CLEFT LIP AND PALATE ANOMALIES/SYNDROMES

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CLEFT LIP AND PALATE ANOMALIES/SYNDROMES

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EDITORIAL
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Editorial: Cleft Lip and Palate Anomalies/Syndromes

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Keywords: cleft lip and palate (CLP), cleft lip and palate deformity, craniofacial abnormalities, genetics, dental anomalies, genome wide association, velopharyngeal (VP) insufficiency, quality of life

Editorial on the Research Topic

Cleft Lip and Palate Anomalies/Syndromes

The topic "*Cleft Lip and Palate Anomalies/Syndromes*" is a Frontiers Research Topic aimed to provide an opportunity for researchers and clinicians from different perspectives and areas to publish recent advances in the understanding of Cleft lip and palate (CLP) anomalies/syndromes.

Cleft lip and palate (CLP) anomalies/syndromes are integrated in different subjects of medicine and dentistry. An abundance of research is ongoing in this field. Any deformities (anatomical or chromosomal) that are initiated during pregnancy, with their effects being detected at birth, are considered as congenital anomalies. Among them, CLP is one of the most common congenital anomalies in the head and neck region and only second to congenital heart disease in the whole body with varied prevalence in different civilizations and races. The World Health Organization (WHO) has recognized and included cleft deformities in their Global Burden of Disease initiative. It is estimated that the overall global prevalence of cleft deformities is one affected individual in every 600 new born babies. Though the exact etiology of CLP is controversial, it is believed that both genetic and environmental factors play an important role. It affects psychological development, causes aesthetic and functional problems such as feeding, speech, hearing, and dental functioning. In addition, it may also contribute in dentoskeletal abnormalities such as maxillary arch constriction (maxillary growth retardation), class III malocclusion, mid facial growth deficiency, congenitally missing and malformed teeth, and other orthodontic anomalies like crowding, rotation, malposition of teeth. Medical, surgical, dental, speech, and allied health in cleft lip and palate anomalies/syndromes are of utmost importance in the clinical, epidemiological and research field. The diverse and complex needs of patients with cleft lip and palate anomalies/syndromes, and the obligatory facilities of many varied professionals warrants a multidisciplinary approach for successful management.

In this topic, studies with genome-wide association study and genetics are presented by Ghazali et al. and Küchler et al.

Ghazali et al. aimed to identify the genetic aberration involved in both Nonsyndromic cleft lip and or without cleft palate (NSCL/P) and hypodontia pathogenesis. Original research with cross-sectional study using genome-wide study copy number variation-targeted CytoScan 750K array carried out on salivary samples are investigated. There were a significant gain and loss of both SKI and fragile histidine triad (FHIT) copy number in NSCL/P with hypodontia compared with the noncleft group were explored.

Küchler et al. explored the association between isolated tooth agenesis and genetic polymorphisms in genes that are crucial for craniofacial and tooth development. They reported, the TT genotype in rs3934908 (SMAD6) was associated with higher chance to present third molar agenesis. BMP2 was also associated in haplotype and diplotype analysis with tooth agenesis.

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In the current topic, studies with human samples using model and cephalometric x-ray were also presented. Two studies investigated oral cleft patients. Haque et al. assessed of 3D digital models using GOSLON Yardstick Index. And explored confounding factors responsible for unfavourable treatment outcome in multi-population children with UCLP. Alam et al. investigated whether the craniofacial sagittal jaw relationship in patients with different type of non-syndromic cleft differed from non-cleft (NC) individuals by artificial intelligence (A.I.)-driven lateral cephalometric (Late. Ceph.) analysis. And the study advocates a decrease in sagittal development (SNA, ANB and Wits appraisal) in different types of cleft compared to NC individuals.

A systematic review from Chen et al. explored giving new insight into current Velopharyngeal Inadequacy-Related Quality of Life Assessment (VPI-related QOL) instrument development, validation, and applicability. In this review, understanding the development and characteristics of different QOL instruments, including their reliability, validity, aim, target, language, and resource, should be important before application in clinic or research.

Human samples were also used to explore the effect of bilateral mandible distraction osteogenesis about the nutrition status of infants with pierre-robin sequence by Jiyau et al. They reported, bilateral mandible distraction osteogenesis surgery has a positive effect on the nutrition status of children with pierre-robin sequence. This effect is mainly reflected by the improvements of the body physical indicators after surgery. And, Yang et al. conducted a preliminary study using computational fluid dynamic analysis, inspiration after posterior pharyngeal flap palatoplasty. Real-time computational fluid dynamics simulation was used to capture the airflow through the ports. Posterior pharyngeal flap palatoplasty is one of the most common-used surgical procedures to correct speech, especially for patients suffering from velopharyngeal insufficiency. They found that the airflow dynamics of the upper airway's inspiration were dependent on the velopharyngeal structure. Although the airflow patterns were similar, the velocities between the one-port and two-port structures were different, which explained why patients after posterior pharyngeal flap palatoplasty breathed harder than before and suggested a one-port structure might be a better choice for secondary velopharyngeal insufficiency reconstruction based on the computational fluid dynamics analyses.

This Research Topic targets the acquisition and dissemination of knowledge regarding all aspects (including etiology, prevention, diagnosis, treatment and its outcome) of cleft lip and palate anomalies/syndromes to share knowledge from both the basic and clinical sciences. Briefly, this Research Topic provided an opportunity for researchers and clinicians from different perspectives and areas to discuss recent advances in the understanding of Cleft Lip and Palate Anomalies/Syndromes. It also will provide readers with new insights and different viewpoints to stimulate further investigations in this broad research field. This Research Topic also achieved its initial aim to have different areas contemplated, such as genome-wide association, genetic polymorphisms in genes involved in craniofacial development and isolated tooth agenesis, 3D digital model assessments, artificial-intelligence based craniofacial assessment, quality of life research, coming together in this article Research Topic.

AUTHOR CONTRIBUTIONS

MA, MM, and KP contributed equally to the conception and writing. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Identification of Copy Number Variation Among Nonsyndromic Cleft Lip and or Without Cleft Palate With Hypodontia: A Genome-Wide Association Study

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Nonsyndromic cleft lip and or without cleft palate (NSCL/P) with the hypodontia is a common developmental abnormality in humans and animals. This study identified the genetic aberration involved in both NSCL/P and hypodontia pathogenesis. A cross-sectional study using genome-wide study copy number variation-targeted CytoScan 750K array carried out on salivary samples from 61 NSCL/P and 20 noncleft with and without hypodontia Malay subjects aged 7–13 years old. Copy number variations (CNVs) of *SKI* and fragile histidine triad (*FHIT*) were identified in NSCL/P and noncleft children using quantitative polymerase chain reaction (qPCR) as a validation analysis. Copy number calculated (CNC) for each gene determined with Applied Biosystems CopyCaller Software v2.0. The six significant CNVs included gains (12q14.3, 15q26.3, 1p36.32, and 1p36.33) and losses (3p14.2 and 4q13.2) in NSCL/P with hypodontia patients compared with the NSCL/P only. The genes located in these regions encoded *LEMD3*, *IGF1R*, *TP73*, *SKI*, *FHIT*, and *UGT2β15*. There were a significant gain and loss of both *SKI* and *FHIT* copy number in NSCL/P with hypodontia compared with the noncleft group ($p < 0.05$). The results supported that CNVs significantly furnish to the development of NSCL/P with hypodontia.

Keywords: cleft lip, cleft palate, hypodontia, DNA copy number variations, genome-wide association study

INTRODUCTION

Tooth agenesis (TA) is one of the most common developmental abnormalities in humans, described by the failure to develop one or more teeth (Ritwik and Patterson, 2018). TA is divided into three categories which are hypodontia, oligodontia, and anodontia (Klein et al., 2013). Hypodontia is the loss of one to five teeth, oligodontia is described as missing six or more teeth, and anodontia refers to the complete loss of tooth growth (Phan et al., 2016). Hypodontia is the most common dentofacial condition in humans (Al-Ani et al., 2017). Hypodontia can occur as an isolated condition without any other recognizable abnormality

called nonsyndromic or associated with other structural deformities known as syndromic (Souza-Silva et al., 2018). The permanent dentition is more regularly affected than deciduous dentition (Abdulgani et al., 2017). A tooth development requires a complex process involving interaction between epithelial and mesenchymal signals, assisted by communication between signaling molecules and genetic pathways (Jussila and Thesleff, 2012). Various factors, such as those from wingless-related integration sites (Wnt), fibroblast growth factor (Fgf), bone morphogenic protein (BMP), and hedgehog (Hh) families, participate in the signaling mesenchymal interaction in tooth growth (Al-Ani et al., 2017). Alteration within one or more signaling pathways attributed to environmental influences (smoking, alcohol intake, chemotherapy, trauma, radiotherapy, and infection) or genetic factors could lead to abnormal tooth development (Rakhshan, 2015; Al-Ani et al., 2017).

The co-occurrence of CL/P (cleft lip with or without cleft palate) and hypodontia was reported frequently in human and animal models (Phan et al., 2016). Congenitally missing teeth or hypodontia is the most common dental anomalies found in CL/P patients (Rahman et al., 2004; Haque and Alam, 2015). Various studies revealed that *MSX1* mutations led to the cleft palate (CP) and TA in humans (Kouskoura et al., 2011; Liang et al., 2012; Leslie and Marazita, 2013). *Msx1*-deficient mice revealed severe craniofacial anomalies, comprising the secondary palatal cleft and missing teeth (Nakatomi et al., 2010). The incidence of hypodontia was higher in more severe CL/P cases and presented as loss in maxillary lateral incisor tooth (Camporesi et al., 2010). The hypodontia among CL/P may significantly impact patient's quality of life in terms of functional decrease, emotional distress, and social prosperity.

The most common orofacial cleft syndrome is the van der Woude syndrome (VWS), which found 2% of all syndromic CL/P, caused by mutation or deletion of the *IRF6* gene in 68% of the cases (Kondo et al., 2002; de Lima et al., 2009). Letra et al. (2012) extensively investigated the role of *IRF6* in the nonsyndromic orofacial cleft (OFCs) with TA situated outside the cleft region in a cohort of 134 Brazilian patients, thereby establishing a borderline-associated *IRF6* marker (rs6588860) in the subgroup of the subjects exhibiting CP and TA. Nine genomic loci and 26 candidate genes were identified in the previous literature leading to the occurrence of those two congenital disabilities (Phan et al., 2016).

The variation in copy number has recently been identified as a significant cause of variation in the structural genome involving both duplications and sequence deletion (Redon et al., 2006). The previous study reported copy number variations (CNVs) in syndromic CL/P with hypodontia patients such as ectodermal dysplasia syndrome, Wolf Hirschhorn syndrome, and DiGeorge syndrome. The CNV in OFCs with TA found had associated with genomic loci such as in 1q21-q25, 1q32, 2q31.2-q32.2, 4p16.3, 8q24, and 16q22. However, the CNV and the related genes for nonsyndromic cleft lip with or without cleft palate (NSCL/P) with hypodontia remained with limited identification (Schinzel and Schmid, 1980; Wong et al., 1999). The causative genes and genomic loci of hypodontia among syndromic CL/P might also lead to the development in nonsyndromic CL/P. Therefore, a genome-wide association study (GWAS) was conducted to identify the contribution of CNV in the development of NSCL/P with hypodontia.

MATERIALS AND METHODS

Study Population

A total of 81 individuals, including 61 NSCL/P cases and 20 noncleft aged 7–13, enrolled in the present comparative cross-sectional study. All the patient samples were recruited from one tertiary hospital in northern and eastern Malaysia between 2016 and 2018. The inclusion criteria for cases were NSCL/P children aged 7–13 years old, and those who had cleft palate only were excluded from this study. The subjects in the comparative group were noncleft children and those without any history of cleft. Noncleft children who have undergone orthodontic treatment were excluded from the study. The sample size for the prevalence of dental anomalies and genetic aberrations were calculated using the single proportion formula based on the prevalence of dental anomalies (Al-Kharboush et al., 2015) and CNV (Simioni et al., 2015). The precision was set at 2% giving a sample size of 42 and six subjects, respectively. However, this study recruited 61 NSCL/P patients and 100 subjects to determine the prevalence of dental anomalies. Dental panoramic tomography was taken for identification and confirmation of the number and morphology of the teeth. Exclusion criteria for the NSCL/P cases were those who had cleft palate only and patients with other syndromes such as ectodermal dysplasia or Axenfeld-Rieger syndromes. This study was approved by the Human Research Ethics Committee of Universiti Sains Malaysia (Reference number: USM/JPEPeM/140357). Both subjects and their guardians had become acquainted with the comprehensive research procedure and signed the informed consent before being enrolled in this study.

DNA Extraction

Saliva samples were collected from each patient and stored in sterile 50-ml conical tubes. Genomic DNA was extracted from saliva samples by following GeneAll Blood SV Mini Kit manual (General Biosystem, Seoul, South Korea), including lysis, binding, washing, and elution.

CytoScan 750K Array and Copy-Number Analysis

The Genome-Wide Human CytoScan 750K Array (Affymetrix, CA United States) was used to analyze genomic alterations according to the manufacturer's protocol. A total of 250 ng of genomic DNA from NSCL/P with hypodontia and control samples (noncleft) were digested with the restriction enzyme *Nsp1*, then ligated to an adapter and PCR amplification using PCR on a single pair of primers that recognized the adapter sequence. The PCR products were analyzed by electrophoresis in 2% agarose using Tris-Borate-EDTA (TBE) to confirm the amplicon size between 150 and 2,000 bp in length. PCR products were combined from each sample and purified using magnetic

beads (Agencourt AMPure, Beckman Coulter, Beverly, MA, United States). The purified PCR products were fragmented using DNase 1 and visualized on 4% TBE agarose gel to confirm that the fragment sizes ranged between 25 and 125 bp. The fragmented PCR products were subsequently end-labeled with biotin and hybridized to the array. The arrays were then washed, stained using GeneChips Fluidics Station 450 and Affymetrix GeneChip Command Console Software, version 1.2, followed by an Affymetrix Chromosome Analysis Suite version 3.1 (CHAS) Affymetrix United States. The data were normalized to baseline reference intensities using 270 HapMap samples and another 90 healthy noncleft in the software to calculate copy number. The Hidden Markov Model (HMM) available within the software package was used to determine the copy number states and their breakpoints. Based on HMM, the log-ratio thresholds were set at ≥ 0.58 and ≤ -1, and used to categorize altered regions as copy number gains (amplification) and copy number losses (deletions). The alterations that only involved at least 25 conservative probes and more than 25 kbp in length were selected to avoid the false-positive CNVs.

Candidates CNV Analysis

This study compared overlapping regions in NSCL/P and noncleft children with or without hypodontia. Hypodontia-specific CNVs were considered potential candidate variants for hypodontia among subjects if they showed a statistically significant difference between patient CNV and CNV or overlapped genes. CNVs correlate with tooth's craniofacial production and morphogenesis or are involved in a process suspected of affecting palatogenesis and odontogenesis.

Real-Time Quantitative PCR Validation

Two statistically significant genes were selected for validation by real-time quantitative PCR (qPCR) analysis on StepOnePlus Real-Time PCR system (Applied Biosystem, Forster City, CA, United States). The target information for the selected genes in the CNV region was entered into Assay Search Tool-Single Tube Taqman® Assay from Life Technologies website[1] to obtain specific primer-probe pair. Two selected sequences were *Homo sapiens SKI* NCBI location Chr 2: 3,652,515-3,736,200, cytoband: 1p36.33 (Hs05780959_cn) and Fragile Histidine Triad Diadenosine Triphosphatase (*FHIT*) NCBI location Chr 3: 60555049–60579400, cytoband 3p14.2 (Hs03472126_cn). CNV performed using TaqMan Genotyping Master Mix for absolute quantitation of copy number using real-time qPCR. *RNase P* was used as endogenous control and NTC (reaction mixture without DNA template) as a negative control. qPCR was run with the following parameters: hold at 95°C for 10 min, followed by 95°C for 15 s and 60°C for 1 min for 40 cycles.

CNC for the gene is identified with Applied Biosystems CopyCaller Software v2.0. Several copies of the target sequence expected in the majority of samples were set at two. Samples were revised to attain optimum experimental conditions where samples are of high quality, copy number and reference assay have amplified, and sample replicates have parallel cycle threshold (CT) and difference of CT (ΔCT) values. The number of copies of the target sequence in every test sample is regulated by relative quantitation (RQ) using the comparative CT ($\Delta\Delta$CT) method. This method quantifies the Ct difference (ΔCt) between target and reference sequences, then compares the ΔCt values of test samples to a calibrator sample known to have two copies of the target sequence. Accepted copy number calls should have a confidence value >95% and Z-score <1.75 (Applied Biosystems CopyCaller Software v2.0).

Statistical Analysis

The analysis was carried out using SPSS, version 26.0. The frequency of the deletion or amplification of each CNV regions was compared between NSCL/P and noncleft with or without hypodontia using Fisher's exact test to identify significant chromosome alterations. Chi-square tests were used to test the significant changes detected by CytoScan 750K Array. Statistical analysis for copy number data was performed using the Mann-Whitney test, and $p < 0.05$ was identified as statistically significant.

RESULTS

Genome-Wide Assessment of CNVs in NSCL/P and Non-cleft With and Without Hypodontia

A total of 81 subjects were recruited in this study, including 61 NSCL/P patients and 20 noncleft subjects. In this NSCL/P, 31 (50.0%) were unilateral cleft lip and palate (UCLP), 13 (21%) were bilateral cleft lip and palate (BCLP), and 17 (27%) were cleft lip only (CL). LUCLP (34%) was the most common cleft type followed by BCLP (21.0%), RUCLP (16.0%), left cleft lip (14.0%), and right cleft lip (13.0%). The mean (SD) age for UCLP, BCLP, CL, and noncleft was 9.3 years (SD 1.8), 9.2 years (2.0), 9.1 years (2.0), and 5.5 years (3.5). Salivary samples from all subjects aged between 7 and 13 years old were subjected to genetic aberration assay using CytoScan 750K array. The subjects comprised four groups, such as NSCL/P with or without hypodontia and noncleft with or without hypodontia. All the samples exhibited chromosomal aberrations. The size of the detected CNVs varied from 25 kb to 2.5 Mb. Of the 721 genomic segments on the 81 samples, the analyses restricted to 558 genomic loci corresponding to the autosomes, including 196, showed amplification, and 362 showed deletion. The current study showed that the highest percentage of amplification occurred in chromosomes 1p, 2q, 12q, and 15q, and deletions observed for chromosomes 1q, 3p, 4q, 6p, and 7q among NSCL/P and noncleft with hypodontia. Identifying a candidate gene for NSCL/P with hypodontia in the CNVs selected regions by searching for copy number losses or copy number gains which involved the genes shared by multiple patients. The most recurrent amplified regions observed on chromosome 1p36.33, 1p36.32, 12q14.3, 14q32.33, and 15q26.3 loci and the most frequent deletion is found on chromosome

[1]http://www.lifetechnologies.com

1q44, 3p14.2, 4q13.2, 6p25.3, and 7q34 among NSCL/P and noncleft with hypodontia.

Six of these unique candidate NSCL/P with hypodontia-specific CNVs (four gains and two losses) were significant, and these CNVs involved a total of eight genes. The six significant CNVs in NSCL/P with hypodontia included four gains (1p36.32, 1p36.33, 12q14.3, and 15q26.3) and two losses (3p14.2 and 4q13.2). The two significant CNVs among noncleft with hypodontia included one gain (1p36.32) and one copy number loss (4q13.2). The fisher exact analysis of the association between NSCL/P subjects in CNVs (gains and losses) are shown in **Table 1**. A total of six of these 334 CNVs were significantly different from NSCL/P only ($p < 0.05$). The significant CNVs included four gains (1p36.32, 1p36.33, 12q14.3, and 15q26.3) and two losses (3p14.2 and 4q13.2) and were found among NSCL/P with or without hypodontia. Besides, a total of 31 CNVs found in the noncleft with hypodontia did not overlap with the number of CNVs among noncleft-only children. Two of these were significantly different from a noncleft-only sample ($p < 0.05$) and are shown in **Table 2**. All the genomic loci that lay within the significant CNVs were employed to identify the phenotype like plausibly pathogenic sequence and genes of interest using Decipher database, OMIM, and Database of Genomic Variants. **Table 3** shows several protein-coding genes identified from the overlapping on the significant copy number gains and losses. Two selected candidates' genes were chosen for validation, *SKI* and *FHIT*, based on the literature (Vieira et al., 2007; Bliek et al., 2008) that the gene pathways were related to any tooth abnormalities, craniofacial deformities, and embryonic development.

Validation of CNVs by Real-Time qPCR

Validation of *SKI* and *FHIT* by CNV assay was performed as a continuous finding of copy number gain and copy number loss found by microarray-based copy number analysis 1p36.33 and 3p14.2 regions in NSCL/P with or without hypodontia. Microarray-based copy number analysis was performed with the Affymetrix CytoScan 750K array and visualized using the Affymetrix Chromosome Analysis Suite version 1.2.2. **Figure 1** shows the 116-kb gain image at 1p36.33 (Chromosome 1: 2021784–2187344; GRCH37/hg19) present in a 10-year-old boy. The region overlaps with *SKI* and *PRKCZ* genes. **Figure 2** shows image of the 26-kb gain at 3p14.2 (Chromosome 3:60,555,048-60,579,400; GRCH37/hg19) present in a 9-year-old girl. The region overlaps with the *FHIT* gene.

Thirty subjects were recruited, including NSCL/P and noncleft hypodontia and normal control (noncleft hypodontia). The number calculated (CNC) for *SKI* and *FHIT* genes were determined. **Figure 3** showed CNC of *SKI* gene in NSCL/P with hypodontia and noncleft with hypodontia compared with the normal control. From the findings, CNC for each individual in NSCL/P with hypodontia and noncleft with hypodontia showed CNC similar to the control group, $2.49 \geq \text{CNC} \geq 1.50$ with one patient expressing more than 2.50 CNC. The CNC in the range of $2.49 \geq \text{CNC} \geq 1.50$ may have a copy number predicted as two. From the 30 subjects, one of the NSCL/P with hypodontia patient showed a copy number predicted (CNP) at three, and the remaining 29 samples had CNP value equal to two.

The scatter plot of the CNC data showed a distinct distribution of copy numbers between all the subjects (**Figure 4**). The straight line showed that the normal copy number fell at two. *SKI* copy number for NSCL/P with hypodontia was found scattered and exceeded two; meanwhile, most SKI copy numbers in normal

TABLE 1 | Association between NSCL/P with or without hypodontia with copy number variations (CNVs).

Variables	NSCL/P with hypodontia	NSCL/P without hypodontia	X^2 statistic (*df*)	*p* value
	No. (%)	No. (%)		
	n = 41	*n* = 20		
CNVs				
Gain 1p36.32				
Gain	16 (39.0)	2 (10)	5.4 (1)	0.020
No gain	25 (61)	18 (90)		
Gain 1p36.33				
Gain	12 (29.3)	1 (5.0)	4.7 (1)	0.030
No gain	29 (70.7)	19 (95.5)		
Gain 12q14.3				
Gain	13 (31.7)	1 (5.0)	5.4 (1)	0.024
No gain	28 (68.3)	19 (95.0)		
Gain 15q26.3				
Gain	12 (29.3)	1(5.0)	4.7 (1)	0.030
No gain	29 (70.7)	19 (95)		
Loss 3p14.2				
Loss	9 (22.0)	0 (0.0)	5.4 (1)	0.024
No loss	32 (78.0)	20 (100.0)		
Loss 4q13.2				
Loss	14 (34.1)	1 (5.0)	5.8 (1)	0.023
No loss	27 (65.9)	19 (95.0)		

TABLE 2 | Association between noncleft with or without hypodontia with copy number variations (CNVs).

Variables	Noncleft with hypodontia	Noncleft without hypodontia	X^2 statistic (*df*)	*p* value
	No. (%)	No. (%)		
	n = 10	*n* = 10		
CNVs				
Gain 1p36.32				
Gain	5 (50.0)	0 (0.0)	6.7 (1)	0.033
No gain	5 (50.0)	10 (100.0)		
Gain 1p36.33				
Gain	2 (20.0)	0 (0.0)	2.2 (1)	0.474
No gain	8 (80.0)	10 (100.0)		
Gain 12q14.3				
Gain	4 (40.0)	0 (0.0)	5.0 (1)	0.087
No gain	6 (60.0)	10 (100.0)		
Gain 15q26.3				
Gain	2 (20.0)	1 (10.0)	0.3 (1)	1.00
No gain	8 (80.0)	9 (90.0)		
Loss 4q13.2				
Loss	5 (50.0)	0 (0.0)	6.7 (1)	0.033
No loss	5 (50.0)	10 (100.0)		

TABLE 3 | Distribution of significant CNVs among NSCL/P and noncleft with and without hypodontia (n = 81).

Chr	Cytoband	CNVs	Start	End	Size (kb)	Marker count	Overlap genes	No of patients (%)
1	p36.32	Gain	329830	3615979	318	150	*TP73, PRDM16*	21 (26)
1	p36.33	Gain	849466	977958	128	70	*SKI, PRKCZ*	15 (19)
12	q14.3	Gain	66724933	66793710	69	56	*LEMD3*	18 (22)
15	q26.3	Gain	99431573	99488091	57	55	*IGF1R*	16 (20)
3	p14.2	Loss	60555048	60579400	93	38	*FHIT*	9 (11)
4	q13.2	Loss	69436558	69571630	129	25	*UGT2β15*	20 (25)

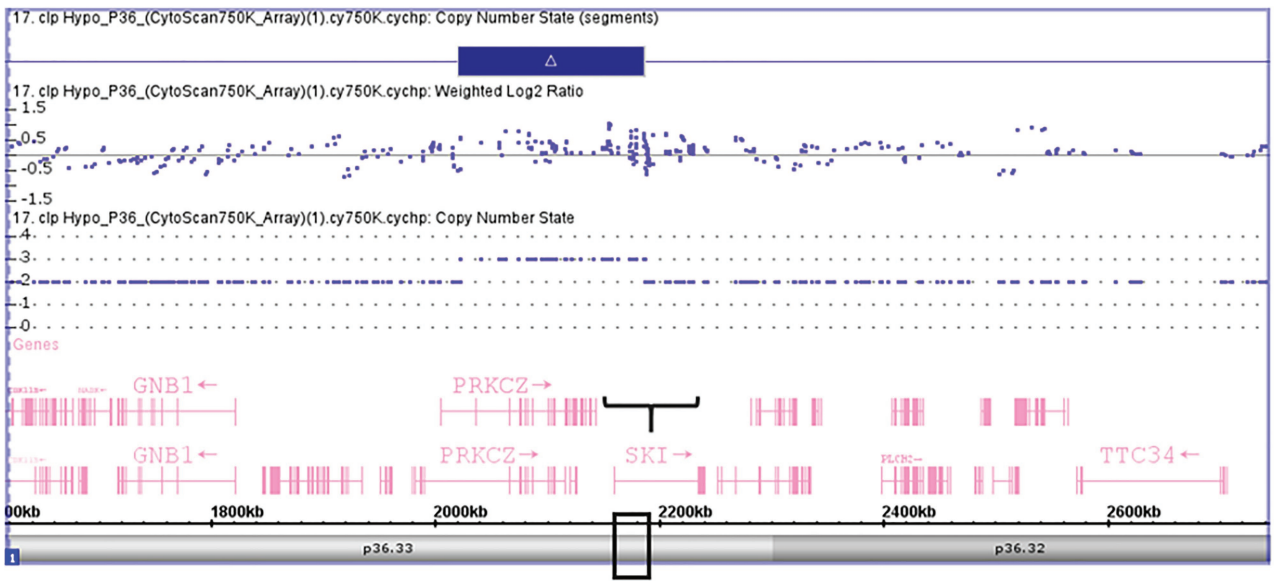

FIGURE 1 | Microarray-based copy number analysis performed with the Affymetrix CytoScan 750K array and visualized using the Affymetrix Chromosome Analysis Suite version 1.2.2. Image of the 116 kb gain at 1p36.33 (Chromosome 1: 2021784-2187344, GRCH37/hg19) present in a boy 10 year-old. The region overlaps with SKI (black box).

controls were dispersed with less than copy number two. There was no significant difference in copy number of *SKI* (p = 0.9) in noncleft with hypodontia (1.98 ± 0.25) compared with the normal control group (1.98 ± 0.07), which means that both groups had normal copy number for *SKI*. However, there was a significant increase in *SKI* copy number in NSCL/P with hypodontia (2.2 ± 0.28, p = 0.02) compared with the normal control group (1.98 ± 0.07). These results revealed that *SKI* copy number gain was present among NSCL/P with hypodontia.

Copy number for *FHIT* was determined for NSCL/P with hypodontia as it showed copy number loss using Cytoscan 750K array. From the findings, the normal control, noncleft with hypodontia, and NSCL/P with hypodontia patients tested had a copy number of 2.49 ≥ CNC ≥ 1.50 except for two patients having CNC < 1.50 (**Figure 5**). Meanwhile, all the NSCL/P with hypodontia patients showed that CNC value did not exceed copy number two, including two samples with a copy number calculated as 1.55. However, scatter plot data had shown a distinct distribution of *FHIT* copy number between all the subjects (**Figure 6**). CNC among NSCL/P with hypodontia were lower than two compared with the normal control while noncleft with hypodontia had more copy number two. A significant decreased of *FHIT* copy number in NSCL/P with hypodontia (2.10 ± 0.231) compared with normal control (2.01 ± 0.069) was confirmed; p = 0.002. However, there was no significant difference in copy number of *FHIT* in noncleft with hypodontia (1.97 ± 0.09) compared with normal control.

DISCUSSION

Genome-Wide Assessment of CNVs in NSCL/P and Noncleft With and Without Hypodontia

In the recent study, 61 NSCL/P and 20 noncleft children were being recruited. A total of 558 genomic loci, including 196 amplification and 362 deletions, were detected among NSCL/P and noncleft with and without hypodontia. This result showed that the copy number loss is higher compared with the copy number gain. These results are highly consistent with those previously described in a systematic analysis study by Conte et al. (2016), with a total of 249 genomic deletions and

FIGURE 2 | Microarray-based copy number analysis performed with the Affymetrix CytoScan 750K array and visualized using the Affymetrix Chromosome Analysis Suite version 1.2.2. Image of the 26 kb loss at 3p14.2 (Chromosome 3:60,555,048-60,579,400; GRCH37/hg19) present in a girl 9 year-old. The region overlaps with FHIT gene (black box).

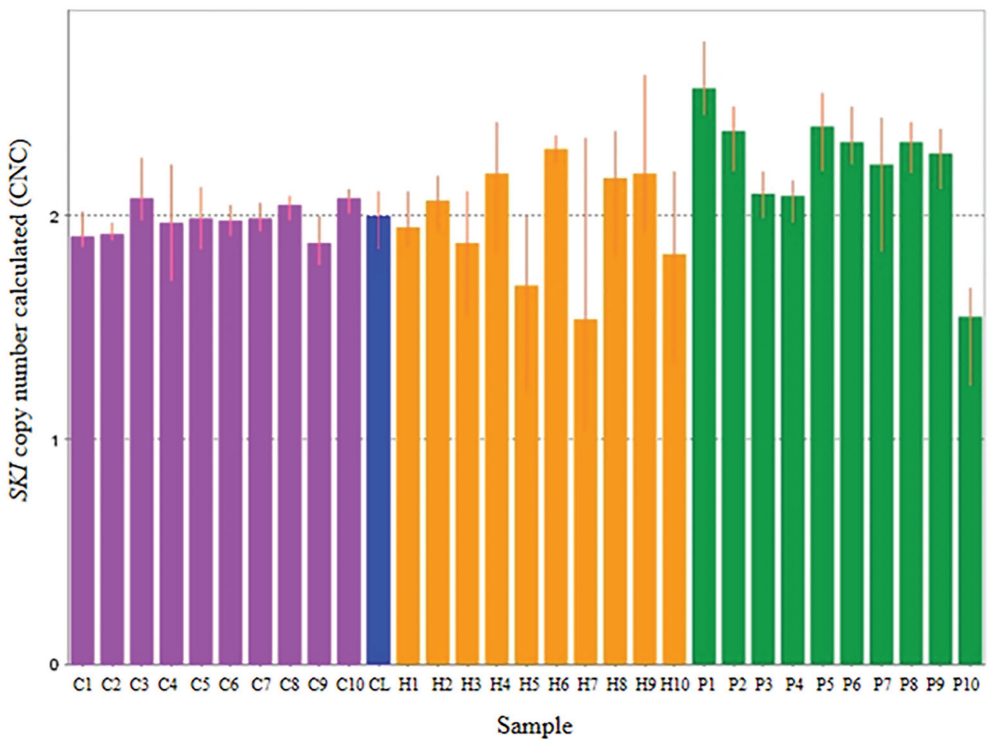

FIGURE 3 | Representative of copy number calculated (CNC) of SKI in normal control (purple bars), non-cleft with hypodontia (yellow bars) NSCL/P with hypodontia (green bars) and calibrator (blue bar).

226 duplications from a cohort of 312 orofacial clefts reported in two publicly accessible databases of chromosome imbalance and phenotype in humans, DECIPHER and ECARUCA. This study supported the finding of CNVs with small and large chromosomal deletions among oral cleft patients and individual patients with multiple congenital disabilities (Phan et al., 2016).

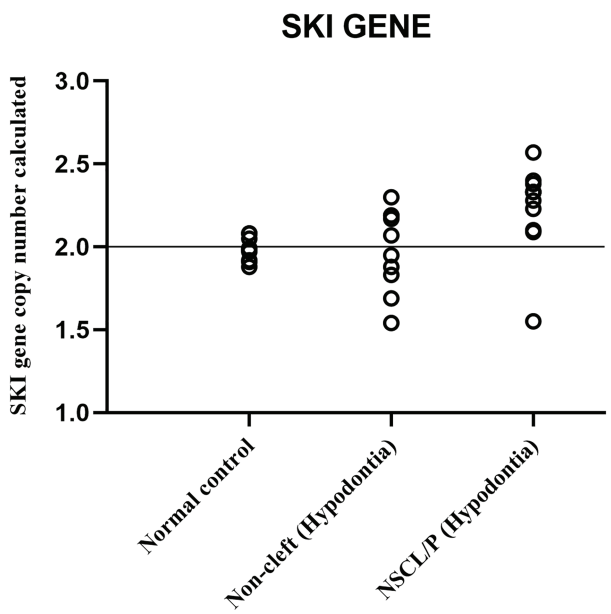

FIGURE 4 | Scatter plot indicated copy number calculated value of SKI among NSCL/P with hypodontia, non-cleft with hypodontia and normal controls.

Our study identified a significant candidate CNVs on 1p36.32, 1p36.33, 3p14.2, 4q13.2, 12q14.3, and 15q26.3 among NSCL/P patients with hypodontia whereby study done by Yu et al. (2017) reported identified 14 novel loci and genetic heterogeneity among NSCL/P only. However, we consider these CNVs important candidates because several of the genes in these regions are functionally linked to normal embryonic and tooth development. Some genomic loci with deletion were reported of TA and syndromic orofacial clefts (OFCs; Phan et al., 2016). Van Der Woude syndrome characterized as cleft lip with or without cleft palate and hypodontia revealed 8 kb deletion or insertion in the region 1q32-q41 (Schutte et al., 2000). An ectodermal dysplasia-like syndrome patient showed a 26-Mb interstitial deletion involving the region 2q31.2-q33.2, with multiple phenotypes, including missing or abnormal teeth with cleft lip and palate (Rifai et al., 2010).

Therefore, quantitative PCR was performed to confirm the data array results. The genes *SKI* and *FHIT* were randomly selected from significant CNVs (1p36.33 and 3p14.2) regions to identify their association with NSCL/P and hypodontia.

Significant Copy Number Gain in *SKI* Among NSCL/P With Hypodontia

The present study showed a significantly increased copy number of the 1p36.33 region, encompassing the *SKI* gene. *SKI* encodes the nuclear proto-oncogene protein homolog of avian sarcoma viral (v-ski) oncogene. These findings are consistent with the previous research that showed *SKI* was the candidate gene for orofacial clefting (Berk et al., 1997); (Lu et al., 2005; Vieira et al., 2005); nevertheless, its role in tooth development remained unclear. The meta-analysis identified six new susceptible loci

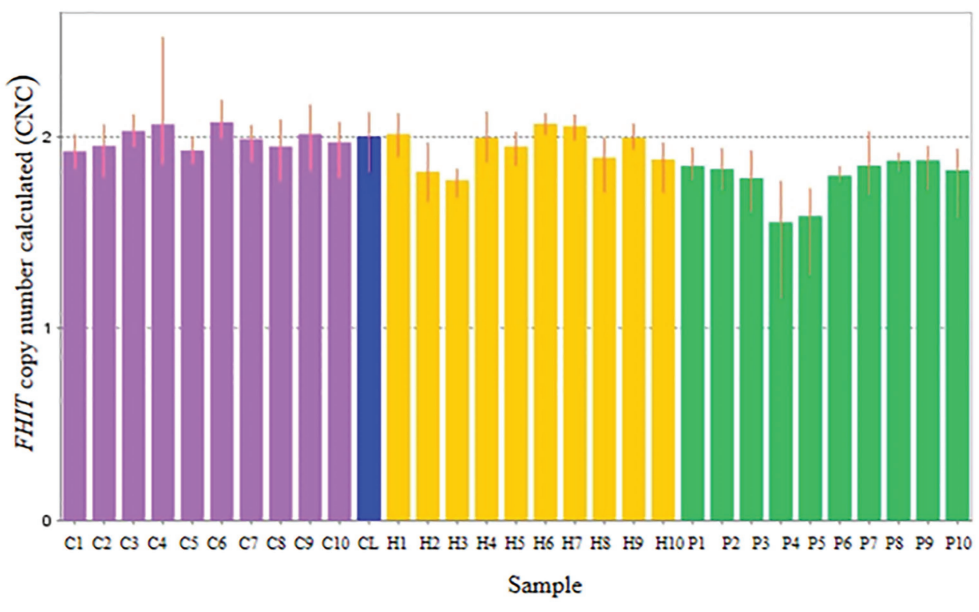

FIGURE 5 | Representative of copy number calculated (CNC) of FHIT in normal control (purple bars), non-cleft with hypodontia (yellow bars) NSCL/P with hypodontia (green bars) and calibrator (blue bar).

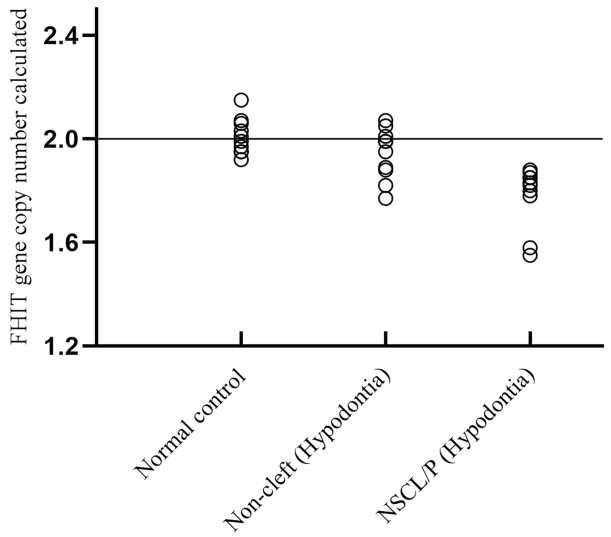

FIGURE 6 | Scatter plot indicated copy number calculated value of FHIT among NSCL/P with hypodontia, non-cleft with hypodontia and normal control.

in European NSCL/P population including 1p36, 2p21, 3p11.1, 8q21.3, 13q31.1, and 15q22.2 (Ludwig et al., 2012). Most previous work investigated the single nucleotide polymorphism (SNP) and mutation of the *SKI* gene compared with the current study, which identified the copy number variation among the subjects. Duplication of the *SKI* gene has not yet been linked to orofacial clefting and tooth development directly. However, we found that *SKI* is involved in several signaling pathways important for the process, such as transforming factor-beta (TGF-β), BMP, and G-protein-coupled receptors (GPCR; Deheuninck and Luo, 2009; Parada and Chai, 2012).

SKI is an essential negative TGF-β signaling regulator, which interacts with mothers against decapentaplegic (SMADs) to suppress TGF-β signaling activity (Yang et al., 2015). *SKI* gene can block *TGF-β* signaling by interfering with the phosphorylation of SMAD2 and SMAD3 by activated *TGF-β* type 1 receptor (Buess et al., 2004). *TGF-β* signaling plays a crucial role in controlling the formation of palates in both palate epithelium and mesenchyme (Iwata et al., 2011). *TGF-β* is also critical for cell proliferation and differentiation in the dental pulp (Niwa et al., 2018). The finding may suggest that copy number gain of *SKI* may alter the building blocks of single proteins (amino acids) in the *SKI* protein. Many of the mutations modify the *SKI* protein region, which binds to SMAD proteins. It assumed that the changed *SKI* proteins could not be attached to SMAD proteins, which allow the unregulated prolongation of *TGF-β* signaling. Excessive *TGF-β* signaling affects gene activity regulation and is likely to interact with palatal and tooth growth. TGF-β3 is one of the main ligands of two serine/threonine kinase receptors, TGFβR1 and TFFβR2, investigated concerning syndromic cleft lip and palate (Loeys et al., 2005).

The *SKI* mutation was also associated with Shprintzen-Goldberg syndrome with distinctive facial features, hypotonia, and intellectual deficiency (Doyle et al., 2012; Schepers et al., 2015).

Significant Copy Number Loss in *FHIT* Among NSCL/P With Hypodontia

A lower 3p14.2 copy number containing a *FHIT* gene was identified among NSCL/P patients with hypodontia. *FHIT* gene is a prominent member of the histidine triad gene family and is considered a tumor suppressor gene. Deleting the FHIT gene has never been reported in disturbing the development of cleft lip and palate and the tooth. Thus, this would be the first finding that revealed *FHIT* dysregulation in patients affected with NSCL/P with hypodontia. The previous result has found a deletion on the similar locus of 3p14.2 in a patient of Waardenburg syndrome type 11A with cleft lip and palate (Lakhanpal et al., 2014). Another study found that the abnormal expression of *FHIT* may be associated with a variety of malignant tumors, such as lung cancer and breast cancer (Pekarsky et al., 2002; Ismail et al., 2011). The mechanism of *FHIT* in triggering NSCL/P and hypodontia formation was unknown. However, it was likely that loss of *FHIT* is involved in the related pathway and transcriptional activities including *TGF-β*, *WNT* signaling, β-activin transcription, and *BMP* (Pekarsky et al., 2002; Weiske et al., 2007; Prosseda et al., 2019). This finding supports the idea that *TGF-β* signaling and canonical *Wnt/β-catenin* pathways play an essential role in the development of tooth and palatogenesis (Tamura and Nemoto, 2016; Niwa et al., 2018; Reynolds et al., 2019). There are also various reports of genetic variations at the *FHIT* locus and loss of *FHIT* protein expression in preneoplasias, proposing a tumor-suppressive role for *FHIT* in the early stages of cancer progression (Waters et al., 2014; Malak et al., 2016; Kiss et al., 2017).

Weiske et al. (2007) reported that *FHIT* related with a lymphoid enhancer-binding factor β-catenin complex by directly binding to the β-catenin, a significant player in the canonical WNT pathway that is decontrolled in numerous form of human cancer. *FHIT* expresses the transcription of target genes such as *cyclin D1*, *AXIN2*, *MMP-14*, and *survivin* when binding to the β-catenin C-terminal domain (Weiske et al., 2007). *AXIN2* likely plays a vital role in regulating beta-catenin stability in the *WNT* signaling pathway (Huraskin et al., 2016). *AXIN2* also has independently associated with tooth agenesis and NSCL/P (Letra et al., 2009). The variations in WNT genes have been recognized in an individual with tooth agenesis (Dinckan et al., 2016). Therefore, the *AXIN2* mutations could lead to an inefficient block of the *WNT* signaling pathway and altered the embryonic development of dental organs and predisposition to cancer (Otero et al., 2019). We hypothesized that reduced induction of *FHIT* might be triggering *AXIN2* mutation and directly producing *WNT* dysregulation in the progression of lip and palate fusion and tooth development and finally leading to abnormal growth.

Validation through copy number analysis has strengthened results from the previous genome-wide association studies.

This current study found a significant gain and loss of copy number of these genes, *SKI* and *FHIT*, respectively, in each of the selected NSCL/P with tooth-missing patients compared with normal groups. Both genes might have a significant contribution to the development of NSCL/P and hypodontia. Different gene copy numbers among individuals and populations reveal gene expression variations (De Smith et al., 2008). CNVs also overlap over 7,000 genes, many of which are essential in biological pathways. Many studies have reported that the number of gene copies has led to a cell modification of the transcription process and make a difference in the expression level (Stranger et al., 2007; Prestel et al., 2010). Hence, evidence for CNVs between genes and potential NSCL/P with hypodontia has been confirmed in this study. Significant copy number gain of *SKI* was observed on the subject members compared with the normal control, so we can conclude that SKI might give a minor contribution to the NSCL/P with hypodontia formation and could trigger the cleft occurrence. In addition, *FHIT* loss was reported to be associated with the NSCL/P with hypodontia development. This novel finding would help to shed light on the regulation of *SKI* and *FHIT* on the orofacial cleft and hypodontia formation. In clinical setting, early complications due to hypodontia can be part of prevention strategies and genetic counseling in a multidisciplinary clinic.

CONCLUSION

In conclusion, this study developed a GWAS study to explore the CNVs among NSCL/P patients and identify potential NSCL/P and hypodontia-related genes. We identified six significant CNVs with four genomic deletions and two duplication, including several genes, such as SKI, FHIT, TP73, LEMD3, IGF1R, and UGT2β15 genes. Our study enhances the reservoir of possible causative genes for NSCL/P and hypodontia genes for genetics studies. It provides a disease link to many of these genes known to contribute to several signaling pathways. Forthcoming human mutation analysis and animal model studies are required to confirm the role of the identified potential causative NSCL/P and hypodontia genes.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: (REPOSITORY: dbVar and ACCESSION ID: nstd202).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Research Ethics Committee of Universiti Sains Malaysia (Reference number: USM/JPeM/140356). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

NG performed all the experiments, designed the methodology, and wrote the original draft. NA contributed to conceptualization, formal analysis, writing-reviewing, and editing the manuscripts. AA performed the data curation and visualization. SS contributed to software analysis and validation process. TPK supervised the research and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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Sagittal Jaw Relationship of Different Types of Cleft and Non-cleft Individuals

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To investigate whether the craniofacial sagittal jaw relationship in patients with non-syndromic cleft differed from non-cleft (NC) individuals by artificial intelligence (A.I.)-driven lateral cephalometric (Late. Ceph.) analysis. The study group comprised 123 subjects with different types of clefts including 29 = BCLP (bilateral cleft lip and palate), 41 = UCLP (unilateral cleft lip and palate), 9 = UCLA (unilateral cleft lip and alveolus), 13 = UCL (unilateral cleft lip) and NC = 31. The mean age was 14.77 years. SNA, SNB, ANB angle and Wits appraisal was measured in lateral cephalogram using a new innovative A.I driven Webceph software. Two-way ANOVA and multiple-comparison statistics tests were applied to see the differences between gender and among different types of clefts vs. NC individuals. A significant decrease ($p < 0.005$) in SNA, ANB, Wits appraisal was observed in different types of clefts vs. NC individuals. SNB ($p > 0.005$) showed insignificant variables in relation to type of clefts. No significant difference was also found in terms of gender in relation to any type of clefts and NC group. The present study advocates a decrease in sagittal development (SNA, ANB and Wits appraisal) in different types of cleft compared to NC individuals.

Keywords: sagittal jaw relationship, cleft lip and palate, cephalometric analysis, wits appraisal, SNA angle, SNB angle, ANB angle, artificial intelligence

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INTRODUCTION

Cleft lip and palate; the second most common developmental abnormalities which extant during birth by presenting anatomical alteration of the lip and/or palate (1). This craniofacial malformation is caused by the effects of both genetic and environmental factors such as maternal smoking and alcohol consumption, stress, viral infection during the first 8 weeks of pregnancy, teratogenic drug etc. (2).

The treatment of cleft lip and palate (CLP) is multidisciplinary where the only purpose is to restore the functional and aesthetic value. Many beneficial approaches have been carried out previously such as: dental arch relationship (3), arch dimension (4), tooth size morphometry (5) and craniofacial morphology (6) to evaluate the outcome of CLP patients.

Atypical sagittal growth of maxilla is a common manifestation in patients with UCLP; acknowledged by many researchers yet all of them came to an understanding that the growth and

TABLE 1 | The angular and linear cephalometric measurements with description.

Measurements	Description
SNA (The sella-nasion-A point)	An angle relates to the antero-posterior position of the maxillary apical base to a line passing through the anterior cranial base.
SNB (The sella-nasion-B point)	An angle relates to the antero-posterior position of the mandibular apical base to a line passing through the anterior cranial base.
ANB (A point- nasion-B Point)	An angle relates to the antero-posterior relationship of the mandible to the maxilla.
Wits appraisal	A linear cephalometric analytic obtained by projecting straight lines from the A and B points, respectively, unto the functional occlusal plane at 90° and measuring the horizontal distance form point AO to BO

TABLE 2 | Sagittal analysis—SNA: Gender, Types of Cleft and Gender times types of cleft two-way ANOVA analysis results.

Gender	Type	Mean	SD	Cleft type	Mean	Multiple comparison			MD	SE	*p*-value	95% CI	
												Lower bound	Upper bound
Male	NC	79.296	3.306	NC	79.795	NC	vs	BCLP	4.841*	1.108	0.000	2.646	7.036
	BCLP	74.654	4.346	BCLP	74.953		vs	UCLP	3.637*	0.961	0.000	1.733	5.541
	UCLP	76.267	4.751	UCLP	76.158		vs	UCL	0.352	1.335	0.792	−2.293	2.997
	UCL	77.709	3.887	UCL	79.443		vs	UCLA	1.752	1.598	0.275	−1.414	4.918
	UCLA	78.542	3.424	UCLA	78.042	BCLP	vs	UCLP	−1.204	1.046	0.252	−3.277	0.869
	Total	76.735	4.429				vs	UCL	−4.489*	1.398	0.002	−7.258	−1.720
Female	NC	80.294	3.641				vs	UCLA	−7.026*	1.704	0.001	−11.905	−2.146
	BCLP	75.253	2.303			UCLP	vs	UCL	−3.285*	1.284	0.012	−5.830	−0.740
	UCLP	76.049	4.170				vs	UCLA	−1.885	1.556	0.228	−4.968	1.198
	UCL	81.177	5.072			UCL	vs	UCLA	1.400	1.811	0.441	−2.188	4.988
	UCLA	77.543	1.139										
	Total	77.920	4.339										
Total	NC	79.843	3.473			*p*-value	PES						
	BCLP	74.819	3.859		Gender	0.387	0.007						
	UCLP	76.160	4.423		Cleft Type	0.000	0.192						
	UCL	79.309	4.638		Gender * Cleft Type	0.654	0.021						
	UCLA	78.209	2.811										
	Total	77.255	4.412										

*SD, standard deviation; MD, mean difference; SE, standard error; CI, confidence interval; PES, partial eta square. * = Significant difference.*

direction of the jaw utterly influenced by the earlier treatment protocol such as time and techniques of primary surgeries (7–12). The detrimental effect of palatoplasty on sagittal growth of maxilla has been widely documented in literature (9) but whether cheiloplasty impedes the growth is still in controversy (8, 13). A number of researchers found that cheiloplasty has an effect on maxillary incisors, alveolar bone and development of maxilla as well whether some researchers claimed no effect on maxillary development (7, 11, 12, 14, 15).

The craniofacial characteristics of CLP can be assessed from cephalogram (16) and cone-beam computed tomography (17) as well. Abundant cephalometric studies have been done on CLP yet restricted to three-dimensionally. Artificial intelligence (A.I.) into dentistry, especially in cleft research is still a new-fangled and robust technique (18–21).

Use of A.I driven Webceph software for the measurements of all variables in cephalogram were the particularities from previous study which is more accurate, precise, robust and reliable compared to manual measurements (18–21).

For the first time, the present study tried to disclose the sagittal development of Saudi Arabian CLP patients, by A. I driven lateral cephalometric analysis and compared it with the non-cleft (NC) individuals and also compared in relation to gender and types of cleft.

MATERIALS AND METHODS

This retrospective study was limited to 123 individuals including 29 bilateral cleft lip and palate (BCLP), 41 unilateral cleft lip and palate (UCLP), 13 unilateral cleft lip UCL, 9 unilateral cleft lip and alveolus (UCLA) and 31 NC individuals with the average age of 13.29 [3.52] (NC), 14.07 [4.73] (BCLP), 14.32 [4.46] (UCLP), 12.78 [4.09] (UCLA), and 13.31 [4.46] (UCL) years, respectively. All the data (medical records and

TABLE 3 | Sagittal analysis—SNB: Gender, Types of Cleft and Gender times types of cleft two-way ANOVA analysis results.

Gender	Type	Mean	SD	Cleft type	Mean	Multiple comparison			MD	SE	*p*-value	95% CI	
												Lower bound	Upper bound
Male	NC	75.739	3.548	NC	76.017	NC	vs	BCLP	0.521	1.139	0.648	−1.736	2.777
	BCLP	74.190	5.326	BCLP	75.496		vs	UCLP	−0.214	0.988	0.829	−2.171	1.744
	UCLP	77.032	3.368	UCLP	76.230		vs	UCL	−0.615	1.372	0.655	−3.334	2.104
	UCL	74.916	4.630	UCL	76.632		vs	UCLA	0.100	1.643	0.951	−3.155	3.355
	UCLA	76.490	5.171	UCLA	75.917	BCLP	vs	UCLP	−0.734	1.076	0.496	−2.866	1.397
	Total	75.643	4.395				vs	UCL	−1.136	1.437	0.431	−3.983	1.711
Female	NC	76.295	3.111				vs	UCLA	−0.421	1.697	0.805	−3.783	2.942
	BCLP	76.803	5.139			UCLP	vs	UCL	−0.402	1.321	0.762	−3.018	2.215
	UCLP	75.429	3.497				vs	UCLA	0.314	1.600	0.845	−2.856	3.484
	UCL	78.348	4.859			UCL	vs	UCLA	0.715	1.862	0.702	−2.974	4.405
	UCLA	75.343	3.585										
	Total	76.225	3.788										
Total	NC	76.044	3.271			*p*-value	PES						
	BCLP	74.910	5.318		Gender	0.399	0.006						
	UCLP	76.250	3.484		Cleft Type	0.943	0.007						
	UCL	76.500	4.871		Gender * Cleft Type	0.201	0.051						
	UCLA	76.108	4.500										
	Total	75.898	4.133										

*SD, standard deviation; MD, mean difference; SE, standard error; CI, confidence interval; PES, partial eta square. * = Significant difference.*

TABLE 4 | Sagittal analysis—ANB: Gender, Types of Cleft and Gender times types of cleft two-way ANOVA analysis results.

Gender	Type	Mean	SD	Cleft type	Mean	Multiple comparison			MD	SE	*p*-value	95% CI	
												Lower bound	Upper bound
Male	NC	3.556	1.800	NC	3.778	NC	vs	BCLP	4.321*	1.047	0.000	2.247	6.396
	BCLP	0.465	4.604	BCLP	−0.543		vs	UCLP	3.851*	0.908	0.000	2.051	5.650
	UCLP	−0.764	3.497	UCLP	−0.073		vs	UCL	0.967	1.262	0.445	−1.533	3.467
	UCL	2.794	5.857	UCL	2.811		vs	UCLA	1.650	1.511	0.277	−1.342	4.643
	UCLA	2.052	4.138	UCLA	2.128	BCLP	vs	UCLP	−0.470	0.989	0.635	−2.430	1.489
	Total	1.092	4.190				vs	UCL	−3.355*	1.321	0.012	−5.972	−0.737
Female	NC	3.999	2.683				vs	UCLA	−2.671	1.561	0.090	−5.762	0.421
	BCLP	−1.551	5.545			UCLP	vs	UCL	−2.884*	1.214	0.019	−5.290	−0.479
	UCLP	0.619	3.004				vs	UCLA	−2.200	1.471	0.137	−5.115	0.714
	UCL	2.828	4.256			UCL	vs	UCLA	0.684	1.712	0.690	−2.708	4.076
	UCLA	2.203	4.580										
	Total	1.695	3.982										
Total	NC	3.799	2.301			*p*-value	PES						
	BCLP	−0.091	4.865		Gender	0.999	.000						
	UCLP	−0.090	3.300		Cleft Type	0.000	0.188						
	UCL	2.810	4.970		Gender * Cleft Type	0.562	0.026						
	UCLA	2.102	3.994										
	Total	1.357	4.094										

*SD, standard deviation; MD, mean difference; SE, standard error; CI, confidence interval; PES, partial eta square. * = Significant difference.*

TABLE 5 | Sagittal analysis—Witts analysis: Gender, Types of Cleft and Gender times types of cleft two-way ANOVA analysis results.

Gender	Type	Mean	SD	Cleft type	Mean	Multiple comparison			MD	SE	*p*-value	95% CI	
												Lower bound	Upper bound
Male	NC	0.831	2.769	NC	0.474	NC	vs	BCLP	3.690*	1.184	0.002	1.344	6.036
	BCLP	−4.233	5.479	BCLP	−3.216		vs	UCLP	3.073*	1.027	0.003	1.038	5.108
	UCLP	−3.804	3.938	UCLP	−2.599		vs	UCL	1.110	1.427	0.438	−1.717	3.937
	UCL	0.326	5.514	UCL	−0.635		vs	UCLA	1.543	1.708	0.368	−1.842	4.927
	UCLA	0.107	5.424	UCLA	−1.068	BCLP	vs	UCLP	−0.617	1.119	0.582	−2.833	1.599
	Total	−2.235	4.979				vs	UCL	−2.581	1.494	0.087	−5.541	.379
Female	NC	0.117	3.678				vs	UCLA	−2.148	1.765	0.226	−5.644	1.348
	BCLP	−2.199	5.031			UCLP	vs	UCL	−1.964	1.373	0.155	−4.684	0.756
	UCLP	−1.395	3.878				vs	UCLA	−1.531	1.663	0.359	−4.826	1.765
	UCL	−1.597	3.169			UCL	vs	UCLA	0.433	1.936	0.823	−3.403	4.268
	UCLA	−2.243	3.425										
	Total	−1.107	3.873										
Total	NC	0.440	3.266			*p*-value	PES						
	BCLP	−3.672	5.350		Gender	0.909	0.000						
	UCLP	−2.629	4.048		Cleft type	0.013	0.106						
	UCL	−0.562	4.515		Gender * cleft type	0.274	0.044						
	UCLA	−0.677	4.764										
	Total	−1.740	4.544										

*SD, standard deviation; MD, mean difference; SE, standard error; CI, confidence interval; PES, partial eta square. * = Significant difference.*

X-rays) of this study were collected from Saudi Board of Dental Residents and approved by the Ethical Committee of Al Rass Dental Research Center, Qassim University (DRC/009FA/20). Non-syndromic cleft individuals with good-quality x-ray images were included whereas any history of craniofacial surgery, bone grafting and orthodontic treatment was excluded from the study. Same age group of healthy non cleft individuals were also included as the control group. Convenient sampling has been done without randomization.

Digital Lateral Cephalogram X-rays were used to measure four different cephalometric parameters (SNA, SNA, ANB and Wits appraisal) to investigate sagittal characteristics of 123 individuals of cleft and non-cleft group. All the cephalometric parameters were measured by one examiner using automated (20, 21) A.I.-driven Webceph software (South Korea). The angular and linear measurements used in this study are detailed in **Table 1**.

Statistical Analysis

Intra-class correlation coefficients were executed to assess the intra-examiner reliability with 20 randomly selected x-rays after 2-weeks interval and found 0.916–0.990 for all measurements which indicates excellent reliability. A two-way ANOVA examination was utilized for gender orientation, types of cleft and gender types of cleft. SPSS 24 (SPSS Inc., Chicago, IL, United States) was used to scrutinize all the data and *p*-value was set as <0.05.

RESULTS

Results of SNA Angle

The mean SNA angle of NC, UCLP, BCLP, UCL and UCLA were 79.795°, 76.158°, 74.953°, 79.443°, and 78.042°, respectively. UCLP ($p < 0.001$) and BCLP ($p < 0.001$) subjects had significantly smaller SNA angles compared to NC subjects. Significant difference also observed in two groups. They are BCLP vs. UCL (*p*-value 0.002) and BCLP vs. UCLA (*p*-value 0.001). However, no significant differences were found in terms of gender.

Table 2 shows the detailed results of SNA angle in relation to gender and types of cleft and NC.

Results of SNB Angle

The mean SNB angle of NC, UCLP, BCLP, UCL, and UCLA were 76.017°, 76.230°, 75.496°, 76.632°, and 75.917°, respectively. No significant differences found in terms of gender and types of cleft (**Table 3**).

Results of ANB Angle

The mean ANB angle of NC, UCLP, BCLP, UCL and UCLA were 3.773°, −0.073°, −0.543°, 2.811°, and 2.128°, respectively. UCLP (*p*-value <0.001) and BCLP (*p*-value <0.001) subjects had significantly smaller ANB angles compared to NC subjects. However, no significant differences were found in terms of gender (**Table 4**).

Results of Wits Appraisal

The mean dimensions of NC, UCLP, BCLP, UCL and UCLA were 0.474, −2.599, −3.216, −0.635, and −1.068 mm, respectively.

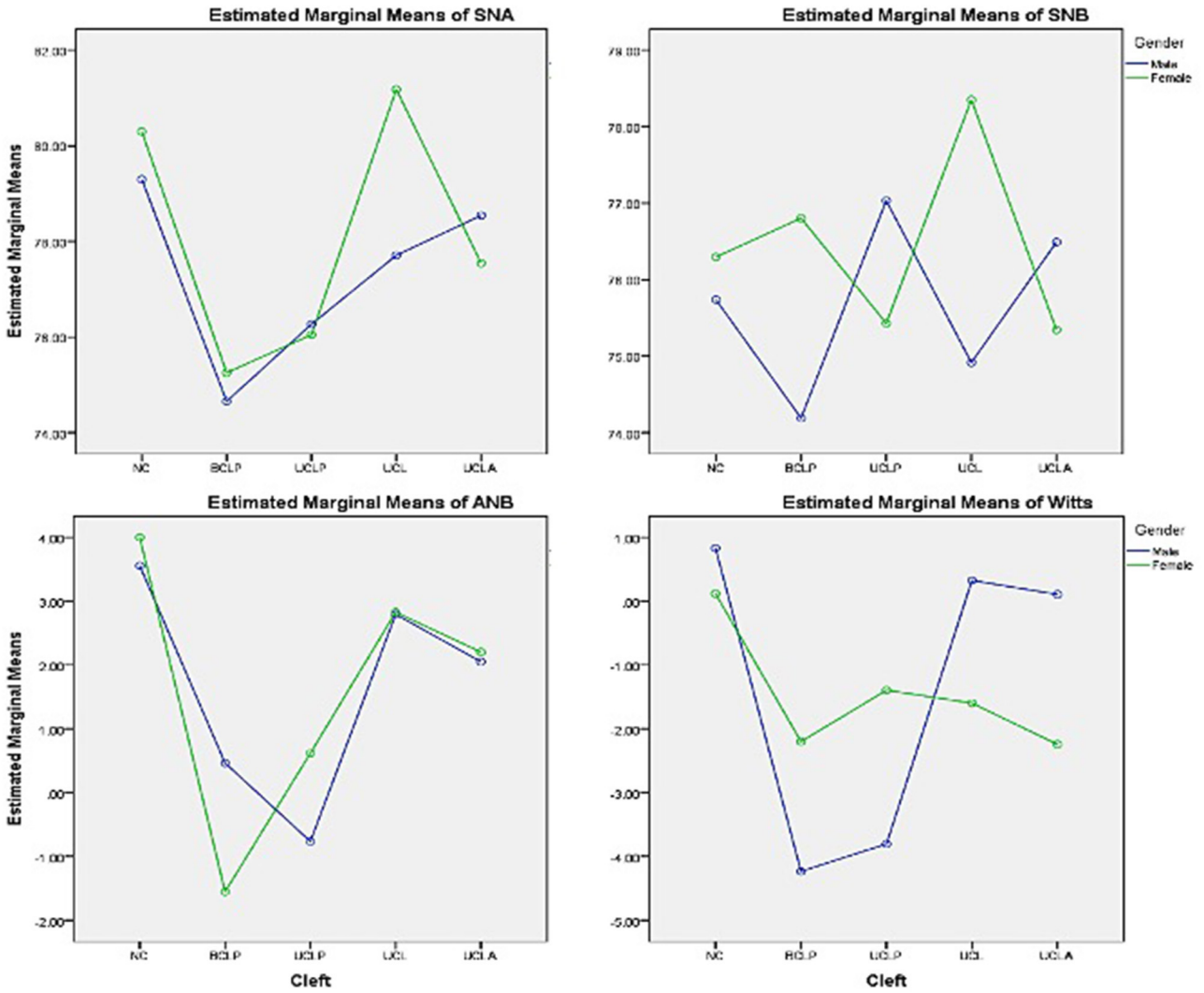

FIGURE 1 | Profile plot of the estimated marginal means of types of cleft and gender*types of cleft.

UCLP (p-value 0.003) and BCLP (p-value 0.002) subjects had significantly smaller Wits appraisal compared to NC subjects. However, no significant differences were found in terms of gender.

Table 5 shows the detailed results of Wits appraisal in relation to gender and types of cleft and NC. The profile plot of estimated marginal means of types of cleft and gender*types of cleft has been shown in **Figure 1**.

DISCUSSION

The prime objective of this paper was to find out and compare the sagittal development among different types of Saudi CLP patients with NC group and also compare it in relation to gender and types of clefts. Even though several researchers were researched on different issues of CLP patients, very few researchers were described on sagittal development of CLP patients. However, no study was documented previously in Saudi population about sagittal development. For the first time, the present work studied the sagittal development among Saudi population exclusively.

In this present study, we measured SNA, SNB, ANB and Wits appraisal of non-syndromic Saudi CLP patients where all the patients completed their primary surgeries (lip surgery and palate surgery) by the first 2 years of life. The maxillofacial growth of a CLP patient is quite convoluted; affected by both congenital and postnatal treatment factors (3). Primary surgeries limited the maxillary growth which leads to maxillary hypoplasia and finally toward Class III malocclusion reported previously (22).

Poor maxillary growth of CLP patients is one of the chief apprehensions of orthodontists for the corrections of the dento-facial discrepancies especially during the period of early adolescence (7). The outcome of the current study may help and support the surgeon to bring out the proper primary surgical techniques and also to the orthodontist to make better treatment

plans for CLP patients. The patient who exhibited smaller SNA, ANB and Wits appraisal specifies the poor maxillary growth may perhaps have need of orthognathic surgery in future for the complete correction of dento-facial divergences (7, 23). Wits appraisal is a supreme linear cephalometric parameter which is used to evaluate and assist an additional information with ANB angle for the assessment of skeletal base discrepancies (24).

In our study we found significant reduction of SNA, ANB and Wits appraisal in cleft individuals compared NC individuals which indicated the maxillofacial growth deficiency. However, SNB was an insignificant variable which is a respectable evidence that cleft does not affect on mandibular growth and coincides with one of the earlier studies (25).

The results of current study also match with the outcome of (26) who assessed 45 lateral cephalograms including both cleft and NC individuals reported significantly decreased SNA and ANB between cleft and NC groups and also did not found any significant difference in SNB angle.

An observational study by Holts et al. (27) using German UCLP, BCLP and NC group showed reduced SNA and ANB angle with minor Class III malocclusion in both UCLP and BCLP groups.

Haque et al. (7) put the idea that Modified Millard techniques of Cheiloplasty and Bardach technique of palatoplasty had noticeable negative effect on anterior segment of maxillae especially on inter canine width by studying maxillary arch dimension of Malaysian UCLP subjects. After analyzing the cephalograms of operated Japanese UCLP subjects, Alam et al. (6, 10) also found altered craniofacial morphology in relation to both postnatal treatment factors and congenital factors.

Lisson et al. (28) compared two centers of German children with BCLP ranging from 10 to 18 years in age: center 1 showed significant reduction of ANB and Wits appraisal and center 2 showed significant reduction of ANB and Wits appraisal and significant increase of SNB angle. The outcome of their study revealed underdeveloped maxilla in both centers even though the time and sequence of palatopasty was the main difference between the centers.

The present study also paid attention to gender disparities with sagittal jaw relationships, however did not get any significant difference. In an earlier Saudi cleft study by Alam and Alfawzan (20) on dental characteristics, did not find any significant relation with gender. Similar findings also reported in Bangladeshi UCLP children (11). In contrast, interestingly Arshad et al. (12) reported significant gender disparities in relation to treatment outcome among Pakistani UCLP children. This different result among different populations indicates the racial bias of UCLP subjects.

Although the present study has reached its aim, however, there were some unavoidable limitations. Because of insufficient data from other centers, this study was conducted from a single center. In a future study we plan to do a multi-center study after collection of sufficient data, especially on UCL and UCLA sample from other centers.

CONCLUSION

In this present study, we observed,

- significant reduction of SNA angle in UCLP, BCLP, UCL and UCLA; ANB angle and Wits appraisal in UCLP and BCLP compared to NC group.
- no significant difference in SNB angle in any cleft and NC group.
- no significant gender inequalities in relation to any type of CLP and NC group.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of Al Rass Dental Research Center, Qassim University (DRC/009FA/20). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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The Assessment of 3D Digital Models Using GOSLON Yardstick Index: Exploring Confounding Factors Responsible for Unfavourable Treatment Outcome in Multi-Population Children With UCLP

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To evaluate dental arch relationship (DAR) using GOSLON Yardstick and also to explore the association between multiple factors (age, gender, UCLP types, UCLP side, Family history of cleft, family history of Class III malocclusion, techniques of cheiloplasty, techniques of palatoplasty) and DAR in children unilateral cleft lip and palate (UCLP) in different populations. Two hundred fifty-five laser scanned 3D digital models (LS3DM) of UCLP children (5–12 years) from Malaysia, Bangladesh, and Pakistan were included. The intra- and inter-examiner agreements were evaluated by kappa statistics, to compare the GOSLON mean score between the populations and to explore the responsible factors that affect DAR, one way ANOVA, and crude logistic regression analysis was used, respectively. The mean GOSLON score was 2.97; 3.40 and 3.09 in Malaysia, Bangladesh, and Pakistan, respectively. Twenty seven, 40, and 30 subjects were in unfavourable (category rating 4 and 5) groups in Malaysia, Bangladesh, and Pakistan, respectively. A significant association was found between techniques of palatoplasty ($p = 0.03$; $p = 0.04$ and $p = 0.04$ in Malaysia, Bangladesh, and Pakistan, respectively) and unfavourable DAR. Different cheiloplasty techniques ($p = 0.04$) and gender ($p = 0.03$) also exhibited noteworthy associations with unfavourable DAR in the Bangladeshi population. Bardach techniques of palatoplasty were significantly associated with unfavourable DAR in all three populations. Moreover, male UCLP and modified Millard techniques of cheiloplasty were also associated with unfavourable DAR in the Bangladeshi population.

Keywords: UCLP, multi populations, treatment outcome, 3D digital models, GOSLON Yardstick

INTRODUCTION

The prevalence of unilateral cleft lip and palate (UCLP) varies between countries with higher rates have been reported in Asians and American Indians which is 1 per 500 births whereas African derivative populations have the lower rates (1 per 2,500 births) (1). Being Asian country, Malaysia, Bangladesh and Pakistan also some extent of high rate of prevalence of cleft lip and palate. In Malaysia, it is found 1 per 941 live births (2) and 1 per 523 live births are reported in Pakistan (3) whereas 3.9:1000 live births are reported in Bangladesh (4) which is relatively higher than other regions in Asia.

Despite variations between these countries, the impact of cleft lip and palate on both aesthetic and functional malformations are equally observed since birth (1, 5). The treatment of UCLP patients is multifaceted, prolonged, and complicated. A series of surgeries is aimed at the treatment of this congenital anomaly for the correction of esthetic and functional development. The manifestation of undeveloped maxillary growth and occurrence of Class III malocclusion is frequent in UCLP patients (6, 7). The growth and development of the maxilla are affected by different techniques of primary lip and palate surgeries. Not only surgeries but also genotype factors have influences on the growth of maxilla reported previously (8, 9).

In this contemporary era of clinical practise, the evaluation of treatment outcomes with reliable and sound evidence is crucial after the early management of UCLP patients. This evaluation provides the suggestion of appropriate orthodontic treatment strategy and surgical methods for the primary repair of CLP thus the entire treatment process could be more successful. The treatment outcome can be evaluated by assessing the dental arch relationship (DAR), craniofacial morphology, maxillary arch dimension, etc. DAR is one of the most ideal measuring tools that can give a complete idea about facial growth and occlusion as well.

Many indices have been established to assess the DAR. Among them, the GOSLON (Great Ormond Street, London, and Oslo) Yardstick (GY) (10), is the most extensively used and relevant index for this determination of evaluating the DAR (8, 9, 11–17). However, only few studies have evaluated both the DAR and effects of multiple factors on the DAR together (8, 9, 12, 13, 17) and the majority of these studies was using plaster dental casts.

Based on the literature search there were few studies done by using 3D digital models (14–16). Thus far, to the best of the author's knowledge, no reported data to date was found on Malaysian, Bangladeshi and Pakistani populations that evaluate the multiple factors (age, gender, family history of cleft, UCLP type and side, techniques of cheiloplasty and palatoplasty) which may influence the treatment outcome using a 3D digital model. Therefore, the present study attempted to evaluate DAR using GY and also to explore the association between multiple factors and DAR of Malaysian, Bangladeshi, and Pakistani UCLP subjects.

MATERIALS AND METHODS

A total of 255 pretreatments orthodontic plaster dental casts of UCLP (involved only unilateral lip and full palate cleft) children from three different populations were selected into this study which consisted of 85 subjects in each population. The dental casts were collected during subjects' first visit to the orthodontist in a renowned hospital separately in Malaysia, Bangladesh, and Pakistan between 2010 and 2013. The subjects' age ranged between 5 and 12 years and who had completed their cheiloplasty and palatoplasty and without bone grafting were included in this study whereas any kind of missing records, syndromic UCLP, bilateral cleft, isolated cleft lip, and cleft palate were not included for further assessment. Those who fulfilled the inclusion and exclusion criteria were selected by a simple random sampling method.

The sample size was calculated on the bases of a ratio of 1 predictor: 20 cases. A total of eight predictors were presented in this prime study. Hence, 160 was the minimum projected sample size for all populations together. Ensuing the inclusion and exclusion criteria, as a final point, 255 samples have been selected for this study. The distribution of all subjects from three populations with multiple factors was shown in **Figure 1**.

The demographic information of the subjects, selection criteria, dependent and independent variables, and statistical analyses detail was given in a flowchart (**Figure 2**).

All the 255 dental casts (both upper and lower jaws) were scanned and converted into LS3DM using the Next Engine laser scanner (Santa Monica) by an experienced craniofacial lab technician following the standard method of scanning of the Next engine laser scanner (**Figure 3**). All scanned data coordinates (in x, y, z) were transferred into STL format (**Figure 4**) and finally, DAR was evaluated using GY index.

Five categories are assessed in GY; 1: excellent; 2: good; 3: fair; 4: poor; 5: very poor which provided the dentofacial growth and development, and also discovered the differences of DAR. Group 1 reflects the excellent treatment outcome which presents a favourable relationship, advantageous skeletal form, a positive overjet, and overbite with the presentation of Angle Class II division 1 malocclusion. Straightforward orthodontic treatment or no treatment is required in this group. Group 2 reflects the good treatment outcome which also shows a favourable relationship with the Class I dental relationship. Straightforward orthodontic treatment is required in this group. Group 3 represents the fair treatment outcome with an edge-to-edge dental relationship. More complex orthodontic treatment is needed in this group. Group 4 reflects the poor treatment outcome presenting an unfavourable facial growth. Reverse overjet of 3–5 mm also shows in this group which point to the confines of orthodontic treatment, where the orthognathic surgery might be required in some cases. Group 5 represents the very poor treatment outcome with an extensive skeletal class III relationship with the need for compulsory surgical treatment.

For further analyses, the groups were collapsed into two groups i.e., favourable and unfavourable groups. Groups 1–3 were in favourable groups and groups 4 and 5 were in the unfavourable group. Subjects in the favourable group may need conventional orthodontics treatment whereas surgical treatment is required with subjects in the unfavourable groups.

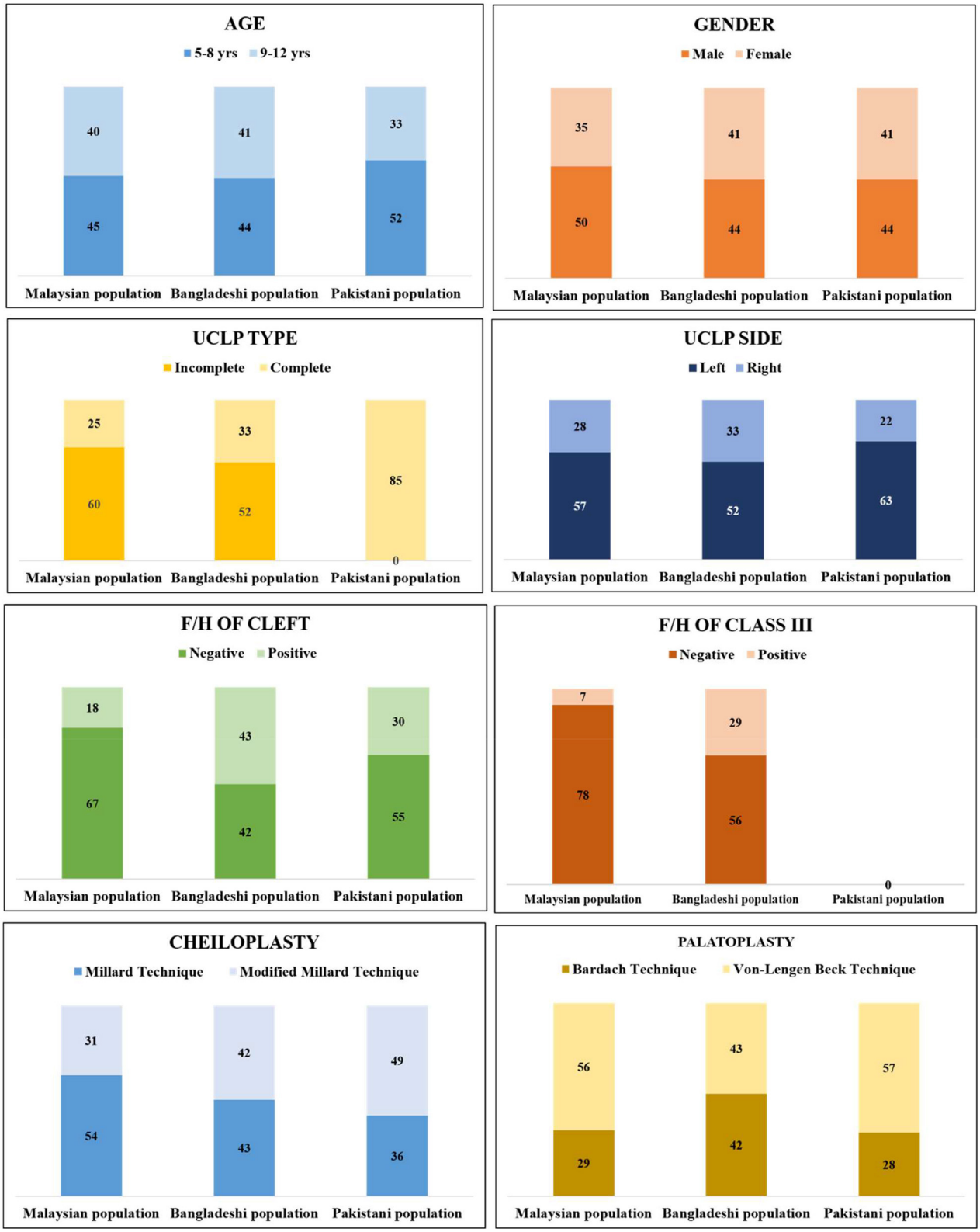

FIGURE 1 | Distribution of all subjects from three populations with multiple factors. (All the Pakistani UCLP subjects were complete type of UCLP. No record was found regarding family history of class III malocclusion in Pakistani UCLP subjects).

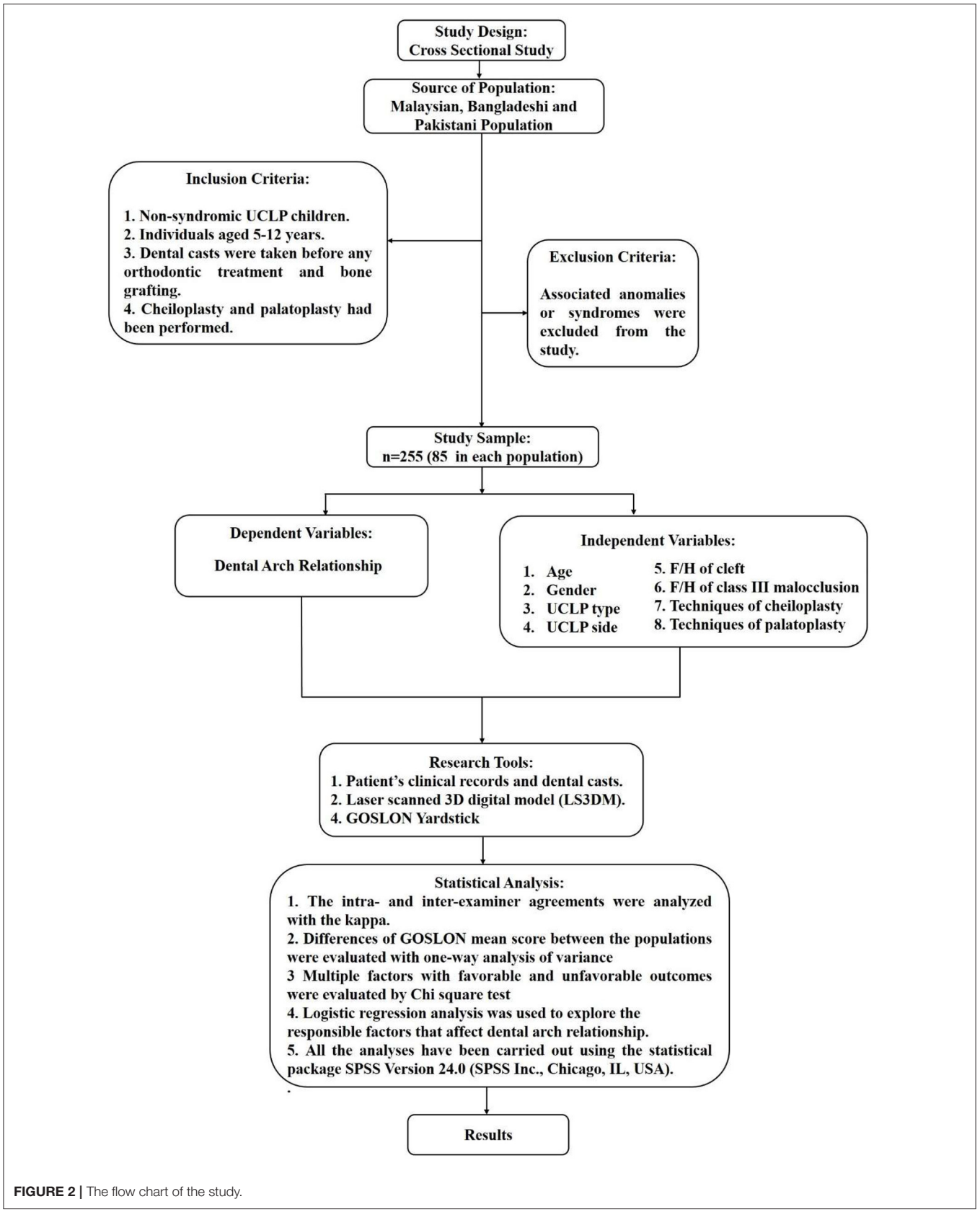

FIGURE 2 | The flow chart of the study.

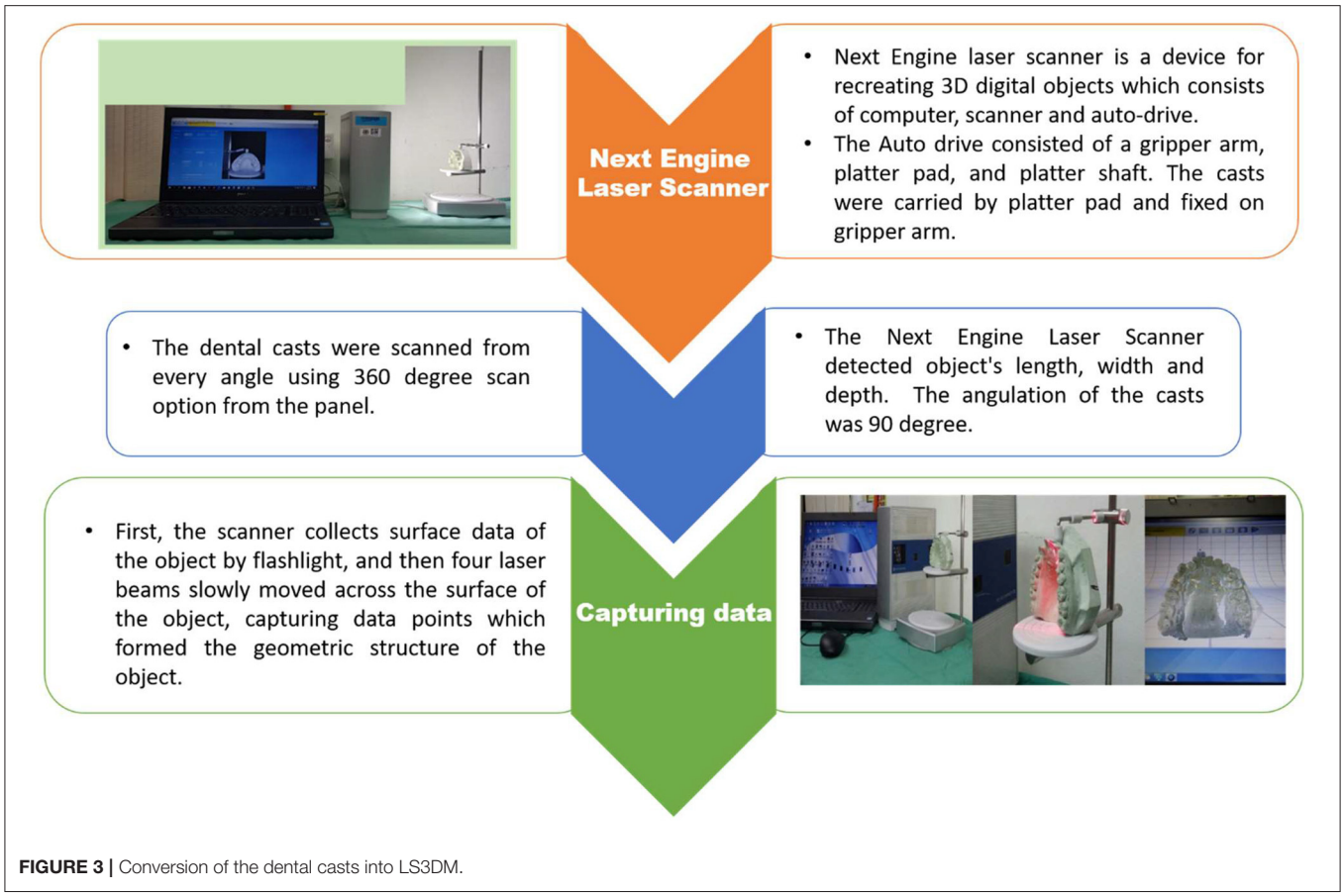

FIGURE 3 | Conversion of the dental casts into LS3DM.

Figure 5 shows five groups of GY model picture in 3D format.

Each population data collected with proper clearance and permission. Ethical consent has been obtained for specific population as approved by the USM Ethics Committee [USM/JEPeM/17100564].

RESULTS

Reliability of GY

The kappa score was 0.70 and 0.63 for intra- and inter-examiner agreements, respectively for GY which recommended good agreements.

Assessment of Treatment Outcome Based on GOSLON Score

Table 1 shows the score distribution and the mean GOSLON score of UCLP subjects of three populations using GY.

Comparison of the Mean GOSLON score Between Three Populations

Table 2 shows mean GOSLON scores for the three populations were compared with ANOVA and followed by *post hoc* Tamhane tests for pairwise comparisons. The only statistically significant difference was between the Malaysian and Bangladeshi populations ($p = 0.01$). Tamhane pairwise tests found that Bangladesh has a higher GY score than Malaysian. Tamhane test was selected due to non-homogenous variance tests (Levene's Test).

Comparison of Factors Between Favourable and Unfavourable Groups

Malaysian Population

Fifty eight and 27 subjects remained in the favourable and unfavourable groups respectively.

Bangladeshi Population

Forty five and 40 subjects reported in favourable and unfavourable groups respectively.

Pakistani Population

Fifty five and 30 subjects remained in favourable and unfavourable groups respectively.

The distribution of the subjects of three populations along with multiple factors using GY are shown in **Table 3**.

Treatment Outcome Associated With Multiple Factors (Favourable vs. Unfavourable DAR)

The crude logistic regression analysis was performed where p-value was set as <0.05 to consider of having significant association with DAR.

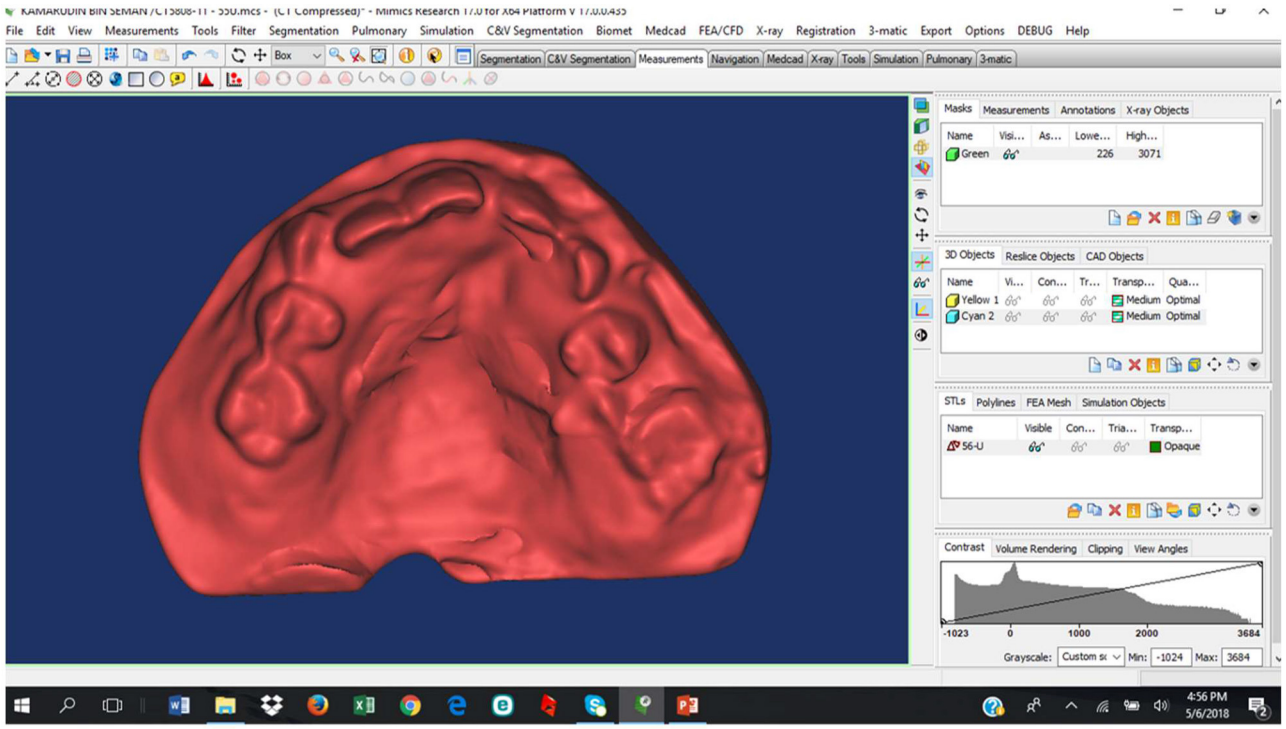

FIGURE 4 | LS3DM in the STL format.

Techniques of palatoplasty ($p = 0.03$) was significantly associated with DAR in Malaysian population where subjects with Bardach technique of palatoplasty had 3.42 times the odds of unfavourable DAR compared to von Langenbeck technique.

In Bangladeshi population, gender ($p = 0.03$), techniques of cheiloplasty ($p = 0.04$) techniques of palatoplasty ($p = 0.04$) were significantly associated with DAR. Male subjects showed 2.93 times the odds ratio to unfavourable DAR than female subjects. The subjects who underwent with the modified Millard technique of cheiloplasty and Bardach technique of palatoplasty had 2.99 and 2.80 times, respectively, the odds to unfavourable DAR compared to the subjects with Millard technique of cheiloplasty and von Langenbeck technique of palatoplasty.

In Pakistani population, techniques of palatoplasty ($p = 0.04$) was significantly associated with DAR where subjects with Bardach technique of palatoplasty had 2.86 times the odds to unfavourable DAR in comparison with von Langenbeck technique.

Table 4 shows a brief result of association of multiple factors on treatment outcome of three different populations.

DISCUSSION

In this present study, the authors evaluated and compared the DAR of UCLP subjects of 5–12 years in three different populations from Asia. Furthermore, the association of multiple factors with favourable and unfavourable DAR between the populations were also explored.

This range of age was chosen as most UCLP patients first received orthodontic treatment at the age of 5–6 years old (18). These UCLP subjects exhibited Class III malocclusions and other dental anomalies, and have yet to undergo alveolar bone grafting by the age of 12 (18). So the selection of this age range may represent the actual knowledge of treatment outcome to the orthodontist as well as to the surgeon. Same age range also documented in some other previous published studies (9, 17, 19).

In this study, Millard technique or modified Millard technique of cheiloplasty was the treatment of choice which was taken place at the age of 3–6 months and correspondingly two different surgical protocols of palatoplasty; Bardach technique or von Langenbeck technique was chosen for the subjects at 12–18 months of age. One skilled, qualified and experienced surgeon from each population performed all the surgeries of three populations.

We assessed 255 LS3DM of non-syndromic UCLP subjects from three populations using GY. The index was found to have good inter- and intra-examiner reliability in the present study which also corresponds with the findings of the earlier studies (20–22).

Treatment Outcome

The mean GOSLON score of Malaysian, Bangladeshi, and Pakistani UCLP subjects was 2.97, 3.40, and 3.01, respectively. In

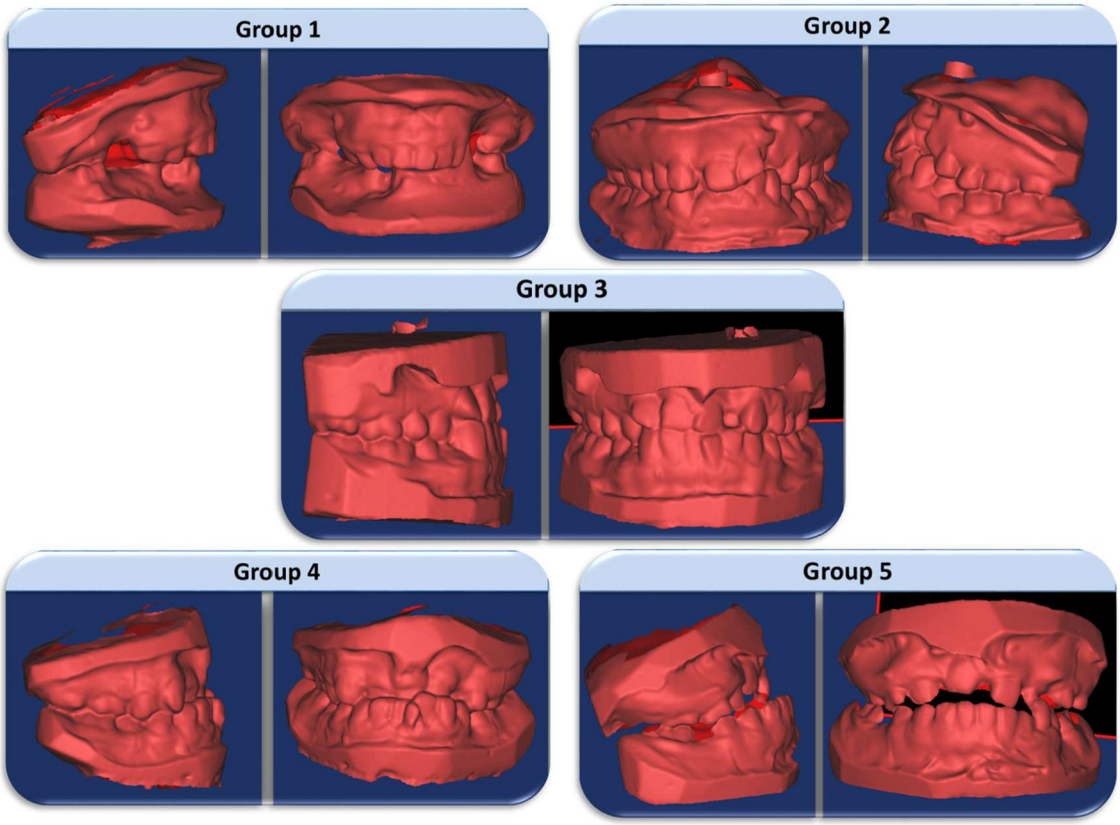

FIGURE 5 | Five groups of GY model picture in 3D format.

TABLE 1 | The score distribution and the mean GOSLON score of UCLP subjects of three populations using GY.

	Malaysia (*n*)	Bangladesh (*n*)	Pakistan (*n*)
Group 1	4	3	4
Group 2	17	11	25
Group 3	44	31	26
Group 4	17	29	19
Group 5	3	11	11
Mean GOSLON Score	2.97	3.40	3.09

TABLE 2 | Comparison of mean GOSLON scores between the three populations.

Population	Mean GOSLON score	SD	Inter-population differences (for $p < 0.05$)
Malaysia	2.97	0.86	Mal vs. Ban
Bangladesh	3.40	1.00	Ban vs. Mal
Pakistan	3.09	1.10	

Mal, Malaysia; Ban, Bangladesh; SD, standard deviation.

the present study, the treatment outcome of Malaysian subjects was good to fair (between groups 2 and 3), representing 71.76% of all cases. Of the leftovers, 4.70% was excellent and 20% was poor and 3.5% was a very poor outcome. Two studies were conducted in Malaysia previously (19, 20). Zreaqat et al. (23) evaluated the treatment outcome among 82 UCLP subjects between 2004 and 2010 and reported a mean GOSLON score of 3.15 with 62% of all cases. On the other hand, 107 UCLP subjects were evaluated by Asif et al. (24) between 2000 and 2012; interestingly this study also found the same mean GOSLON score of 3.15 represented with 68% of all cases. The same GOSLON score of previous studies indicated a similar treatment outcome which could be due to the use of the same surgical technique/protocol. Both of the studies used plaster dental casts in their research.

The mean GOSLON score of Bangladeshi UCLP was 3.40. The treatment outcome of Bangladeshi subjects was fair to poor outcome representing 70.59% of all subjects. Only one study reported a mean GOSLON score of 3.238 with 68% of all subjects (25) for DAR in the Bangladeshi UCLP subjects using plaster dental casts.

The mean GOSLON score of Pakistani UCLP was 3.01. The treatment outcome of most Pakistani subjects of our study was fair; representing 30.59% of all cases. The remaining

TABLE 3 | Distribution of subjects with multiple factors in favourable and unfavourable groups using GY in Malaysian, Bangladeshi and Pakistani UCLP children.

Variable	Tx outcome of Malaysian population, *n* (%)		Tx outcome of Bangladeshi population, *n* (%)		Tx outcome of Pakistani population, *n* (%)	
	Favourable	Unfavourable	Favourable	Unfavuorable	Favourable	Unfavuorable
Age						
5–8 years	29 (34.1)	16 (18.8)	21(24.7)	23 (27.1)	36 (42.2)	16 (18.8)
9–12 years	29 (34.1)	11 (12.9)	24 (28.2)	17 (20.0)	19 (22.4)	14 (16.5)
Gender						
Male	38 (44.7)	12 (14.1)	19 (22.4)	25 (29.4)	24 (28.2)	20 (23.5)
Female	20 (23.5)	15 (17.6)	26 (30.6)	15(17.6)	31 (36.5)	10 (11.8)
UCLP Side						
Left	40 (47.1)	17 (20.0)	30 (35.3)	22 (25.9)	43 (50.6)	20 (23.5)
Right	18 (21.2)	10 (11.8)	15 (17.6)	18 (21.2)	12 (23.5)	10 (11.8)
UCLP type						
Incomplete	46 (54.1)	14 (16.5)	29 (34.1)	23 (27.1)	N/A*	N/A*
Complete	12 (14.1)	13 (15.3)	16 (18.8)	17 (20.0)		
F/H of Cleft						
Negative	44 (51.8)	23 (27.1)	24 (28.2)	18 (21.2)	37 (43.5)	18 (21.2)
Positive	14 (16.5)	4 (4.7)	21 (24.7)	22 (25.9)	18 (21.2)	12 (14.1)
F/H of Class III						
Negative	54 (63.5)	24 (28.2)	32 (37.6)	24 (28.2)	N/A**	N/A**
Positive	4 (4.7)	3 (3.5)	13 (15.3)	16 (18.8)		
Cheiloplasty						
MT	41 (48.2)	13 (15.3)	17 (20.0)	26 (30.6)	23 (27.1)	13 (15.3)
MMT	17 (20.0)	14 (16.5)	28 (32.9)	14 (16.5)	32 (37.6)	17 (20.0)
Palatoplasty						
BT	14 (16.5)	15 (17.6)	18 (21.2)	25 (29.4)	13 (15.3)	15 (17.6)
VLT	44 (51.8)	12 (14.1)	27 (31.5)	15 (17.6)	42 (49.4)	15 (17.6)

UCLP, unilateral cleft lip and palate; F/H Cleft, Family history of cleft; F/H C-III, Family history of class III malocclusion; MT, Millard technique; MMT, modified Millard technique; BT, Bardach Technique; VLT, Von-Lengenbeck Technique.

**all the Pakistani UCLP subjects were complete type of UCLP.*

***no record was found regarding family history of class III malocclusion in Pakistani UCLP subjects.*

subjects were as follows 4.70% was excellent, 29.41% was good, 22.35% was poor and 12.94% was a very poor outcome. The current findings are consistent with the results of Arshad et al. (12); which is the only study found in the literature on the Pakistani population.

All these previous studies in Malaysia, Bangladesh, and Pakistan were done using plaster dental casts separately. Taking the advantage of the 3D digital model (26, 27) our study evaluated DAR in multi-population UCLP subjects where the results were comparable with those evaluation performed on plaster dental casts.

There have been many studies done about UCLP with GY in other populations. Different populations showed different results. For example, a recent multicenter study, found the mean GOSLON score ranged from 2.58 to 3.07 among three centres. They also reported one stage palatoplasty showed a low GOSLON score (better outcome) than two-stage palatoplasty (28). Another multicenter study reported a range of 3.16–3.70 mean GOSLON scores among different cleft centres between 1985 and 2000 on Turkish populations (16). Their findings are comparable to our Bangladeshi and Pakistani outcome, keeping in mind that the surgeons involved in the treatment of UCLP patients still practise the same protocols of surgery in such populations. Eighty percent of the UCLP patients were fair to poor outcomes (GOSLON 3 and 4) in a Japanese population (9) while a study of 66 UCLP cases in Western Australia demonstrated a total GOSLON score of 3.17 (29).

These differences might be for the disparities in different techniques of cheiloplasty and palatoplasty and/or the experience of the surgeons. The current findings of the study demonstrated that the treatment outcome in the three populations was comparable. The Malaysian subjects presented comparatively favourable outcomes than the other two populations and Bangladeshi subjects tended more toward unfavourable outcomes. It should be noted that presenting different outcomes in different populations and races of treatment outcomes based on the DAR seemed to be attributable to surgical procedures, but the racial difference in the craniofacial morphology also deserves consideration.

TABLE 4 | Logistic regression analysis of multiple factors with treatment outcome (Favourable vs. unfavourable group) using GY in three population.

Independent variable	Exp (B)	95% CI		*p*-value
		Lower	Upper	
Malaysian population				
Age	1.34	0.43	4.19	0.61
Gender	0.59	0.20	1.71	0.33
UCLP Side	0.53	0.16	1.73	0.30
UCLP Type	0.39	0.12	1.25	0.11
F/H of Cleft	2.03	0.44	9.41	0.37
F/H of C-III	0.42	0.07	2.38	0.32
Cheiloplasty	0.04	0.13	1.23	0.11
Palatoplasty	3.42	1.09	10.78	**0.03***
Bangladeshi population				
Age	1.29	0.47	3.52	0.62
Gender	2.93	1.09	7.85	**0.03***
UCLP Side	0.44	0.015	1.32	0.15
UCLP Type	1.15	0.37	3.60	0.81
F/H of Cleft	1.05	0.37	2.96	0.93
F/H of C-III	0.60	0.21	1.76	0.36
Cheiloplasty	2.99	1.07	8.38	**0.04***
Palatoplasty	2.80	0.47	7.80	**0.04***
Pakistani population				
Age	0.55	0.21	1.47	0.23
Gender	2.31	0.86	6.17	0.09
UCLP Side	0.75	0.25	2.24	0.60
F/H of Cleft	0.72	0.26	1.99	0.52
Cheiloplasty	0.90	0.33	2.44	0.84
Palatoplasty	2.86	1.05	7.76	**0.04***

**F/H, family history. *p > 0.05.*

Effects of Multiple Factors on DAR

The present study explored the factors that may be associated with the unfavourable DAR in three populations. Gender (male/female), side of cleft (left/right), type of cleft (complete/incomplete), the presence of a family history of cleft and class III malocclusion in the family, palatoplasty, and cheiloplasty were the independent variables. Crude logistic regression analysis was used to assess the association between each factor and DAR.

In this study, we found more males (59, 52, and 52% in Malaysia, Bangladeshi and Pakistani, respectively) than females. The results correspond to the outcomes with the previous studies (1, 12). Moreover, male UCLP subjects were significantly associated with unfavourable DAR in the Bangladeshi population though Malaysian and Pakistani UCLP subjects did not show any significant associations. Contrariwise, Arshad et al. (12) reported female gender had more unfavourable DAR in Pakistani UCLP subjects previously. Yet the actual cause of this phenomenon is still unconvinced (30).

All the subjects in the present study were between 5 and 12 years old. Fifty two percent of subjects were in the early mixed dentition period in the Malaysian population and 52 and 61% were in Bangladeshi and Pakistani populations, respectively. Left-sided clefts were observed more in all the populations. The prevalence of the left-sided cleft were 57, 52, and 63% in Malaysia, Bangladesh, and Pakistan, respectively. The higher prevalence of left-sided cleft cases than the right side was also reported in the literature (13). The majority of Malaysian (70%) and Bangladeshi (61%) subjects consist of the incomplete type of UCLP while all the subjects from Pakistan were complete UCLP. Anatomically, when a failure of fusion occurred between the hard and soft tissue structures of the soft palate, hard palate, alveolus, and lip refers to the complete type of UCLP. Habitually complete type of UCLP treatment is quite complex rather than incomplete UCLP. However, this variable was not a statistically significant factor. Alam et al. (31) reported no significant association between age, side, and type of UCLP in a recent Japanese UCLP study which is constant with our findings.

The choice of different techniques of cheiloplasty depends on the surgeon's preferences and different cases as well. Sometimes cheiloplasty is carried out alone or sometimes goes together with primary palatoplasty. However, noticeable developmental retardation was reported when the performances are completed together (32). In this study, the Millard technique was found to be common for lip surgery in Malaysian subjects while the modified Millard technique was more common in Bangladeshi and Pakistani UCLP subjects.

Millard technique of cheiloplasty significantly showed a favourable outcome of DAR than the modified Millard technique in Bangladeshi subjects. Adetayo et al. (2) compared the Millard and Tennison–Randall's techniques of cheiloplasty among Nigerian UCLP subjects and found no significant differences. Both techniques showed a favourable outcome of DAR. Two types of modified Millard techniques were performed in Japanese UCLP subjects where Modified Millard with vomer flap was significantly associated with unfavourable DAR than modified Millard only (17). Due to the rotation advancement methods of the modified Millard technique, the development of tension is attributed which tends toward unfavourable growth patterns (33). Kuijpers-Jagtman and Long, (34) stated that the greater lip tension is anticipated to cause dentoalveolar contraction more willingly than skeletal changes. Nevertheless, the skeletal changes comprising an anterior portion of the maxilla in anteroposterior and transverse dimension has also been reported (35–38). However, lip length was not considered in the present study, which could justify the use of a modified Millard technique. Till to date, it is still doubtful which surgical technique provides the best outcome either for lip repair or for palate repair. The aim of the surgery, differences in the severity of the cases, the surgeon's experience, expertise, and preferences may affect the outcome of the surgery as well.

The primary aim of palatoplasty is to restore function and phonetics. Common traditional surgery techniques like von Langenbeck, Bardach technique, V-Y pushback were used to achieve these goals. In this study, all the subjects were treated with either the Bardach technique or von Langenbeck technique palatoplasty. Bardach techniques of palatoplasty was

identified as a factor that resulted in unfavourable DAR among all populations.

The unfavourable effect of palatoplasty on speech, maxillary growth, upper dental arch, and dental occlusion has been extensively documented. Consistent comparative results of different methods are seldom documented. Abundant confounding factors i.e., the defect size, extension of the defect, time of surgery and prominently the growth response makes valuation difficult (3).

An earlier study reported that minor scar formation was the main cause of the better outcomes of the von Langenbeck technique (39). Yet some researchers concluded their studies with no significant difference in the outcomes of different surgical techniques (40, 41). Sato et al. (42) found favourable outcomes among Brazilian UCLP subjects who had used the von Langenbeck technique of palatoplasty. Its favourable effects on outcomes have also been discussed in the previous study (43).

In contrast, the Bardach technique forms scar which would be responsible for the growth restriction. Fistula formation has also been associated as a drawback of this technique when performed to repair larger defects (44). However, Rossell-Perry et al. (45), reported no significant differences between twoflap (Bardach technique) and oneflap palatoplasty on DAR. Moreover, the patient treated with the Bardach technique achieved more normal speech (46). Altered surgical methods are employed to determine the outcome on DAR, and it can be stated from the studies that the treatment outcome of UCLP subjects is influenced by the surgical technique used.

Because of the disagreement between the outcomes of the studies, it is recommended that the correlation between treatment outcome (DAR) and the effect of multiple factors be better explored.

The present study provided information that postnatal treatment factors are associated with favourable and unfavourable DAR in all three populations. These findings could warrant a modification of management protocols to ensure improvement in future cleft outcomes.

The present study has achieved its aim by getting precise informative findings however it has some unavoidable limitations. Obtaining data from a single centre was one of the limitations of this study therefore the findings may not be generalizable. A multi-centre and prospective study design may provide more insights of the attributes of the variability of subjects.

CONCLUSION

The present study revealed that,

Malaysian Population

1. The mean GOSLON score was 2.97
2. von Langenbeck technique of palatoplasty significantly associated with favourable DAR among Malaysian UCLP children.

Bangladeshi Population

1. The mean GOSLON score was 3.40
2. Female subjects, Millard technique of cheiloplasty and von Langenbeck technique of palatoplasty significantly associated with favourable DAR among Bangladeshi UCLP children.

Pakistani Population

1. The mean GOSLON score was 3.09
2. von Langenbeck technique of palatoplasty significantly associated with favourable DAR among Pakistani UCLP children.

DATA AVAILABILITY STATEMENT

The original contributions generated for this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the study was approved by the Ethics Committee of the Hospital Universiti Sains Malaysia (HUSM) [USM/JEPeM/17100564]. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SH, MK, and MA designed the study, performed the data collection, data analysis and interpretation, wrote the manuscript and reviewed the manuscript. SH, MK, MA, and WW performed the data collection, data analysis and interpretation, and wrote the manuscript. All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Exploring the Association Between Genetic Polymorphisms in Genes Involved in Craniofacial Development and Isolated Tooth Agenesis

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Tooth agenesis is a common congenital anomaly in humans and is more common in oral cleft patients than in the general population. Many previous studies suggested that oral cleft and tooth agenesis share a similar genetic background. Therefore, this study explored the association between isolated tooth agenesis and genetic polymorphisms in genes that are crucial for craniofacial and tooth development. Panoramic radiographs, anamnesis, and genomic DNA from 273 patients were included. Patients were classified as tooth agenesis present, when at least one permanent tooth was congenitally missing. Patients with syndromes and oral cleft were excluded. Only unrelated patients were included. The genetic polymorphisms in *BMP2* (rs235768 and rs1005464), *BMP4* (rs17563), *RUNX2* (rs59983488 and rs1200425), and *SMAD6* (rs3934908 and rs2119261) were genotyped by real-time polymerase chain reaction. Genotype and allele distributions were compared between the tooth agenesis phenotypes and controls by Chi-square test. Haplotype and diplotype analysis were also performed, in addition to multivariate analysis (alpha of 0.05). A total of 86 tooth agenesis cases and 187 controls were evaluated. For the rs235768 in *BMP2*, patients carrying TT genotype have higher chance to present tooth agenesis [$p < 0.001$; prevalence ratio (PR) = 8.29; 95% confidence interval (CI) = 4.26–16.10]. The TT genotype in rs3934908 (*SMAD6*) was associated with higher chance to present third molar agenesis ($p = 0.023$; PR = 3.25; 95% CI = 1.17–8.99). *BMP2* was also associated in haplotype and diplotype analysis with tooth agenesis. In conclusion, genetic polymorphisms in *BMP2* and *SMAD6* were associated with isolated tooth agenesis.

Keywords: genetic polymorphisms, craniofacial development, dental anomaly, tooth agenesis, single nucelotide polymorphisms

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INTRODUCTION

Isolated tooth agenesis (or congenitally missing teeth) is one of the most common congenital defects in humans, which affects approximately 20% of the average worldwide population (Vastardis, 2000). Tooth agenesis can be classified into two main types: non-syndromic and syndromic. Non-syndromic tooth agenesis involves a congenitally missing permanent or primary tooth or teeth in an isolated form without any other major birth defects, such as oral cleft and syndromes. Isolated tooth agenesis occurs in both aches (maxilla and mandible) and can affect any type of teeth, although the most commonly affected teeth are third molars, maxillary lateral incisors, and premolar (Küchler et al., 2008a,b). Syndromic tooth agenesis refers to congenitally missing teeth associated with syndromes and oral clefts (such as cleft lip, clef palate, and cleft lip with palate) (Lu et al., 2016). Tooth agenesis is often observed in individuals with oral clefts (in the cleft area and also in non-cleft areas) and their non-affected family members (Küchler et al., 2011). Several observational epidemiological and genetic studies suggest that oral clefts and isolated tooth agenesis share a similar genetic background (Phan et al., 2016).

The bone morphogenetic protein (BMP) family, comprising an extensive group of phylogenetically conserved growth factors, such as BMP2 and BMP4, which plays an important role during tooth development (Zhang et al., 2005; Saadi et al., 2013; Taşlı et al., 2014; Yuan et al., 2015). Interestingly, genetic polymorphisms in *BMP2* and *BMP4* have been associated with both, isolated tooth agenesis (Antunes et al., 2012; Gong et al., 2015) and oral clefts (Antunes et al., 2013; Saket et al., 2016; Bahrami et al., 2020). Mothers against decapentaplegic homolog 6 (SMAD6) belongs to the SMAD family, which are important signaling pathway proteins during craniofacial development (Estrada et al., 2011; Suzuki et al., 2020). SMAD6 is known to inhibits BMP signaling in the nucleus by interacting with transcription repressors (Wu et al., 2016). Another molecule with a crucial role in craniofacial development is Runt-related transcription factor (RUNX2). This gene has been identified as essential for tooth formation (Camilleri and McDonald, 2006). Therefore, this study explored the association between isolated permanent tooth agenesis and genetic polymorphisms in genes that are crucial for tooth and craniofacial development.

MATERIALS AND METHODS

Sample

The study protocol was reviewed and approved by the local Ethics Committee (no. 01451418.3.0000.5419). Informed consent was obtained from all participating individuals or parents/legal guardians during the dental appointment and the assent was also obtained from children. The guideline Strengthening the Reporting of Genetic Association (STREGA) was followed for this study (Little et al., 2009).

Pre-dental treatment records including anamnesis and panoramic radiographs from patients undergoing dental treatment in universities and private clinics in Curitiba, Paraná state and Ribeirão Preto, São Paulo state (both cities located in Brazil) were evaluated. The sample consisted of biologically unrelated individuals aged 8–43 years old. The exclusion criteria included patients younger than 7 years of age, patients with syndromes, oral clefts, history of facial trauma or facial surgery, and records with missing radiographs in the tooth agenesis and control group.

Determination of Tooth Agenesis Phenotype

The control and tooth agenesis cases were identified by the assessment of panoramic radiographs and treatment records by two trained dentists. All panoramic radiographs were examined using the same protocol and in all cases tooth agenesis was clearly evident from the panoramic radiographs alone (Küchler et al., 2008a,b). The inclusion criterion in the tooth agenesis group was that at least one permanent tooth was affected. Tooth agenesis was defined based on the age of subjects and when initial tooth formation should be visible in the radiographs (Küchler et al., 2008a,b). All controls had all permanent teeth, including third molars. Patients with tooth extraction were excluded.

Tooth agenesis cases were also divided into third molar agenesis and other permanent tooth agenesis subgroups for the analysis.

Selection of Genetic Polymorphisms, DNA Extraction, and Genotyping

The selection of the genes was initially based on the screening of previously published studies suggesting that these genes are involved in the maxilla, mandible, and tooth development phenotypes. Then, potentially functional genetic polymorphisms were screened from the dbSNP database[1] and SNPinfo,[2] based on the following criteria: minor allele frequency (MAF) $\geq$10% in the global population, and classification of the genetic polymorphisms as potentially functional (for altering amino acid sequence of the protein product, or for occurring in the proximal promoter of the gene and potentially influencing gene expression, or previously associated with craniofacial phenotypes). The characteristics of the selected genetic polymorphisms are presented in **Table 1**.

For the genotyping analysis genomic DNA was used. The DNA was isolated from buccal epithelial cells by a rinse of saline solution. Briefly, the tubes with saliva were centrifuged and supernatant was discarded. Extraction solution (Tris–HCl 10 mmol/L, pH 7.8; EDTA 5 mmol/L; SDS 0.5%, 1 mL) and proteinase K (100 ng/mL) were added to the tube. Ammonium acetate also was added to remove non-digested proteins and the solution centrifuged. DNA was precipitated with isopropanol and washed with ethanol. DNA was then resuspended and quantified by spectrophotometry (NanoDrop 1000; Thermo Scientific, Wilmington, DE, United States) (Küchler et al., 2012).

The selected genetic polymorphisms were blindly genotyped *via* real-time polymerase chain reaction (PCR) StepOne^{TM}

[1]http://www.ncbi.nlm.nih.gov/snp/

[2]http://snpinfo.niehs.nih.gov/

TABLE 1 | Characteristics of the selected genetic polymorphisms and previously reported associations with oral phenotypes.

Gene	Chromosome	Genetic polymorphism	Base change	Function	MAF	References
Bone morphogenetic protein 2 (*BMP2*)	20p12.3	rs1005464	A/G	Intron	0.194	Previously associated with dental crowding (Ting et al., 2011) and mandibular retrognathism (Küchler et al., 2021).
		rs235768	A/T	Missense (Arg > Ser)	0.676	Previously associated with mandibular retrognathism (Küchler et al., 2021)
Bone morphogenetic protein 4 (*BMP4*)	14q22.2	rs17563	A/G	Missense (Val > Ala)	0.454	Previously associated with isolated tooth agenesis (Antunes et al., 2012; Gong et al., 2015). May be a risk factor for oral clefts in Brazilians (Antunes et al., 2013; Bahrami et al., 2020)
Runt-related transcription factor 2 (*RUNX2*)	6p21.1	rs59983488	G/T	Upstream	0.179	This polymorphism was associated with maxillary protrusion (Küchler et al., 2021)
		rs1200425	A/G	Intron	0.448	Previously associated with skeletal malocclusion phenotypes (Küchler et al., 2021)
SMAD family member 6 (*SMAD6*)	15q22.31	rs2119261	C/T	Intron	0.419	Associated with the shape of the palatine rugae pattern (Silva-Sousa, 2021)
		rs3934908	C/T	Intron	0.436	Borderline association with the palatine rugae length asymmetry (Silva-Sousa, 2021).

Obtained from databases: http://www.thermofisher.com and http://www.ncbi.nlm.nih.gov.
Arg, arginine; Ser, serine; Val, valine; Ala, alanine.

using TaqMan™ technology (Applied Biosystems). The TaqMan technology uses extremely sensitive allele-specific probes (VIC™ and FAM™ dyes were used for the alleles). A negative control template was included in every reaction plate. In addition, 10% of samples were randomly selected for repeated analysis and the results showed 100% concordance. DNA samples that failed to be genotyped were excluded from further analyses. The success rates were as follows: rs235768 (*BMP2*) = 86.0%; rs1005464 (*BMP2*) = 84.9%; rs17563 (*BMP4*) = 84.2%; rs59983488 (*RUNX2*) = 85.5%; rs1200425 (*RUNX2*) = 83.8%; rs3934908 (*SMAD6*) = 84.9%; rs2119261 (*SMAD6*) = 84.2%.

Statistical Analysis

Hardy–Weinberg Equilibrium (HWE) was assessed for each genetic polymorphism by Chi-square test.[3] Chi-square test was also used to compare the allele and genotype distribution according to tooth agenesis phenotypes. Prevalence ratio (PR) and 95% confidence intervals (CI) were obtained. PLINK[4] was used to compare haplotype frequencies between groups using Fisher's exact test.

Multivariate Poisson regression adjusted by gender and ethnicity was done to evaluate genotypes in the co-dominant model and also diplotypes. Diplotype is a combination of two haplotypes that may be evaluated by an interaction term (Gatlin et al., 2009; Guo et al., 2017). Poisson regression was performed using SPSS Statistics Version 25.0 (IBM Corp., New York, NY, United States).

The significance level was determined as $p < 0.05$.

[3] wpcalc.com/en/equilibrium-hardy-weinberg
[4] zzz.bwh.harvard.edu/plink

RESULTS

A total of 273 individuals (116 males and 157 females) was included. Eighty-six were included in the tooth agenesis group and 187 in the control group. In the tooth agenesis group, 53 individuals presented third molar agenesis (61.6%) and 42 (48.4%) presented other types of missing teeth. Gender and ethnicity were not associated with tooth agenesis ($p > 0.05$) (**Supplementary Table 1**).

Table 2 shows the allele distribution according to the groups. The allele T of the rs235768 in *BMP2* was associated with higher chance to present tooth agenesis in comparison with control group ($p < 0.001$; PR = 3.45; 95% CI = 2.54–4.70). In the subgroup analysis, the T allele was also associated with higher chance to present third molar agenesis ($p < 0.001$; PR = 4.30; 95% CI = 2.83–6.52) and other agenesis ($p < 0.001$; PR = 4.48; 95% CI = 2.78–7.21). The allele C of the rs3934908 in *SMAD6* was associated only with higher chance to present third molar agenesis ($p = 0.036$; PR = 1.43; CI 95% = 1.00–2.09).

Table 3 presents the haplotype frequency comparisons between groups. All haplotypes formed by the rs235768 and rs1005464 polymorphisms in *BMP2* were associated with tooth agenesis and tooth agenesis subgroups ($p < 0.001$). The haplotypes T–G and T–A were more frequent in tooth agenesis cases, while the haplotypes A–G and A–A were more frequent in controls.

Table 4 shows the genotype distribution among groups in the co-dominant model. The rs235768 in *BMP2* was associated with tooth agenesis in univariate and multivariate analysis ($p < 0.001$). The genotype TT in rs3934908 in *SMAD6* was associated with an increased chance to present third molar agenesis ($p = 0.023$; PR = 3.25; CI 95% = 1.17–8.99).

TABLE 2 | Allelic distribution between groups.

Gene	Genetic polymorphism	Allele	Control	Tooth agenesis	*p*	PR (95% CI)	Third molar agenesis	*p*	PR (95% CI)	Other agenesis	*p*	PR (95% CI)
BMP2	rs235768	A	233	42	**<0.001**	3.45 (2.54–4.70)	25	**<0.001**	4.30 (2.83–6.52)	20	**<0.001**	4.48 (2.78–7.21)
		T	91	104			65			50		
	rs1005464	G	249	112	>0.999	1.01 (0.72–1.42)	67	0.457	1.05 (0.68–1.62)	55	0.480	0.94 (0.56–1.58)
		A	73	30			21			15		
BMP4	rs17563	A	184	75	0.182	1.15 (0.87–1.51)	50	0.477	1.03 (0.71–1.50)	38	0.338	1.12 (0.73–1.72)
		G	134	67			38			32		
RUNX2	rs59983488	G	260	116	0.326	0.89 (0.60–1.31	73	0.467	0.93 (0.57–1.53)	56	0.344	0.82 (0.44–1.54)
		T	58	22			15			10		
	rs1200425	G	183	90	0.105	0.81 (0.61–1.09)	54	0.226	0.84 (0.56–1.24)	46	0.079	0.69 (0.43–1.11)
		A	135	50			32			22		
SMAD6	rs3934908	C	175	71	0.165	1.16 (0.88–1.52)	38	**0.036**	1.43 (1.00–2.09)	38	0.434	1.06 (0.70–1.61)
		T	145	73			50			34		
	rs2119261	C	179	81	0.390	0.94 (0.71–1.25)	51	0.415	0.93 (0.64–1.36)	40	0.383	0.90 (0.58–1.40)
		T	141	59			37			28		

Fisher's exact tests were performed. All comparisons were performed with control group as reference. Bold forms means statistical difference. PR, prevalence ratio.

TABLE 3 | Haplotypes frequency comparisons between groups.

Genes	Genetic polymorphism	Haplotypes	Control	Tooth agenesis	*p*	Control	Third molar agenesis	*p*	Control	Other agenesis	*p*
BMP2	rs235768 and rs1005464	A–G	52.2	26.5	**<0.001**	51.9	24.0	**<0.001**	51.4	25.6	**<0.001**
		A–A	19.5	1.5	**<0.001**	19.8	2.0	**<0.001**	20.2	2.9	**<0.001**
		T–G	25.1	52.2	**<0.001**	25.4	52.0	**<0.001**	25.8	52.9	**<0.001**
		T–A	3.1	19.5	**<0.001**	2.8	21.7	**<0.001**	2.3	2.9	**<0.001**
RUNX2	rs59983488 and rs1200425	G–G	49.4	53.3	0.444	49.6	51.0	0.828	50.0	57.7	0.259
		G–A	32.4	30.4	0.683	32.1	31.5	0.914	31.8	26.5	0.411
		T–G	8.2	11.3	0.290	7.9	11.7	0.266	7.5	10.9	0.369
		T–A	9.9	4.8	0.072	10.1	5.6	0.197	10.5	4.6	0.143
SMAD6	rs3934908 and rs2119261	C–C	34.4	33.2	0.825	34.2	30.5	0.515	33.9	34.2	0.965
		C–T	20.4	15.3	0.197	20.4	12.6	0.094	20.8	17.2	0.508
		T–C	21.6	24.5	0.493	21.7	27.3	0.266	22.0	24.6	0.649
		T–T	23.5	26.8	0.454	23.5	29.4	0.254	23.1	23.9	0.896

PLINK compares the frequencies between expected number of haplotypes by Fisher's exact test. Bold forms means statistical difference.

Table 5 shows the diplotype analysis. The diplotype analysis of the studied genetic polymorphisms in *BMP2* demonstrated that individuals carrying TT + AA genotypes (rs235768–rs1005464) had a higher chance to present tooth agenesis (p = 0.016; PR = 5.33; 95% CI = 1.36–20.83), third molar agenesis (p = 0.013; PR = 10.15; 95% CI = 1.62–63.29), and other agenesis (p = 0.020; PR = 7.64; 95% CI = 1.38–42.16) than the control individuals.

DISCUSSION

Dental development results from several interactions acting synergistically and antagonistically, leading to tooth epithelium and mesenchyme formation. The process is regulated by different mechanisms involving the expression of several genes (Nieminen, 2009). Mutations and/or genetic polymorphisms in one or more genes involved in the earlier stages of dental development

TABLE 4 | Genotypic distribution between groups.

Geno	Control	Tooth agenesis	p^u	p^m	PR^m (95% CI)	Third molar agenesis	p^u	p^m	PR^m (95% CI)	Other agenesis	p^u	p^m	PR^m (95% CI)
rs235768 (*BMP2*)													
AA	78 (48.1)	8 (11.0)	Ref.			4 (8.9)	Ref.			4 (11.1)	Ref.		
AT	77 (47.5)	26 (35.6)	**0.045**	**0.007**	2.76 (1.32–5.79)	17 (37.8)	**0.009**	**0.011**	3.89 (1.36–11.14)	12 (36.3)	0.067	0.068	2.77 (0.92–8.29)
TT	7 (4.3)	39 (53.4)	**<0.001**	**<0.001**	8.29 (4.26–16.10)	24 (53.3)	**<0.001**	**<0.001**	14.02 (5.34–36.80)	20 (55.5)	**<0.001**	**<0.001**	13.47 (5.06–35.83)
rs1005464 (*BMP2*)													
GG	98 (60.9)	44 (62.0)	Ref.			25 (56.8)	Ref.			22 (62.8)	Ref.		
AG	53 (32.9)	24 (33.8)	>0.999	0.876	0.96 (0.64–1.46)	17 (38.6)	0.587	0.546	1.17 (0.69–2.01)	11 (31.4)	>0.999	0.731	0.88 (0.44–1.77)
AA	10 (6.2)	3 (4.2)	0.755	0.550	0.73 (0.26–2.01)	2 (4.5)	>0.999	0.766	0.82 (0.23–2.92)	2 (5.7)	>0.999	0.790	0.83 (0.22–3.13)
rs17563 (*BMP4*)													
AA	55 (34.6)	23 (32.4)	Ref.			16 (36.4)	Ref.			13 (37.1)	Ref.		
AG	74 (46.5)	29 (40.8)	0.869	0.965	1.01 (0.63–1.60)	18 (40.9)	0.699	0.929	0.97 (0.53–1.76)	12 (34.3)	0.509	0.353	0.70 (0.34–1.46)
GG	30 (18.9)	19 (26.8)	0.333	0.247	1.33 (0.81–2.17)	10 (22.7)	0.817	0.811	1.08 (0.54–2.16)	10 (28.6)	0.476	0.416	1.34 (0.65–2.76)
rs59983488 (*RUNX2*)													
GG	106 (66.7)	51 (73.9)	Ref.			33 (75.0)	Ref.			24 (72.7)	Ref.		
GT	48 (30.2)	14 (20.3)	0.189	0.163	0.68 (0.39–1.16)	7 (15.9)	0.114	0.066	0.46 (0.20–1.05)	8 (24.2)	0.533	0.801	0.90 (0.42–1.92)
TT	5 (3.1)	4 (5.8)	0.480	0.319	1.43 (0.70–2.94)	4 (9.1)	0.228	0.120	1.88 (0.84–4.18)	1 (3)	>0.999	0.836	1.20 (0.20–7.24)
rs1200425 (*RUNX2*)													
GG	51 (32.1)	29 (41.4)	Ref.			17	Ref.			16 (47.0)	Ref.		
AG	81 (50.9)	32 (45.7)	0.272	0.411	0.83 (0.54–1.28)	20	0.452	0.504	0.81 (0.45–1.47)	14 (41.2)	0.154	0.275	0.50 (0.15–1.71)
AA	27 (17.0)	9 (12.9)	0.287	0.336	0.73 (0.39–1.37)	6	0.613	0.569	0.78 (0.34–1.80)	4 (11.8)	0.284	0.130	0.50 (0.20–1.22)
rs3934908 (*SMAD6*)													
CC	45 (28.1)	17 (23.6)	Ref.			8 (18.2)	Ref.			9 (25.0)	Ref.		
CT	85 (53.1)	37 (51.4)	0.734	0.604	1.20 (0.59–2.42)	22 (50.0)	0.519	0.320	1.58 (0.63–3.95)	20 (55.5)	0.829	0.715	1.19 (0.46–3.05)
TT	30 (18.8)	18 (25.0)	0.304	0.153	1.84 (0.78–4.32)	14 (31.8)	0.056	**0.023**	3.25 (1.17–8.99)	7 (19.4)	0.786	0.508	1.47 (0.46–4.70)
rs2119261 (*SMAD6*)													
CC	45 (28.1)	19 (27.1)	Ref.			13 (29.5)	Ref.			9 (26.5)	Ref.		
CT	89 (55.6)	43 (61.4)	0.744	0.476	1.27 (0.65–2.47)	25 (56.8)	>0.999	0.686	1.18 (0.53–2.62)	22 (64.7)	0.677	0.459	1.41 (0.56–3.50)
TT	26 (16.3)	8 (11.4)	0.636	0.366	0.61 (0.21–1.75)	6 (13.6)	0.791	0.694	0.79 (0.24–2.55)	3 (8.8)	0.527	0.397	0.49 (0.09–2.54)

p^u was obtained by Fisher's exact test. p^m and prevalence ratio (PR) were obtained by Poisson regression adjusted by gender. All comparisons were performed with control group as reference. Bold forms mean p-values < 0.05.

TABLE 5 | Diplotype analysis with *BMP2* gene SNPs by Poisson regression adjusted by gender and ethnicity.

			Control vs. agenesis		Control vs. third molar agenesis			
SNPs	**Reference diplotype**	**Diplotypes**	**PR (95% CI)**	***p*-Value**	**PR (95% CI)**	***p*-Value**	**PR (95% CI)**	***p*-Value**
rs235768 and rs1005464	AA + GG	AT + GA	1.04 (0.30–3.60)	0.949	1.68 (0.33–8.55)	0.527	1.17 (0.25–5.38)	0.836
		TT + AA	5.33 (1.36–20.83)	**0.016**	10.15 (1.62–63.29)	**0.013**	7.64 (1.38–42.16)	**0.020**

PR, prevalence ratio.
Bold forms mean p-values < 0.05. Other genes were not associated.

could potentially lead to tooth agenesis. Therefore, in the present study, we replicated genotype–phenotype associations previously observed (Antunes et al., 2012; Gong et al., 2015) and also investigated the association of some novel genes and polymorphisms with isolated tooth agenesis.

In the past decades, innumerous evidence suggests the association between tooth agenesis and oral clefting [revised by Phan et al. (2016)]. Phan et al. (2016) systematically investigated the currently available literature to investigate co-occurrence of tooth agenesis and oral clefts to gain insight into the molecular mechanisms underlying their dual involvement in the development of teeth and facial primordia. The authors concluded that not only the disrupted gene, but even the location of the mutations within the gene can lead to diverse phenotypes, ranging from the isolated form of tooth agenesis to the syndromic one for of oral clefts. In fact, genes involved in oral clefts and in the syndromic form of tooth agenesis are known as a useful approach to select candidate genes for the isolated forms of tooth agenesis (Vieira, 2003). Another important approach to select candidate genes for isolated tooth agenesis is based on the identification of the genes expressed in dental development. So far, more than 300 genes are listed in the database created to catalog genes expressed in different stages of dental development.[5] Therefore, the genes were selected in our study based on their previous associations and their expression and role in dental development.

Genetic polymorphisms are DNA sequence variations occurring in the genome that are characterized by the existence of at least two variants (Botstein and Risch, 2003). They are involved in many phenotypic differences observed in clinical practice. The selection of the genetic polymorphisms studied here was based on their MAF due to our limited sample size. Previous associations with craniofacial phenotypes were also taken into consideration (as shown in **Table 1**) and also the function of the genetic polymorphism.

Two genetic polymorphisms (rs17563 and rs235768) selected here are missense variations located within the coding region and produce amino acid changes. The rs17563 in *BMP4* studied here replaced the amino acid valine by alanine at position 152 of the protein. This genetic polymorphism has been widely investigated in oral cleft research. A recent systematic review and comprehensive meta-analysis investigated case-control studies with 2,058 oral cleft cases and 2,557 controls. In their overall analysis, no significant association between the rs17563 polymorphism and the risk of oral cleft was observed, however, their subgroup analysis demonstrated that rs17563 was associated with oral cleft risk in Chinese and Brazilian populations (Bahrami et al., 2020). The polymorphism rs17563 was also associated with isolated tooth agenesis in Brazilians and Chinese (Antunes et al., 2012; Gong et al., 2015). Although we also investigated Brazilians, the lack of association observed in our study may be explained by the fact that these previous studies excluded third molars. Although we also performed a stratified analysis excluding third molars, the sample size could lead to a false-negative result, once it is well-known that BMP4 is important for tooth development and the BMP4 expression pattern coincides with the bud-to-cap stage transition in tooth development (Saadi et al., 2013).

The other missense variant studied here was the rs235768, which is located in BMP2 resulting in an arginine to serine replacement. *BMP2*, another important member of the *BMP* family involved in regulating tooth initiation, can induce human tooth germ cells to differentiate into odontogenic and osteogenic cells (Zhang et al., 2005; Taşlı et al., 2014). In animal models, *BMP2* expressed in the presumptive dental epithelium (Neubüser et al., 1997) could result in the arrest of tooth development after knockdown (Yuan et al., 2015). In fact, our study demonstrated interesting results in both studied genetic polymorphisms in BMP2. To carry the T allele increases the risk to present tooth agenesis. The haplotype and diplotype analysis also showed that rs235768 interacts with the intronic variant rs1005464 and is involved in the risk for any type of tooth agenesis, including third molar agenesis.

Third molars are the most common congenitally missing teeth, followed by premolars and maxillary lateral incisors (Polder et al., 2004). Although third molar agenesis is a phenotype highly prevalent in humans, its etiology has been poorly explored so far. A recent study evaluated a large sample of same sex twins (172 monozygotic and 112 dizygotic) and concluded that a dominant factor for third molar agenesis is genetics (Trakinienė et al., 2018). However, the genes involved in third molar agenesis are still unexplored. Our results suggest that genes/genetic polymorphisms involved in the agenesis of other tooth types could be candidate for third molar agenesis studies.

Mothers against decapentaplegic homolog 6 is known to interact with BMP signaling in the nucleus by interacting with transcription repressors (Wu et al., 2016), including BMP2 (Li et al., 2003). SMAD6 is important for craniofacial development

[5]http://bite-it.helsinki.fi

(Estrada et al., 2011; Timberlake et al., 2016). A previous study identified rare *SMAD6* and common *BMP2* alleles involved with craniosynostosis in humans (Timberlake et al., 2016). In our study, genotype and allele distribution of the intronic variant rs3934908 in *SMAD6* was associated with third molar agenesis.

Runt-related transcription factor is well known to be involved in tooth development (Camilleri and McDonald, 2006). RUNX2-deficient mice show an arrest of molar tooth development at the early cap stage, suggesting that RUNX2 is required for the progression of tooth development from the cap stage to the bell stage (D'Souza et al., 1999) and therefore is a candidate for isolated tooth agenesis. In our study, some borderline association was observed for the studied genetic polymorphisms in *RUNX2*, suggesting that future studies should investigate the association of variations in this gene with isolated tooth agenesis in a larger population.

Although our study provides some interesting information regarding the genes involved in the etiology of isolated tooth agenesis, it has some obvious limitations. The limited sample size could lead to a type II error in the analysis of genetic polymorphisms with small effect. Also, the number of selected genetic polymorphisms does not cover the studied genes. Additionally, future studies should also evaluate the role of these genetic polymorphisms in tooth agenesis risk in oral cleft individuals.

In conclusion, our study suggested that genetic polymorphisms in *BMP2* and *SMAD6* are involved in a higher chance to present isolated tooth agenesis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Local Ethics Committee (no. 01451418.3.0000.5419). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

EK and CK conceptualize the study. EK, MM, and RS designed and organize the sample recruitment. GM-V and AS performed the sample collection. CR, GM-V, and AS-S performed the laboratory analysis. CR, AS-S, and EP analyzed the data. CR, EK, GM-V, AS-S, and CK wrote the manuscript. EK, PP, and CK funding support. All authors read and approved the final version of the manuscript.

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Study on the Effect of Bilateral Mandible Distraction Osteogenesis About the Nutrition Status of Infants With Pierre-Robin Sequence

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Objective: By comparing and studying the changes of food intake, weight, body length, BMI, blood albumin level and other indicators of infants with Pierre-Robin Sequence (PRS) before and after Bilateral Mandible Distraction Osteogenesis operation, to explore the effect of distraction osteogenesis on PRS patients about the improvement of nutrition status.

Methods: The children with PRS who admitted to the Oral and Maxillofacial Surgery Department of Guangzhou Women and Children's Medical Center from July 2015 to December 2020 were selected. All patients accepted bilateral mandible distraction osteogenesis surgery, and the pre- and post-operative indicators were recorded, such as food intake, weight, length, blood albumin level and others. BMI was calculated based on the indicators mentioned above, and comparative statistical analysis was performed.

Results: 1. All patients were fed with whole milk before the first surgery, and the average calorie per kg was 91.8 kcal/kg, significantly lower than the standard (100–150 kcal/kg), suggesting the overall nutritional intake of PRS patients is low; 2. *t* tests for independent samples were used to analyze the pre-operative and post-operative indicators. The WFA percentile increased from 14.16 ± 2.17 to 15.01 ± 1.85% ($P = 0.0048$), WFA *z* score increased from −2.40 ± 0.18 to −1.90 ± 0.14 after the surgery ($P = 0.0010$), LFA percentile increased from 20.04 ± 3.48 to 33.67 ± 4.29% ($P = 0.0098$), LFA z score increased from −2.09 ± 0.19 to −1.42 ± 0.23 ($P = 0.0009$), BMI *z* score increased from −1.95 ± 0.22 to −1.39 ± 0.16 ($P = 0.0408$), ALB raised from 37.06 ± 0.51 to 42.85 ± 0.30 g/L ($P < 0.001$), which indicating that the physique of patients improved after the distraction osteogenesis surgery, mainly was reflected by the lifting of weight and length growth curves; the body shape also improved, indicating that the patients' nutrition status after the surgery is also improved.

Conclusion: Bilateral mandible distraction osteogenesis surgery has a positive effect on the nutrition status of children with PRS. This effect is mainly reflected by the improvements of the body physical indicators after surgery.

Keywords: Pierre-Robin Sequence (PRS), distraction osteogenesis (DO), nutrition status, weight for age (WFA), length for age (LFA), blood albumin

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INTRODUCTION

Pierre-Robin Sequence (PRS) is a group of congenital malformations characterized by micrognathia, glossoptosis, with or without a cleft palate. The patients usually suffer from breathing difficulties and disorders of eating and swallowing due to the small, dysplastic mandible pushing the tongue backward, even attach to the pharyngeal wall, resulting in narrowing of the airway and oropharyngeal cavity, in the newborn and infancy time. Therefore, the growth and development of the patients are often affected, and even could be life threatening in severe cases. There are researches confirming that the nutrition status of patients with PRS without suitable treatment was at the middle-low position compared to the same age group, and the proportion of combination with severe malnutrition is about 53% depending on different statistical methods and populations. Around 50–100% of children with PRS also suffer from varying degree of feeding difficulties, relying on gastric tube, gastrostomy and even parenteral nutrition to sustain living (1, 2). Bilateral mandible distraction osteogenesis (BMDO) surgery has been considered as the first choice for the treatment of severe PRS. After BMDO treatment, the mandible is efficiently lengthened, and the oropharyngeal cavity and airway are enlarged. As a result, difficulties in breathing and eating which are present before surgery are greatly eased (3). According to the above, we reasonably speculate that with the improvement of breathing and eating, the nutrition status and physical level of the patients will also be significantly improved.

In order to analyze the influence of BMDO on the nutrition status of PRS patients, we selected the weight, length, food intake, and blood albumin level of the patients before and after the operation as indicators. We retrospectively analyzed 100 cases of PRS infants who underwent BMDO surgery in our hospital from 2015 to 2020. The changes in the nutritional status before and after surgery furthermore proved the necessity of BMDO surgery for PRS patients, and our result helped us to discuss the choice of surgery indicators from another aspect.

MATERIALS AND METHODS

A total of 100 PRS patients treated with BMDO in the Oral and Maxillofacial Surgery Department of Guangzhou Women and Children's Medical Center from 2015.6 to 2020.12 were selected. Their age was between 15 d and14 m before the first surgery. The data and general conditions of the patients are shown in **Table 1**.

Inclusion criteria of the cases in this study: 1. The diagnosis of PRS is clear, that is, infants with micrognathia, glossoptosis, with or without cleft palate, have not undergone other maxillofacial surgery in the past; 2. General conditions can tolerate surgery under general anesthesia; 3. Distractor implantation and removal operations were completed in our hospital, and the pre- and post-operative data were collected completely.

All patients underwent bilateral mandible distraction osteogenesis under general anesthesia. The incisions were parallel and approximately 1.5 cm away from the lower edge of the mandible near the mandibular angle. The osteotomy lines were designed according to the pre-operative three-dimensional CT model to represent the distraction angle and direction in the simulated operation as much as possible. Distraction started at a speed of 1.2 mm/day after a 48–72 h latency period. In order to avoid excessive movement of the mandible affecting the formation of new bone and the displacement of bone segments, all patients were fed by gastric tube during the distraction period. It takes about 1–2 weeks for the segments to reach the ideal position, when the patients were gradually trained to use a spoon for milk intaking, and the gastric tube was removed when the feeding situation meet patients' daily need. In order to promote the formation and hardening of the new bone and avoid loading of the mandible, all patients' caregivers were told not to use the pacifiers and use spoon for feeding until the removal of the distractor. The physical and biochemical indicators were measured and recorded before two operations, and *t* tests for independent sample were performed to compare the changes of indicators before and after BMDO.

The measurements in this study included weight (kg), body length (cm), milk volume (ml), blood albumin level (g/L), and calculated the related indexes such as Weight for age (WFA), percentile and *z* score, Length for age (LFA), percentile and *z* score, Weight For Length (WFL), percentile and *z* score, BMI and its percentile and *z* scores. All physical indicators were compared with the standard values of normal infants of same age (4). See **Table 2** for more details.

TABLE 1 | Baseline characteristics ($n = 100$).

Gender, *n*	
Male	44
Female	56
Born weight, kg, mean (SD)	2.91 ± 0.53
Gestation, weeks, mean (SD)	38.05 ± 2.15
Admission age, months, mean (SD)	
First	1.28 ± 1.32
Secondary	6.02 ± 2.67
Admission weight, kg, mean (SD)	
First	3.55 ± 0.88
Secondary	6.14 ± 0.92
Admission length, cm, mean (SD)	
First	52.92 ± 3.73
Secondary	63.85 ± 4.48
Admission BMI, mean (SD)	
First	13.06 ± 2.66
Secondary	15.16 ± 1.88

SD, standard deviation.

RESULTS

1. All patients were fed with total milk before the first surgery. Due to the large age span, there was a big difference in the exact volume of milk intake, so we take the average calorie per kg as the parameter. The average intake was 91.8 kcal /kg, lower than the standard (100∼120 kcal/kg) (5) (**Table 3**).

TABLE 2 | Comparative statistics of independent sample *t* test about physical indicators before and after BMDO.

Group		*n*	Mean	Standard deviation mean	*t*	Sig.	95% Confidence interval	
							Lower limit	Upper limit
WFL percentile (%)	1	64	31.4016	3.7554	−0.152	0.5054	−10.8022	9.2574
	2	73	32.1740	3.4220				
WFL *z* score	1	82	−1.1899	0.2481	0.109	0.9141	−0.5638	0.6297
	2	88	−1.2228	0.1721				
WFA percentile (%)	1	65	14.1631	2.1677	−0.297	0.0048**	−6.4456	4.7606
	2	72	15.0056	1.8486				
WFA *z* score	1	98	−2.4022	0.1809	−2.154	0.0010***	−0.9544	−0.0418
	2	91	−1.9042	0.1441				
LFA percentile (%)	1	62	20.0435	3.4772	−2.468	0.0098**	−24.5573	−2.7005
	2	69	33.6725	4.2901				
LFA *z* score	1	82	−2.0851	0.1900	−2.179	0.0009***	−1.2598	−0.0621
	2	88	−1.4242	0.2333				
BMI for age	1	54	18.0259	3.1768	−1.772	0.0789	−15.8501	0.8773
	2	73	25.5123	2.7727				
BMI *z* score	1	82	−1.9450	0.2190	−2.062	0.0408*	−1.0922	−0.0237
	2	88	−1.3870	0.1631				
ALB (g/L)	1	100	37.0550	0.5067	−9.878	0.0000****	−6.9514	−4.6350
	2	110	42.8482	0.2953				

Group 1: First admission, before BMDO; Group 2: second admission, after BMDO; WFL, weight for length; WFA, weight for age; LFA, length for age; ALB, blood albumin.
**P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.*

2. After BMDO, most statistical indicators improved, the WFA percentile increased from 14.16 ± 2.17 to 15.01 ± 1.85% (P = 0.0048), WFA z score increased from −2.40 ± 0.18 to −1.90 ± 0.14 (P =0.0010), LFA percentile increased from 20.04 ± 3.48 to 33.67 ± 4.29% (P =0.0098), as the z score rose from −2.09 ± 0.19 to −1.42 ± 0.23 (P =0.0009). BMI z score increased from −1.95 ± 0.22 to −1.39 ± 0.16 (P = 0.0408). The changes of indicators above all suggested that the physical levels of patients improved after surgery, especially the body length (**Figure 1**).
3. Blood albumin level: Before the first surgery, the average blood albumin level in venous blood of all patients was 37.06 ± 0.51 g/L, which was significantly lower than the normal value (40–55 g/L) according to the nutrition status analysis of healthy children in the same age (6). This result indicated that the pre-operative nutrition status was generally poor. The average level of albumin in the venous blood before the second surgery was 42.85 g/L, which was significantly improved from the results before (P < 0.0001). Combined with other results of our study, the improvement mentioned above suggested that the body composition (nutrition status) of PRS patients accepted BMDO has improved compared with that before surgery (**Figure 2**).

TABLE 3 | Feeding statistics before BMDO.

	N	Minimum value	Maximum value	Meverage	Standard deviation
Milk (ml)	93	80.00	900.00	478.3441	180.79093
Milk per kg (ml)	93	25.81	258.06	137.0931	44.45625
Kcal per kg	93	17.29	172.90	91.8524	29.78569

DISCUSSION

Malnutrition is an imbalance situation between nutritional needs and intake, leading to insufficient accumulation of energy, protein or micronutrients that may have a negative impact on growth, development, and other related results (7). It is one of the common symptoms appeared in patients with PRS. The causes of malnutrition in PRS are various, which may be related to the high energy consumption of respiratory work caused by upper airway obstruction, or to feeding difficulty (FD) caused by micrognathia and glossoptosis (8). The latter one may also be a direct result of respiratory dysfunction. According to the latest research results, some scholars believe that the abnormal development of tongue muscle due to mandible dysplasia is also one of the new reasons worth paying attention to. The incidence of feeding difficulties can be up to 91% in patients with PRS (9), and even if the impact of cleft palate is considered, PRS patients are more likely to have eating and breathing problems than children with ordinary cleft palate (10). According to statistics, 45.8% of patients with PRS need enteral nutritional support (11). Marston et al. (12) reviewed PRS patients who accepted BMDO surgery in 2006, 2009, and 2012 in hospitals across the United States in a 2018 study. Of the 276 children, 17.4% needed gastrostomy to improve food intake, and 16.7% needed the support of total

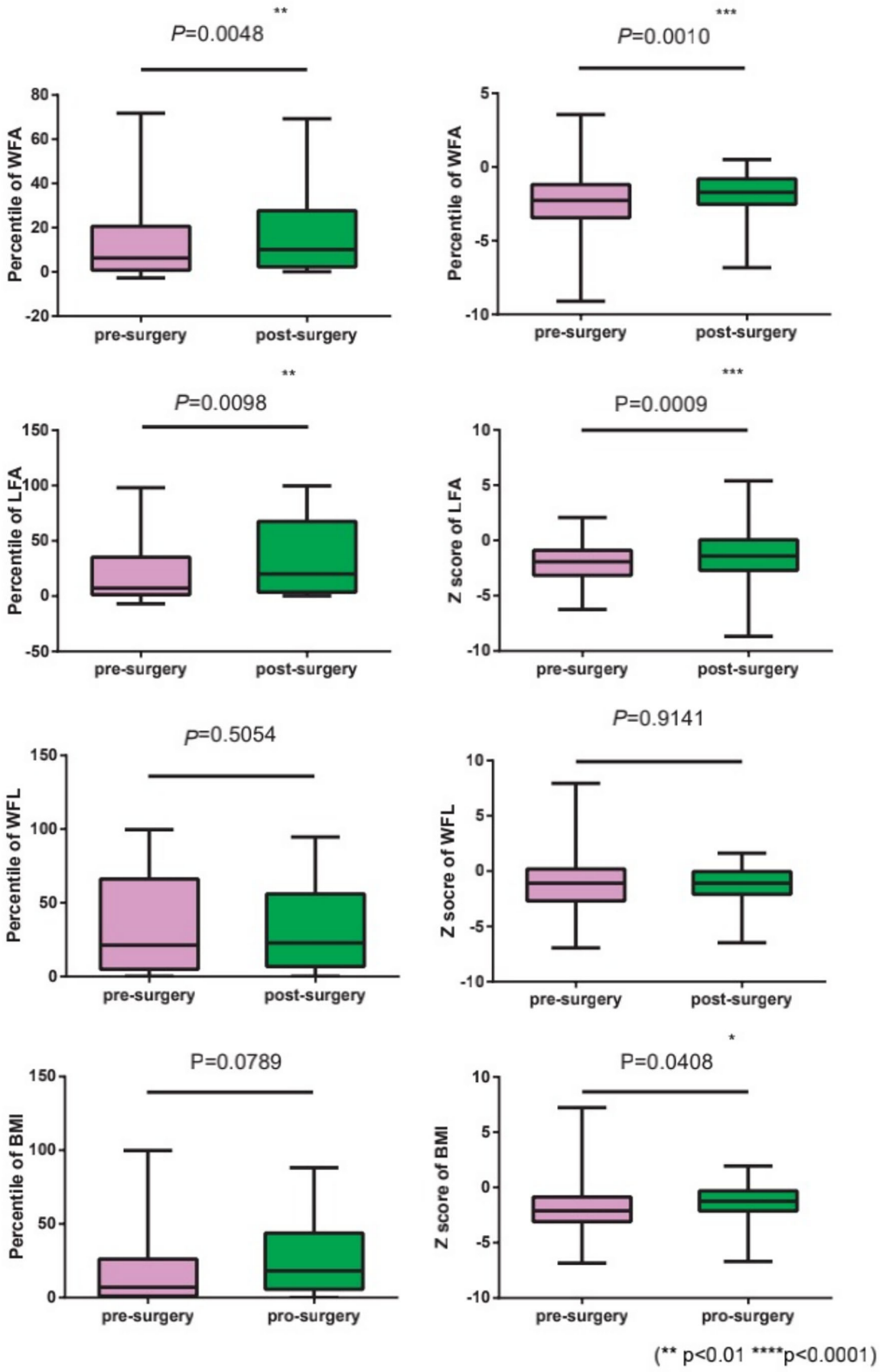

FIGURE 1 | Independent sample comparison statistics of pre- and post-operative body index. *$P<0.05$, ***$P<0.001$.

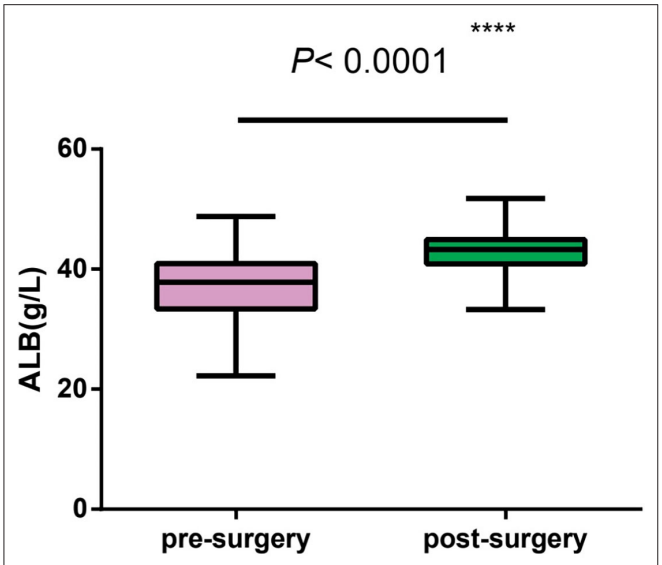

FIGURE 2 | Independent sample comparison statistics of pre- and post-operative blood albumin levels.

parenteral nutrition. The study by Susarla et al. (1) compared two different treatment methods of PRS, tongue-lip adhesion and distraction osteogenesis, and found that for the children with improved breathing who accepted Tougue-lip Adhesion (TLA), the improvement of swallowing, weight gaining and nutrition status were still not satisfacted; this result may be related to the change of the inherent physiological function structure of the lips and tongue, but it also reflected from another angle that it is not appropriate to only consider the solution of airway obstruction. Whether distraction osteogenesis should be performed or not, the overall nutritional risk of patients should be taken into consideration as well. Although distraction osteogenesis has been considered as an ideal treatment to improve the quality of life and prognosis of PRS patients, its main purpose is still for solving upper airway obstruction. Some doctors still have doubts and questions about the surgery (9) due to the difficulty of surgery, anesthesia, scar and other complications.

According to the author's experience and previous case reports (13). There was no difference in the incidence of post-operative complications for PRS patients accepted surgery in neonatal or non-neonatal period. However, early surgery is conductive to the catch-up of weight, and BMDO within 3 months after birth reduces the need for feeding intervention in PRS patients (14). Resuming oral eating and chewing exercise as soon as possible can also promote the catch-up growth of the mandible (15, 16). Studies have pointed out that repeated airway infections between the age of 2–3 months have a long-term negative impact on growth and development (17). In addition, PRS children accepted BMDO at early age were less likely to have psychomotor development delay and severe malnutrition. Which is also helpful to completing the cleft palate surgery that should be arranged before the age of 1 year, so as to improve their pronunciation (18).

Association and the American Society of Parenteral Nutrition came to a consensus in 2015 (19), and confirmed a set of basic indicators for diagnosing and recording malnutrition in the pediatric population (1 month to 18 years old), recommending energy intake, energy demand and growth indicators to assess nutrition status, generally including length-for-age (LFA), weight-for-age (WFA), and body mass index (BMI) for age. Growth rate is defined as the rate of weight or length/height change over time. This rate of change can be explained as an early sign of a healthy or unhealthy response to the nutritional environment (20). During the growth period, infants need to gain weight at a relatively balanced rate to maintain a relatively stable position on the growth curve, and excessive weight gain and weight loss have been pointed out "relatively independent and more closely related to mortality than other malnutrition indicators (such as BMI for age)" (21). The consensus also pointed out that during the treatment of children, the weight may not change with the changes in body composition at the same time, so the BMI index may not be accurate enough as the only nutritional evaluation index, and the degree of malnutrition may also be underestimated for newborns and small-month-old infants at the first administration to hospitals. Based on previous studies (6, 22), for every 1 g/L decrease in serum albumin concentration, the mortality rate of hospitalized children increased by 137%, and the staying time in the ICU and ward were increased by 28 and 71%, respectively. The blood albumin level can be used as a mature independent indicator for the nutrition status evaluation of children with disease. Therefore, indicators such as height/length and blood albumin level are more suitable for the definition and evaluation of chronic malnutrition (23). This is also why we use LFA, WFA, BMI and blood albumin levels as the evaluation standards in our study.

In previous researches on PRS, researchers have focused more on the improvement of upper airway obstruction and dyspnea symptoms. The study on the nutritional status mainly focused on the changes in weight and body length followed by the airway improvement. More people believed that the improvement of nutritional status is a result of the alleviation of airway problems (24–26). However, some scholars believe that nutritional changes should be studied independently with airway changes, because breathing and swallowing difficulties in PRS patients may not only be related to anatomical variation (such as micrognathia and glossoptosis etc.), but also to abnormal laryngeal development, neurological disorders, and various other associated syndromes (27). For this part of the patients, high-calorie feeding should be applied as soon as possible (28), instead of waiting for the relief of airway symptoms to bring an improvement in nutrition status; the nutritional risk of PRS may not be accompanied by dyspnea, since the symptoms can appear immediately after birth, or may be delayed (29). Previous studies have mostly used to evaluate the changes in weight, length, BMI index pre- and post-operatively without the observation of blood albumin levels' change. Compared with hemoglobin (HGB), serum albumin is relatively less affected by inflammation, blood sampling methods, and has a long half-life period, which is more suitable for defining chronic malnutrition. Our research is the only study to evaluate the changes of serum albumin and body index in PRS patients, and serum albumin levels increased significantly

without treatment of albumin transfusion after BMDO, proving that even in some children whose weight gain was not obvious after surgery, the protein nutrition level was also improved. Meanwhile, recovering and maintaining a good nutrition status may accelerate the recovery of airway permeability during growth and development by promoting the development of neuromuscle maturity and coordination (28).

Our research has the following limitations: 1. It is a retrospective study without prospective design. Many physical indicators such as upper arm circumference, blood lipids, blood albumin precursors and others that can further reveal the nutrition status of patients were not included in the routine examinations before surgery; 2, Children with syndromic and non-syndromic PRS were not distinguished and statisted separately, but even in children with syndromic PRS, that is, combined with other neuroendocrine or genetic chromosomal defects, also achieved significant nutritional improvement after BMDO. It suggested that BMDO surgery can help improve PRS patients' food intake in all aspects; 3, There is no control group. Theoretically speaking, the control group should be designed as children with confirmed PRS but without accepting BMDO. However, since PRS may be associated with or without cleft palate that can easily cause eating and swallowing difficulties, it was difficult to distinguish whether the eating difficulties in children without BMDO were related to the micrognathia or the cleft palate. Therefore, this kind of patients were not included in our study. In the follow-up study, prospective design can be carried out to include more evaluation indicators and parameters that affect the nutrition status of PRS patients, such as days of gastric tube use, gastric tube utilization rate and post-operative choking rate, to clarify the relationship between surgery, nutrition and airway.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LJ and SJ contributed equally to conception and design of the study. LJ organized the database and wrote the first draft of the manuscript. SJ performed the statistical analysis. CY and LF reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Case Report: Anesthetic Management and Electrical Cardiometry as Intensive Hemodynamic Monitoring During Cheiloplasty in an Infant With Enzyme-Replaced Pompe Disease and Preserved Preoperative Cardiac Function

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Introduction: Pompe disease is caused by deficiency of the lysosomal enzyme acid α-glucosidase, which results in cardiac and muscular complications that can jeopardize perioperative outcomes. We report a 4-month-old infant with Pompe disease receiving cheiloplasty under general anesthesia with the aid of peripheral nerve blocks and intensive hemodynamic monitoring.

Case Description: This case report describes a 4-month-old full-term Taiwanese female infant who presented with left unilateral cleft lip and palate in the prenatal examination. She was diagnosed with infantile-onset Pompe disease after acidic α-glucosidase (GAA) gene sequencing. She also received enzyme replacement therapy (ERT) 15 days after birth and regular ERT every other week. Cheiloplasty was performed under general anesthesia uneventfully, and peripheral nerve blocks were adopted for analgesia. Intensive hemodynamic monitoring using electrical cardiometry technology (ICON®) and pulse contour analysis (FloTrac system) were applied during the operation. No adverse effects were observed, and the wound healed well. Therefore, the patient was discharged 4 days after surgery.

Conclusion: With the availability of ERT, severe organ dysfunction in infantile-onset Pompe disease patients is no longer common. However, moderate cardiac depression can still occur while increasing inspiratory pressure and deepening the anesthesia level despite a normal preoperative echocardiogram report. Therefore, careful, gradual titration

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is desirable. Furthermore, electrical cardiometry can detect hemodynamic changes more instantaneously and reliably than pulse contour analysis. In addition, we suggest taking advantage of the peripheral nerve block as a part of balanced anesthesia to alleviate the cardiac suppression caused by general anesthesia.

Keywords: Pompe disease, cardiomyopathy, hypotonia, anesthesia, impedance cardiography, pulse wave analysis

INTRODUCTION

Pompe disease, an autosomal recessive disorder, is caused by deficiency of the lysosomal enzyme acid α-glucosidase, which metabolizes glycogen in lysosomes (1, 2). Glycogen that is not able to be metabolized is stored in the organs, especially the heart, skeletal muscle, and liver, which causes hypertrophic cardiomyopathy, hypotonia, and hepatomegaly (3). Patients with infantile-onset Pompe disease may appear normal but exhibit hypotonia and feeding difficulties later. They may also present with atelectasis and respiratory distress due to the compression of the bronchi by an enlarged heart (4). In the past, the life expectancy of these infants was typically <1 year because of cardiorespiratory failure. Sudden death due to ventricular outflow tract obstruction was previously reported (3). Although the generalized use of enzyme replacement therapy (ERT) with recombinant acid α-glucosidase protein, which can improve cardiac function and increase survival rate (5), has reduced these deaths dramatically (2), patients receiving general anesthesia are still at high risk due to the higher incidence of cardiac arrest and respiratory insufficiency.

Here, we report a female infant with infantile-onset Pompe disease who underwent cheiloplasty under general anesthesia and peripheral nerve blocks. In addition, we applied intensive hemodynamic monitoring using electrical cardiometry technology (ICON®) and pulse contour analysis (FloTrac system) intraoperatively. This study was approved by the Taipei Medical University Joint Institutional Review Board (N202104013). We have obtained the written informed consent from her parents. De-identification was done according to Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule safe harbor method.

CASE DESCRIPTION

This case report describes a 4-month-old full-term Taiwanese female infant weighing 5.5 kg. She was born through spontaneous delivery, and her birth weight was 2,645 g, which was small for the gestational age of 38 weeks. Cleft lip and palate were observed in the patient in the prenatal examination. After birth, she presented symptoms of mild hypotonia without dysphagia or dysarthria. Occasional shortness of breath was noticed (**Figure 1A**). Laboratory data revealed abnormally high aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine kinase (CK), which were 125 U/L (normal range: <32 U/L), 48 U/L (normal range: <33 U/L), and 872 U/L (normal range: 26–192 U/L), respectively. The enzyme activity test of GAA was 0.04 μM/h (normal range: >0.80 μM/h).

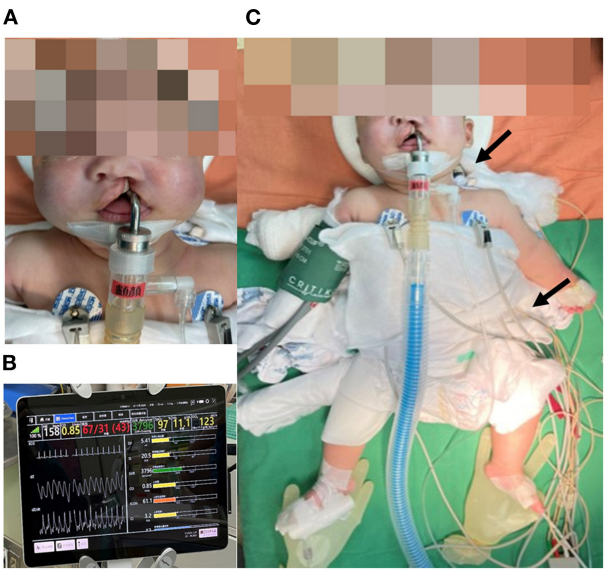

FIGURE 1 | (A) The patient presented with left complete cleft lip and palate. Hypotonia and macroglossia were not observed, which may be related to regular ERT beginning at 15 days old. **(B)** Electrical Cardiometry Technology (ICON®) calculated and provided instant SV, SI, SVR, CO, ICON, and CI during the operation. **(C)** ICON® sensors were located at the left side of the neck and thorax. Arrows represent the sites of electrode patches. ERT, enzyme replacement therapy; SV, stroke volume; SI, stroke volume index; SVR, systemic vascular resistance; CO, cardiac output; ICON, index of contractility; CI, cardiac index.

X-ray showed cardiomegaly (**Supplementary Figure 1A**). Left ventricular mass index (LVMI) was 105.49 g/m$^{2.7}$, which was over the 95th percentile for her age (<6 months old) (6, 7). GAA gene sequencing was performed, and the patient had two pathogenic heterozygous mutations, c.424_440del17 (p.Ser142Leufs*29) and c.1935C>A (p.Asp645Glu). One heterogenous pseudodeficiency mutation, p.Gly576Ser, was also noticed. Her cross-reactive immunological material (CRIM) status was positive linked to c.1935C>A (p.Asp645Glu). All above findings indicated the diagnosis of Pompe disease. She received regular ERT at an initial dosage of 20 mg/kg intravenous infusion every other week beginning at 15 days old until 2 days before surgery. Preoperative echocardiography revealed preserved LV systolic function with minimal tricuspid regurgitation. Patent ductus arteriosus (PDA) and patent foramen ovale (PFO) were closed. LVMI was 65.5 g/m$^{2.7}$, which was in the 75th percentile for her age (<6 months old) (6, 7). Her AST, ALT, and CK were decreased to 56, 33, and 160 U/L before the surgery, respectively.

TABLE 1 | Comparison of hemodynamic parameters between electrical cardiometry technology (ICON) and pulse contour analysis (FloTrac).

Time after anesthesia started (min)	NIBP (mmHg)	ABP (mmHg)	*ICON CO(L/min)	*ICON SVR (dyn·s/cm^5)	*ICON SVV (%)	**FloTrac CO (L/min)	**FloTrac SVR (dyn·s/cm^5)	**FloTrac SVV (%)
45	58/30 (42)	59/28 (39)	0.79	3,842	10	14.9	152	10.3
75	63/34 (47)	66/33 (46)	0.8	3,838	8	15.7	180	21.1
105	63/32 (45)	63/32 (44)	0.81	3,993	9	15.6	174	8.8
135	63/32 (45)	66/32 (44)	0.82	4,013	7	15.6	176	9.6
165	63/31 (44)	67/31 (43)	0.83	3,596	7	15.9	167	6.8
195	62/31 (47)	68/31 (44)	0.84	3,861	8	16.0	173	10.1

NIBP, non-invasive blood pressure; ABP, arterial blood pressure; CO, cardiac output (normal range: 0.82–1.52 L/min); SVR, systemic vascular resistance (normal range: 2,870–5,331 dyn·s/cm^5), SVV, stroke volume variation (normal range: 5–15%). NIBP and ABP are manifested as systolic blood pressure/diastolic blood pressure (mean arterial pressure).

**ICON: electrical cardiometry technology.*

***FloTrac: pulse contour analysis.*

The patient was admitted for scheduled cheiloplasty due to left complete unilateral cleft lip and palate. Upon arriving in the operating room, pulse oximetry, and electrical cardiometry technology (ICON®; Osypka Medical GmbH, Berlin, Germany) (**Figures 1B,C**) were applied before induction. Intravenous induction was performed with thiamylal 30 mg (5.4 mg/kg), cisatracurium 1 mg (0.18 mg/kg), dexamethasone 1 mg (0.18 mg/kg), and atropine 0.05 mg (0.01 mg/kg). Subsequently, a non-invasive blood pressure cuff and electrocardiography were applied. Intubation was successfully performed with Pentax AWS-s100 (PENTAX Medical, Hamburg, Germany; video-assisted laryngoscope) on the first attempt. Additionally, we placed a radial arterial line and FloTrac system (Edwards Lifesciences, Irvine, CA, USA) for hemodynamic monitoring. Peripheral nerve blocks with 0.4125% ropivacaine was performed as a part of combined anesthesia and for perioperative analgesia, including bilateral infraorbital nerve block (1.4 mL on each side), bilateral external nasal nerve block (0.5 mL on each side), and nasopalatine nerve block (0.1 mL). Anesthesia was maintained with sevoflurane 1.8–3.1% and 49–65% oxygen concentration in the air. The initial ventilator setting was inspiratory pressure (P_{Insp}): 13 cm H_2O, positive end-expiratory pressure (PEEP): 0 cm H_2O, respiratory rate (RR): 30 breaths per minute, and inspiratory-to-expiratory time (I:E ratio): 1:1.5. Our goal was to maintain mean arterial pressure (MAP) above 45 mmHg and heart rate (HR) between 90 and 160 bpm. We focused on five parameters of ICON®: index of contractility (ICON), systolic time ratio (STR), thoracic fluid content (TFC), stroke volume variation (SVV), and systemic vascular resistance (SVR). We focused on three parameters of FloTrac: cardiac output (CO), SVV, and SVR. We compared CO, SVV, and SVR between these two hemodynamic monitors (**Table 1**). Due to the age limitation (8), the data from FloTrac deviated from the physiological range and were too inaccurate to reflect the clinical condition.

During early maintenance, we titrated the inhalational gas concentration from 0.7 to 1.0 minimal alveolar concentration (MAC) before the skin incision, and we observed that the hemodynamic parameters on ICON® changed accordingly. ICON and SVR dropped gradually in 1 min, whereas CO increased. However, 3 min later, the decline in ICON and SVR was significant. CO remained at the same level, causing a decrease in arterial blood pressure. Therefore, we cautiously decreased the inhalational gas concentration. Detailed changes in ICON, SVR, and CO are displayed in **Supplementary Figure 2**. Fifty minutes after anesthesia induction, we changed the ventilator setting and increased P_{Insp} from 13 to 15 cm H_2O. Arterial blood pressure dropped soon after. This combined with the observation that SVV was occasionally above the normal range suggested fluid deficiency; therefore, we prescribed bolus normal saline (3 mL) with no apparent improvement. The ventilator setting was adjusted back to the initial setting, and the inhalational gas concentration was slowly titrated to 0.6 MAC. Eighty minutes after anesthesia induction, we also increased the normal saline infusion rate, as SVV was above the normal range and TFC was low; however, MAP was stable and in the normal range. No additional analgesic agent was prescribed during maintenance. At the end of surgery, we prescribed neostigmine 0.125 mg and glycopyrrolate 0.025 mg as neuromuscular blocking agent reverse medication before extubation. Extubation was uneventful with normal respiratory function. She was discharged 4 days later without adverse events.

After 3 months of follow-up, the patient was able to roll over, lie prone on the forearms, and reach out for objects, and she had a social smile. However, she could not maintain a sitting position unassisted, indicating slower development potentially than other infants of the same age. In addition, mild hypotonia was still observed compared with other infants. The girl infant also underwent another surgery in our hospital several months later. Unlike the previous surgery, she was more tolerant of inhalational gas concentration titration, which may represent improved cardiac function under regular ERT. After receiving ERT treatment for half a year, her anti-alglucosidase-α antibody revealed positive. Therefore, hydrocortisone was added in the following ERT courses. Her anti-alglucosidase alfa antibody will be followed in the next few months to see if additional immunosuppressive agents are needed.

DISCUSSION

This case presented several unique aspects. First, the surgery was complex. The entire cheiloplasty procedure took approximately 4 h to complete. Furthermore, no study has described Pompe disease patients receiving cheiloplasty before. In addition, resolving surgical site bleeding and avoiding coughing or straining during emergence from anesthesia and extubation may be difficult in this surgery, and Pompe disease could aggravate these difficulties. Second, we used ICON® as an intensive hemodynamic monitoring method. Few facilities will or are able to perform stress test for cardiac function assessment for infants. As mentioned in our study, these patients might have a normal static cardiac echocardiography report, but are not normal enough to face stress from surgery and general anesthesia. In previous studies, Sanders et al. (9) and Lotfy et al. (10) had suggested ICON® to be a useful trend monitor. Also, with its safe and easy applicable method and comprehensive hemodynamic parameters, it turns out to be a practical hemodynamic monitor. Additionally, a randomized controlled trial (10) recruited 42 pediatric patients who underwent hepatoportoenterostomy, and CO reliability was compared across electrical bioimpedance cardiometer, ICON®, and transoesophageal Doppler (TED), and the results showed that ICON® use was effective intraoperatively. However, due to inadequate sample sizes, more comparative studies are required to provide accurate data and evidence in infants. Third, peripheral nerve blocks were employed as a part of combined anesthesia to provide ideal analgesic effects. Utilizing peripheral nerve block in patients undergoing cleft lip and palate can prevent the patients from complications of respiratory depression and airway obstruction, which is induced by opioid (11, 12). Also, the patients are allowed to wake up pain free, and closure of the suture lines can be maintained (13, 14). In this case, impaired liver function was noted since birth which may result in poor opioid metabolism function. Also, decreased dosage of opioid was suggested by Racca et al. in concern of negative effect to cardiac and respiratory systems (15). The use of peripheral nerve blocks can provide opioid free anesthesia for Pompe disease patients thus create a safe practice. On the other hand, potential risks of peripheral nerve blocks include nerves, vessels injury and local anesthetics overdose (16).

In Pompe disease patients, the diagnostic challenge is that many diseases share similar symptoms and signs of Pompe disease. Diseases such as hypothyroidism, congenital muscular dystrophy, and other glycogen storage diseases, are needed to be differentiated. And the final diagnosis of Pompe disease was made due to laboratory data, gene sequence, and the organs being influenced (cardiac, liver, and muscle) (1).

Previously reported cases of infantile-onset Pompe disease patients who received general anesthesia were listed in **Table 2**. We performed a database search of PubMed®, using keywords of Pompe disease and anesthesia. Reference lists were also manually searched to include all the related studies. In these studies, most underwent a short procedure. Thirteen patients underwent central venous catheter insertion or muscle biopsy, and the remaining three patients underwent elective intubation, bronchoscopy, and bilateral inguinal hernia repair. Only 2 of the 16 patients received ERT, but bradycardia with wide QRS complexes and non-sustained ventricular tachycardia were still reported after induction and during the early phase of maintenance anesthesia, respectively. Among the 14 patients without ERT, 10 had complications such as bradycardia, torsade de pointes, ventricular fibrillation, and cardiac arrest. None of these patients involved intensive intraoperative hemodynamic monitoring methods.

The cause of arrhythmia of infantile-onset Pompe disease patients may be due to the intracardiac accumulation of glycogen inducing progressive hypertrophic cardiomyopathy and diastolic dysfunction. Therefore, venous return is obstructed and cardiac output decreases. Besides, certain anesthetics cause significant decrease in systemic vascular resistance or diastolic blood pressure, which result in decreased coronary perfusion and arrhythmia (18). Early diagnosis and enzyme replacement therapy resulted in inapparent cardiac dysfunction in our case, which prevented arrhythmia from occurring. During the whole procedure, no arrhythmia episode was presented in our case. However, mild TR in echocardiography and lower tolerance to anesthetics intraoperatively were still observed. Other concern in intubation was due to macroglossia in Pompe disease patients, but this phenomenon was not obvious in our case. With the help of video-assisted laryngoscope, intubation underwent smoothly. Regarding to atony in Pompe disease patients, we reduced the dose of muscle relaxant and also contacted intensive care unit for the risk of confronting difficult extubation. Finally, no related problem was confronted.

It might be possible for the Pompe disease girl infant to receive the surgery later, after receiving more courses of enzyme replacement therapy. However, many studies suggested to perform primary cleft lip repair between the ages of 3 and 6 months since it is proper timing, allows subsequent treatments to perform and also for better establishing lip competence (20, 21). Concerns of early surgical intervention in high-risk patients may be raised; however, with early enzyme replacement therapy in our case, cardiac and other organ functions seem to be tolerable to surgical stress and anesthetics.

Thiamylal, atropine, and cisatracurium were administered as induction agents and initially maintained under 2.2% sevoflurane. The selected hypnotic was thiamylal, which reduces SVR but increases the heart rate and is theoretically able to maintain CO. However, the use of barbiturates as intravenous induction agents is uncommon in the literature. Cisatracurium was selected as the neuromuscular blocking agent, as it is metabolized through Hoffman elimination and is thus unrelated to liver and renal function. Suxamethonium was not considered due to the associated risks of rhabdomyolysis and hyperkalemia in Pompe disease patients (19). Pompe disease, as a kind of metabolic myopathy, safe anesthesia in suspected myopathy suggested by Trevisan et al. (22) should be considered due to the risk of malignant hyperthermia. Although few events had been reported (23), additional attention should be paid to the changes of body temperature and end-tidal carbon dioxide ($EtCO_2$) if halogenated inhalational anesthetics was chosen. After anesthesia induction, we utilized peripheral nerve blocks as additional analgesia. Only two studies

TABLE 2 | Summary of reported cases of infantile-onset Pompe disease patients who underwent general anesthesia.

References	Age	LV mass index (g/m^2)	ERT (+/− −)	Procedure	Agents	Complications
DeSena et al. (17)	3 m	N/A	− −	Central venous catheter insertion	Sevoflurane, vecuronium	Ventricular fibrillation after vecuronium infusion
Wang et al. (18)	23 m	125	+ (every other week from age 6 m to 17 m)	Elective intubation	Thiopental, succinylcholine, vecuronium	Bradycardia with wide QRS complexes and variable atrioventricular conduction after induction agent infusion
	2 y	76.8	+	Bronchoscopy, stomaplasty	Ketamine, sevoflurane, succinylcholine	Paroxysmal supraventricular tachycardia followed by nonsustained ventricular tachycardia under 2.4% sevoflurane (5 min after induction)
	8 m	704	− −	Central venous catheter insertion	Propofol, fentanyl	Bradycardia and ventricular fibrillation after intubation
	14 d	59.3	− −	Central venous catheter insertion	Propofol	Bradycardia (−21% to −31% from baseline) after propofol infusion
	4 m	191	− −	Central venous catheter insertion, muscle biopsy	Sevoflurane, propofol, 40% nitrous oxide	Bradycardia, desaturation, and ventricular fibrillation after propofol infusion for maintenance
	5 m	446	− −	Central venous catheter insertion, skin biopsy	Sevoflurane, propofol, nitrous oxide, rocuronium	Torsade de pointes VT on 2% sevoflurane in nitrous oxygen and oxygen maintenance (16 min after induction)
	2 m	253 (examined after this episode)	− −	Bilateral inguinal hernia repair	Sevoflurane	Ventricular fibrillation after sevoflurane induction
	2 m	233	− −	Muscle biopsy	Sevoflurane, nitrous oxide	Ventricular fibrillation and ventricular tachycardia after sevoflurane and nitrous oxygen induction
	8 m	363	− −	Muscle biopsy, percutaneous gastrostomy, tunneled venous catheter placement	Etomidate, fentanyl, rocuronium	Hypotension and ventricular fibrillation under sevoflurane and nitrous oxide (14 min after induction)
Ing et al. (19)	5.2 ± 3 m	366	− −	Central venous catheter insertion, muscle biopsy	Thiopental, sevoflurane, fentanyl, rocuronium	None
	5.2 ± 3 m	191	− −	Central venous catheter insertion, muscle biopsy	Sevoflurane, nitrous oxide, propofol, rocuronium	Cardiac arrest under continuous maintenance infusion of propofol and 40% nitrous oxide in oxygen (shortly after induction)
	5.2 ± 3 m	240	− −	Central venous catheter insertion, muscle biopsy	Sevoflurane, nitrous oxide, fentanyl, rocuronium	None
	5.2 ± 3 m	362	− −	Central venous catheter insertion, muscle biopsy	Ketamine, nitrous oxide, sevoflurane, fentanyl, rocuronium	None
	5.2 ± 3 m	221	− −	Central venous catheter insertion, muscle biopsy	Ketamine, nitrous oxide, fentanyl, rocuronium	None
McFarlane and Soni (4)	5 m	N/A	− −	Hickman line insertion, marrow aspiration, liver biopsy, muscle biopsy	Nitrous oxide, halothane 2%, suxamethonium	Bradycardia and cardiac arrest after increasing halothane concentration and intubation

LV, left ventricular; ERT, enzyme replacement therapy; N/A, data not found in the literature; y, years of age; m, months of age; d, days of age.

have reported on infantile-onset Pompe disease patients who underwent muscle biopsy using peripheral nerve blocks but were without endotracheal intubation (16, 24). In our case, the patient received cheiloplasty, which requires general anesthesia. Sevoflurane is generally used in children with Pompe disease due to its advantage of rapid emergence. However, it can also simultaneously decrease SVR and myocardium contractility, thereby decreasing coronary perfusion pressure (17). Therefore, with the help of peripheral nerve blocks, the concentration of sevoflurane can be decreased, lowering the risk of inadequate coronary perfusion and arrhythmia.

We further analyzed the data of ICON, ventilator settings and the sevoflurane concentration using the Pearson correlation coefficient. A negative correlation between ICON and peak inspiratory pressure was observed ($R = -0.212$, $p = 0.0026$), while a positive correlation between ICON and inhalational gas concentration was noted ($R = 0.2732$, $p < 0.0001$). However, **Figure 2A** demonstrates that an increased inhalational gas concentration resulted in a drop of ICON value instead of a positive elevation. The positive correlation between ICON and inhalational gas concentration should be interpreted carefully. Because when icon decreased, and blood pressure consequently dropped, we tended to adjust the inhalational gas to a lower concentration. The positive correlation might be explained by our management rather than reflecting the actual relationship between these 2 parameters. Generally, an increase in peak inspiratory pressure and inhalational gas concentration would induce a drop in ICON; however, the titration of inhalational gas preserved ICON and CO at first. Later, an acute drop in ICON occurred, accompanied by a compensatory decrease in SVR and an increase in CO. Although sevoflurane is thought to be a safe anesthetic in Pompe disease patients, we should still keep concentrations low to prevent hypotension (18). One case report concluded that the increased risk of arrhythmia in patients with infantile-onset Pompe disease is likely related to a simultaneous decrease in SVR and myocardium contractility, decreasing coronary artery perfusion pressure (17). According to Shekerdemian et al. (25), positive pressure ventilation has been proved to reduce cardiac output because of reduced right ventricular filling. During inspiration phase of positive pressure ventilation, intrathoracic pressure increased results in elevated right atrium pressure, and eventually forms a decreased right ventricular filling and cardiac output. What's more, pediatric patients have a lower chest wall compliance compared to adult patients, so more intrathoracic pressure is needed to expand the same volume of chest cage in pediatric patients, which contributing to greater degree of cardiac output reduction. This phenomenon was also observed clinically by Gullberg et al. (26, 27) that the decreased mean airway pressure would increase cardiac output in neonates and infants. Therefore, in addition to minimizing the risk at induction and early maintenance related to anesthetic agents, extra attention must be focused on setting a suitable anesthetic depth and inspiratory pressure. We did not use electroencephalography (EEG) or alternative monitors such as entropy to evaluate her central nervous system function. This is because those monitors would interfere with the surgeon and contaminated the surgical field. No neurologist's visit was arranged preoperatively or postoperatively due to absence of newly onset neurological symptoms. However, the sequential performance of EEGs before and after the head and neck surgery could be taken into consideration as in cardiac surgery to exclude the potential embolus or small cerebral stroke in infant (28).

We also noticed a correlation between two contractility parameters in ICON®: STR and ICON. STR equates to the pre-ejection period (PEP) divided by the left ventricular ejection time (LVET). Theoretically, low STR correlates with high ejection fraction. ICON represents the acceleration of the blood in the aorta. After analysis, we found that STR was related to ICON. Moreover, STR could react faster than ICON when a large variation occurred (**Figure 2B**; to exhibit the same trend of changes, the data is presented as 1/ICON and STR). A possible explanation for this phenomenon is that STR is related to ejection fraction (EF), but ICON is related to blood speed and acceleration. Hence, once cardiac suppression occurs, STR will reflect this event faster than ICON. However, the accuracy of STR is dependent on adequate fluid status in patients. In circumstances of inadequate fluid status, ICON may still be more reliable.

Fluid is another factor influencing perfusion, and we monitored fluid status through the SVV and TFC parameters of ICON® in this case. Mahmoud et al. (29) reported that TFC could be used to guide the fluid removal rate and amount in patients undergoing hemodialysis. The results showed that compared with the hemodynamically stable group, the hypotension group had lower TFC, indicating that they were hypovolemic. In our case, considering that SVV was more than the normal range and that TFC was lower initially, hypovolemia was potentially present. However, the infant's MAP could be compensated within a relatively stable range, which indicates that this infant's fluid supplement could not be guided by mean arterial pressure (**Figure 2C**).

Once Pompe disease is suspected, genetic screening, laboratory tests, electrocardiography, and image surveys should be conducted immediately. ERT should also be arranged as soon as the diagnosis has been made. Recent studies have revealed that with ERT intervention to prevent hypertrophic cardiomyopathy and a thorough preoperative evaluation, anesthesia management is much safer in infantile-onset Pompe disease patients (2, 17). A previous study reported a 13-year-old girl with juvenile Pompe disease who received ERT and underwent kyphoscoliosis corrective surgery (23). Fluid maintenance involved 450 mL of blood and 2.3 L of a lactated ringer under central venous pressure guidance, and the estimated blood loss was 1.1 L, which seemed to be acceptable in the general population. However, the patient experienced pleural effusion after surgery. One review article also noticed different types of arrhythmias occurred in some infantile-onset Pompe disease patients receiving ERT (30). Therefore, despite adequate ERT, mild degeneration in cardiac function can still occur. In our case, a similar condition was recorded. The patient was more sensitive to sevoflurane and changes in peak inspiratory pressure compared with other children, as a higher concentration of sevoflurane and a higher peak inspiratory pressure resulted in an apparent drop in ICON (**Figure 2A**) and, consequently, stroke volume index (SI). Otherwise, even

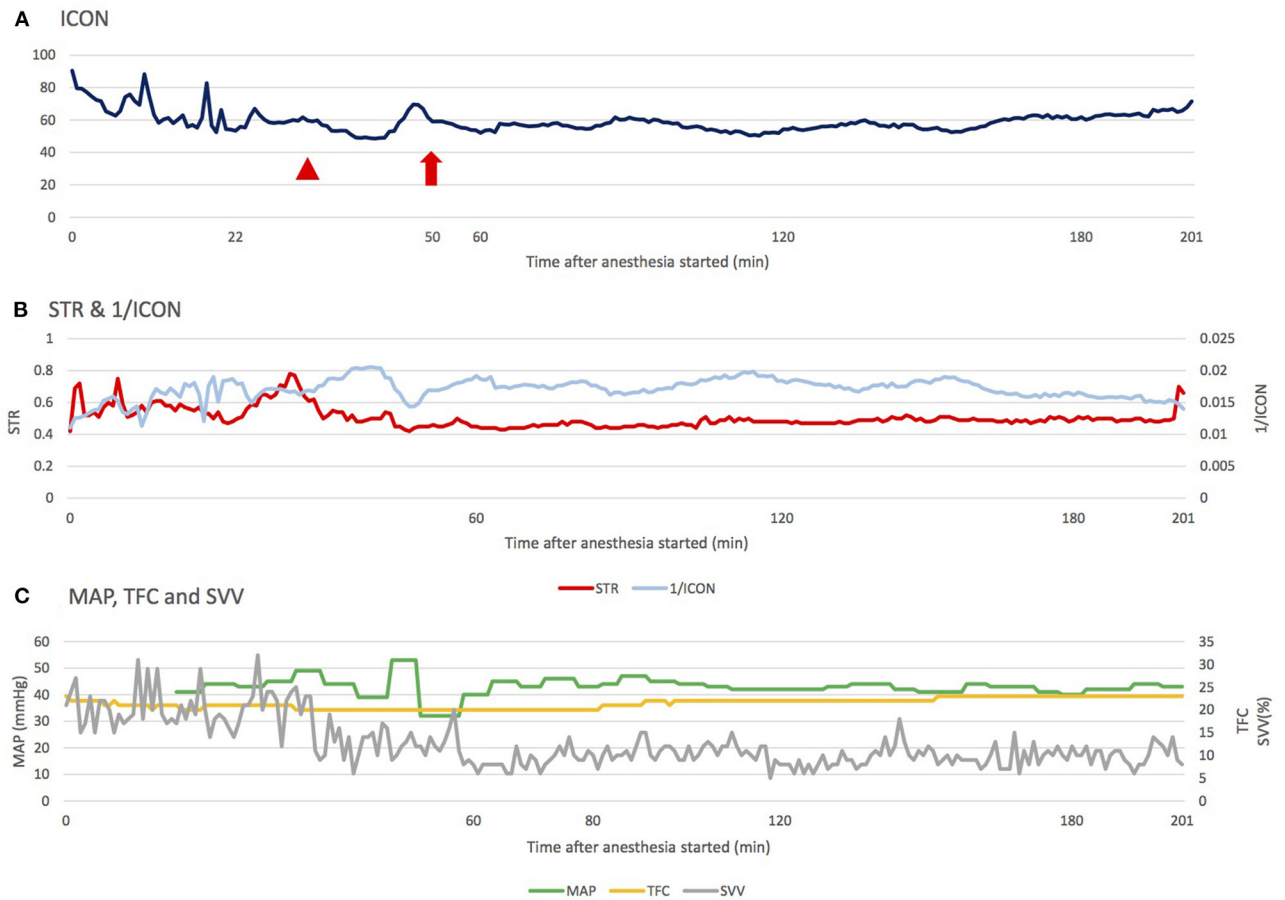

FIGURE 2 | The changes of ICON, STR, MAP, TFC, and SVV during the operation. **(A)** During maintenance, ICON was mainly affected by the setting of inhalational agent concentration and inspiratory pressure in the ventilator. The arrowhead indicates the increased sevoflurane concentration before incision; the arrow indicates the alteration of ventilator setting: P_{Insp} from 11 to 13 cm H_2O, respiratory rate from 30 to 28 breaths per minute (normal range of ICON: 45.0–75.0). **(B)** Changes in STR and 1/ICON were compatible, and STR seemed to react earlier, providing anesthesiologists a faster reference to adjust the anesthesia. **(C)** Fluid is another factor influencing perfusion. Regardless of the trend of change in TFC and SVV, MAP was able to compensate in a relatively stable range (Normal range of TFC: 25–35, SVV: 5–15%). ICON, index of contractility; STR, systolic time ratio; TFC, thoracic fluid content; MAP, mean arterial pressure; P_{Insp}, inspiratory pressure.

in the era of ERT, there were still some limitations in treating Pompe disease, such as great expense, immune response due to high amounts of exogenous enzyme, progression of muscle weakness even responding well to ERT initially (30), limited effects to central nervous system due to blood brain barrier (31), and white matter abnormalities/ventricular enlargement in brain MRI (32). Some alternative therapies have been developed, like gene therapy (33, 34), enzyme enhancement therapy (35), and substrate reduction therapy (36). Further studies were required to determine the benefits of these treatments in decreasing perioperative risks for Pompe disease.

Our study has some limitations. First, we lacked strong evidence to clarify and support the accuracy of ICON® intraoperatively in infants. More comparative studies may be needed to prove its use and validity in infants. Second, ICON® depends on the theory of electrical bioimpedance cardiometry, which is interfered by electrocautery. In our case, ∼10% of the data were of low quality and therefore excluded. Third, since the perioperative complication rate has dropped dramatically after the spread of ERT, cases with cardiac complications which we mentioned above were not fully comparable with current cases.

CONCLUSION

The recent consensus suggests that early diagnosis and treatment with ERT can improve hemodynamic stability. However, with regular ERT, lower tolerance to anesthetic agents was still noted in this case. Peripheral nerve blocks can reduce the required dose of analgesic agents and make the processes of surgery and emergence safer and faster. For infants with existing cardiac pathophysiologic changes, ICON® may be considered, as the trend of hemodynamic parameters can facilitate the advanced identification of potential problems, especially STR. Future studies should focus on ICON® in infant perioperative monitoring to validate the safety and multiple parameters in this non-invasive hemodynamic monitoring method.

DATA AVAILABILITY STATEMENT

The raw data presented in the study are deposited in figshare repository. doi: 10.6084/m9.figshare.16955032.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Taipei Medical University-Joint Institutional Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

M-CL and H-CT had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. D-MN helped us in acquisition of relevant data. All authors made substantial contributions to the study conception and acquisition of data, or analysis and interpretation of data, involved in drafting the article or revising it critically for important intellectual content, and approved the final version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.729824/full#supplementary-material

Supplementary Figure 1 | (A) The girl infant's X-ray at 16 days old, which revealed cardiomegaly. **(B)** The electrocardiography of the girl infant at 15 days old, which revealed sinus tachycardia compared with other infants at the same age. Heart rate: 188 beats per minute; RR interval: 339 milliseconds (ms); PR interval: 56 ms; QRS duration: 99 ms; QT interval: 255 ms.

Supplementary Figure 2 | Inhalational gas concentration was titrated from 0.7 to 1.0 MAC before skin incision (red arrows indicate the time of titration). ICON and SVR dropped gradually in 1 min, and CO increased. However, ICON and SVR dropped significantly 3 min later. By contrast, CO remained at the same level. MAC, minimal alveolar concentration; ICON, index of contractility; SVR, systemic vascular resistance; CO, cardiac output.

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Self-Consciousness of Appearance in Chinese Patients With Cleft Lip: Validation of the Chinese Derriford Appearance Scale 59 (DAS 59) Instrument

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Objective: To develop a reliable and valid Chinese version of the Derriford Appearance Scale 59 (DAS 59) instrument for assessing the self-consciousness of appearance in Chinese patients with cleft lip.

Methods: The original DAS 59 instrument was translated into Mandarin, back-translated, and culturally adapted among the Chinese population, following the protocol of the original DAS 59. The validation of the Chinese DAS 59 instrument was estimated on 443 adult participants including 213 subjects with a history of cleft lip with/without palate (CL/P, study group) and 230 normal subjects without facial appearance concern (control group). The reliability was estimated by Cronbach's α coefficient and Guttman's split-half coefficient. Content validity was tested using the Spearman correlation coefficient, while discriminant validity was tested by the Mann–Whitney U test.

Results: The overall internal consistency of Chinese DAS 59 was excellent; Cronbach's α was 0.951 (α = 0.965 and 0.959 in the study and control groups, respectively). Further, Guttman's split-half coefficient was excellent in the study group (0.935) and control group (0.901). The validity of content was good with an acceptable correlation between all the items and domains. The construct validity through the discrimination was good with a statistically significant difference in most domains between the two groups. Patients with CL/P had more concern about the general self-consciousness and social self-consciousness of appearance. They also showed a good self-concept score.

Conclusion: The Chinese version of DAS 59 demonstrated acceptable reliability and good construct and discriminant validity. It can be used for the research and assessment of the psychological state and quality of life for Chinese patients with cleft lip as well as other appearance problems and concerns.

Keywords: Derriford Appearance Scale, reliability, quality of life, validation, cleft lip

INTRODUCTION

Cleft lip with/without palate (CL/P) is a common congenital developmental malformation of the oral and maxillofacial region, accompanied by severe physical defects and various psychological problems (1). The psychological state of CL/P patients can be different from that of normal people (2). Hence, psychological intervention is also a key part of CL/P sequential therapy, which shows a crucial role in the treatment and quality of life (QoL) of patients with CL/P (3).

Physical attractiveness is commonly estimated as a prediction for the individual character and is esteemed independent of other traits. Consequently, physical beauty could affect upon how others see people, with the end goal that alluring people get special treatment during youth and adulthood in most social circumstances. Disfigurement and deformity caused by congenital malformations, diseases, trauma, and burns in addition to their treatments lay apparently various individuals at a social drawback with a danger of heavy trouble and social brokenness (4–6). Therefore, the psychological aspects of deformity should be estimated well, as regular social communication for those with appearance issues is a foundation of unalleviated stress, anxiety, and pain. Moreover, it can be used with surgical interventions to test variations from baseline to post-operation stage, like the benefit inferred from pre- and post-operative photographs (7).

The primary objective of CL/P treatment is to improve appearance and function to get better QoL. To decide if CL/P therapy achieves its proposed target, investigations more often depend on estimating and depicting the professionally reported results, for example, the clinical marks of surgical competence or aesthetics dependent on expert perception (8, 9). Estimating clinician-reported results is critical to set up the clinical adequacy of treatment and guarantee beneficial practice. Nonetheless, relying on clinician-reported results implies significantly less familiarity with the patient-reported results of repair at the end of cleft treatment pathway (10, 11). As of late, there has been a more prominent acknowledgment of the demand concerning the patient's perception in deciding the genuine result of surgery after the treatment of patients with oral cleft (8, 12). However, there is still a lack of comprehensive, valid, and reliable tools that could help in understanding the psychological status of patients with CL/P.

In the past, the investigation regarding this scope was restricted by the lack of a suitable effect measure to estimate individual fulfillment and health-associated QoL. Later on, various psychometric instruments have been developed to evaluate the psychological influence and alteration of manifestation such as the Appearance Schemas Inventory (13), Body Image Avoidance Questionnaire (14), and Body Dysmorphic Disorder Examination (15); however, most of them were criticized for weak content validity, a limited range of applicability, or restricted psychometric development (7). Most of the aforementioned tools did not develop obviously to estimate the range of symptomatology that is applicable to the wide scope of hardships experienced by individuals living with issues of appearance. Thereby, such measures reported lower response to the nature of the dysfunctions and the seriousness of the misery that the involved individuals experience (7).

There is a demand for the establishment and validation of an instrument to estimate patient-reported outcomes in terms of satisfaction, psychosocial prosperity, and health-associated QoL in Chinese patients with cleft lip. Among the patient-reported outcome instruments, the CLEFT-Q is an instrument that was developed internationally for children and young adults with CLP (16, 17). It includes 12 independently functioning scales that evaluate different concerns in terms of appearance, facial function (speech), and health-related QoL. The Derriford Appearance Scale (DAS) was designed by Carr et al. (7) to measure the psychosocial adjustment in patients with appearance issue. The original DAS had shown sustainable reliability and validity and had been utilized in a wide range of populations, including those who had (no) appearance concern in both general and clinical individuals. Two forms of DAS had been developed, one with 24 items, which is mainly composed for daily application in clinical work and another with 59 items, which is intended for research and deep estimation (18, 19). The DAS had also exhibited good psychometric features when translated and approved into different cultural nations (20–23).

Prior to applying any psychometric measure to various cultural group settings, it ought to be translated, approved, and adjusted to regional cultural and social requirements. Thus, the present study was sought to establish a valid and reliable Chinese version of the Derriford Appearance Scale 59 (DAS 59) instrument for assessing the self-consciousness of appearance in Chinese patients with cleft lip.

MATERIALS AND METHODS

Translation of DAS 59 Instrument

The Chinese translation of the DAS 59 instrument was conducted according to the protocol mentioned in the original DAS (7). A native Chinese-speaking surgeon, who was additionally familiar with English, translated the DAS 59 tool into Chinese. A board of Chinese mother-tongue specialists was gathered, which involved five surgeons who lived in an English-native country for more than 1 year. The committee talked about some disputable words utilized in the translation. A backward translation of the Chinese DAS 59 tool into English was conducted by an individual with experience in English linguistics who was not aware with the health-related quality of life (HRQoL) inventories. A specialist board-checked the backward-translated English edition and reconciled it again with the first English one. The last Chinese DAS 59 tool was pretested with a suitable group of patients with/without appearance concerns (40 patients with CL/P and 40 normal subjects) to confirm the readability. After a minor adjustment by the board, the final Chinese DAS 59 tool was developed.

The Chinese DAS 59 Instrument

Similar to the original DAS 59 (7), the Chinese DAS 59 is formulated as a sequence of 59 statements and questions with reply categories in a Likert format from 1 to 5 to assess the frequency of symptoms (1, almost never.... 5, almost always)

and levels of distress (1, not at all distressed.... 5, extremely distressed). It is intended for use in subjects aged ≥16 years. An introductory part gathers the features of appearance aspect to which the respondent raises most sensitivity. This is meant as the respondent "feature" in the body of the instrument. The instrument also detects any other aspects of appearance to which the person might also have distresses. A total of 57 items assess the range of psychological discomfort and dysfunction, and 2 items assess physical distress and physical dysfunction. The instrument was developed to be utilized by clinical and scientific experts from relevant fields of plastic surgery, dermatology, clinical psychology, and psychiatry.

Simple and concise guidelines are given on how to accomplish the DAS 59, which is composed as a self-report instrument to be finished without others' intervention. The DAS 59 creates six aspects of psychological discomfort and dysfunction (total score and five domain scores) in addition to an aspect of physical discomfort and dysfunction. The five domains are (1) General self-consciousness of appearance (GSC); (2) Social self-consciousness of appearance (SSC); (3) Sexual and body self-consciousness of appearance (SBSC); (4) Negative self-concept (NSC); and (5) Facial self-consciousness of appearance (FSC). The greater the scale, the higher the severity of discomfort and dysfunction. Total scale and domain scores are achieved by adding the scores of independent items as indicated by the instructions presented in a guide that supplies the original DAS 59 (24).

Sample Size Calculation

The required sample for internal consistency of the Cronbach's alpha was computed by utilizing Bonnett's formula (25) with an alpha of 0.05 and a power of 90%, 133 participants would be required. Ethical approval was obtained from the ethical committee of West China Hospital of Stomatology, Sichuan University (No. WCHSIRB-D-2016-084R1), and the guidelines from the Declaration of Helsinki were followed.

Study Participants

A total of 218 adult patients with a history of CL/P who visited the center of cleft lip and palate, West China Hospital of Stomatology, Sichuan University from March 2018 to October 2019 were enrolled as a study group. A control group of 230 normal adults who did not have appearance-related concerns and were free of any previous history of cosmetic-intended surgical interventions were conveniently enrolled from outpatient clinics in the same area of the study. Therefore, the Chinese DAS 59 instrument was delivered to a total of 448 participants after being instructed about the objective of the project and singed an informed consent form. The participants completed the Chinese DAS 59 independently within 15 min under the guidance of an assistant who did not interfere with the privacy of the subjects. Finally, there were 443 participants who completed the instrument, accounting for 98.9% of delivered reports; 213 in the study group (129 males and 84 females) aged between 16 and 64 years (mean age = 22.53 ± 7.87 years) and 230 in the control group (96 males and 134 females) aged between 18 and 65 years (mean age = 28.47 ± 10.42 years).

TABLE 1 | Reliability and internal consistency of Chinese DAS 59 using Cronbach's alpha.

Domain	Item no.	Study group	Control group	Overall
General self-consciousness of appearance	17	0.942	0.891	0.923
Social self-consciousness of appearance	20	0.940	0.909	0.928
Sexual and bodily self-consciousness of appearance	9	0.775	0.780	0.774
Negative self-concept	5	0.876	0.846	0.885
Facial self-consciousness of appearance	4	0.750	0.686	0.723
Physics	2	0.716	0.916	0.798
No factor	2	0.507	0.457	0.479
Total	59	0.965	0.959	0.961

Statistical Analysis

IBM SPSS version 25 (IBM Corp., Armonk, NY, USA) was utilized to do the analysis. The reliability of the DAS 59 instrument was tested by the Cronbach's alpha coefficient and Guttman's split-half coefficient. It was regarded sustainable when the alpha was ≥0.70 and satisfactory for those ≥0.60 (26). The validity of content was tested by estimating the correlations between the items and also the domains using the Spearman correlation coefficient. Discriminant validity was evaluated by assessing the variations between the two groups using the Mann–Whitney U test. The reports were not considered if there are two or more missing values. If a report was presented with one missed value, such value was compensated by the mean score of the relevant item (26).

RESULTS

Reliability

The Chinese DAS 59 instrument revealed excellent reliability and internal consistency as demonstrated by a total excellent Cronbach's alpha coefficient (0.961, **Table 1**) and Guttman's split-half coefficient (0.931, **Table 2**). The five main domains of DAS 59: GSC, SSC, SBSC, NSC, and FSC, have revealed good reliability as revealed by the total Cronbach's alpha values of 0.923, 0.928, 0.774, 0.885, and 0.723, respectively.

The Guttman's split-half coefficient also confirmed the good reliability of these five main domains as shown in **Table 2**.

Validity

The face validity of Chinese DAS 59 was good as confirmed by the expert committee and was further confirmed by a review of literature. The content validity was also good as revealed by the significant correlation between almost all the items and domains (**Table 3**). Discriminant validity was proven by the significant variations between the study and control groups (**Table 4**).

TABLE 2 | Reliability and internal consistency of Chinese DAS 59 using Guttman's split-half coefficient.

Domain	Item no.	Study group	Control group	Overall
GSC	9	0.909	0.819	0.879
SSC	10	0.898	0.835	0.874
SBSC	5	0.731	0.660	0.708
NSC	3	0.842	0.789	0.860
FSC	2	0.744	0.678	0.711
Physics	1	0.716	0.916	0.798
No factor	1	0.507	0.457	0.479
Total	31	0.935	0.901	0.913

TABLE 3 | Spearman's correlation coefficient between domains of Chinese DAS 59.

Domain	GSC	SSC	SBSC	NSC	FSC	Physics	No factor
GSC	1.000	0.869**	0.784**	0.127**	0.635**	0.616**	0.632**
SSC	0.869**	1.000	0.803**	0.077	0.710**	0.637**	0.620**
SBSC	0.784**	0.803**	1.000	0.047	0.781**	0.637**	0.587**
NSC	0.127**	0.077	0.047	1.000	0.023	0.069	0.068
FSC	0.635**	0.710**	0.781**	0.023	1.000	0.566**	0.502**
Physics	0.616**	0.637**	0.637**	0.069	0.566**	1.000	0.457**
No factor	0.632**	0.620**	0.587**	0.068	0.502**	0.457**	1.000

** *Correlation is significant at the 0.01 level (two-tailed).*

TABLE 4 | Discriminant validity of Chinese DAS 59.

Domain	*P*-value
GSC	0.684
SSC	0.025
SBSC	0.002
NSC	0.000
FSC	0.000
Total score	0.638

Statistical significance at 0.05 level.

TABLE 5 | Total and domain scores of DAS 59 scale in patients with cleft lip.

Domain	Min	Q1	Q2	Q3	Max	Mean (SD)
GSC	17	30	41	52	83	41.8 (14.7)
SSC	20	27	39	54	85	41.1 (16)
SBSC	9	13.5	19	24	41	19.5 (7.4)
NSC	5	13	16	20	25	16.1 (5)
FSC	4	4.5	7	10	18	7.8 (3.4)
Physics	2	2	3	5	10	3.7 (1.9)
No factor	2	3	4	6	10	4.3 (1.8)
Total score	59	103	130	164.5	244	134.3 (40.8)

Self-Consciousness of Appearance in Patients With Cleft Lip

Tables 5, **6** show the scores of DAS 59 responses for patients with cleft lip. Overall, patients with cleft lip have shown high concerns regarding their appearance, particularly in terms of the general self-consciousness of appearance and social self-consciousness of appearance. On the other hand, they had shown a good degree of positive self-concept.

DISCUSSION

More recently, the QoL has turned into a valuable parameter in evaluating the viability of clinical interventions. An evaluation of the results relevant to QoL is critical in aesthetic surgery, like CL/P treatment, since patient satisfaction is the transcendent element by which achievement is characterized. Until a later time, it was hard to validate these disputes in an objective design. The previous period, nonetheless, has realized a blast of concern in QoL evaluation devices as a substitute tool for generally profiting from health interventions (27). A progression of tools relevant to health-associated QoL are presently accessible; a large portion of them are instruments with non-specific expressions, not explicitly made for subjects going through aesthetic surgery, and they might misjudge specific impacts of body modifications coming from these interventions.

In the modern time, investigations have started to consider the construction of self-perception and its connection to corrective clinical treatments (28). Experimental proof reflected by some developing studies proposes that aesthetic patients reveal self-perception disappointment at baseline and improvements in self-perception after the operation. Estimating patient-centered outcomes have become progressively critical in aesthetic and reconstructive surgical procedures.

The DAS is a tool developed to precisely and reliably assess the variety of the QoL after cosmetic and reconstructive surgical interventions. The DAS was particularly intended to assess the psychosocial change in individuals who have reported cosmetic issues. This instrument has shown sustainable reliability and validity and had been used in a wide range of people, including those with/without appearance concerns.

The present study describes the translation and validation of the Chinese form of the DAS 59 instrument and its application to patients with CL/P. The translation procedure was performed, following the protocol mentioned in the original DAS. The Chinese DAS enrolled a clinical population represented by cleft lip patients and normal population without appearance concerns. The total internal consistency was excellent ($\alpha = 0.96$) and was quite close to the alpha value reported in the original DAS 59 (7). All the items have demonstrated good correlation, revealing a good homogeneity. Further, domains also exhibited sustainable internal consistency and correlation.

The construct validity of the Chinese DAS 59 was tested through discrimination between the involved groups. Discriminant validity was confirmed by significant variations among the CL/P subjects and normal population. In this context, the social self-consciousness of appearance, sexual

TABLE 6 | Score of domain's items of DAS 59 scale in patients with cleft lip.

Domain's items	Min	Q1	Q2	Q3	Max	Mean (SD)
General self-consciousness of appearance						
1. Self-consciousness of "feature"	1	3	3	4	5	3.1 (1.1)
8. Taking a special interest in others' "features"	1	1.5	3	3	5	2.5 (1.1)
10. Avoiding photography	1	2	3	4	5	2.8 (1.2)
12. Being hurt by others' comments	1	2	3	4	5	3 (1.3)
15. Raising subject of the "feature" in conversation before others do	0	1	2	3	5	2.3 (1.1)
17. Being irritable at home	1	1	2	3	5	2.3 (1.1)
27. Feel unattractive	1	1	2	3	5	2.4 (1.2)
28. Feel unlovable	1	1	2	3	5	2 (1.1)
30. Feel embarrassed	1	1	2	3	5	2.3 (1.2)
31. Feel inferior	1	1	2	3	5	2.5 (1.3)
34. Distress when others stare	1	1	2	3	5	2.3 (1.2)
35. Distress when others make remarks	1	1	3	3	5	2.6 (1.3)
36. Distress when others ask about the "feature"	1	1	3	3	5	2.6 (1.3)
38. Distress when seen in a particular view	1	1	2	3	5	2.5 (1.3)
41. Distress when "feature" seen in a mirror/window	1	1	2	3	5	2.3 (1.3)
42. Distress when meeting strangers	1	1	2	3	5	2.2 (1.2)
58. How hurt do you feel?	1	1	2	3	5	2.3 (1.1)
Social self-consciousness of appearance						
2. Avoiding children in the street	1	1	2	3	5	2 (1.2)
3. Difficulty making friends	1	1	2	3	5	2.3 (1.3)
5. Avoiding school/college/work	1	1	1	3	5	2 (1.2)
6. Avoiding pubs/restaurants	1	1	1	3	5	1.8 (1.1)
7. Avoiding parties/discos	1	1	2	3	5	2.2 (1.3)
13. Avoiding department stores	1	1	1	3	5	1.9 (1.1)
14. Avoid leaving the house	1	1	2	3	5	2.3 (1.2)
16. Closing into a shell	1	1	2	3	5	2.1 (1.2)
18. Being misjudged	1	1	3	3	5	2.5 (1.2)
19. Previous avoidance of school/college/work	0	1	1	3	5	1.9 (1.2)
20. Feeling an embarrassment to friends	1	1	2	3	5	2.1 (1.1)
21. Feeling a freak	1	1	2	3	5	2 (1.2)
22. Worrying about sanity	1	1	1	2	5	1.7 (1.1)
29. Feel isolated	1	1	2	3	5	2.4 (1.3)
32. Feel rejected	1	1	2	3	5	2.3 (1.2)
33. Feel useless	1	1	2	3	5	1.9 (1.1)
39. Distress when going to school/college/work	1	1	2	3	5	2.2 (1.2)
40. Distress when on public transport	1	1	1	3	5	1.9 (1.1)
47. Distress when not being able to go to social events	1	1	2	3	5	2 (1.2)
50. Distress when not being able to go to pubs/restaurants	1	1	1	3	5	1.8 (1.1)
Sexual and bodily self-consciousness of appearance						
4. Avoiding undressing in front of partner	1	1.5	3	3.5	5	2.7 (1.3)
9. Avoiding communal changing rooms	1	1	3	3	5	2.4 (1.2)
23. Adverse effect on sex life	1	1	2	3	5	2 (1.2)
24. Adverse effect on marriage	1	1	2	3	5	2.2 (1.3)
37. Distress when going to the beach	1	1	2	3	5	2.1 (1.2)
43. Distress from being unable to wear favorite clothes	1	1	2	3	5	2.3 (1.3)
45. Distress from being unable to go swimming	0	1	1	3	5	1.8 (1.1)
46. Distress from being unable to play games	1	1	2	3	5	2 (1.1)
49. Distress from being unable to look in the mirror	1	1	1	3	5	1.9 (1.2)
Negative self-concept						
52. How confident do you feel?	1	2	3	4	5	3.1 (1.2)

(Continued)

TABLE 6 | Continued

Domain's items	Min	Q1	Q2	Q3	Max	Mean (SD)
54. How secure do you feel?	1	2	3	4	5	3.1 (1.2)
55. How cheerful do you feel?	1	3	3	4	5	3.3 (1.2)
56. How normal do you feel?	1	3	3	4	5	3.5 (1.2)
57. How masculine/feminine do you feel?	1	3	3	4	5	3.2 (1.3)
Facial self-consciousness of appearance						
11. Avoid getting the hair wet	1	1	2	3	5	2.2 (1.2)
44. Distress from being unable to change hairstyle	1	1	2	3	5	2.1 (1.2)
48. Distress from being unable to answer the front door	1	1	1	3	5	1.8 (1)
51. Distress from being unable to go out in windy weather	1	1	1	3	5	1.8 (1.1)
Physics						
25. Cause physical pain	1	1	1	2	5	1.7 (1)
26. There are physical limitations	1	1	1	3	5	2 (1.2)
No specific factor						
53. How irritable do you feel?	1	1.5	3	3	5	2.5 (1.2)
59. How hostile do you feel?	1	1	1	3	5	1.8 (1.1)

and bodily self-consciousness of appearance, negative self-concept, and facial self-consciousness of appearance have good discriminating validity between two groups. Meanwhile, it can be reflected in the patients with CL/P that maxillofacial defects have obvious influence on social life and other aspects of clinical patients. However, in terms of the general self-consciousness of appearance, and physical influence, there was no obvious variation between the two groups. These findings could be referred to some various demographic characteristics between the two groups such as occupation and education level.

Patients with cleft lip showed almost high concerns about their appearance and associated psychosocial aspects as revealed by their self-reported scores in DAS 59 responses. Their concerns were more prominent in the aspect of general self-consciousness of appearance (41.8 $\pm$ 14.7), particularly in the items of self-consciousness of "feature," taking a special interest in others' "features," avoiding photography, being hurt by others' comments, distress when others make remarks, and distress when others ask about the 'feature'. Moreover, they also gave an apparent concern about their social self-consciousness of appearance (41.1 $\pm$ 16), particularly in the items of being misjudged, difficulty making friends, avoiding parties/discos, avoid leaving the house, feel isolated, feel rejected, and distress when going to school/college/work. On the other hand, patients with cleft lip still showed an apparent degree of positive self-concept as shown in their responses of items under the negative self-concept domain.

More and less, the current study further confirms that the DAS 59 could be a valuable tool for assessing the viability of cosmetic and reconstructive intervention and going further to analyze the motivations underlying the demand of appearance-related interventions. Although the present study included an acceptable sample size, it is still not enough for a more comprehensive analysis of the DAS 59 instrument. Hence, the present cohort did not investigate the factorial construction of the Chinese DAS 59. In addition, the present study had only estimated the validation of DAS 59 in a limited range of clinical samples (CL/P). Therefore, a multicenter research that includes a larger sample size with a wide diversity of clinical population will be necessary to get a more robust result and thorough exploring of the different aspects underlying the DAS 59.

CONCLUSION

A reliable and valid Chinese form of DAS 59 was established to assess the psychological impact in individuals with appearance concerns. Patients with cleft lip have shown a high concern regarding their appearance, especially to the general and social self-consciousness of appearance. Thereby, the Chinese DAS 59 instrument could be useful in assessing and understanding the psychological and psychosocial distress of Chinese patients with cleft lip. The present Chinese DAS 59 instrument could be approved more by further validation and improvement study.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Ethical Review Board of West China Hospital of Stomatology, Sichuan University (approval no. WCHSIRB-D-2016-084R1). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

KS, SS, HL, PP, NC, and TC contributed to the collection of data. KS, SS, HL, NZ, and TC analyzed the data. KS, SS, HL, HH, YW, and CG contributed to writing and revising the article. BS, HH, YW, and CG supervised the research. All authors contributed to the article and approved the submitted version.

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Anxiety in Chinese Patients With Cleft Lip and/or Palate: A Preliminary Study

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Objectives: To preliminarily analyze factors that affected the prevalence of anxiety in Chinese patients with cleft lip and/or palate (CL/P).

Methods: The Generalized Anxiety Disorder Scale (GAD-7) was used to screen anxiety in Chinese CL/P patients. Non-CL/P individuals were also included as the control group. Sociodemographic and clinical data consisting of diagnosis, gender, only child or not, monthly household income, and current family location were collected to analyze possible factors that could affect the anxiety of this patient population.

Results: One hundred forty-two and 78 valid questionnaires were collected from the study and control groups, respectively. The mean GAD-7 score of the study group (3.092 ± 3.381) was significantly lower than the control (3.987 ± 2.505). Moreover, the proportion of patients presenting with moderate-severe anxiety was larger in the study group than in the control group (6.6 vs. 0.0%). Statistically significant differences in GAD-7 scores were observed between the study and control groups when the patient was the only child, living in an urban area, or the monthly household income was between 1,000 and 5,000 yuan.

Conclusion: Although the severity of anxiety in Chinese CL/P patients was not severer than those without CL/P, there was a relatively high incidence of moderate-severe anxiety in CL/P patients, while the only child, current family location and the monthly household income played significant roles in affecting anxiety psychology.

Keywords: cleft lip and/or palate, anxiety, GAD-7, psychology, Chinese CL/P patients

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INTRODUCTION

Cleft lip and palate (CL/P) is one of the most common congenital craniofacial abnormalities, characterized by failure of normal fusion of the palate and lip at the midline during embryonic development, resulting in a clinically obvious deformity of the newborn (1), bringing unignorable appearance malformations and increasing risk for various psychological diseases like loneliness, low self-esteem, social disorders, and mood disorders to patients (2). Although there lies a strong association between psychological wellbeing and appearance, and between psychological wellbeing and social adjustment (3), some other studies demonstrated few differences in psychological status between CL/P patients and those who without appearance malformations (4). It reminds us of the need for further studies.

As a common mental illness, anxiety refers to a feeling of nervousness, impatience, and restlessness about things, accompanied by physical symptoms such as palpitation, chest distress, and hyperventilation (5). It has been reported that compared with individuals without CL/P, CL/P patients are easily faced with relatively higher severity of anxiety (6). As anxiety could affect the life quality of the patients, identifying factors influencing anxiety status in Chinese CL/P patients would provide the theoretical basis for psychological intervention and improve the quality of cleft care. Thus, in this study, we sought to preliminarily investigate the characteristics of anxiety in Chinese CL/P patients by applying the Generalized Anxiety Disorder Scale (GAD-7) to analyze the effect of CL/P on anxiety and the possible related influencing factors.

MATERIALS AND METHODS

Subjects

Chinese Patients with a history of cleft lip and (or) cleft palate who visited West China Hospital of Stomatology, Sichuan University, between July 2019 and January 2021 were enrolled in the study group. Inclusion criteria of the study group were as follows:

- Patients with complete or incomplete unilateral or bilateral non-syndromic cleft lip and (or) palate.
- Patients who underwent cleft lip repair and (or) palatoplasty.
- Patients aged 10 years or above (7).

Through the distribution of questionnaires in the general population, inclusion criteria of the control group were as follows:

(1). Individuals with no significant facial defects or other major diseases.

(2). Individuals aged 10 years or above (7).

Sociodemographic and clinical data were collected for analysis, including diagnosis, gender, only child or not, monthly household income, and current family location.

The research protocol was reviewed and approved by the Ethics Committee of West China Hospital of Stomatology, Sichuan University (No. WCHSIRB-D-2016-084R1). Written informed consents were acquired from all the patients or their parents.

Measurement of Anxiety Status

The Generalized Anxiety Disorder 7-Item Scale (GAD-7) was used to assess patient anxiety status. Seven items were used to assess the frequency of anxiety symptoms over 2 weeks on a 4-point Likert scale ranging from 0 (never) to 3 (nearly every day). The total score of GAD-7 ranged from 0 to 21, with increasing scores indicating more severe functional impairments due to anxiety. The total score was calculated for each patient and interpreted as follows: normal (0–4), mild (5–9), moderate (10–14), and severe (15–21).

As one of the most widely used assessment scales applied for screening, diagnosis, and severity assessment of anxiety disorders (8), GAD-7 has been transadapted into Chinese culture and validated (9). It can serve as an effective tool for anxiety screening in Chinese adolescents (7).

In present study, all subjects were given questionnaires in Chinese version and guided by trained volunteers to make sure every item was fully understood. Each subject completed the questionnaire independently.

Statistical Analysis

SPSS 23.0 (IBM Corp., Armonk, NY, USA) is used for statistical analyses, and the count data was represented by mean ± standard deviation (SD). Independent samples-T test was used to compare the two independent variables within groups and the independent variables between groups, while one-way ANOVA was used to compare more-than-two independent variables within groups when the compliance with normal distribution is tested by Shapiro-Wilk. The Kruskal-Wallis test was typically used when the normality assumption was violated. P-values < 0.05 were statistically significant, while P-values < 0.01 were highly statistically significant.

RESULTS

Participant Characteristics

A total of 145 GAD-7 (142 valid and 3 invalid) questionnaires were collected from the study group (Mean age: 17.2 ± 5.8 years), while a total of 78 questionnaires were collected from the control group (Mean age: 18.5 ± 5.2 years). No significant difference in age was found between the two groups. The demographic characteristics of both groups were shown in **Table 1**.

TABLE 1 | The demographic characteristics of the study group and control group.

Variables	The study group		The control group	
	Number	Percentage	Number	Percentage
1. Diagnosis				
Cleft lip and palate	81	57.0%		
Cleft lip	38	26.8%		
Cleft palate	23	16.2%		
2. Gender				
Male	87	61.3%		
Female	55	38.7%		
3. Only child				
Only child	45	31.7%	45	57.7%
Non-only child	97	68.3%	33	42.3%
4. The monthly household income				
Under 1,000 yuan	27	19.0%	2	2.6%
1,000–5,000 yuan	73	51.4%	27	34.6%
5,000–10,000 yuan	17	12.0%	28	35.9%
More than 10,000 yuan	18	12.7%	21	26.9%
5. Current family location				
Rural	73	51.4%	17	21.8%
Urban	69	48.6%	61	78.2%

Anxiety Status in the Study and Control Groups

The mean GAD-7 scores in the study and control groups were 3.092 and 3.987 points, respectively, and the scores difference was statistically significant (**Table 2**). Moreover, the proportion of moderate-severe anxiety in the study group was larger than in the control group (6.6 vs. 0.0%) (**Table 3**).

The Analyses of the Factors That Influenced GAD-7 Scores

Sociodemographic and clinical data consisting of diagnosis, gender, only child or not, monthly household income, and current family location did not influence the anxiety status between subgroups in both study and control groups. However, analysis between groups showed a statistically significant difference in subjects that were an only child and lived in an urban location, while a highly statistically significant difference was demonstrated in subjects with a total family income between 1,000 and 5,000 yuan (**Table 4**).

DISCUSSION

It has always been assumed that facial appearance is vital for healthy psychosocial development (10). Given that CL/P patients experience difficulties in feeding, speech dysfunction, and cosmetic defects from birth, their psychological development could be affected (11). However, in contrast, other studies demonstrated no significant difference in psychological status between CL/P patients and non-CL/P individuals (12, 13). In the setting of the fact that research on the incidence of anxiety and its influencing factors of CL/P patients in China were considerably unclear, the present research aimed to gain a deeper insight into the prevalence of anxiety in Chinese CL/P patients and identify potential influencing factors.

Interestingly, we found that the average GAD-7 score of Chinese CL/P patients was lower than that of the control group. Possible explanations were as follows: There were studies that claimed CL/P patients reported better emotional wellbeing and overall self-worth than healthy people, and the impact of CL/P was not worse than having other concerns around appearance (14, 15), given that CL/P reportedly strengthened the individual's vulnerability to disadvantageous psychological experiences when faced with emotional difficulties and negative self-perception, as most of the patients were observed to perform and adapt well when confronted with adversities (16, 17). What's more, as the severity of anxiety could be divided into normal, mild anxiety, moderate anxiety and severe anxiety on the basis of GAD-7 score, we found that the proportion of moderate-severe anxiety in the study group was larger than that in the control group, indicating that CL/P disease might pose a severer negative effect on patient's anxiety. Nonetheless, further studies with larger sample sizes should be conducted to substantiate the potential negative effect exerted by CL/P.

The heterogeneity of cleft types could explain the differences observed in our study. Although the difference was not statistically significant, higher GAD-7 scores were found in patients diagnosed with cleft palate (CP) or cleft lip and palate (CLP) than those with cleft lip (CL), consistent with previous studies. As for CLP patients, they faced more significant challenges in social interactions and communications than other types of CL/P resulting from significant speech difficulties and language delays (18), potentially contributing to the worse psychological status. Additionally, it had been investigated that children with CP showed severer problems with parent- and teacher-reported depression, anxiety, and other psychological difficulties than children with CL or CLP, partially explained by greater speech problems and subsequently poorer learning disorder rather than facial defects because of its morphological abnormities and functional defects.

We also found that female patients scored slightly higher in GAD-7 than males while the difference was not statistically significant, indicating the possibility that female patients experienced relatively worse psychological conditions to some

TABLE 2 | The GAD-7 scores of the study group and control group.

	Mean ± SD	Median	Range	Interquartile Range	95% Confidence interval for mean		*P*-value
					Lower bound	Upper bound	
The study group	3.092 ± 3.381	2.000	17.000	3.000	2.531	3.653	
The control group	3.987 ± 2.505	4.000	9.000	4.000	3.423	4.552	
							0.016*

$P < 0.05$ by independent samples-T test.

TABLE 3 | Severity of anxiety in the study and control groups.

	Total	Normal		Mild anxiety		Moderate anxiety		Severe anxiety	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
The study group	142	110	77.5%	24	16.9%	6	4.2%	2	1.4%
The control group	78	46	59.0%	32	41.0%	0	0.0%	0	0.0%

TABLE 4 | Factors affecting anxiety status of Chinese patients with CL/P.

Variables	The study group		The control group		*P*-value (study vs. control)
	Mean ± SD	*P*-value	Mean ± SD	*P*-value	
1.Diagnosis					
Cleft lip and palate	3.123 ± 3.455	0.967			
Cleft lip	2.974 ± 3.234				
Cleft palate	3.174 ± 3.499				
2.Gender					
Male	3.046 ± 3.316	0.841			
Female	3.164 ± 3.511				
3.Only child					
Non-only child	3.412 ± 3.665	0.081	3.636 ± 2.421	0.292	0.729
Only child	2.326 ± 2.597		4.244 ± 2.560		0.017*
4.The monthly household income					
Under 1,000 yuan	4.037 ± 4.808	0.064	4.000 ± 5.659	0.839	0.992
1,000–5,000 yuan	2.493 ± 2.631		4.296 ± 2.826		0.004**
5,000–1,0000 yuan	4.529 ± 3.105		3.964 ± 2.099		0.513
Over 1,0000 yuan	3.167 ± 3.792		3.619 ± 2.439		0.656
5.Current family location					
Urban	2.696 ± 3.331	0.181	3.787 ± 2.430	0.183	0.037*
Rural	3.471 ± 3.471		4.706 ± 2.710		0.175

*$P < 0.05$, **$P < 0.01$ by independent samples-T test or one-way ANOVA.*

extent. As gender differences have been frequently studied when it came to psychology, it has been demonstrated that females were more likely to experience appearance dissatisfaction (19) and more at risk for emotional distress than males with the same diagnosis due to their higher awareness of sentiment and sensitivity (20).

Moreover, a statistical difference in patients who were an only child was found between CL/P patients and the general. For the sake of reasonable explanation, we found that as parents with children or children with siblings suffering from severe diseases such as cancer were more likely to break the family's normal function and dynamic balance (21), dysfunctional family predicted higher levels of anxiety and other emotional disorders in adolescents, receiving more negative feedback and discouragement of emotional expression (22). However, opposite outcomes have been reported in our study. It's worth noting that even though China's one-child policy ended in 2015, it was in place for over three decades and was mainstream in Chinese families, contributing to the psychosocial differences between groups of only children vs. children with siblings. We found that non-only-children with CL/P scored higher than only-child patients, implying that having siblings might be a risk correlated with anxiety, depression and other psychological disorders (23), partially explained by the so-called resource dilution model (24), meaning that increase in numbers of children bringing about a reduction in available resources from parent to child (25). As for children in general, opposite outcomes have been reported, indicating the complex predictive effects on anxiety in an only child vs. a non-only child group.

In terms of household income, CL/P patients with a monthly household income between 5,000 and 10,000 yuan were documented to have the highest GAD-7 scores, followed by a monthly household income lower than 1,000 yuan. A statistically significant difference in scores was found between groups in subjects with monthly household income between 1,000 and 5,000 yuan, as the control group scored higher than the study group. Given that the general consensus from most published studies was that the severity of anxiety and depression was negatively associated with the household income (26, 27), socioeconomic deprivation could be intricately linked to mental illness. Although low family income implied poor access to healthcare, less educational opportunities, increased risk of unemployment and eventually giving rise to mental illnesses, CL/P patients tend to exhibit stronger resilience in the face of adversity, with enhanced ability to cope and manage stress and negative experiences compared to the general population due to their craniofacial anomaly (17), which might result in a lower degree of anxiety caused by unsatisfied economic conditions. Anyway, a deeper study should be implemented in the future to investigate the specific relationship between economic conditions and CL/P patients' psychological status.

Higher GAD-7 scores were obtained between groups in subjects from rural areas, which could be partly explained by the negative role of their residential location that could influence their self-worth and self-knowledge (28). It is thought that living in undeveloped areas is an essential factor in deciding one's inner awareness and it can cause more psychological distress (29). In our study, a statistical difference in GAD-7 scores was also found

between CL/P patients and people without CL/P of the urban area, while the latter scored much higher.

Several limitations need to be noted in our study. The participants of our study were recruited from a limited geographic area and the study was conducted in only one specialized hospital of stomatology in western China. Also, the sample size of our study was not large enough. In addition, there was the outbreak of The 2019 Coronavirus Disease (COVID-19) epidemic during our collection of data, posing a challenge to psychological resilience upon both Chinese society and the international community. Our results might be influenced by the outbreak and prevalence of COVID-19.

CONCLUSION

Herein, given the limitations of this study, we preliminarily provided some evidence on the significance of various causes of anxiety in Chinese CL/P patients. Most importantly, we found that the severity of anxiety in Chinese CL/P patients was not severer than those without CL/P.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of West China Hospital of Stomatology, Sichuan University (No. WCHSIRB-D-2016-084R1). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ZX and CY contributed equally to this work. ZX, CY, YZ, YY, WX, and YZ contributed to the collection of data. ZX, CY, YZ, and TC analyzed the data. ZX, CY, BS, HH, and CG contributed to writing and revising the paper. BS, HH, and CG supervised the research. All authors contributed to the article and approved the submitted version.

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Velopharyngeal Inadequacy-Related Quality of Life Assessment: The Instrument Development and Application Review

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Objective: For the patient-reported outcome (PRO) measures related to patients with velopharyngeal inadequacy (VPI), different quality of life (QOL) instruments have been developed. The present systematic review was designated to identify current VPI-related QOL instrument development, validation, and applicability.

Methods: Pubmed, Cochrane, Embase, Web of Science, and EBSCO databases were searched in January 2022. "Velopharyngeal" or "palatopharyngeal" and "quality of life" or "life quality" were searched in title, abstract, and keywords. This study followed Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines. Two investigators independently reviewed abstracts and full texts of the identified literature. An established checklist was used to evaluate the measurement properties of each identified instrument.

Results: A total of 375 articles and 13 instruments were identified, which can be divided into nine types of families according to their development procedures. Developmental and measurement characteristics, evidence of conceptual model, content validity, reliability, construct validity, scoring, interpretation, respondent burden, and presentation for all instruments were shown.

Conclusion: The patient's self-report assessment and parent-proxy assessment are both valuable. The conclusion that any QOL instrument is absolutely the best for patients with velopharyngeal inadequacy could not be drawn. Understanding the development and characteristics of different QOL instruments, including their reliability, validity, aim, target, language, and resource, should be important before application in clinic or research.

Keywords: velopharyngeal inadequacy, quality of life, instruments, patient-report outcomes, patient-report outcome questionnaire

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INTRODUCTION

Velopharyngeal inadequacy (VPI) is the generic term for denoting three types of abnormal velopharyngeal function, namely, velopharyngeal insufficiency which is caused by structural etiologies, velopharyngeal incompetency which is incurred with neurogenic etiologies, and velopharyngeal mislearning which is related to functional etiologies (1). Velopharyngeal inadequacy occurs at high frequency among patients with post-operative cleft palate and patients with non-cleft palate functional velopharyngeal inadequacy, both of which are caused by multiple reasons. The causes can be divided into congenital and acquired (**Table 1**) (2–9). As orofacial clefts, like cleft palate, are among one of the most common congenital disabilities worldwide (10, 11), the problems caused by velopharyngeal inadequacy, such as speech and swallowing problems, remain a significant challenge to clinicians.

Speech therapy, prosthetic appliances, and surgery can help restore the velopharyngeal inadequacy (12). Objective measurements like nasopharyngoscope and imaging evaluation for the measurement of velopharyngeal gap size and nasalance are usually used for post-operative assessment. However, anatomic change and improvement cannot guarantee functional recovery, let alone solve social and emotional problems that come with the disability. Speech evaluation is commonly applied for function tests which are mostly based on the experience of speech therapists. It is possible to use the automatic evaluation system to assist in diagnosing specific speech problems (13, 14). Despite this, speech evaluation still cannot illustrate the feelings of the patients.

Tools are strongly needed in understanding the patients' perceptions. For the patient-reported outcome (PRO) measures related to patients with velopharyngeal inadequacy, different quality of life (QOL) instruments have been developed during the past two decades. However, clinicians or researchers may find it challenging to choose the appropriate instrument for their study and presume that published instruments all have appropriate measurement properties. A checklist developed by Francis DO was designed to help identify components that are considerably crucial to the construction of PRO measures. This particular checklist was applied to evaluate VPI-related QOL instruments in this study (**Table 2**) (15). This study aims to perform a comprehensive review of VPI-related QOL instruments and provide a pragmatic approach to assessing the QOL of patients with velopharyngeal inadequacy.

TABLE 1 | Causes of velopharyngeal inadequacy.

Congenital causes	Acquired cuases
1. Submucous cleft palate	1. Palatoplasty
2. Van Der Woude syndrome	2. Le Fort I maxillary advancement
3. Kallmann syndrome	3. Adenoidectomy
4. Pierre Robin sequence	4. Adenotonsillectomy
5. DiGeorge syndrome	5. Tonsillectomy
6. Kabuki makeup	6. Uvulopalatopharyngoplasty
7. Hemifacial microsomia	7. Trauma
8. Klippel–Feil syndrome	8. Oral or pharyngeal cavity tumors
9. Down syndrome	
10. Mosaic trisomy 8	
12. Irregular adenoids	
12. Hypertrophic tonsils	
13. Velocardiofacial syndrome	
14. Unilateral hypoplasia of the velum and pharynx	

MATERIALS AND METHODS

This review was conducted with reference to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines (16). The research protocol was censored and approved by the Ethic Committee of West China Hospital of Stomatology, Sichuan University (Approval No. WCHSIRB-D-2016-084R1).

Search Strategy

A comprehensive search was conducted through literature databases, including Pubmed, Cochrane, Embase, Web of Science, and EBSCO host. The search of literature was conducted in January 2022. No publication date limit was set during the literature search. "velopharyngeal" or "palatopharyngeal" and "quality of life" or "life quality" were searched in title, abstract, and keywords.

Study Selection

Abstracts for all studies identified in the literature search were independently reviewed by two investigators. Those meeting the predetermined screening criteria were advanced to full-text review. Inclusion criteria were as follows: 1. Research is on human subjects; 2. The participants include the patients with velopharyngeal inadequacy; and 3. The study mentioned at least one kind of instrument for QOL. Articles lacking adequate information in their title or abstract for determining eligibility were also included in the full-text review phase. Only the articles describing the development and validation of each instrument with the original version were included for analysis, and the translated versions or modified versions were excluded.

Data Extraction and PRO Measures Assessment

First, one reviewer assessed each study's methods using a criteria checklist developed *a priori* (15). Another reviewer completed the evidence table which has been thoroughly discussed between the three authors to compare the characteristics of QOL measurements. Then, the two reviewers checked each other's results and dealt with the ambiguities. If they were unable to reach a consensus, the third author was consulted.

The checklist was designed to help reviewers identify components crucial to constructing patient-reported outcome (PRO) measures. Measurement properties, including conceptual model, content validity, reliability, construct validity, scoring

TABLE 2 | Checklist of key characteristics to consider when evaluating a patient-reported outcome (PRO) measure[a].

Characteristic	Score
Conceptual model	
1. Has the PRO construct to be measured been specifically defined?	
2. Has the intended respondent population been described?	
3. Does the conceptual model address whether a single construct or scale or multiple subscales are expected?	
Content validity	
4. Is there evidence that members of the intended respondent population were involved in the PRO measure's development?	
5. Is there evidence that content experts were involved in the PRO measure's development?	
6. Is there a description of the method by which items or questions were determined?	
Reliability	
7. Is there evidence that the PRO measure's reliability was tested?	
8. Are reported indexes of reliability adequate?	
Construct validity	
9. Is there reported quantitative justification that a single scale or multiple subscales exist in the PRO measure?	
10. Is the PRO measure intended to measure change over time? If yes, is there evidence of both test-retest reliability and responsiveness to change? Otherwise, award 1 point if there is an explicit statement that the PRO measure is not intended to measure change over time.	
11. Are there findings supporting expected associations with existing PRO measures or with other relevant data?	
12. Are there findings supporting expected differences in scores between relevant known groups?	
Scoring and interpretation	
13. Is there documentation of how to score the PRO measure?	
14. Has a plan for managing or interpreting missing responses been described?	
15. Is information provided about how to interpret the PRO measure scores?	
Respondent burden and presentation	
16. Is the time to complete reported and reasonable? Or, if it is not reported, is the number of questions appropriate for the intended application?	
17. Is there a description of the literacy level of the PRO measure?	
18. Is the entire PRO measure available for public viewing?	

[a]*Instructions: Please indicate in the Score column whether or not the information provided in the citation or source document meets each criterion (0 indicates criterion not met and 1 indicates criterion met).*

and interpretation, respondent burden, and presentation for all instruments were evaluated.

Data Synthesis

Meta-analysis was not applicable for data aggregation due to the heterogeneity of studies in constructs, methods, and intended purposes. Efforts were still made to summarize some useful regular patterns for clinical practice.

RESULTS

Literature Search and Screening

The literature search and screening flow diagram were shown in **Figure 1** (16). A total of 375 articles were identified. After exclusion of duplicates, 180 articles remained. Eventually, 13 instruments were identified, including KIDSCREEN, PedsQL 4.0 Generic Core Scales (Pediatric Quality of Life Inventory 4.0), KINDL, Child Oral Health Impact Profile (COHIP), Child Oral Health Impact Profile-Short Form (COHIP-SF), Velopharyngeal Insufficiency Quality of Life (VPIQOL), Velopharyngeal Insufficiency Effects on Life Outcomes instrument (VELO), Pediatric Voice Outcome Survey (PVOS), Pediatric Voice-Related Quality-of-Life survey (PVRQOL), Voice Handicap Index (VHI), Pediatric Voice Handicap Index (pVHI), 9-item Voice Handicap Index (VHI-9i), and Swallowing Quality of Life questionnaire (SWAL-QOL). According to the development procedure, these instruments could be divided into nine types of families (**Figure 2**). Among these instruments, only VPIQOL and VELO were specifically designed for patients with velopharyngeal inadequacy (17–20).

Developmental and Measurement Characteristics

The number of participants involved in different studies ranged from 29 to 22,295 (**Supplementary Table 1**). In the instrument development, the proportion of females was the lowest for SWAL-QOL (21.5%), followed by PVRQOL with 40%. The percentage of females in VHI development was the highest (60.32%). The other instruments had balanced proportions of females and males at around 50%. A total of 10 instruments were designed for children and their parents, in which the mean age of patients ranged from 8.7 to 13.2. Three instruments were designed without age restriction, in which the mean age of participants ranging from 45 to 66.1. The United States of America (USA) had an active role in developing instruments, and participated in the development of 11 instruments. Multiple European countries jointly developed KIDSCREEN and VHI-9i, while the KINDL instrument originated from Germany.

These data regarding the developmental characteristics of instruments could be used for the quality evaluation of

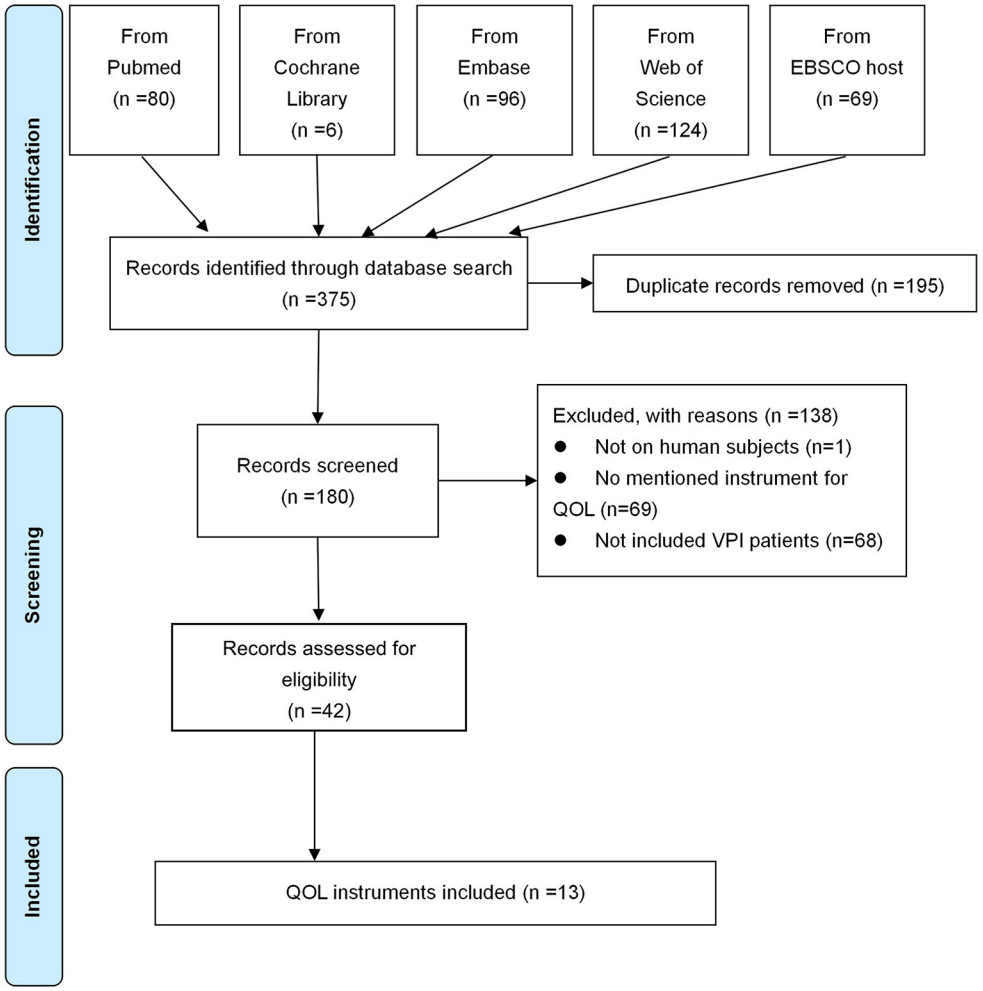

FIGURE 1 | Literature searching and screening flow diagram. QOL, quality of life; VPI, velopharyngeal insufficiency.

evidence (**Supplementary Table 1**). Type 1 to type 3 instruments, including KIDSCREEN, PedsQL 4.0, and KINDL, were designed for the general health of pediatrics and they can be used on healthy children and children with acute or chronic diseases (21–23). Type 4 instruments (COHIP and COHIP-SF) aimed to assess the oral-facial wellbeing of school-age children (24–29). Type 5 instruments (VPIQL and VELO) were developed for patients with velopharyngeal inadequacy (17, 18). Type 6 to type 8 instruments (PVOS, PVRQOL, VHI families) focused on dysphonia problems (30–36). Lastly, type 9 instruments (SWAL-QOL) concentrated on dysphagia problems (37–39). Due to the different study populations, the distribution of pathology and mean age were varied between different types. The sample of KIDSCREEN, which came from a European project called "Screening and Promotion for Health-related Quality of Life in Children and Adolescents - A European Public Health Perspective" that included 13 European countries, was the biggest among all the instruments. The sample of PedsQL 4.0, which came from the State's Children's Health Insurance Program (SCHIP), was the second largest. Other instrument data were hospital-based, and only KIDSCREEN, PedsQL 4.0, and COHIP-SF were population-based.

Measurement aims, target populations, and item characteristics of these instruments were shown in **Table 3**. As a measure of PRO, QOL was evaluated based on the patients' experience and perception. For many patients with velopharyngeal inadequacy, the causes are congenital (**Table 1**) (11). Considering the development of their cognition, it was hard for young children to evaluate their QOL by themselves. Hence, the caregivers' proxy QOL assessment was essential for such situations. Here, the concept "patient" in PRO did not only refer to the patients' selves, but also included the parents. In the usage of the QOL instruments for patients with velopharyngeal inadequacy, the youngest children ranged from 2 (PedsQL 4.0, PVOS and PVRQOL) (22, 30, 31) to 3 years old (VELO and pVHI) (18, 36). However, for those self-report QOL instruments for VPI, the youngest age was 4 years old (KINDL) (23), followed by 5 (PedsQL 4.0 and VPIQOL) (17, 22)

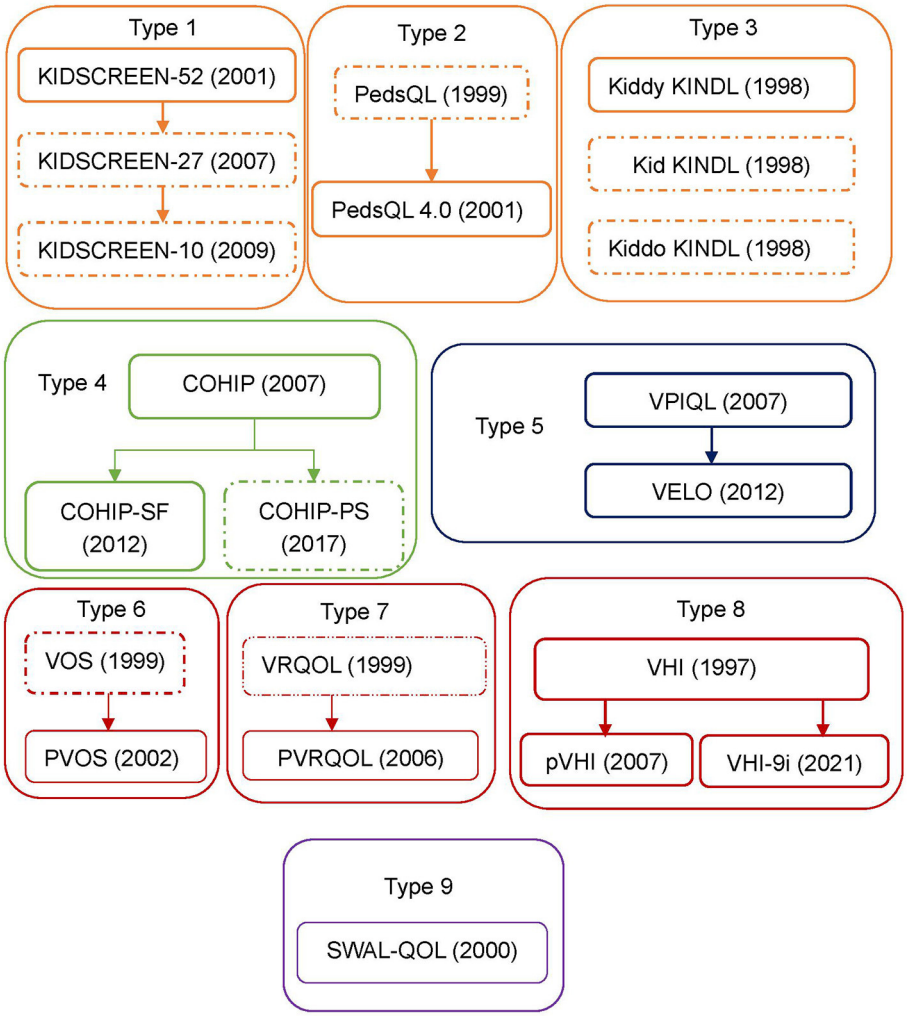

FIGURE 2 | Instruments types applied for VPI patients. Development procedures and types of the instrument applied to patients with velopharyngeal inadequacy. Thirteen instruments that have been applied to patients with velopharyngeal inadequacy are identified. These can be divided into 9 types according to the development procedure. A dotted box would mean that the instrument has not been applied to patients with velopharyngeal inadequacy. PedsQL, pediatric quality of life inventory; PedsQL 4.0, pediatric quality of life inventory 4.0 generic core scales; KINDL, german generic quality of life instrument for children; COHIP, child oral health ompact profile; COHIP-SF, child oral health ompact profile-short form; COHIP-PS, child oral health ompact profile-preschool version; VPIQOL, velopharyngeal insufficiency quality of life; VELO, velopharyngeal insufficiency effrets of the outcomes instrument; VOS, voice outcome survey; PVOS, pediatric voice outcome survey; VRQOL, voice-related quality-of-life survey; PVRQOL, pediatric voice-related quality-of-life survey; VHI, voice handicap index; pVHI, pediatric voice handicap index; VHI-9i, 9-item voice handicap index; SWAL-QOL, swallowing quality of life questionnaire.

and 8 years old (KIDSCREEN, VELO, COHIP, COHIP-SF) (18, 21, 24, 29).

Pediatric Voice Outcome Survey (PVOS) was the shortest instrument, with only 4 items. In contrast, KIDSCREEN-52 contains as many as 52 items and needs 15–20 min to complete. Most of the item numbers range from 10 to 40. Due to this, there were several types of instruments developed a short-form version to reduce the time burden (KIDSCREEN-27, KIDSCREEN-10, PedsQL 4.0, COHIP-SF, VELO, VHI-9i) (29, 34, 40–42).

In general, the majority of instruments were thoroughly discussed by experts and/or patients in the generating and modified procedure. The pilot study was common in a validation test. No noticeable gender difference was found. Except for 3 instruments (VELO, VHI, and SWAL-QOL), most instruments applied on patients with velopharyngeal inadequacy were designed only for children.

Measures Assessment

Conceptual Model

Besides PVOS and PVRQOL instruments which intend to measure a single concept, most instruments clearly defined their construct and respective target population. The health definition from WHO and the conceptualization of health-related QOL were the most commonly used methods (21, 23, 24, 43).

TABLE 3 | Measurement aims, target populations, and item characteristics of instruments for the quality of life (QOL) of patients with velopharyngeal inadequacy.

Instrument	Development year	Measurement aim	Target population	Language version	No. of Items or domains	Response options	Domains	Reporter and versions
KIDSCREEN	2001	To assess children's and adolescents' subjective health and wellbeing	Healthy and chronically ill children and adolescents from 8 to 18 years	English German Dutch Spanish Portuguese French Czech Polish Hungarian Swedish Greek Persian Japanese Italian Korean Latvian Russian	KIDSCREEN-52: 52 items and 10 domains KIDSCREEN-27: 27 items and 5 domains KIDSCREEN-10: 10 items and 1 domain	5-point Likert-type	Physical-, psychological wellbeing, moods and emotions, self-perception, autonomy, parent relations and home life, social support and peers, school environment, social acceptance (bullying), financial resources	Children self-report: KIDSCREEN-52 KIDSCREEN-27 KIDSCREEN-10 Parent proxy-report: KIDSCREEN-52 KIDSCREEN-27 KIDSCREEN-10
PedsQL 4.0 (PedsQL 4.0 generic core scales)	2001	To measure health related quality of life in children and adolescents ages 2–18	Healthy school and community populations, as well as pediatric populations with acute and chronic health conditions between 2 and 18	English UK-English Spanish Japanese Chinese Thai Hungarian Brazilian Korean and so on (Total of 92 kinds of languages)	Core: 23 items, 4 domains	5-point Likert-type	Physical functioning, Psychosocial health: emotional functioning, social functioning, school functioning	Child self-report: 5–7 years; 8–12 years; 13–18 years. Parent proxy-report: 2–4 years; 5–7 years; 8–12 years; 13–18 years.
KINDL (Generic core instrument)	1998	To access health-related quality of life in children and adolescents aged 3 years and older	Healthy and ill children and adolescents between 3 and 17 years of age	Abrabic Bulgarian Chinese Danish Dutch English French German Greek Iranian Italian Japanese Korean Nepalese Norwegian Polish Portuguese Russian Serbian Sinhala Spanish Swedish Turkish Vietnamese	The original one had 40 items and 4 domains. The latest one has 19–24 items and 6 domains for children, 24–46 items and 6 domains for parents.	3 or 5-point Likert-type	Generic core instrument	Children self-report: 4–6 years (Kiddy) 7 to 13 years (Kid) 14–17 years (Kiddo) Patient proxy-report: 3 to 6 years (Kiddy) 7–17 years (Kid/Kiddo)

(Continued)

TABLE 3 | Continued

Instrument	Development year	Measurement aim	Target population	Language version	No. of Items or domains	Response options	Domains	Reporter and versions
COHIP (Child Oral Health Impact Profile)	2007	To assess oral-facial wellbeing in school-age children	Children aged 8–15	English Spanish French Arabic Chinese Korean Dutch Amharic Persian	34 items 5 domains	5-point Likert-type	Oral health, functional wellbeing, social/emotional wellbeing, school environment, self-image	Patients and parents
COHIP-SF (Child Oral Health Impact Profile-Short Forms)	2012	To assess oral-facial wellbeing in school-age children with a short form	Children aged 8–15	English Japanese Indonesian Arabic Chinese German	19 items 5 domains	5-point Likert-type	Oral health, functional wellbeing, social/emotional wellbeing, school environment, self-image	Patients and parents
VPIQOL (Velopharyngeal Insufficiency Quality of Life)	2007	To assess alterations of QOL in children aged 5–17 years with VPI	5 to 17 years children with velopharyngeal insufficiency	English	48 items (43 for patients); 7 domains (6 for patients)	5-point Likert-type	Speech limitations, swallowing problems, situational difficulty, emotional impact, perception by others, activity limitations and caregiver impact	Patients and parents
VELO (VPI Effects on Life Outcomes instrument)	2012	To measure and follow QOL in patients with VPI	Velopharyngeal insufficiency	English Chinese Spanish Nepali Portuguese Dutch	23 items (26 for parents); 5 domains (6 for parents)	5-point Likert-type	Speech limitation, swallowing problems, situational difficulty, emotional impact, perception by others, caregiver impact	Patients and parents (Parent Proxy Assessment divided the patients with VPI into those 9 years or younger and those 10 years and older)
PVOS (Pediatric voice outcome survey)	2002	To measure the VR-QOL in the pediatric population	Children and adolescents with voice concerns specific to congenital- or neonatal-acquired lesions (sample age 2 to 18)	English Turkish	4 items	3 and 5-point Likert-type	NA	Parents

(Continued)

TABLE 3 | Continued

Instrument	Development year	Measurement aim	Target population	Language version	No. of Items or domains	Response options	Domains	Reporter and versions
PVRQOL (Pediatric Voice-Related Quality-of-Life survey)	2006	To assess voice changes over time in the pediatric population	Pediatric with voice disorders (sample age 2 to 18)	English Arabic Turkish Brazilian Chinese Serbian	10 items	6-point Likert-type	NA	Parents
VHI (Voice Handicap Index)	1997	To quantify the psychosocial consequences of voice disorders	Adult voice disorder patients (sample mean age 52.3)	English Korean Czech Norwegian Croatian Japanese French Chinese Arabic Italian Portuguese Turkish Greek Spanish Hebrew Dutch German Swedish Russian Marathi Slovak Finnish Persian Serbian Danish Latvian Lebanese	30 items 3 domains	5-point Likert-type	Emotional, functional, physical	Patients
pVHI (Pediatric Voice Handicap Index)	2007	To quantify the impact of a voice disturbance on the child's social, emotional, and functional wellbeing	Dysphonia on the pediatric population (children younger than 3 were excluded)	English Persian Turkish Chinese Arabic Danish Italian Portuguese French Hebrew Korean Malayalam Spanish Dutch Polish	23 items 3 domains	5-point Likert-type	Emotional, functional, physical	Parents
VHI-9i (nine-item Voice Handicap Index)	2009	To quantify the psychosocial consequences of voice disorders with short form	Voice disorder patients	English Dutch French German Italian Portuguese Swedish	9 items	5-point Likert-type	Emotional, functional, physical	Patients

(Continued)

TABLE 3 | Continued

Instrument	Development year	Measurement aim	Target population	Language version	No. of Items or domains	Response options	Domains	Reporter and versions
SWAL-QOL (Swallowing Quality of Life questionnaire)	2000	To measure treatment variations and treatment effectiveness.	Adult dysphagia patients (mean age was 65.8)	English French Norwegian German Persian Italian Dutch Swedish Chinese Greek	44 items 11 domains	5-point Likert-type	Burden, eating duration, eating desire, symptom frequency, food selection, communication, fear, mental health, social, fatigue, sleep	Patients; A close family member; Interviewers

NA, not available, the instrument has no subdomains.

Content Validity

Most of the earliest instruments in each type contained patient and expert participation. Only VHI was developed based on patients' opinions. The focus group was the most common method. During the modification of the original instruments, fewer studies involved target patients. Four instruments did not specify who developed the instrument (pVHI, PVOS, PVRQOL, and VHI-9i). Three instruments provided limited information about the development of items (KINDL, VPIQOL, and PVRQOL).

Reliability

Except for VPIQOL, all the other instruments were tested and demonstrated adequate reliability. VPIQOL did not provide any information about reliability determination (17).

Construct Validity

Construct validity dimension was one of the most demanding criteria to meet, especially for longitudinal validity. Only five instruments (KINDL, COHIP, VELO, PVOS, and PVRQOL) provided sufficient information to assess both test-retest reliability and responsiveness to change. Longitudinal validity was crucial for analyzing cohort study data and measuring intervention effect, which was particularly compromised by test-retest reliability and responsiveness validity. Test-retest reliability could guarantee the baseline stability, and responsiveness validity can measure the change before and after the intervention. The dimensionality was justified for 8 instruments (KIDSCREEN, PedsQL 4.0, KINDL, COHIP, COHIP-SF, VELO, VHI-9i, and SWAL-QOL) by factor analysis. In contrast, the criteria of convergent validity and known group validity were easy to meet. There were four instruments that failed to meet the convergent validity (VPIQOL, PVOS, VHI, and pVHI), and one instrument was unable to test the distinguish validity (PVRQOL).

Scoring and Interpretation

Compared with other dimensions, this dimension was the most difficult one to achieve. A total of 9 instruments clearly explained the scoring approach or algorithm (KIDSCREEN, PedsQL 4.0, KINDL, COHIP, VPIQOL, VELO, PVOS, PVRQOL, and VHI-9i), four of which described the plan for missing data (KIDSCREEN, PedsQL 4.0, KINDL, and COHIP). Five instruments provided information on how to interpret the scores (KIDSCREEN, PedsQL 4.0, KINDL, COHIP, and VHI-9i). The result also suggested that scoring and interpretation was the most neglected dimension during instrument development.

Respondent Burden and Presentation

In this dimension, all the instruments were available for public viewing. Seven instruments (KIDSCREEN, PedsQL 4.0, COHIP, COHIP-SF, VELO, VHI-9i, and SWAL-QOL) discussed the number of questions and retained a reasonable result. Five instruments (PedsQL 4.0, COHIP, COHIP-SF, VELO, and SWAL-QOL) described the literacy level.

Among the 6 dimensions, reliability and conceptual model were the two easiest critera to meet, while construct validity and scoring and interpretation were two hardest to meet (**Figure 3**). COHIP (18/18) met the most criteria, followed by PedsQL 4.0 (17/18), KIDSCREEN (16/18), VELO (15/18), KINDL, and SWAL-QOL (14/18). VPIQOL (8/18) and PVRQOL (8/18) met the least criteria. Plan for missing data was the most challenging criteria for the instrument, and only four instruments mentioned it. Longitudinal validity, literacy level, and scaling description took the place of second hardest among all criteria, with only five instruments who fulfilled it. Except for VPIQOL, all the other instruments had been demonstrated with considerably good reliability.

DISCUSSION

The importance of QOL has been increasingly embodied (3). Similar to other chronic diseases, velopharyngeal inadequacy has a long course and is difficult to fully recover from. For a long time, surgical outcome evaluations have been patient-centered rather than patient-reported. QOL is a patient-reported outcome which can measure the experience of the target population and give patients the right to participate in the therapy. QOL assessment is also the only way to measure the patients' subjective feelings like depression, pain, satisfaction, and so on. Therefore, the treatment should pay attention to QOL improvement as much as surgical outcomes. Patients themselves, along with their caregivers, are

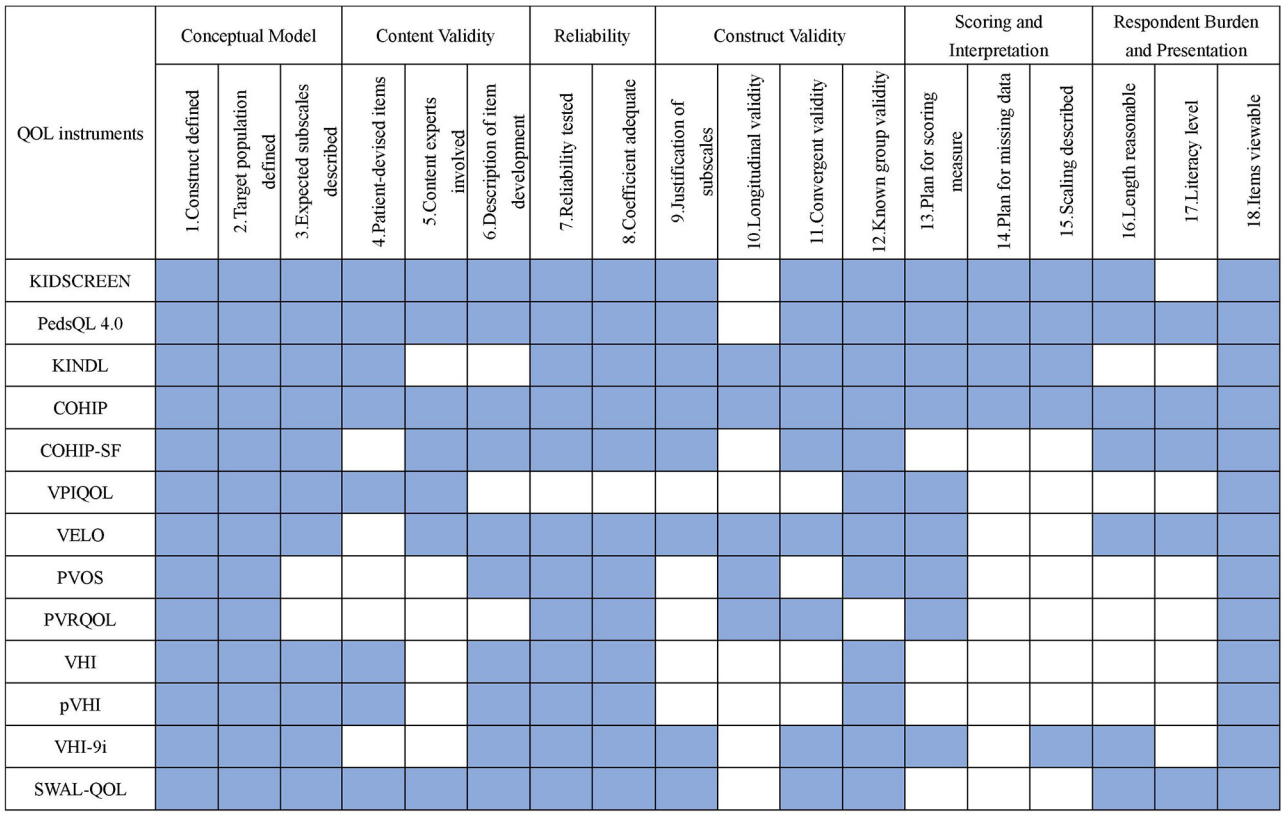

FIGURE 3 | Summary comparison of measurement properties among identified instruments. Blue indicate the criterion is met. PedsQL 4.0, pediatric quality of life inventory 4.0 generic core scales; KINDL, german generic quality of life instrument for children; COHIP, child oral health ompact profile; COHIP-SF, child oral health ompact profile-short form; VPIEQOL, velopharyngeal insufficiency quality of life; VELO, velopharyngeal insufficiency effrets of the outcomes instrument; PVOS, pediatric voice outcome survey; PVRQOL, pediatric voice-related quality-of-life survey; VHI, voice handicap index; pVHI, pediatric voice handicap index; VHI-9i, 9-item voice handicap index; SWAL-QOL, swallowing quality of life questionnaire.

enough to evaluate the QOL of the patients. Meanwhile, QOL should not be limited to the patient as circumstance of the disease influences the QOL of the whole family. In addition, there are some instruments pay attention to the QOL of family members (44, 45).

Results clearly showed the developmental characteristics, measurement aims, target population, item characteristics, and measurement properties of velopharyngeal inadequacy-related QOL instruments. Based on the results, this discussion section of the present systematic review tried to answer the following questions: (1) Who should be responsible for assessing the QOL of patients with velopharyngeal inadequacy? (2) How to choose the appropriate instrument? and (3) How can the QOL result apply for practice?

The opinion that the patient should evaluate his/her own QOL is a dominant one. Self-reported measurement can promote the treatment effects and encourage cooperation from the patients. It is also helpful and beneficial for clinical practice to more accurately reflect the patients' perception more accurately (46). One study showed that the parent-reported QOL outcomes could not provide further information regarding a child's QOL (47). Some others hold the opposite opinion in that the parent's view should be regarded as more important. Only parents can make a comprehensive and long-term evaluation of the consequence of illness (30).

The age of 6 marks the beginning of abstract thinking and self-concept (48). By the age of 11 or 12, children start to have a clear understanding of some complex emotions, such as worry, shame, and jealousy. Their self-concept acquires sophisticated dimensions, such as romantic appeal and popularity with peers. Children develop the concept of time at about the age of 8 when their recall period starts to lengthen and their understanding of the frequency of events begins to emerge. A. Jokovic et al. recommended age-specific QOL instruments for children aged 6–14. He proposed that instruments should be grouped into the following ages: 6–7-, 8–10-, and 11–14-year-olds (49). From the above, QOL is crucial and valuable no matter from the patient's self-report or the patient's parent-report. For school-aged children and adolescents, the self-report QOL instrument is the best choice. However, the caregivers are also the target population. Caregivers are usually dissatisfied with the children's QOL and therapy effect (31). It is also essential to "cure" the caregivers, make them have a reasonable expectation,

and promote their cooperation. For children younger than 5 years old, the caregivers are highly recommended to be the ones to accomplish the assessment. Instruments, including PedsQL 4.0, KINDL, PVOS, PVRQOL, and pVHI, are great options. For children aged 5–7 years old, choice can be made from KIDSCREEN, PedsQL 4.0, andKINDL for self-report. For school-age children (8 and above), VPIQOL, VELO, and COHIP-SF can be used for self-report.

Besides the age of the target population, the research objective should be one important factor to consider for choosing instruments. If we want to compare general and oral health between patients with velopharyngeal inadequacy and healthy people, a generic instrument like KIDSCREEN, PedsQL 4.0, KINDL, COHIP, or COHIP-SF could be better. VPIQOL and VELO are designed for measuring the specific VPI-related health problems. If we focus on the voice problem, we can choose VHI, pVHI, PVOS, or PVRQOL. If we focus on the swallowing problem, SWAK-QOL is the right choice. If we want to measure the therapeutic change, we have to choose an instrument that has a good test-retest reliability and responsiveness validity, such as KINDL, COHIP, VELO, PVOS, and PVRQOL.

Language is another influencing factor for choosing an instrument. Most of the instruments are developed in English. If we want to translate the English version instrument and use it, we would first have to do the validation research in the target population. Applying the transferred and validated instruments could save time and labor. The earlier developed instruments have a bigger chance of being translated and tested with various language versions. Among these instruments, PedsQL 4.0 has the largest number of language versions which could be widely applied in most countries (**Table 3**). In addition, if we want to apply an instrument to a rural area, the literacy level should be taken into account.

Time burden can limit the clinical application. Therefore, time burden plays a crucial role in choosing instruments. With respect to comprehensiveness, generally speaking, long instruments with more items could provide more information. In terms of acceptance and practicability, short instruments are more appropriate to use. PVOS is the shortest instrument, with only 4 items, compared to the longest instrument, KIDSCREEN, with 52 items. Pilot tests can be applied before deciding on the instrument, like recording the time and assessing the feedback from the target population. The sampling set and the number of working staff should also be considered. It is difficult for a busy clinic or other hand-shorted places to handle a long instrument.

The first included article was published in 2004 with the PVOS instrument, which aimed to assess the outcome of surgery for velopharyngeal insufficiency (50). In contrast, few studies have recently applied PVOS. This might be due to its simplicity. The VELO instrument has the dominant place in the recent 3 years (20, 51–61), followed by the type 8 family (VHI, pVHI, and VHI-9i) (57, 61–64). The VELO instrument is specially designed for patients with velopharyngeal insufficiency who accounted for the majority of patients with velopharyngeal inadequacy, thereby enabling its widespread. The VHI instrument has the longest history, which indicates the primary place of voice-related QOL for velopharyngeal inadequacy QOL.

Finally, how can the QOL result be applied to practice? QOL result is one of the therapeutic effect indexes which can indicate the health outcome. The outcome is likely to be influenced by patient and medical treatment factors. After adjusting the patient factors as confounders, the variation of outcomes can be attributed to the difference of treatment effect, which is important for treatment assessment, comparison, and improvement (3, 65–67). Apart from this, the distribution of the health outcomes of patients with velopharyngeal inadequacy can be used for estimating health service demands to provide evidence for health resource allocation.

Some questions, such as the following, still remain unexplored and can be used as directions for future studies of VPI-related QOL measures: (1) What's the relationship between QOL results and other therapeutic indexes? (2) How big is the difference between patients with velopharyngeal inadequacy and their caregivers? Does the difference change with age? and (3) How many changes in the scores can suggest the treatment is effective?

CONCLUSION

Quality of Life (QOL) is an essential index to measure treatment effects. Patient self-reported assessment and caregiver proxy assessment are both valuable. The choice of QOL measure instrument should be made according to research aim, target population, language requirement, time, and labor resources.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

NC and HH contributed to the collection of data, writing, and revising the article. NC analyzed the data. BS and HH supervised the research. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Inspiration After Posterior Pharyngeal Flap Palatoplasty: A Preliminary Study Using Computational Fluid Dynamic Analysis

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Posterior pharyngeal flap palatoplasty (PPF) is one of the most commonly used surgical procedures to correct speech, especially for patients suffering from velopharyngeal insufficiency (VPI). During PPF, surgeons use the catheter to control the lateral velopharyngeal port on each side. Airway obstruction and sleep apnea are common after PPF. To understand the air dynamics of the upper airway after PPF, we used computational fluid dynamics (CFD) to demonstrate the airflow. In our previous study, we have revealed the expiration process of the upper airway after PPF and shown the features of how PPF successfully restores the oral pressure for speech. In this study, we focus on examining the inspiration process. Normal airway structures were included. For the normal velopharyngeal structure, one cylinder was applied to each model. For recapitulating the velopharyngeal structure after PPF, two cylinders were used in each model. The ports for borderline/inadequate closure, which can help the oral cavity get the required pressure, were chosen for this study. A real-time CFD simulation was used to capture the airflow through the ports. We found that the airflow dynamics of the upper airway's inspiration were dependent on the velopharyngeal structure. Although the airflow patterns were similar, the velocities between one-port and two-port structures were different, which explained why patients after PPF breathed harder than before and suggested that the one-port structure might be a better choice for secondary VPI reconstruction based on the CFD analyses.

Keywords: cleft palate, velopharyngeal closure, posterior pharyngeal flap, computational fluid dynamics, palatoplasty complications

INTRODUCTION

A typical speech requires the soft palate to rise and make contact with the posterior pharyngeal wall to close off the nasal cavity from the mouth. Velopharyngeal insufficiency (VPI) happens when this contact is loose, or sometimes no contact exists. Under these circumstances, air can leak into the nose, causing hypernasal vocal resonance and nasal emissions (1). Although there is no gold standard for surgical repair of VPI, posterior pharyngeal flap palatoplasty (PPF) is one of the most commonly used surgical procedures to correct speech, especially for patients suffering from VPI (2–4). The superiorly based pharyngeal flaps were the most commonly performed procedure, ideal for patients with good lateral pharyngeal wall movement but poor palate movement (5).

During PPF, surgical adhesion of the soft palate to the posterior pharyngeal wall is performed, and a 4-mm-diameter catheter is recommended for controlling the velopharyngeal port on each side (6–8). Although PPF is a reliable surgical maneuver for palatal reconstruction, a few unavoidable complications are bothering patients postoperatively, including airway obstruction and sleep apnea. As a result, patients may need further surgery to adjust the ports to correct these problems (9). A 4-mm-diameter catheter represents an approximate 12.5 mm^2 area, and according to its flexibility, it can help control the ports up to 10 mm^2. The concept of controlling the size of the port in 10 mm^2 was based on Warren's pressure-flow device outcomes, which demonstrated that inadequate closure happened when the velopharyngeal port area was more extensive than 20 mm^2 (10–12). Because the one-port structure was changed to the two-port structure, the final size of the port area, which is 10 mm^2, was just divided from 20 mm^2. However, based on recent computational fluid dynamic (CFD) analyses for velopharyngeal conditions, the airflow dynamics were much more complicated (13–17). The velopharyngeal ports' size was calculated to be more than 13.34 mm^2 when inadequate closure occurred, and different velopharyngeal closure patterns led to different airflow dynamics (13, 15). Meanwhile, the reasons for airway complications have been related to tonsils, high flaps, vertical advancement donor site closure method, and velocardiofacial syndrome (18). How the airflow is changed and the comparison of the airflow before and after PPF has never been revealed. Understanding the changes can help avoid airway complications and improve VPI care.

Our previous study has demonstrated the process of airflow before the speech (airflow from the lung to the oral cavity) in the upper airway after PPF (14). In this study, to understand the inspiratory process's airflow after PPF, we again applied real-time CFD to demonstrate the upper airway's air velocity and pressure. The models for normal velopharyngeal closure and velopharyngeal closure after PPF were shown before (14). The velopharyngeal ports were replaced by one cylinder and two cylinders, and inspirations with different velopharyngeal ports were recapitulated. We tried to find the differences in the airflow between the two kinds of structures.

MATERIALS AND METHODS

Study Individuals and Airway Reconstruction

Study individuals and airway model reconstructions were described in our previously published study (14). The normal airway structures of seven individuals (three men and four women, age, 20–31 years), with no notable abnormalities (such as sleep-related symptoms or sleep apnea), were included. For the normal velopharyngeal structure, one cylinder (radius, 2.82 mm; height, 4.5 mm) was applied to each model (**Figure 1A**). Two cylinders (radius, 2.00 mm; height, 4.5 mm) were applied to each model to recapitulate the velopharyngeal structure after posterior pharyngeal flap palatoplasty (**Figure 1B**). Using the cylinder as the replacement allows us to control the variables by changing its radius. ANSYS Discovery Live (DL) (ANSYS Inc., Canonsburg, PA, United States) was used for model manipulation under both circumstances. The cylinders' inferior areas were perpendicular to the trachea's posterior wall and crossed the anterior edge of the atlas. Each cylinder was tangent to the airway's posterior wall. The distance between the two cylinders was set at 4 mm based on the shape of the PPF. The ports for borderline/inadequate closure, which can help the oral cavity get the required pressure, were chosen (14).

Airflow Dynamic Simulation of Inspiration

The real-time CFD simulation was performed under laminar, steady-state airflow at 35℃ in the inspiration direction (19, 20). The nasal walls were non-slip and rigid. The gauge pressure was 0 Pa at the proximal end of the airway (**Figure 1C**). The inspiratory airflow of the inlet condition at the nostrils was 200 ml/s (21). The time of each process was set at 0.1 s for comparison. The fidelity of calculation was set to the maximum in the software. The airflow pressure and velocity of the whole airway and the ports, as well as the airflow pressure at the half-site of the entire airway (**Figure 1D**), were demonstrated by ANSYS Discovery Live (ANSYS Inc.). The scale bar cannot be fixed in this study because the real-time computational fluid dynamics were transient, and the scale bar was changing with time.

Statistics

For testing the difference between PPF (two ports) and normal velopharyngeal closure (one port), the velocity and pressure at the orifice areas at the end of the calculation process (0.1s) were used. A paired *T*-test was applied to compare the velocity and pressure levels at the orifices between two manipulations of the same individuals. *P*-values <0.05 were considered to be significant.

The research protocol was censored and approved by the Ethics Committee of West China Hospital of Stomatology, Sichuan University (Approval No. WCHSIRB-D-2016-084R1). Individual participants could not be identified during or after data collection. Written informed consent was acquired from all the individuals enrolled in this study.

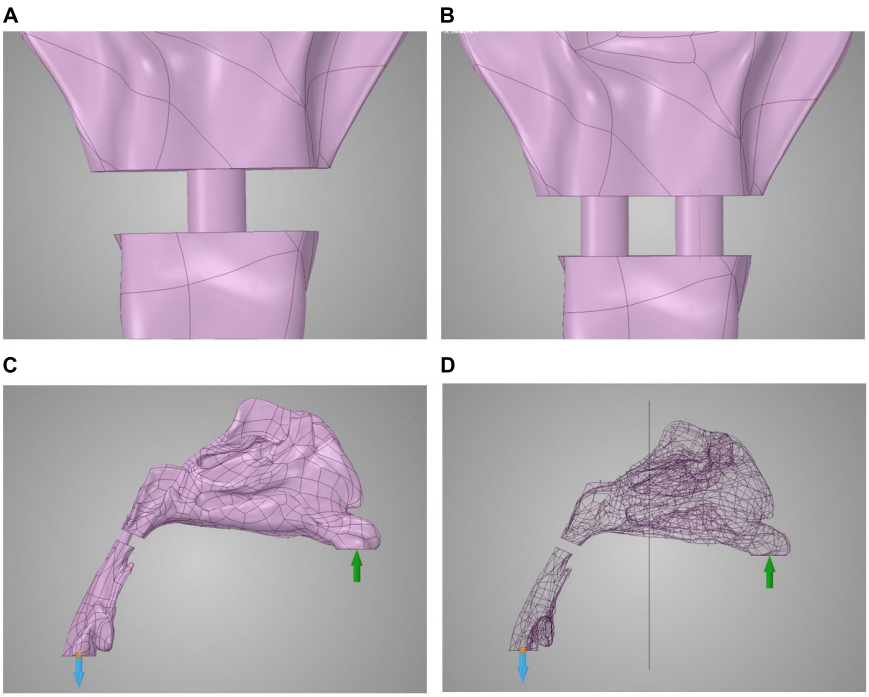

FIGURE 1 | Model reconstruction of normal velopharyngeal closure and velopharyngeal closure after PPF. **(A)** One cylinder for one port under normal velopharyngeal closure, **(B)** two cylinders for the two ports under velopharyngeal closure after posterior pharyngeal flap palatoplasty, **(C)** the boundary set, and **(D)** the plane to divide the whole airway for calculating the airflow pressure in the nasal cavity. The green arrow represents the input of airflow, and blue arrow represents the output of airflow.

RESULTS

Airflow Velocity Patterns Through the Upper Airways

Computational fluid dynamics demonstrated airflow velocity patterns. **Figure 2** shows the airflow velocity through the upper airway. The inspiration process of the one-port and two-port velopharyngeal closures showed no significant difference according to the airflow velocity patterns in the same individual. All the individuals showed a slight velocity increase from the nasal vestibule to the region of three turbinates, and the velocity would decrease when getting into the turbinates. The velocity was lowest in the nasopharynx. The highest velocity happened at the velopharyngeal port.

Differences can be found between different individuals. In individuals 3 and 5 (**Figures 2Cc,Ee**), the main airflow was found to flow through the inferior turbinate. In the other five individuals (**Figures 2Aa,Bb,Dd,Ff,Gg**), the middle turbinate was the main airflow path.

Airflow Pressure Patterns Through the Upper Airways

Figures 3, **4** show the airflow pressure patterns of seven individuals. There was no significant difference between one-port and two-port structures in the same individual, except for individual 4. The nasal cavity pressure was significantly higher than the airway below the velopharyngeal port in individuals 1, 2, 5, and 7 (**Figures 3Aa,Bb,Ee,Gg**). In individuals 3 and 6, the highest pressure happened at the nasal vestibule to the turbinates.

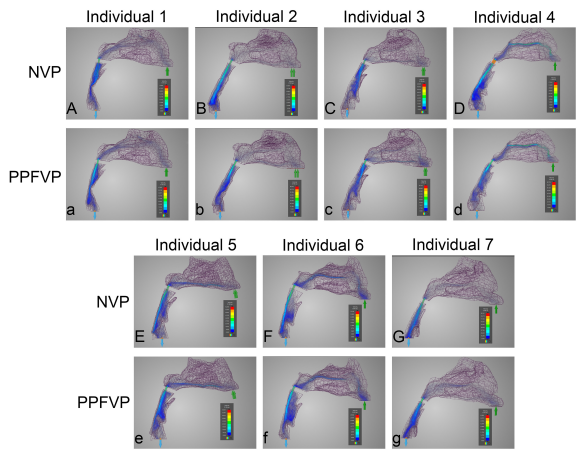

FIGURE 2 | Airflow velocity patterns through the upper airways. The airflow velocity patterns of seven individuals were demonstrated. The color of the airflow was used to show the velocity changing in the same model. NVP, normal velopharyngeal closure (one port); PPFVP, velopharyngeal closure after posterior pharyngeal flap palatoplasty (two ports). The scale bar cannot be fixed because of the real-time simulation process in which the scale bar was changing with time.

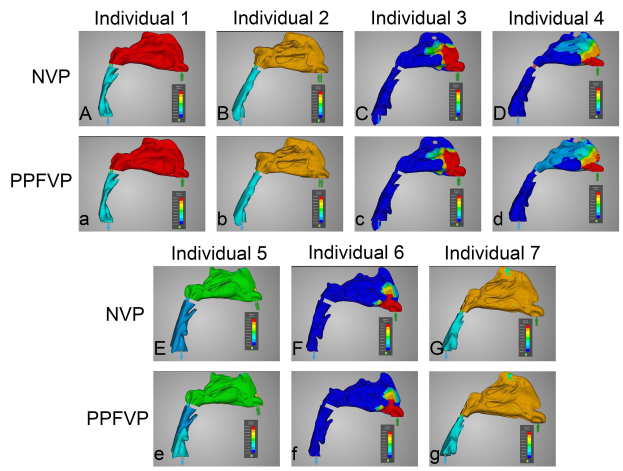

FIGURE 3 | Airflow pressure patterns through the upper airways. The airflow pressure patterns of seven individuals were demonstrated. The color of the airflow was used to show the pressure changing in the same model. NVP, normal velopharyngeal closure (one port); PPFVP, velopharyngeal closure after posterior pharyngeal flap palatoplasty (two ports). The scale bar cannot be fixed because of the real-time simulation process in which the scale bar was changing with time.

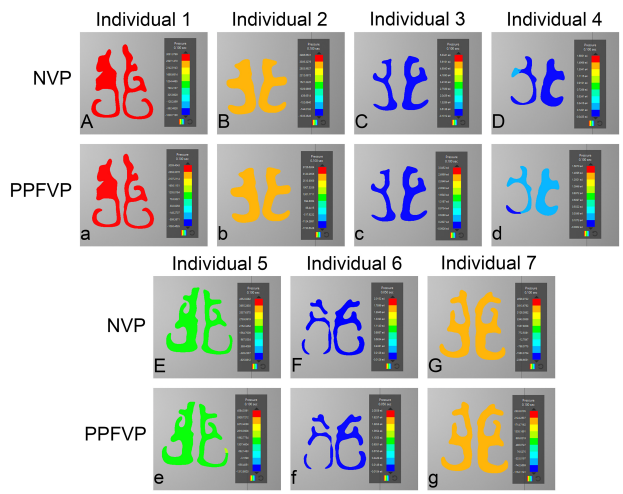

FIGURE 4 | Airflow pressure patterns through the turbinates. The airflow pressure patterns through the turbinates of seven individuals were demonstrated. NVP, normal velopharyngeal closure (one port); PPFVP, velopharyngeal closure after posterior pharyngeal flap palatoplasty (two ports). The scale bar cannot be fixed because of the real-time simulation process in which the scale bar was changing with time.

The difference between one-port and two-port structures was found in individual 4 (**Figures 3Dd**, **4Dd**).

The Difference in the Inspiration Process Between One-Port and Two-Port Structures

Table 1 shows the velocity and pressure of each individual at the velopharyngeal port simulated by CFD. The paired *T*-test was used to compare the velocity and pressure at the orifice between one-port and two-port structures (**Figure 5** and **Supplementary Table 1**). The velocity at the velopharyngeal port of the one port was significantly different from the two ports. The pressures at the velopharyngeal port were the same between the two situations.

DISCUSSION

For decades, PPF palatoplasty has been applied to secondary VPI for restoring speech and correcting abnormal hypernasal vocal resonance and nasal emissions (1, 22). After PPF, the two-port fixed velopharyngeal structure replaces the normal one-port movable velopharyngeal structure. Before the surgery, the velopharyngeal port was too large to close or did not function normally to guarantee enough oral pressure for speech when VPI happened (23, 24). Thus, PPF helps narrow down the connecting tunnel between nasal and oral cavities and divides one large tunnel into two smaller tunnels. The oral pressure under the two-port circumstances could reach the required magnitude much more accessible than before (14). However, the weaknesses of this surgical method are apparent. Airway obstruction is one of the most significant problems caused by PPF, affecting the patients' quality of life (9).

As imaging and computational tools have been improved over the past decades, CFD has become an efficient method for researching patient-specific airway models (25). It has been applied to study cleft palate-related airflow (13–15, 26). CFD can provide visible outcomes that demonstrate the real-time airflow and precisely calculate the fluid parameters. Those parameters can be compared between samples quickly. Thus, we again applied CFD to help us understand the fluid dynamic characteristics of the upper airways of patients with PPF.

The mechanism of PPF, which was to connect the posterior pharyngeal wall physically, was clear (1). The port area was the most important because it directly influences the surgical outcomes. It was also reported that the size of the pharyngeal flap (large, medium, and narrow) could be decided by preoperative velar and pharyngeal movement (27), which in turn could affect the postoperative outcomes. If the ports were too small, the patients would find it difficult to breathe with the nose as the VP ports cannot be changed (28). Then they prefer to breathe with their mouth. It could cause more problems such as abnormal

TABLE 1 | Velocity and pressure at the orifice of the VP port of each individual.

Individual	Normal VP closure (one port)		VP closure after PPF (two ports)	
	Velocity (m/s)	Pressure(Pa)	Velocity (m/s)	Pressure(Pa)
1	8.48	404.58	5.51	548.63
2	10.21	586.22	6.74	370.4
3	6.14	55.36	3.3	139.89
4	9.1	213.26	5.41	192.31
5	7.96	318.61	6.64	225.51
6	3.71	54.71	2.68	12.45
7	9.94	363.97	6.44	149.44

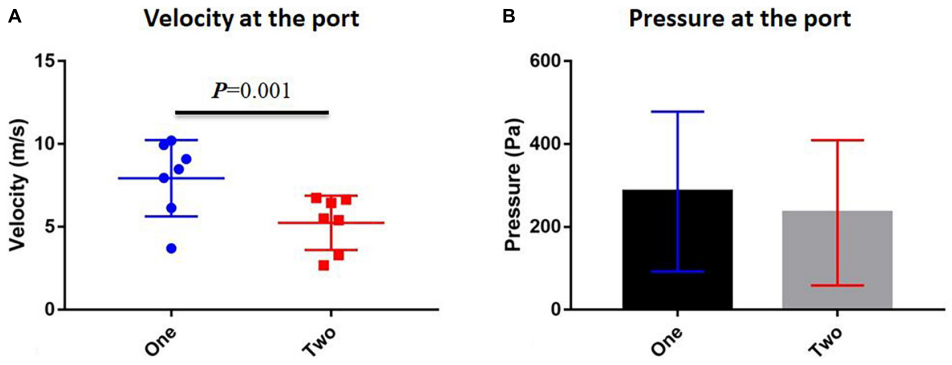

FIGURE 5 | Comparison between one-port and two-port velopharyngeal closures. **(A)** Velocity at the velopharyngeal port and **(B)** pressure at the velopharyngeal port. Error bars are S.D.

maxillofacial growth (29). A large port might not guarantee appropriate oral pressure for speech. According to results from a pressure-flow measurement model by Dr. Warren's team (10–12), the area of two ports should be fixed at 10 mm^2 (8).

Our previous study has found that the area should be around 9.67 mm^2, similar to Warren's recommendation of 10 mm^2 (14). However, the air dynamics were proven to be changed with the two ports when we demonstrated the speech process in the two-port velopharyngeal structure. The speech process was like an expiration process, and significant differences were revealed. The inspiration process should also be analyzed to comprehensively understand the air dynamic changes in the upper airway and those complications such as nasal obstruction. This study focused on this purpose and performed real-time CFD simulation to elucidate the inspiration process characteristics of the two-port velopharyngeal structure.

As we controlled the ports not based on the area but the velopharyngeal function status, the ports' total areas were different under different manipulations of the airway model from the same patient sample. We had demonstrated the reasons in our study that the ports' areas could not be simply divided as the whole airflow system had been changed after changing the structure (14). When we performed the PPF to correct secondary VPI, we hoped the surgery could help the patient's velopharyngeal structure to function like ordinary people. Thus, studying and comparing the airflows of one-port and two-port structures when the velopharyngeal functions are the same should be the right clue.

Interestingly, there was no significant difference in the inspiration airflow patterns between one-port and two-port structures. The phenomena were explainable as we controlled the velopharyngeal closure condition. In this study, these two kinds of manipulations, such as one port and two ports, of the same model were used in the areas under which the airway model worked as a borderline/inadequate closure. The total areas were different between the two manipulations. In other words, the one-port and two-port velopharyngeal closures were in the same condition to gather enough oral pressure. The airflow through the same upper airway should show no significant difference regardless of the port's shape or size when the functions were the same. It needed to be clarified and emphasized that under the same velopharyngeal closure condition as borderline/inadequate closure, the total areas of the ports of one-port and two-port velopharyngeal structures were different, which again proved that the dynamic airflow mechanism of the upper airway is complicated and the two ports cannot be divided from the one port.

The velocity at the orifice showed a significant difference between one-port and two-port groups. The velocities of the two-port structure were lower than those of the one-port structure. It could help us understand why patients suffered airway obstruction and had to use mouth breathing rather than nasal breathing (30). The volume of breathed air should be maintained at a stable magnitude, but under the two-port structure, the airflow velocity decreased, and patients needed more endeavor to get enough air for breathing. That also explained why patients would find it hard to breathe with their nose. Thus, it might suggest that surgeons used a surgical maneuver to remain only at one port and still give the patient the necessary oral pressure for speech. For example, the lateral pharyngeal flap would exclusively remain one port while narrowing down the velopharyngeal port (31). Our study suggested that the one-port structure might help patients feel better than the two-port structure while still guaranteeing the required oral pressure for speech. Anyway, this hypothesis needed further clinical studies, such as speech-based outcomes for the lateral pharyngeal flap, and specific analyses to confirm.

In summary, we first used the simplified models to define the airflow areas for different VP conditions, which built the foundation for the following several CFD analyses (13). We compared different VP closure patterns and found that the patterns could affect the VP conditions (15). Based on this study, it was suspected that many factors could influence airflow dynamics in the airway. PPF was one operation that would permanently change the airway structure, and because of the remained small ports, patients always felt it difficult to breathe with the nose. Thus, we again used the CFD to first check the VP conditions in different port areas with the two ports (14). In addition, in this study, to further show the whole process of the patients after PPF, the inspiration process was

tested. Our study demonstrated the airflow of the inspiration comprehensively and supplemented our latest publication to show the whole speech process of patients after PPF palatoplasty. It could help the surgeon understand the PPF maneuver better and provide important physiological and clinical insights into the velopharyngeal port after PPF palatoplasty. With the help of CFD and our simplified models, we successfully demonstrated the cause of those problems in VPI patients.

Some shortcomings remained in our models. For example, due to the need for demonstrating how the changing of port areas affects the airflow and a lack of a more realistic port structure, the two ports were simplified as two cylinders. Due to the simplification, we did not select the patient samples with VPI, but the validation should be done in the future. This study had to follow our last publication about the expiration of PPF and applied the same manipulations to the models. Moreover, the inspiration rate could not stay constant under real circumstances, although this is negligible due to the recording's short duration. The setting of acquiring the data at 0.1 s was used to compare the airflow differences between structures, which can be changed according to the purpose of the study and should be validated with clinical measurement for the time of getting enough oral pressure before the speech. The airway resistance should be analyzed, and the validation of this CFD methodology by comparing the model predictions with actual surgical outcomes should be completed in future studies. Different times of daily life also could affect patients' inspiration, such as at rest or during exercise and sleeping or awake so that the inlet condition might change. ANSYS Discovery Live is a good example for clinical use, which shows directly to the patients how the airflow through their airway quickly; however, if increased accuracy and high-fidelity of the calculated outcomes such as pressure or velocity are required, CFD software based on finite elements is recommended.

CONCLUSION

Airflow dynamics of inspiration in the upper airway were found to be dependent on the velopharyngeal structure. Although the airflow patterns were similar, the velocities between one-port and two-port structures were different, which explained why patients after PPF breathed harder than before and suggested the one-port structure might be a better choice for secondary VPI reconstruction.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethic Committee of West China Hospital of Stomatology, Sichuan University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CY, JaL, and HL contributed equally to this study and analyzed the data. CY, JaL, HL, NC, and XY contributed to the collection of data. CY, JaL, HL, NC, XY, BS, and HH contributed to writing and revising this study. BS, JtL, and HH supervised the study. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.823777/full#supplementary-material

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