formation in mice. Food and Chemical Toxicology. 2012; 50: 2270–2274.

[42] Chromy BA, Gonzales AD, Perkins J, Choi MW, Corzett MH, Chang BC, *et al*. Proteomic analysis of human serum by two-dimensional differential gel electrophoresis after depletion of high-abundant proteins. Journal of Proteome Research. 2004; 3: 1120–1127.

[43] Swietlik JJ, Sinha A, Meissner F. Dissecting intercellular signaling with mass spectrometry-based proteomics. Current Opinion in Cell Biology. 2020; 63: 20–30.

[44] Sosa-Acosta P, Melani RD, Quiñones-Vega M, Melo A, Garcez PP, Nogueira FCS, *et al*. Proteomics of ZIKV infected amniotic fluids of microcephalic fetuses reveals extracellular matrix and immune system dysregulation. Proteomics—Clinical Applications. 2022; 16: e2100041.

[45] Ntai I, Toby TK, LeDuc RD, Kelleher NL. A method for label-free, differential top-down proteomics. Methods in Molecular Biology. 2016; 80: 121–133.

[46] Guo Y, Yu D, Cupp-Sutton KA, Liu X, Wu S. Optimization of protein-level tandem mass tag (TMT) labeling conditions in complex samples with top-down proteomics. Analytica Chimica Acta. 2022; 1221: 340037.

[47] Quiñones-Vega M, Velásquez E, Sosa-Acosta P, Melo A, Garcez PP, Nogueira FCS, *et al*. Proteomic profiles of Zika virus-infected placentas bearing fetuses with microcephaly. Proteomics—Clinical Applications. 2022; 16: e2100042.

[48] Wang C, Zhai S, Yuan X, Zhang D, Jiang H, Qiu L, *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. Environmental Toxicology and Pharmacology. 2019; 72: 103270.

[49] Hou Y, Xu Y, Fu D, Ren Z, Tao Y, Zhao H. Quantitative proteomic analysis of nonsyndromic orofacial cleft patient serum. Oral Diseases. 2023; 29: 206–210.

[50] Jia S, Zhang Q, Wang Y, Wang Y, Liu D, He 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.

[51] Nagalla SR, Canick JA, Jacob T, Schneider KA, Reddy AP, Thomas A, *et al*. Proteomic analysis of maternal serum in down syndrome: identification of novel protein biomarkers. Journal of Proteome Research. 2007; 6: 1245–1257.

[52] Sutcliffe MJ, Burgoyne PS. Analysis of the testes of H-Y negative XOSxrb mice suggests that the spermatogenesis gene (Spy) acts during the differentiation of the A spermatogonia. Development. 1989; 107: 373–380.

[53] Wang X, Yang X, Huang P, Meng X, Bian Z, Meng L. Identification of maternal serum biomarkers for prenatal diagnosis of nonsyndromic orofacial clefts. Annals of the New York Academy of Sciences. 2022; 1510: 167–179.

[54] Yu B, Zhang B, Wang J, Wang QW, Huang RP, Yang YQ, *et al*. Preliminary proteomic-based identification of a novel protein for Down's syndrome in maternal serum. Experimental Biology and Medicine. 2012; 237: 530–539.

[55] Zuanazzi D, Arts EJ, Jorge PK, Mulyar Y, Gibson R, Xiao Y, *et al*. Postnatal identification of zika virus peptides from saliva. Journal of Dental Research. 2017; 96: 1078–1084.
[56] Szabo GT, Tihanyi R, Csulak F, Jambor E, Bona A, Szabo G, *et al*. Comparative salivary proteomics of cleft palate patients. The Cleft Palate-Craniofacial Journal. 2012; 49: 519–523.
[57] Cho CJ, Smith CR, Diamandis EP. Amniotic fluid proteome analysis from down syndrome pregnancies for biomarker discovery. Journal of Proteome Research. 2010; 9: 3574–3582.
[58] Datta S, Turner D, Singh R, Ruest LB, Pierce WM, Knudsen TB. Fetal alcohol syndrome (FAS) in C57BL/6 mice detected through proteomics screening of the amniotic fluid. Birth Defects Research Part A: Clinical and Molecular Teratology. 2008; 82: 177–186.
[59] Tsangaris GT, Karamessinis P, Kolialexi A, Garbis SD, Antsaklis A, Mavrou A, *et al*. Proteomic analysis of amniotic fluid in pregnancies with down syndrome. Proteomics. 2006; 6: 4410–4419.
[60] Grassia V, Lombardi A, Kawasaki H, Ferri C, Perillo L, Mosca L, *et al*. Salivary microRNAs as new molecular markers in cleft lip and palate: a new frontier in molecular medicine. Oncotarget. 2018; 9: 18929–18938.
[61] Rabe A, Gesell Salazar M, Völker U. Bottom-up community proteome analysis of saliva samples and tongue swabs by data-dependent acquisition nano LC-MS/MS mass spectrometry. Methods in Molecular Biology. 2021; 16: 221–238.
[62] Gebara N, Scheel J, Skovronova R, Grange C, Marozio L, Gupta S, *et al*. Single extracellular vesicle analysis in human amniotic fluid shows evidence of phenotype alterations in preeclampsia. Journal of Extracellular Vesicles. 2022; 11: e12217.
[63] Bhatti G, Romero R, Gomez-Lopez N, Chaiworapongsa T, Jung E, Gotsch F, *et al*. The amniotic fluid proteome changes with gestational age in normal pregnancy: a cross-sectional study. Scientific Reports. 2022; 12: 601.
[64] Sachs L, Serr DM, Danon M. Prenatal diagnosis of sex using cells from the amniotic fluid. Science. 1956; 123: 548.
[65] Darbinian N, Darbinyan A, Sinard J, Tatevosian G, Merabova N, D'Amico F, *et al*. Molecular markers in maternal blood exosomes allow early detection of fetal alcohol spectrum disorders. International Journal of Molecular Sciences. 2022; 24: 135.
[66] Schmitt K, Curlin JZ, Remling-Mulder L, Aboellail T, Akkina R. Zika virus induced microcephaly and aberrant hematopoietic cell differentiation modeled in novel neonatal humanized mice. Frontiers in Immunology. 2023; 14: 1060959.
[67] Pang H, Jiang Y, Li J, Wang Y, Nie M, Xiao N, *et al*. Aberrant NAD+ metabolism underlies Zika virus-induced microcephaly. Nature Metabolism. 2021; 3: 1109–1124.
[68] Tingaud-Sequeira A, Trimouille A, Salaria M, Stapleton R, Claverol S, Plaisant C, *et al*. A recurrent missense variant in EYA3 gene is associated with oculo-auriculo-vertebral spectrum. Human Genetics. 2021; 140: 933–944.
[69] Riedhammer KM, Burgemeister AL, Cantagrel V, Amiel J, Siquier-Pernet K, Boddaert N, *et al*. Suleiman-El-Hattab syndrome: a histone modification disorder caused by TASP1 deficiency. Human Molecular Genetics. 2022; 31: 3083–3094.
[70] Kato K, Miya F, Oka Y, Mizuno S, Saitoh S. A novel missense variant in CUL3 shows altered binding ability to BTB-adaptor proteins leading to diverse phenotypes of CUL3-related disorders. Journal of Human Genetics. 2021; 66: 491–498.
[71] Magini P, Smits DJ, Vandervore L, Schot R, Columbaro M, Kasteleijn E, *et al*. Loss of SMPD4 Causes a Developmental Disorder Characterized by Microcephaly and Congenital Arthrogryposis. American Journal of Human Genetics. 2019; 105: 689–705.
[72] Reilly L, Seddighi S, Singleton AB, Cookson MR, Ward ME, Qi YA. Variant biomarker discovery using mass spectrometry-based proteogenomics. Frontiers in Aging. 2023; 4: 1191993.
[73] Révész Á, Hevér H, Steckel A, Schlosser G, Szabó D, Vékey K, *et al*. Collision energies: optimization strategies for bottom-up proteomics. Mass Spectrometry Reviews. 2023; 42: 1261–1299.
[74] Zhou M, Lantz C, Brown KA, Ge Y, Paša-Tolić L, Loo JA, *et al*. Higher-order structural characterisation of native proteins and complexes by top-down mass spectrometry. Chemical Science. 2020; 11: 12918–12936.
[75] Chen B, Brown KA, Lin Z, Ge Y. Top-down proteomics: ready for prime time? Analytical Chemistry. 2018; 90: 110–127.
[76] Messner CB, Demichev V, Bloomfield N, Yu JSL, White M, Kreidl M, *et al*. Ultra-fast proteomics with Scanning SWATH. Nature Biotechnology. 2021; 39: 846–854.
[77] Schoof EM, Furtwängler B, Üresin N, Rapin N, Savickas S, Gentil C, *et al*. Quantitative single-cell proteomics as a tool to characterize cellular hierarchies. Nature Communications. 2021; 12: 3341.
[78] Cong Y, Motamedchaboki K, Misal SA, Liang Y, Guise AJ, Truong T, *et al*. Ultrasensitive single-cell proteomics workflow identifies >1000 protein groups per mammalian cell. Chemical Science. 2020; 12: 1001–1006.
[79] Brunner A, Thielert M, Vasilopoulou C, Ammar C, Coscia F, Mund A, *et al*. Ultra-high sensitivity mass spectrometry quantifies single-cell proteome changes upon perturbation. Molecular Systems Biology. 2022; 18: e10798.
[80] Mahdessian D, Cesnik AJ, Gnann C, Danielsson F, Stenström L, Arif M, *et al*. Spatiotemporal dissection of the cell cycle with single-cell proteogenomics. Nature. 2021; 590: 649–654.
[81] Mund A, Coscia F, Kriston A, Hollandi R, Kovács F, Brunner A, *et al*. Deep visual proteomics defines single-cell identity and heterogeneity. Nature Biotechnology. 2022; 40: 1231–1240.
[82] Bhatia HS, Brunner AD, Öztürk F, Kapoor S, Rong Z, Mai H, *et al*. Spatial proteomics in three-dimensional intact specimens. Cell. 2022; 185: 5040–5058.

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