THE OROFACIAL CLEFT PHENOME

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THE OROFACIAL CLEFT PHENOME
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By

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To the loves of my life, Chong, Smee, Matt, Muffin, and Boof.
“If you are not prepared to be wrong, you will never come up with anything original.”
Sir Kenneth Robinson
(1950 – 2020)

“It is more important to know what sort of person has a disease, than to know what sort of disease a person has.”
Hippocrates of Kos
(circa 460 BCE – 375 BCE)
Cleft lip and/or cleft palate, a visible birth defect of the face and mouth, is as old as the beginnings of humankind. Yet, it remains as one of the greatest unsolved medical mysteries. The birth defect stems from oral and facial malformations in the very early stages of the developing embryo in the womb, in the first few months after conception. When something goes wrong during the early period of oral and facial development, the upper lip, roof of the mouth, and teeth become malformed, often with different combinations of defects and degrees of severity.

The cupid’s bow of a cleft upper lip is split up by one or two gaps. In the old days, the common name for cleft lip was harelip as the defect resembled a hare’s split upper lip. Cleft palate is a gap or hole in the roof of the mouth, which resembles bone destruction in the mouth from syphilis, and that misled medical theories and practices. Even though surgical repair of cleft lip evolved from 390 B.C.E., cleft palate repair was not attempted until 1816. Professor Girolamo Fabrizi d’Acquapendente, better known in the medical world as the Father of Embryology, ushered in the dawn of understanding into the origins of cleft lip and palate by his work on the developing foetus in “De Formato Foetu” (c.1600). The striking variations in the physical defects of individuals with isolated cleft lip and/or palate stirred a bone of contention as to the cause and effect. There are two brigades of thought on this. The first brigade contended the disorder was from complex combinations of many gene mutations and environmental factors that tipped the balance towards malformations in the developing foetus. The other brigade mounted fresh evidence on family inheritance of mutated genes as well as new gene mutations causing cleft lip with or without cleft palate that are not inherited from either parent.

The Father of Medicine, Hippocrates of Kos, once said, “It is more important to know what sort of person has a disease than to know what sort of disease a person has.” Not all individuals with the same mutated genes have similar risks to cleft malformations. There are different tallies of babies born with cleft defects from different ancestral backgrounds. Pervasiveness of cleft births was similar to their ancestors that did not change with relocation to new countries. The gender of babies born with clefts mattered as well. It was more common for boys than girls to be born with cleft malformations. Babies born with cleft lip were more likely to be males whereas babies with cleft palate were more commonly females. Unusual patterns of teeth and slow developing teeth implicated their involvement in the disorder. The
unique dental patterns distinguished teeth and their development in individuals with cleft malformations. These were possible biological markers or biomarkers to identify differences in those with orofacial clefts. In children without cleft malformations, the chances of having malformed, missing or slow teeth are small. Teeth form from special cells called neural crest cells that are found next to the spinal cord in the developing foetus. These cells migrate to the head in many segments that unite to form the face, jaws and teeth. It stands to reason that teeth can be affected when the tooth-bearing parts of the jaw fail to form or are improperly joined together. Teeth next to the cleft defect in the jaw bone may be malformed, smaller than normal, or missing, although there are also reports of teeth located away from the cleft defect that are malformed or missing. It is uncertain if the remote dental defects are inherent, part of the cleft disorder, or due to disturbed development from the effects of early surgery to repair the lip and palate defects.

To avoid the confusion from a variety of reasons for abnormal dental development, it is important to distinguish people of different ancestries, males and females, individuals with overlapping cleft-types, or effects from before and after surgery that contribute to the disorder. This series of research seeks to establish the frequency and type of dental biomarkers in infants and children with orofacial clefts from different ancestries, with and without overlapping cleft defects, with and without surgery. We found extra baby incisors on the same side of the cleft defect in unoperated infants with cleft lip only. The extra incisor was surmised to be a disturbance in tooth formation after the segments of bone in the rudimentary upper jaw were fused. No extra nor missing teeth were found in unoperated infants with isolated cleft palate, which indicated tooth development was probably separate from palate formation. Dental development was slow in all unoperated infants with different types of cleft defects, slower in the group with cleft lip than the group with cleft palate. Males were slower in tooth development than females. Slow tooth development also occurred in operated children with cleft lip and palate between 5 to 9 years of age. As these children grew older, tooth formation came up to speed between 9 to 13 years of age and paralleled that of children without clefts. In more than two thirds of children with cleft lip and palate, the permanent upper incisors adjacent to the cleft defect were found to be missing or small.

The research findings provided us with clues to clarify the biological mechanisms in people with different cleft malformations. Accurate findings are important in identifying at-risk individuals by precise matching of biological traits. Detailed information is needed for precision medicine, which offers us the best possible individualised approach to plan, prevent and treat those who are at risk of and affected by this disorder.
ABSTRACT

Introduction
The global average for prevalence in births with orofacial clefts is 9.92 per 10,000. Variability in prevalence could be as much as a seven-fold difference in different ethnicities. Precise phenotyping and subphenotyping were essential in understanding the orofacial cleft phenome. Accurate characterizations to identify biomarkers in different cleft phenotypes would refine the diagnosis to advance personalised medicine in future prevention and treatments.

Aims
To establish the descriptive epidemiology of infants from different ancestries born with orofacial clefts, and to determine the primary and secondary dental anomalies and maturity in infants and children with different cleft-types.

Materials
Study I. Birth Defects Registry records of population live births of multiethnic infants in Singapore, with syndromic and non-syndromic orofacial clefts born in 2003 to 2012.


Methods:
I. Retrospective population-based study of cleft-associated live births of different ethnicities to determine prevalence, trends, heterogeneity in cleft malformations, anomalies associated with cleft defects, and infant mortality rate.

II and III. Retrospective population-based study of Northern European Danish infants with isolated unilateral cleft lip and isolated cleft palate to determine primary and secondary dentition anomalies and longitudinal dental maturity at 2 and 22 months of age.

IV and V. Retrospective case-control cohort study of Singaporean children with unilateral cleft lip and palate to determine secondary dentition anomalies and longitudinal dental maturity at 5 to 9 years and 9 to 13 years.
Results:

I. The overall population prevalence of cleft live births was 16.72 per 10,000 with a flat trendline over ten years. Ethnic-specific prevalence varied: Chinese, 17.17; Malay, 16.92; Indian, 10.74; and mixed ethnicities, 21.73. The infant mortality rate was 4.76%.

II and III. There were no primary nor secondary dental anomalies in unoperated infants with isolated cleft palate. There were primary and secondary dental anomalies in infants with unilateral cleft lip. Dental maturity was delayed in infants of both cleft-types.

IV and V. A high frequency of secondary dentition anomalies was detected in children with unilateral cleft lip and palate. Dental maturity was delayed and asymmetric with greater delay on the cleft side that normalised during adolescence.

Conclusions:

The prevalence of cleft live births was ethnic-specific and the mortality rate of infants with clefts was higher than the population norm. Infants with isolated cleft palate had no primary nor secondary dentition anomalies. Primary and secondary dental anomalies were present in infants and children with unilateral cleft lip, with and without cleft palate. Delayed dental maturity was present in infants and children with clefts.
LIST OF SCIENTIFIC PAPERS

Yow M, Jin A, Yeo GSH.
Submitted to Population Health Metrics.

II. Dental subphenotypes in infants with orofacial clefts – a longitudinal population-based retrospective radiographic study of the primary and secondary dentitions.
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III. Deep orofacial phenotyping of population-based infants with isolated cleft lip and isolated cleft palate.
Yow M, Hermann NV, Wei Y, Karsten A, Kreiborg S.

IV. Secondary dentition characteristics in children with non-syndromic unilateral cleft lip and palate: a retrospective study.
Tan E, Kuek MC, Wong HC, Ong S, Yow M.
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V. Longitudinal dental maturation of children with complete unilateral cleft lip and palate: a case-control cohort study.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CHASQ</td>
<td>Cleft Hearing, Appearance and Speech Questionnaire</td>
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<tr>
<td>CLEFT-Q</td>
<td>Cleft-specific Patient-reported Outcome Questionnaire</td>
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<tr>
<td>CLP</td>
<td>Cleft lip and palate</td>
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<td>CP</td>
<td>Cleft palate</td>
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<tr>
<td>FGF</td>
<td>Fibroblast growth factor</td>
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<tr>
<td>IPDTOC</td>
<td>International Perinatal Database of Typical Oral Clefts</td>
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<tr>
<td>MFT</td>
<td>Multifactorial threshold</td>
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<tr>
<td>OFC</td>
<td>Orofacial cleft</td>
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<tr>
<td>PROMS</td>
<td>Patient-reported Outcome Measures</td>
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<tr>
<td>QOL</td>
<td>Quality of life</td>
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<tr>
<td>SHH</td>
<td>Sonic hedgehog</td>
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<tr>
<td>TGF-β</td>
<td>Transforming growth factor-beta</td>
</tr>
<tr>
<td>UCL</td>
<td>Unilateral cleft lip</td>
</tr>
<tr>
<td>UCLP</td>
<td>Unilateral cleft lip and palate</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<td>WNT</td>
<td>Wingless-related integrated site</td>
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1 INTRODUCTION

1.1 CLEFT LIP AND/OR PALATE

1.1.1 Epidemiology

Cleft lip and/or palate is one of the most common craniofacial birth defects. The global average in prevalence of births with cleft lip with and without cleft palate was 9.92 per 10,000, reported by the Working group of the International Perinatal Database of Typical Oral Clefts [IPDTOC Working Group, 2011]. There were considerable variations in prevalence by geographical location with as much as a sevenfold difference between the highest and lowest reported rates (Fig.1). Comparative studies of groups with cleft lip and/or cleft palate demonstrated immigrants in new countries had similar prevalence of cleft births as the population in the old country [Ching et al., 1974; Leck et al., 1995; Croen et al., 1998; Kirby et al., 2000]. Ancestral genes are implicated but the aetiologies and biological pathways have yet to be elucidated.

Orofacial clefts phenotypes are heterogeneous and vary by cleft-types commonly classified as syndromic and non-syndromic orofacial clefts. There are grouped into three principal classes: cleft lip with or without cleft alveolus (cleft primary palate); cleft palate (cleft secondary palate); combination of cleft lip and cleft palate (cleft primary and secondary palate involvement) [Fogh-Andersen, 1942]. Non-syndromic cleft phenotypes are the most common that account for 85% of overall cleft-types. The frequency of syndromic orofacial
cleft phenotypes is less, 15% of overall cleft-types. Syndromic cleft phenotypes are associated with a recognisable spectrum of co-occurring disorders. There are more than 300 identified syndromes associated with oral clefts [Mossey et al., 2012].

1.1.2 Embryology

Upper lip and palate development begin from the 4th intrauterine week of gestation. Paired facial swellings, the medial nasal and lateral nasal processes, develop on the frontonasal process of neural crest origin [Sperber et al., 2010]. By the 6th week, the upper lip and primary palate are formed from fusion of the medial nasal processes and paired maxillary processes [Jiang et al., 2006]. At about the same time, paired maxillary processes enlarge and form at the primitive mouth. Outgrowths of the maxillary processes begin to develop by the side of the tongue. By the end of the 8th week and beginning of the 9th week, palatal extensions of the paired maxillary processes rise above the descending tongue to meet and fuse in the midline with each other and the medial nasal processes. The fusion of the primary and secondary palates closes off the nasal from the oral cavity. Palatal development is completed by the 12th intrauterine week [Bush et al., 2012].

Odontogenesis occurs at about the same time as facial development. The dental epithelium forms at 6 weeks intrauterine in the primitive mouth. The inner process or dental lamina invaginate into the mesenchyme to form teeth, whereas the outer process or vestibular lamina breaks down to form the vestibule, thus separating the tooth-bearing areas from the lips and cheeks [Hovorakova et al., 2018]. Two cell types contribute to tooth development: the oral epithelium that give rise to the ameloblasts and enamel of the crown; and the neural crest cells that give rise to the dental papilla and tooth follicle to form dentine, pulp and periodontal ligament to complete tooth formation. The development of the maxillary lateral incisor is notable in that the tooth is formed by two components of the dental epithelium, from fusion of the maxillary process and medial nasal process [Ooé, 1957]. If fusion of the medial nasal and the maxillary processes is disrupted, the consequence is a cleft dentoalveolus. The defect in fusion may involve one or both components of the dental epithelium that form the maxillary lateral incisor tooth. When this happens, a number of developmental tooth anomalies may occur, viz. tooth agenesis, microdontic tooth, supernumerary tooth, or talon cusp formation involving the maxillary lateral incisor. This explains why lateral incisor anomalies commonly co-exist with cleft malformations of the dentoalveolus in the primary palate.
1.1.3 Phenotypes

Physical traits in individuals with cleft lip and/or palate are heterogeneous. The clinical presentations are non-standardised and highly variable such that individual descriptions are necessary for the clinical phenotype. The orofacial cleft phenotypes originate from defects of developmental processes in the first branchial arch and the broad classifications of cleft lip, cleft palate, and cleft lip with cleft palate were thought to be genetically distinct [Fogh-Andersen, 1942; Whitaker et al., 1981; Harville et al., 2005; Grosen et al., 2010]. Global rate for the cleft phenotypes was 9.92 per 10,000 with sexual dimorphism associated with different cleft phenotypes. The cleft lip with or without cleft palate phenotype was more frequently found in males and the cleft palate only phenotype was more common in females. Laterality in individuals with cleft lip was not a random feature and presented more often on the left side: the global norm was 63.1% left-sided and 36.9% right-sided. Similarly, the cleft lip with cleft palate phenotype occurred more frequently on the left than right side, 58.9% and 41.1%, respectively. Bilateral cleft lip with involvement of both sides was less common, 10.3% [IPDTOC Working Group, 2011].

1.1.4 Subphenotypes

Subphenotypes were associated with a spectrum of traits that could be both obvious and hidden. The less visible traits reported were anomalies in the brain structure, lip prints, dentition and development, submucous clefts of the lip, dentoalveolus and palate, bifid uvula, and velopharyngeal insufficiency.

1.1.4.1 Craniofacial subphenotype

Many studies reported findings of the differences in the craniofacial structure of individuals with and without orofacial clefts. The syndromic craniofacial phenotype at one end of the spectrum was more obvious than the non-syndromic phenotype. Both intrinsic and extrinsic factors could bear upon the eventual outcomes of the craniofacial structure due to the long-term developmental nature of orofacial formation. The cause and effect of the orofacial cleft facies were complex and unclear. Intrinsic dysplasia and extrinsic environmental influences had bi-directional effects that could modulate craniofacial growth concurrently or consequentially [Hermann et al., 2000; Kreiborg et al., 2006]. Functional adaptations to breathe, effects of inflammation and/or infections or the possibility of iatrogenesis from early surgery, these factors all play a role to a larger or lesser extent in affecting the outcomes of craniofacial structure [Jensen et al., 1983; Kemaloğlu, 1999; Peltomäki, 2007; Rajion, 2012].
Growth potential in the mandible was found to be normal in unoperated infants with isolated cleft palate soon after birth. However, the infants had shorter mandibles that never caught up in length despite a normal growth rate. There was a trend in reduced mandibular size with increased severity of palatal defect in the infants [Eriksen et al., 2006]. The orofacial cleft phenotype in infants was distinctive despite the heterogeneity in cleft defects. In the Danish population-based cohort of infants with isolated cleft lip with and without cleft palate, and isolated cleft palate, all cleft phenotypes were characterised by bimaxillary retrognathism with increased transverse widths of the maxilla and nasal cavity. The size of the pharyngeal airway was reduced and related to the short and retrognathic mandible [Kreiborg et al., 2006].

1.1.4.2 Dental subphenotype

Orofacial development is spatiotemporally regulated. There is coordinated timing of development in differentiation and/or fusion processes. The heterogeneity of cleft phenotypes could be demonstrated as anatomical defects linked to time-dependent disruptions in embryonic and/or foetal development. Based on this premise, the variability in severity of dental subphenotypes were ascribed to differentiation and/or fusion defects that corresponded to the timing of embryonic maldevelopment [Luijsterburg et al., 2014]. Due to the close association of cleft malformations and the frequency of dental anomalies, particularly tooth agenesis, developmental pathways regulated by common genes were speculated to be the cause. This led to the discovery of candidate genes for cleft malformations associated with tooth agenesis [Phan et al., 2016]. Their involvement implicated overlapping orofacial development and odontogenesis, and greater cleft severity was associated with increased dental anomalies. Individuals with bilateral clefts were frequently associated with higher frequencies in odontogenic defects than those with unilateral clefts [Dixon et al., 2011]. Dental maturation was delayed and the delay was greater on the cleft side than the non-cleft side in individuals with unilateral clefts [Eerens et al., 2001; Slayton et al., 2003; Aizenbud et al., 2005].

Higher frequencies of odontogenic anomalies were found in the secondary dentition more so than in the primary dentition. Individuals with cleft defects involving the palate also presented with tooth agenesis at and remote from the cleft region. The prevalence of odontogenic anomalies increased along with the severity of the cleft defect. Postnatal development of the primary, mixed or permanent dentitions could be confounded by several environmental factors during a child’s growth and development. Heterogeneous characteristics of the dentition in individuals with clefts may represent abnormal development with multiple aetiologies that included infections or iatrogenic disruptions during early surgery [Kirkham, 1931; Jensen et al., 1983; Jugessur et al., 2009; Dentino et al., 2012; Phan et al., 2016; Korolenkova et al., 2018].
Biologically relevant groupings with careful distinctions would improve the power of genotype-phenotype precision in future research and diagnosis [Cox et al., 2018].

1.1.5 Dental caries subphenotype

Dental caries is reportedly higher in children with clefts than without clefts [Wells, 2014]. Previous reports have indicated a high prevalence of tooth anomalies in both primary and secondary dentitions that increased risks of dental caries individuals with orofacial clefts [Worth et al., 2017]. Increased susceptibility to tooth decay was attributed to a host of extrinsic and intrinsic factors related to the tooth subphenotype [Chu et al., 2016]. The interaction of several components was necessary to initiate dental decay, viz. tooth-surface, intraoral biofilm, substrate from the diet, salivary quality and quantity, and time (Fig. 2).

Figure 2. Inter-relationship of the multifactorial components in dental caries.

Caries prevention involved maintenance of a balance of the factors at play. An imbalance in any one of the components predisposed to dental decay [Selwitz et al., 2007; Aas et al., 2008]. Disrupted functions of the salivary glands and abnormal tooth development were found to be associated with a mutated gene, IRF6, a known candidate gene of syndromic and non-syndromic cleft lip and palate phenotypes. Dental-specific IRF6-cKO, demonstrated in a murine model, substantially increased caries risk from defective tooth-patterning and amelogenesis in conjunction with salivary gland dysplasia that reduced salivary flow and protein production [Chu et al., 2016; Tamasas et al., 2016]. Other risk factors for dental caries in individuals with clefts were malaligned and/or hypoplastic teeth at the cleft site, circumoral tightness and stiffness of the scarred upper lip that reduced food shedding and access to the toothbrush, and orthodontic appliance interventions that modified biofilms on teeth. Individuals with clefts are at risk of dental caries due to a multitude of factors, and as such, it will be prudent to identify early strategies for caries prevention in this group.
1.1.6 Associated anomalies

Associated anomalies are congenital defects characterized by functional, structural, morphological, or positional anomaly of a single or part of an organ co-occurring with the cleft malformation before birth. They can be recognized during prenatal assessments or at birth, although some structural or functional defects are detected a year after birth or even later [Impellizzeri et al., 2019].

The prevalence of associated systemic anomalies in individuals with clefts vary from as low as 1.5% to as high as 63% [Shprintzen et al., 1985]. The frequency of associated anomalies differed in different cleft phenotypes. It was reported to be lowest in individuals with cleft lip defects, between 7.6 to 41.4%, as opposed to a higher frequency in individuals with cleft lip and palate defects, ranging from 21.1 to 61.2%. Individuals with cleft palate have the highest frequency of associated systemic anomalies, 22.2 to 78.3% [Maarse et al., 2012].

Defects of the heart, musculoskeletal system and nervous system were the most commonly detected associated anomalies in the cleft palate phenotype. The order of defects in descending frequency found in the cleft lip with cleft palate phenotype was the heart, nervous system, and musculoskeletal system [Genisca et al., 2009]. One of the most commonly associated musculoskeletal anomalies involved defects in cervical vertebral fusion that affected more than 50% of Swedish children with non-syndromic cleft lip and/or palate compared to children without clefts in a treatment centre [Karsten et al., 2019]. Due to advanced medical technology in routine obstetric screening, associated anomalies were diagnosed more often in individuals with orofacial clefts. Detection of anomalies in prenatal screening predicts presence of chromosomal abnormalities that require further tests and postnatal follow-up for timely and appropriate interdisciplinary management [Maarse et al., 2011].

1.1.7 Genetics, Epigenetics, and Environment

1.1.7.1 Genetics

In individuals with OFC syndromes, a recognisable cluster of physical and developmental anomalies is found. There are more than 350 syndromes associated with orofacial clefting documented in the Online Mendelian Inheritance in Man [OMIM®]. In individuals with syndromic clefts, 75% of them have a mutation at a single genetic locus transmitted by Mendelian inheritance. The most common syndromic cleft condition is the van der Woude syndrome that accounted for 2% of all cleft phenotypes [Kondo et al., 2002]. The syndromic orofacial cleft phenotypes were more likely to have cleft palate or cleft lip with cleft palate rather than cleft lip malformations [Leslie et al., 2013].
More individuals, 70%, are affected by non-syndromic than syndromic clefts. The aetiology is not straightforward as the disorder is not purely of genetic origin. With less than 50% concordance in monozygotic twins, variable expressivity and low penetrance, the cause of non-syndromic cleft phenotypes was postulated to be multiple genetic variants and environmental factors that occurred at different time-points during embryogenesis and foetal development. Much knowledge in the causal genes of non-syndromic cleft phenotypes was driven by gene discoveries in individuals with syndromic clefts [Rahimov et al., 2012; Luijsterburg et al., 2014; Beaty et al., 2016].

With the use of complex genetic mapping, more than 30 candidate genes were identified in the non-syndromic cleft phenotypes [Genetics Home Reference]. Candidate genes, grouped by protein families, molecular functions, and biological functions, were different for the CP and CLP phenotypes. The CP phenotype was associated with the T-box, collagen-α chain, and TGF-β gene families whereas the CLP phenotype was associated with several, viz. heparin-binding FGF, patch-related, zinc-finger, neurotransmitter gated ion channel, tyrosine protein kinase, WNT-related, acyltransferase, intra-flagellar transport 140/170-related, transferase-related, and tropomyosin gene families. There was an overlap for both phenotypes in the homeobox domain [Funato et al., 2017; Leslie et al., 2017]. Genes associated with the tooth-agenesis cleft phenotype came from five major families: WNT, FGF, BMP, TGF and PAX [Phan et al., 2016] that encoded proteins for neural crest development in face, palate, dentoalveolus and teeth formation. In families of non-syndromic cleft individuals, de novo single gene mutations were identified. Pathogenic alleles were found in five genes, CTNND1, PLEKHA7, PLEKHA5, ESRP2, and CDH1 that were responsible for the deregulation of the epithelial adhesion pathway in the cause of non-syndromic cleft lip with and without cleft palate phenotypes [Cox et al., 2018].

1.1.7.2 Epigenetics

In addition to genetics, epigenetics, which could be heritable, modified gene activity that affected the cleft phenotype at the cellular or organ level. It involved risk factors and complex mechanisms that could explain the inconsistencies in penetrance and expressivity of the OFC genotype-phenotype [Beames et al., 2020]. Distinguishable methylation profiles were found in the three cleft-types of cleft lip only, cleft palate only, and cleft lip and palate. A much greater methylation profile difference was detected between the cleft lip only group and the cleft palate only group. The cleft lip with cleft palate group was more similar to the cleft lip only group [Sharp et al., 2017]. While much progress has been made in identifying the genetic basis of
individuals with cleft lip and palate, not much is known about the mechanisms of epigenetics that affected the variability in phenotypic expressions.

1.1.7.3 Environment

Environmental or behavioural risk elements were implicated in the complex interaction of genetics and epigenetics in the causation of cleft lip and palate. A number of environmental factors was implicated as contributory to the multifactorial nature of orofacial cleft malformations: tobacco use, alcohol consumption, diet, pharmacological consumption, occupational/domestic exposures, infections, and maternal conditions [Garland et al., 2020; Martinelli et al., 2020]. Tobacco, in all its forms of consumption, exposed users to nicotine in cigarettes, e-cigarettes, cigars, hookah, and snus or snuff. Maternal exposures to first or second-hand smoke were associated with significant 1.5-fold increase in pregnancy outcomes of infants born with non-syndromic orofacial clefts. Mothers consuming dissolvable tobacco in snus or snuff had similarly high odds of having babies born with orofacial clefts [Gunnerbeck et al., 2014]. A weaker association of pregnancy outcomes with orofacial clefts was found in the alcohol consumption group [Bille et al., 2007; Kummet et al., 2016].

1.1.7.4 Maternal condition

Maternal conditions that increased risks of orofacial clefts in the developing foetus were high maternal age, hypertension, preeclampsia, and diabetes mellitus [Gunnerbeck et al., 2014]. Pre-gestational and gestational diabetes in pregnant mothers were significantly related to infants born with cleft palate and cleft lip with or without cleft palate. Pre-gestational diabetes was independent of increased body mass index (BMI) in the association of risks of infants born with defects, whereas gestational diabetes in combination with a BMI of 25 kg/m² or greater was associated with increased risks of isolated or multiple birth defects [Correa et al., 2008]. Medication especially topiramate, used in maternal epilepsy, was associated with increased risks in pregnancy outcomes of infants with cleft lip with or without cleft palate [Alsaad et al., 2015]. Maternal habitus and increased abdominal fat were associated with increased risks in foetal anomaly development. The risk of neural tube defects in foetal development increased by almost two-fold in pregnancies of women with greater BMI [Rasmussen et al., 2008].

1.1.8 Multidisciplinary management

The principal objectives in treatment of an individual with orofacial clefts were to achieve functional hearing, speech, eating, swallowing, and facial balance. A harmonious facial appearance was important for social acceptance and integration into the community.
Interdisciplinary management to repair and habilitate individuals with clefts required a high level of expertise and coordination of long-term healthcare services, and there could be lifelong implications depending on the severity of the cleft defect.

Coordination of care was demanding and included the patients and families. Support services were required for feeding, and importantly, in coping skills to manage psychosocial challenges and expectations of growth and treatment outcomes. Depending on the severity of the cleft malformation, the coordinated and sequenced presurgical-surgical procedures, postsurgical evaluations, and long-term assessments, could be onerous with several surgeries and secondary revisions. Adequate support tailored to the needs of the patient and family was necessary to prevent treatment-fatigue in parents/caregiver and patients. An integrated team of trained multidisciplinary experts for long-term coordinated care of the patient was essential for good outcomes [American Cleft Palate-Craniofacial Association, 2018].

1.1.9 Burden of care
The number of treatment procedures that children with orofacial clefts could undergo varied as according to the severity of the cleft defect, the child’s age and needs, associated anomalies or other birth defects, and the expectations of the patients and families.

1.1.9.1 Burden of treatment
Depending on individual needs, the treatment protocol can span the period of time from soon after birth until the late teens or young adulthood. Surgery to repair a cleft lip is usually done in the first few months of life. If the infant does not meet the surgical “rule of 10s” in age (10 weeks), weight (10 pounds), and haemoglobin (10 grams), primary surgery is delayed until the infant is fit for surgery. Surgery to repair a cleft palate is recommended within the first 18 months of life or earlier for speech development [de Ladeira et al., 2012]. In coordination with primary surgical care, most children undergo a host of other treatments: early dental check-ups and care, speech therapy, audiology and sleep-disordered breathing assessments, otolaryngological interventions, alveolar bone grafting, orthodontic treatment, orthognathic surgery, and prosthodontics for malformed or missing teeth. Primary surgeries of the upper lip and soft palate to reposition and repair the disrupted lip and oropharyngeal muscles are necessary for normal swallowing and speech development. Secondary surgical revisions may be required to enhance nasolabial appearance although there was controversy over the effectiveness of secondary surgeries in improving primary surgical outcomes. More than half of the patients who underwent secondary revisions did not achieve improved outcomes in nasolabial appearance [Trotman et al., 2007; Long et al., 2011]. Subjectivity in appearance
assessments and socioeconomic circumstances played a role in the variable experiences that could increase the burden of secondary surgical care [Sitzman et al., 2015; 2020].

1.1.9.2 Burden of healthcare expenditure

The goals in care of individuals with clefts were both function and cosmesis driven. The multitude of healthcare visits, assessments, multidisciplinary treatment sessions, primary and secondary surgeries, time, travel, and expenses, constituted an immense burden of care that could take a toll on the patient and family’s wellbeing in the long term. In countries with no socialised healthcare, a lifetime in private healthcare insurance expenditure for an individual with orofacial cleft was estimated to be $101,000 [Boulet et al., 2009]. Healthcare costs for children with clefts were eight times higher than that of unaffected children due to higher consumption of hospital services [Boulet et al., 2009; Wehby et al., 2012].

1.1.10 Patient-reported outcomes

With growing emphasis on patient-centred care, patient-reported outcome measures (PROMS) are increasingly being used in determining healthcare outcomes. Measures focus on the concerns of patients to evaluate the effectiveness of different treatments. They are particularly useful in identifying alternative care. Patients are taken on board as integral members of the healthcare team to participate in formulating health policies, assessing treatment and outcomes, and establishing patient-safety guidelines [Rivera et al., 2019].

The Cleft Hearing, Appearance and Speech Questionnaire (CHASQ), is an example of a PROMS questionnaire developed for self-reported outcome measures of individuals with cleft lip and palate. In comparing Swedish and British individuals with cleft lip and palate between 9 to 20 years of age, both groups had similar CHASQ scores reflecting satisfaction in all three domains assessed [Stiernman et al., 2020]. In the cleft-specific patient-reported outcomes (CLEFT-Q) of individuals with cleft lip and palate from 12 countries, the need for further treatment was indicated by older females with visible facial scars [Klassen et al., 2018]. The findings from PROMS were at odds with professional opinions on secondary surgery [Sitzman et al., 2015, 2020]. It is evident that value-added care is only possible by patients and professionals coming together to understand what are the concerns important to patients that impact their lives, and the professional perspectives on what can be realistically achievable with treatment.
1.1.11 Quality of life

Quality of life (QOL) is defined by the World Health Organisation (WHO) as “an individual’s perception of their position in life embedded in a cultural, social, and environmental context.” [WHO QOL Group, 1998]. The multi-dimensional aspects of the WHO QOL questionnaire involve subjective assessments of one’s own health, functional, and socioemotional wellbeing, satisfaction with the care given, and their sense of self [Skevington et al., 2004] that take into consideration the physical, psychological, social, family, and environmental aspects of QOL. Multiple impaired functions of individuals with orofacial clefts place them at risk of lower QOL. Coping challenges from having facial differences have significant impact on wellbeing, especially those with severe dentofacial discrepancies recommended for surgical interventions. Other factors affecting functional wellbeing were speech and dental development [Broder et al., 2014]. There were many QOL implications for individuals with clefts throughout their lives, chief among which was the significant expenditure in time and cost on healthcare use [Wehby et al., 2010]. The psychological health of children and adults with cleft lip and/or palate was significantly affected in the psychosocial domain [de Queiroz et al., 2015].
2 AIM

General:

a) To establish the descriptive epidemiology of infants born with OFC of different ancestries in a multiethnic country.

b) To determine the longitudinal prevalence and patterns of primary and secondary dentitions in infants with orofacial clefts.

Specific:

Study I. Establish the orofacial cleft live birth prevalence in the resident population groups with different ancestries, heterogeneity in cleft-types, and mortality rate of infants with orofacial clefts.

Study II and III. Investigate dental anomalies and maturity of the primary and secondary dentitions in unoperated and operated infants with isolated unilateral cleft lip, and unoperated infants with isolated cleft palate.

Study IV and V. Investigate dental anomalies and maturity of the secondary dentition in a cohort of operated children with isolated unilateral cleft lip and palate.
3 MATERIALS AND METHODS

3.1 STUDY GROUPS
Study I. Registry data of resident population-based cleft live births (N=608) in a multiethnic country, Singapore.

Study II and III. Registry data of population-based cleft live births of Northern European ancestry in Denmark: unoperated infants (2.3 months) with isolated unilateral cleft lip and isolated cleft palate (UCL, n=183; CP, n=83). Longitudinal follow-up of infants (22 months) with operated isolated unilateral cleft lip (UCL, n=111) and unoperated isolated cleft palate (CP, n=81).

Study IV and V. National Dental Centre Singapore registry of consecutively treated cohort of children with unilateral cleft lip and palate (5 to 9 years, N=60; 9 to 13 years, N=55).

3.2 CONTROL GROUPS
Study I. Registry data of resident population-based live births (N=363,633) in a multiethnic country, Singapore.

Study II and III. The London Atlas of Human Dental Development and Eruption.

Study IV and V. National Dental Centre Singapore’s registry of radiographs of children with no orofacial clefts (5 to 13 years, N=115) with radiographs for dental treatment purposes.

3.3 METHODS
3.3.1 Study I
The International Classification of Diseases 9th Edition (ICD-9) with Extension of the British Paediatric Association (BPA) Classification of Diseases (1979) Coding of Birth Defects [ICD-9, 1979] was used for subjects registered from 2003 to 2011. Individuals registered from 2012 onwards were coded using the International Classification of Diseases 10th Edition (ICD-10) Chapter XVII Royal College of Paediatric Child and Health Extension [ICD-10, 2004]. Extractions from the Registry’s database were done by using the following codes in ICD-9: 749, and in ICD-10: Q35-Q37. Cleft laterality (side of the cleft), submucous cleft, bifid uvula and grading of the cleft defects were not classifiable by the codes and could not be recorded. The count of infants with clefts was by live pregnancy outcomes of Singaporean mothers grouped by ethnicity and registered by Immigration and Checkpoints Authority as resident
citizens or permanent residents living in Singapore in the period 2003 to 2012. Live births of foreigners in Singapore and Singaporeans who did not reside in the country were excluded. Stillbirths and abortions (spontaneous and elective) were also excluded.

Resident population-based data were compiled from the Singapore National Birth Defects Registry. The database was compiled from multiple sources: the cytogenetics and histopathology laboratories, neonatal wards and maternity hospitals, medi-claims, birth defects, death certificates with reported congenital anomalies, stillbirths and abortuses (spontaneous and elective). The study population data sources comprised government organizations, public and private healthcare institutions. The data from 2003 to 2012 was compiled from multiple sources: cytogenetics and histopathology laboratories, neonatal wards and maternity hospitals, medi-claims, birth defects, death certificates with reported congenital anomalies, stillbirths and abortuses (spontaneous and elective). All data were anonymised by the Registry before study analysis.

3.3.2 Study II and Study III

Grading cleft defect severity:

![Classification of unilateral cleft lip severity](image)

Grading of cleft lip severity (Fig. 3) was by the extent of involvement of the upper lip: Grade 1 - up to one-third of the lip height from the lower vermilion border of the upper lip; Grade 2 - greater than one-third and up to two-thirds of the upper lip height; Grade 3 - greater than two-thirds to subtotal of the upper lip height; Grade 4 - the total upper lip height.

Grading of cleft palate severity (Fig. 4) was by the extent of involvement of the secondary palate: Grade 1 - soft palate only; Grade 2 - up to one-third of the palate from the posterior; Grade 3 - greater than one-third and up to subtotal of the palate from the posterior; Grade 4 - the total length of the palate up to the incisive foramen.
The primary dentition and longitudinal secondary dentition formation were evaluated by retrospective records and radiographs for anomalies in tooth-number and crown morphology. Dental maturation was assessed by tooth formation stages (Fig. 5 and Fig. 6).

**Staging dental maturity of infants at 2 and 22 months of age:**

![Figure 5. Simulated radiographic stages of incisal maturation. Adapted from Moorrees et al., 1963.](image)

![Figure 6. Simulated radiographic stages of molar maturation. Adapted from Moorrees et al., 1963.](image)

Definition of tooth maturation stages [Moorrees et al., 1963]: Ci - initial cusp formation (not assessed as this stage is easily missed or mis-identified in the radiographs); Cco - coalescence of cusps; Coc - cusp outline complete; Cr½ - crown half completed with dentine formation; Cr¾ - crown three-quarters completed; Crc - crown completed with defined pulp roof; Ri - initial root formation with diverged edges.

**3.3.3 Study IV and Study V**

**Staging dental maturity of children at 5 to 13 years of age:**

Secondary dentition formation and maturity were evaluated by retrospective records and radiographs for anomalies in tooth-number and crown morphology. Dental maturation was assessed by tooth formation stages (Fig. 7) as according to the definition of tooth maturation stages [Demirjian et al., 1973]: Stage A - cusp tips are calcified but are not fused yet; Stage B - calcified cusps are united so an outlined occlusal surface is well defined; Stage C - enamel
formation is complete at the occlusal surface. Dentinal deposition has commenced. The outlines of the pulp chamber are curved; Stage D - crown formation is complete to the cementoenamel junction. The pulp chamber in the uniradicular teeth is curved being concave toward the cervical region. In the molars, the pulp chamber has a trapezoid form. The pulp horns are beginning to differentiate. Root formation is seen; Stage E - the walls of the pulp chamber are straight. The pulp horns are more differentiated. The root length is less than the crown height. In molars, the radicular bifurcation is visible; Stage F - the walls of the pulp chamber now form an isosceles triangle. The apex ends in a funnel shape. The root length is equal to or greater than the crown height. In the molars, the bifurcation has developed sufficiently to give roots a distinct outline with funnel shaped endings; Stage G - the walls of the root canal are now parallel and its apical end is still partially open (distal root in molars); Stage H - the apical end of the root canal is completely closed (distal root in molars). The periodontal ligament has a uniform width around the root and the apex.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Molar</th>
<th>Bicusp</th>
<th>Cusp</th>
<th>Incisor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><img src="image1" alt="Molar A" /></td>
<td><img src="image2" alt="Bicusp A" /></td>
<td><img src="image3" alt="Cusp A" /></td>
<td><img src="image4" alt="Incisor A" /></td>
<td>Cusp tips are calcified but are not fused yet.</td>
</tr>
<tr>
<td>B</td>
<td><img src="image5" alt="Molar B" /></td>
<td><img src="image6" alt="Bicusp B" /></td>
<td><img src="image7" alt="Cusp B" /></td>
<td><img src="image8" alt="Incisor B" /></td>
<td>Calcified cusps are united so an outlined occlusal surface is well defined.</td>
</tr>
<tr>
<td>C</td>
<td><img src="image9" alt="Molar C" /></td>
<td><img src="image10" alt="Bicusp C" /></td>
<td><img src="image11" alt="Cusp C" /></td>
<td><img src="image12" alt="Incisor C" /></td>
<td>Enamel formation is complete to the occlusal surface. Dentinal deposition has commenced. The outlines of the pulp chamber are curved.</td>
</tr>
</tbody>
</table>
| D     | ![Molar D](image13) | ![Bicusp D](image14) | ![Cusp D](image15) | ![Incisor D](image16) | Crown formation is complete to the cementoenamel junction. The pulp chamber in the uniradicular teeth is curved being concave toward the cervical region.
In the molars, the pulp chamber has a trapezoid form. |
| E     | ![Molar E](image17) | ![Bicusp E](image18) | ![Cusp E](image19) | ![Incisor E](image20) | The walls of the pulp chamber are straight. The pulp horns are more differentiated.
The root length is less than the crown height. In molars, the radicular bifurcation is visible. |
| F     | ![Molar F](image21) | ![Bicusp F](image22) | ![Cusp F](image23) | ![Incisor F](image24) | The walls of the pulp chamber now form an isosceles triangle.
The apex ends in a funnel shape. The root length is equal to or greater than the crown height. In the molars, the bifurcation has developed sufficiently. |
| G     | ![Molar G](image25) | ![Bicusp G](image26) | ![Cusp G](image27) | ![Incisor G](image28) | The walls of the root canal are now parallel and its apical end is still partially open (distal root in molars). |
| H     | ![Molar H](image29) | ![Bicusp H](image30) | ![Cusp H](image31) | ![Incisor H](image32) | The apical end of the root canal is completely closed (distal root in molars). The periodontal ligament has a uniform width around the root and the apex. |

Figure 7. Stages of secondary dentition maturation. Adapted from Demirjian et al., 1973.

Study casts, dental panoramic, anterior maxillary occlusal, and periapical radiographs of the patients were examined for cleft-sidedness, congenitally missing permanent teeth, supernumerary teeth, microodontic, and macrodontic teeth in the anterior maxillary region, presence of malformed permanent cleft-sided lateral incisor and its morphology (peg-shaped, conical shaped, canine-formed), positions of the permanent lateral incisors relative to the cleft side and presence of rotated cleft-sided central incisors.
3.3.4 Statistical analysis

Study I – Prevalence denominator was population live births per 10,000. Linear regression was used in trend tests and the significance level was set at 5%.

Study II and III – Descriptive statistics of mean, standard deviation, median, minimum and maximum values, and frequencies and percentages for categorical variables. Cohen’s kappa coefficient was used to measure agreement in dental maturity assessments. Fisher’s exact test was used for associations between dental development stages by gender and grades of severity as well as to test for statistical differences in frequency of dentitional anomalies between male and female infants with UCL and between the UCL group and controls. The McNemar test was used in analysing agreement of dental anomalies in the two dentitions and dental maturity between the groups with UCL and CP. All tests performed were two-sided. The significance level was set at 5%.

Study IV and V – Paired t-test was used to evaluate the comparison in the mean dental age delay between the groups with cleft and no cleft. Two-sample t-test and Kruskal-Wallis test were used to test for significant gender or ethnic differences. The dental age delay of complete UCLP subjects with and without hypodontia was compared using the Mann-Whitney U test to determine if hypodontia affected the dental development. Spearman’s correlation coefficient evaluated the relationship between the severity of hypodontia and dental age delay. Poisson regression analysis with corrected multiplicative dispersion factor was used to compare the risk of asymmetric tooth pairs between the groups with and without clefts. The significance level was set at 5%.

3.3.5 Error of the Method

Study I – Ascertainment of every case-entry was matched against existing records in the system. The merging functions and contradiction modules checked, verified, and handled inconsistencies to resolve discrepancies and duplication. Field visits were conducted by the Registry coordinators at the medical records offices of restructured and private hospitals for data collection. Annual audits were done to standardise the definitions of data items and abstraction rules to ensure standards of consistency and accuracy in data collection. Inter-rater reliability audits of similar cases abstracted by the Registry coordinators were checked for levels of agreement.
Study II and III – Accuracy in establishing dental age for dental maturity was tested for reliability and precision by two orthodontists using the radiographs of 38 infants. Intra-assessor and inter-assessors’ determination of dental maturity ages were established by Cohen’s kappa coefficient for strengths of agreement, 0.9286 and 0.7994, respectively. Detection of dentition anomalies were repeated for all cases with perfect agreement.

Study IV and V – The Demirjian’s Dental Development system was used to assess secondary dentition maturity. Intra-examiner reliability in assessing tooth formation by test-retest with 60 teeth and analysed using the Cohen’s kappa coefficient, the strengths of agreement for each tooth ranged from 0.70 to 1.00. Detection of dentition anomalies were repeated for all cases with perfect agreement.

3.3.6 Ethical Considerations

The ethical implications in the use of registry data relevant to the studies are discussed here. The four principles in principlism [Gillon, 2003; Yan et al., 2004; Buchanan, 2008; Beauchamp et al., 2013] will be used in the discussion of the ethics involved in registry-based research.

The four key principles are beneficence, non-maleficence, autonomy, and justice. These are the pillars in upholding the standards of practice by which all healthcare professionals are held accountable to. They form the framework and values for thought processes and decision-making in adopting appropriate practices to do good, avoid harm, support and respect the independence of individual’s actions and rights, and fair play.

The first principle of beneficence, to do good, was well considered in all the studies. The individuals were unaware of the situations and use of their medical, dental, and health information. It is possible for distress and discomfort to be experienced by individuals if they are informed of research findings of predictions in health risks and reduced life expectancy outcomes. The ethical stand is to exercise sensitivity in dissemination of information, particularly if it is by mass media and not a one-on-one. Pre-emptive public counselling should be made accessible to mitigate any negative impacts from discomfiture of individuals on receiving uncertain news. To uphold the principle of beneficence, patient-support programmes building on the knowledge from the research will prepare future safeguards of population health and psychological wellbeing of those affected.
The second principle of non-maleficence, to avoid harm, was addressed by ways to prevent breaches in confidentiality of personal data. Data extraction, anonymization and aggregation were done by a trusted party to preserve the privacy of individuals and confidentiality of personal medical information in all studies. Further safeguards were implemented by way of securing secondary data in a password-protected, non-networked computer within the institutions or encrypted data storage devices used by members of the research team.

The third principle triggers a controversial point as there is lack of informed consent with no leeway for autonomy in the use of registry data for research. It can be argued as to what degree of sacrifice is acceptable in limiting an individual’s freedom of choice in giving up rights to privileged information to help others. The ethical standing on this issue is to weigh the benefits of the greater good versus autonomy. If a gain in knowledge of health-giving from research outweighs multiple chronic health issues, shortened life span, and onerous burden of care, then the ethic of civic virtue in pursuing the greater common good should be upheld.

The fourth principle of justice is that of fair play. This principle ensures the interests of the vulnerable groups of infants and children are looked after. If studies of the infants and children’s records were done without merits or benefits, that would have been unjust scrutiny. The principle of justice is upheld in all the studies as the gain in knowledge has the potential for applications in present and future healthcare practices to improve the diagnoses, treatments and pregnancy outcomes.

The Hippocratic Oath calls for obligations to provide health benefits with minimal harm. The four keys of principlism render a foundation by which healthcare professionals can fulfil the obligatory responsibilities to improve population health. The emphasis in research ethics using human subjects and their data is all-important and must be taken seriously in this undertaking.
4 RESULTS

4.1 STUDY I

Prevalence per 10,000 for live births of all clefts, isolated clefts with no associated malformations, cleft lip, cleft palate, cleft lip with cleft palate were 16.72, 8.77, 6.85, 3.16, and 6.71, respectively. Prevalence stratified for gender and ethnicities were: male, 17.72; female, 15.78; Chinese group, 17.17; Malay group, 16.92; Indian group, 10.74; and mixed ethnicity group, 21.73 (Fig. 8). Infants with isolated clefts, non-isolated clefts with other malformations, and syndromic clefts were 52.5%, 42.1% and 5.4%, respectively (Table 1). Upward trend in infants with clefts was not significant (p=0.317) (Fig. 9). The mortality rate in infants with clefts was 4.76%.

![Graph showing prevalence of clefts over years by ethnicity.](image)
In infants with UCL, the frequencies of dental anomalies were high in both primary (38.3%) and secondary (18.0%) dentitions. No primary or secondary dentition anomalies were observed in infants with CP. Longitudinal dental anomalies in the UCP group were dissimilar (p=0.003). Risk differences involved primary supernumerary teeth (p=0.0001) and talon cusp formation (p=0.0001), and secondary tooth-agenesis (p=0.001) of the maxillary lateral incisor.
corresponding to side of the cleft lip when compared with the control (Table 2). Delayed primary and secondary dental maturation occurred in the UCL and CP groups, greater in infants with UCL (p<0.0001) (Table 3). Primary and secondary dental maturation featured sexual dimorphism with greater delay in males (UCL, p<0.0001; CP, 0.0001>p=0.001). The effect of cleft severity on dental maturation was significant in infants with UCL (p=0.0361) and CP (p=0.0175) in the primary but not secondary dentition. The findings underscore the importance of accurate deep phenotyping to decipher confounding variants in the genotype-phenotype driven precision medicine.

Figure 10. Distribution of dental anomalies – comparison of infants with UCL and control.

<table>
<thead>
<tr>
<th>Primary Dentition</th>
<th>UCL group Frequency N=111 (100%)</th>
<th>Control Frequency N=4,564 (100%)</th>
<th>UCL group comparison with control</th>
<th>p-value</th>
<th>Risk difference</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agenesis</td>
<td>0 (0.0)</td>
<td>25 (0.6)</td>
<td>1.000</td>
<td>-0.005</td>
<td>-0.008, 0.027</td>
<td></td>
</tr>
<tr>
<td>Supernumerary</td>
<td>35 (31.5)</td>
<td>26 (0.6)</td>
<td>&lt;0.0001*</td>
<td>0.310</td>
<td>0.224, 0.405</td>
<td></td>
</tr>
<tr>
<td>Microdontia</td>
<td>1 (0.9)</td>
<td>0 (0.0)</td>
<td>0.195</td>
<td>0.007</td>
<td>-0.003, 0.047</td>
<td></td>
</tr>
<tr>
<td>Talon Cusp</td>
<td>6 (5.4)</td>
<td>0 (0.0)</td>
<td>&lt;0.0001*</td>
<td>0.054</td>
<td>0.020, 0.114</td>
<td></td>
</tr>
<tr>
<td>Fusion</td>
<td>1 (0.9)</td>
<td>39 (0.9)</td>
<td>0.619</td>
<td>0</td>
<td>-0.010, 0.041</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>43 (38.7)</td>
<td>98 (2.1)</td>
<td>&lt;0.0001*</td>
<td>0.366</td>
<td>0.275, 0.463</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primary Dentition</th>
<th>Male UCL group Frequency N=74 (66.7%)</th>
<th>Female UCL group Frequency N=37 (33.3%)</th>
<th>Male and female comparison Frequency N=71 (65.5%)</th>
<th>p-value</th>
<th>Risk difference</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agenesis</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1.000</td>
<td>0</td>
<td>-0.095, 0.049</td>
<td></td>
</tr>
<tr>
<td>Supernumerary</td>
<td>22 (29.7)</td>
<td>13 (35.1)</td>
<td>0.666</td>
<td>-0.054</td>
<td>-0.260, 0.142</td>
<td></td>
</tr>
<tr>
<td>Microdontia</td>
<td>0 (0.0)</td>
<td>1 (2.7)</td>
<td>0.333</td>
<td>-0.027</td>
<td>-0.141, 0.394</td>
<td></td>
</tr>
<tr>
<td>Talon Cusp</td>
<td>3 (4.1)</td>
<td>3 (8.1)</td>
<td>0.398</td>
<td>-0.041</td>
<td>-0.185, 0.363</td>
<td></td>
</tr>
<tr>
<td>Fusion</td>
<td>1 (1.4)</td>
<td>0 (0.0)</td>
<td>1.000</td>
<td>0.014</td>
<td>-0.084, 0.073</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>26 (35.1)</td>
<td>17 (46.0)</td>
<td>0.305</td>
<td>-0.108</td>
<td>-0.316, 0.101</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Secondary Dentition</th>
<th>Male UCL group Frequency N=74 (66.7%)</th>
<th>Female UCL group Frequency N=37 (33.3%)</th>
<th>Male and female comparison Frequency N=71 (65.5%)</th>
<th>p-value</th>
<th>Risk difference</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agenesis</td>
<td>5 (6.8)</td>
<td>5 (13.5)</td>
<td>0.297</td>
<td>-0.068</td>
<td>-0.230, 0.061</td>
<td></td>
</tr>
<tr>
<td>Supernumerary</td>
<td>1 (1.4)</td>
<td>0 (0.0)</td>
<td>1.000</td>
<td>0.014</td>
<td>-0.084, 0.073</td>
<td></td>
</tr>
<tr>
<td>Microdontia</td>
<td>5 (6.8)</td>
<td>5 (13.5)</td>
<td>0.297</td>
<td>-0.068</td>
<td>-0.230, 0.061</td>
<td></td>
</tr>
<tr>
<td>Talon Cusp</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1.000</td>
<td>0</td>
<td>-0.095, 0.049</td>
<td></td>
</tr>
<tr>
<td>Fusion</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1.000</td>
<td>0</td>
<td>-0.095, 0.049</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>11 (14.9)</td>
<td>45 (24.3)</td>
<td>0.295</td>
<td>-0.095</td>
<td>-0.282, 0.073</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Infants with UCL: Comparison of risk difference between the UCL group risk and control risk in primary dentition anomalies and comparison between male and female risk in the primary and secondary dentition anomalies using the Fisher’s exact test. Risk difference is the difference between UCL risk and control risk i.e. UCL percentage value minus control percentage value for each anomaly.

(If one female had agenesis and microdontia in the secondary dentition and counted once in overall).
4.3 STUDY IV & V

Of the 60 patients studied, 63.3% had hypodontia, 21.7% had supernumerary teeth, 69.6% had microdontia, and 12.5% had macrodontia in the secondary dentition. All of the cleft-sided permanent lateral incisors had associated anomalies, with a large proportion (43.1%) missing; and when present in 31 subjects, the majority (90.3%) was positioned distal to the cleft. Most of the cleft-sided permanent central incisors were rotated if present, and prevalent at 86.7% (Fig.11). Delayed dental maturation was found in the 5 to 9-year-old children with UCLP compared to controls by 0.55 years (standard deviation: 0.75) (p<0.001). There was no significant difference between the dental maturation of children with UCLP and controls in the 9 to 13-year-old group (P=.744). The risk of developing asymmetric tooth-pairs in the group with UCLP at both age groups were significantly higher than the control group (p<0.001) (Table 4).

Table 3. Comparison of UCL and CP groups: chronological age and dental maturity.

<table>
<thead>
<tr>
<th></th>
<th>UCL group</th>
<th>CP group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chronological age in months</td>
<td>Dental maturity in months</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Female</td>
<td>65 (35.5)</td>
<td>2.4 (3.4)</td>
</tr>
<tr>
<td>Male</td>
<td>138 (64.5)</td>
<td>2.4 (5.1)</td>
</tr>
<tr>
<td>Total</td>
<td>138 (100.0)</td>
<td>2.4 (5.1)</td>
</tr>
</tbody>
</table>

*Equivalent to 20 fetal weeks.

Table 4. Comparison of cleft group and control: chronological age, dental age, and asymmetric tooth-pair formation.
Figure 11. Summary of prevalence of dental anomalies in children with unilateral cleft lip and palate.
5 DISCUSSION

5.1 POPULATION SURVEILLANCE

Public health surveillance is defined by the WHO as the “continuous, systematic collection, analysis and interpretation of health-related data needed for the planning, implementation, and evaluation of public health practice” [WHO, 2012]. The purpose in population surveillance of infants born with orofacial cleft malformations is to monitor population reproductive health outcomes and healthcare resource planning to manage affected outcomes. An infant with a complete orofacial cleft malformation faced a slew of long-term challenges with chronic functional, physical, mental health and general health problems [Berg et al., 2016]. The health of every birth mattered and every birth of an infant with orofacial cleft malformation placed a burden on the family, community, and healthcare resources [Boulet et al., 2009; Galloway et al., 2017]. Most countries showed minor variations in up or downtrends in the prevalence of orofacial cleft births over the years. The mild fluctuations could be random due to diagnosis and documentation, coverage and reporting, and age of registration [Jensen et al., 1988; Grosen et al., 2010; Klintö et al., 2020].

5.2 DETECTION AND DIAGNOSIS

Advances in medical imaging made it possible for prenatal detection of foetuses with orofacial clefts. Extraoral and large clefts were easier to detect than intraoral clefts. As cleft malformations can be obvious or occult, different diagnostic tools are necessary. Ultrasonography in two or three-dimensional views, magnetic resonance imaging, computed tomography, and radiography, have been variously used to diagnose hidden malformations, e.g. subepithelial lip clefts, submucosal primary and secondary cleft palates, and the extent of the anomalies. Postnatal imaging that complement clinical diagnosis are routinely used in assessments for developmental or functional deficits in formulating treatment plans. Imaging protocols are routine in investigations for the follow-up of developmental and other associated anomalies in individuals with orofacial clefts [Abramson et al., 2015].

In addition to morphological diagnosis, cytogenomics testing form a part of the comprehensive diagnosis in individuals with OFC. Chromosome microarray analysis and detailed molecular analysis are proposed in conjunction with precise phenotyping for the provision of personalised genetic service in predicting risks of recurrence for orofacial clefts [Cox et al., 2018; Lustosa-
Mendes et al., 2020]. Genes were associated in the predictability of different cleft phenotypes that could be developed for future diagnostics [Huang et al., 2019].

5.3 CRANIOFACIAL AND DENTAL DEVELOPMENT

Orofacial and dental structures formation in the early stages of human development were tightly sequenced and coordinated under the control of genes in concert with the environment and epigenetics. Perturbations in embryogenesis led to maldevelopment in the embryo and foetus [Sperber et al., 2010]. Various biological pathways in the formation of the head and teeth were affected by genetic mutations that modified gene functions in four main pathways of development, viz. *FGF*, *SHH*, *WNT*, and *TGF-β* pathways [Oliver et al., 2020]. The clinical implications in disruption of these pathways were phenotypes with craniofacial anomalies, orofacial clefts and tooth anomalies.

Orofacial structures, in particular the mandible, were found to be smaller in individuals with orofacial cleft malformations. The growth potential was normal although the vector of growth was different in the CP and the UCL phenotypes [Hermann et al., 2002; Kreiborg et al., 2006]. By the same token, prenatally developing teeth were smaller than that of infants with no clefts and dental maturation was delayed [Hermann et al., 2012; Hermann et al., 2017]. Delayed dental development and dental anomalies were common findings in individuals with orofacial clefts. The amount of delay was variable, ranging from 0.2 to 0.9 years with a mean of 0.6 years [van Dyck et al., 2019]. The maxillary lateral incisor tooth at the cleft site was the most vulnerable tooth for dental anomalies and delayed development. This was due to its position in the dental arch at the convergence of the maxillary and medial nasal processes during fusion [Hovorakova et al., 2018].

The evidence was conflicting with regards to the effects of cleft severity, cleft laterality and gender on dental maturation [van Dyck et al., 2019]. Findings were variously confounded by sampling, heterogeneity or homogeneity of the study samples, methodologies used in dental age ascertainment, and control groups for comparison. Due to slow development and different morphology of teeth in individuals with OFC, there were inherent technical errors in establishing dental age and the challenge was establishing the accuracy and reliability of the findings [AlQahtani et al., 2010; Jayaraman et al., 2013; Pinchi et al., 2018].
5.4 TREATMENT

Depending on the severity of the individual’s OFC and the cleft team’s protocol, the intensity of team interactions, evaluations and interdisciplinary treatment procedures were variable. They encompassed multidisciplinary expertise from genetics, paediatrics, paediatric dentistry, orthodontics, audiology, speech, surgery, viz. craniofacial, plastic, oral and maxillofacial, and otolaryngologic, psychological and social services [American Cleft Palate-Craniofacial Association, 2018]. Treatment outcomes are hard to predict due to different malformations, variations in development, individual responses to various treatments, team protocol, and experience of the cleft team. Treatment impacts the wellbeing of all involved, the patient, the family unit, and the caregiver, particularly when treatment trajectories are long and spans the developmental duration of a child over 18 years [Sischo et al., 2017]. During the protracted period of treatment to deliver the objectives of care, it is important to assess patients, families, and caregivers to ensure the goal in helping the patients to achieve a good quality of life was not defeated by the burden of care [Alansari et al., 2014].

5.5 PREVENTION

The effectiveness of prevention programmes in births with orofacial clefts was questioned. The anticipated downtrend in prevalence of births with orofacial clefts was not evidenced after decades of folic acid use in preparation for pregnancy. To the contrary, some reports showed periconceptional intake of folic acid could actually increase the risk of cleft births, in particular, births of infants with the cleft lip and alveolus phenotype [Rozendaal et al., 2013]. Risk reduction was in the decreased number of infants with associated anomalies and not in infants born with orofacial clefts [Czeizel et al., 2013; Gildestad et al., 2015]. Considerable heterogeneity between studies confounded the findings and recommendations could not be substantiated in the use of folic acid for the prevention of births with orofacial clefts [Zhou et al., 2020]. The controversy continues and prevention remains uncertain.

The orofacial cleft disorder is poorly understood without correct and comprehensive data. Consequent to that, diagnosis and treatment planning are incomplete with improvised treatment along the way, and the prevention programme is ineffective. Without specialised manpower planning and training, resource allocation for healthcare and healthcare facilities becomes skewed and ineffectual. Reliable and correct data are challenging to attain but essential in the evaluation of the multiplex problems in individuals with orofacial clefts. The
way forward is personalised medicine over the traditional patient-management approach by generalised empirical evidence. Each individual does not mirror the mean as the mean is a lonely place to be [J.R.E. Mills, personal communication, 22 June 1987]. For appropriate initiatives and treatment to be implemented, it behooves all researchers in the orofacial field to gather representative data from precise phenotyping for accurate interpretations in the translation to precision medicine for upstream healthcare.

5.6 STUDY I

Population prevalence of live births with orofacial clefts

Prevalence for cleft live births of the resident population in Singapore, without stillbirths and abortuses, was 16.7 per 10,000. It was 1.7 times higher than the global average of 9.92 per 10,000. There trend was flat with no significant upward trend (p=0.317). In the decade from 1993 to 2002, the prevalence was 18.7 per 10,000 with significant upward trend that included stillbirths and abortuses of residents, non-residents, and foreigners in Singapore [Tan et al., 2008]. Prevalence in both decades were at the higher end of the global range from 2.89 to 23.85 [IPDTOC Working Group, 2011].

Ethnic-specific prevalence

Singapore is an immigrant country with forebears from Malaysia, China and India. The group with Indian ancestry had the lowest prevalence, 10.74 per 10,000. Prevalence in the groups with Chinese and Malay ancestries was almost similar, 17.17, 16.92 per 10,000, respectively. The cleft prevalence of the ethnic groups resembled those in the old countries of their forebears. This corroborates with ethnic-specific prevalence may be linked to ancestral genes [Mukhopadhyay et al., 2020]. The group with the highest prevalence of 21.73 per 10,000 is the mixed ethnicity group. In comparison between the two decades, the prevalence of this group doubled over two decades, from 1.16 per 10,000 (from 1993 to 2002) to 21.73 per 10,000 (from 2003 to 2012). The group with mixed ethnicities was very heterogeneous with Southeast Asian ancestries from Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Thailand, and Vietnam. Population demographics in this group varied year by year due to changing immigration patterns, and late registration of older subjects with clefts could be the reason for the higher prevalence value in this group.
Gender and cleft-types

Males had higher prevalence than females. The former was predisposed to cleft lip and palate or cleft lip only, whereas females tended to have cleft palate only. The role of gender in different cleft phenotypes was postulated to be due to the timing of embryological development, and slower palatal development could result in more cleft palate defects in females [Burdi et al., 1969]. Gender also represented a substantial estimated attributable fraction in non-modifiable factors for the population with OFC [Raut et al., 2019]. The multifactorial threshold (MFT) model predicted greater genetic liability within a population with higher overall cleft prevalence that predisposed to increased frequencies of severe cleft although the evidence was not conclusive [Mitchell et al., 2002].

Associated malformations

Syndromic clefts were more commonly associated with CP defects (55.6%) and half of infants with non-syndromic CP (50.2%) were associated with other malformations. The associated anomalies of infants with CP in the present study involved mostly the musculoskeletal system and heart anomalies. The findings concurred with other reports on the high frequency of associated anomalies with CP cases that should be routinely examined for additional malformations [Maarse, 2011]. The infant mortality rate of infants born with clefts was 4.8%. The majority of infants born with OFC who perished within the first year had associated anomalies. This was more than double the population infant mortality rate of 2.1%.

Limitations

The findings in this study are based on diagnostic data soon after birth. The high probability of under-reporting of cleft live births and associated malformations is likely and due to early registrations. Palatal defects can prove to be challenging to diagnose and associated comorbidities easily missed in small infants. Continual examination and diagnosis for data updates to the registry will improve accuracy in ascertainment of data.

5.7 STUDY II & III

Dental subphenotypes in infants with orofacial clefts

Longitudinal samples from population-based cohorts of Northern European ancestry with two non-overlapping phenotypes of isolated cleft lip and isolated cleft palate were used to minimise the confounding effects of gender, ancestry, overlapping cleft-types, and treatment effects on the developing dentitions.
**Dental anomalies in the primary and secondary dentitions**

The types of dental anomalies in the primary and secondary dentitions were different. Supernumerary primary teeth did not form secondary supernumerary teeth. The lack of concurrence in longitudinal dental anomalies of the primary and secondary dentitions associated with clefts suggested disturbed development that was time-dependent. Cleft lip was relatable to differentiation defects, and cleft palate was mostly due to fusion defects. In this study, the significant association of unilateral cleft lip and maxillary lateral incisors anomalies could well be a combination of late-stage differentiation and fusion defects of the lip and dental epithelium, respectively. The significant occurrence of missing permanent maxillary lateral incisors with unilateral cleft lip could be under the control of genes, epigenetics and/or the environment that could include treatment effects [Kirkham, 1931; Jugessur et al., 2009; Dentino et al., 2012; Phan et al., 2016; Korolenkova et al., 2018].

There were no missing teeth in the primary dentition although there were missing permanent maxillary lateral incisors in infants with greater cleft lip severity of Grades 3 and 4, and in frequencies of 12.1% and 60.0%, respectively. The greater prevalence of missing teeth in the primary and secondary dentitions could also be attributed to different ancestral genes, epigenetics, and environmental effects or iatrogenesis.

**Dental Maturation**

Male infants, in both groups with UCL and CP, were significantly delayed in dental maturation compared to females. This could be related to normal sexual dimorphism of human biological development. Biological variation of dental maturation in children with no clefts was also similar in the control samples of the London Atlas [AlQahtani et al., 2010]. Females, in general, were found to be advanced in dental development compared to males [Stack, 1960] that could not be attributed to hormonal differences alone [Garn et al., 1959].

Delayed dental maturation in the primary and secondary dentitions occurred in both groups with UCL and CP, which increased with cleft severity in infants with UCL and CP in the primary dentition but not in the secondary dentition. There was sexual dimorphism in delayed dental maturation that was more pronounced in males. The delay in dental maturation was greater in infants with UCL compared to infants with CP.

In unoperated infants with clefts, cleft severity grades were inadequate to distinguish subphenotypic heterogeneity in infants with UCL and CP. Dental anomalies, including deviations in the number of primary teeth and malformations of primary teeth were traits that
defined the unoperated infants with UCL but not the unoperated infants with CP. Delayed
dental maturation in the primary dentition characterised both the unoperated UCL and CP
subphenotypes shortly after birth. The knowledge derived from this study of unoperated and
operated infants with OFC provided information for guidance in patient/parent counselling,
patient-management, planning of facilities, training of specialized manpower, and enlarging
the knowledge base of the OFC phenome. The availability of comprehensive phenotypic data
for integration with genomic data would facilitate precision medicine in developing strategies
for future treatment and prevention.

Limitations

The main limitation in this study was the retrospective study design without matched
population-based controls for comparison of dental anomalies and dental maturation. As it was
not possible for a prospective control groups of infants without OFC, the best available
historical controls were employed for comparisons in this study. Another limitation was in
using a series of tooth stages to determine the overall dental maturation age. This was based on
radiographic findings of unerupted teeth at one time-point, a “snap-shot” that was fitted into
pre-determined age categories rather than establishing the continuous dental maturation age of
individual teeth. Due to inherent delays in dental development, secondary tooth agenesis could
have been overestimated whereas supernumerary teeth and talon cusp formation were possibly
underestimated. Due to the inherent delay in tooth development, the frequency of secondary
dental anomalies could be higher if the study samples were re-examined at an older age.

5.8 STUDY IV & V

Dental anomalies and maturation in the permanent dentition

Children with unilateral cleft lip and palate were found to be associated with higher prevalence
of dental anomalies such as hypodontia (63.3%), supernumerary teeth (21.7%), and
abnormalities in tooth size, 69.6% had microdontia, and 12.5% had macrodontia. All of the
cleft-sided permanent lateral incisors had associated anomalies, with a large proportion
(43.1%) missing; and when present in 31 subjects, the majority (90.3%) was positioned distal
to the cleft. Most of the cleft-sided permanent central incisors were rotated if present, and
prevalent at 86.7%.

Delayed dental maturation of the secondary dentition was found in the 5 to 9-year-old children
with unilateral cleft lip and palate compared to controls by 0.55 years (standard deviation: 0.75)
(p<0.001). There was no significant difference between the dental maturation of children with
UCLP and controls in the 9 to 13-year-old age group (p=0.744). The group with unilateral cleft
lip and palate had higher risk of asymmetrically developing tooth pairs than the control group for both age groups (p<0.001).

**Limitations**

Tooth maturation stages are more obvious and more easily staged with shorter durations of tooth development in the younger age group than the older age group. There could be much more variation in dental maturation in the older age group as there were potentially more extrinsic postnatal environmental factors, infections and/or iatrogenesis that could have exerted their influence on the development of teeth with age. With tooth development slowing down, especially during the last stages of root development, assessment of tooth formation stages became challenging. There was uncertainty in accuracy of establishing root length in individuals with clefts as it was reportedly shorter than normal [Hunter, 1975]. The assessment of root length was all important in the Demirjian’s method of determination of dental maturation age [Demirjian et al., 1973]. In the older age group of children with clefts, usually two incompletely formed teeth were available for assessment, the second premolar and molar. With slow dental development, shorter root length in children with clefts, and uncertainty in timing of root closure, an overestimation was likely to be a recurrent systematic error in the older group of children.
6 CONCLUSION

6.1 STUDY I
Prevalence of OFC was ethnic-specific and sexually dimorphic in Singapore with no rising trend. There was a twofold difference in prevalence in the ethnic groups with the highest and lowest frequencies. Prevalence was higher in males than females. Infants with cleft palate only were the most common compared to the cleft lip with cleft palate, and cleft lip only phenotypes. Mortality rate of infants with OFC was double that of the population’s infant mortality rate.

6.2 STUDY II & III
In unoperated Danish infants of Northern European descent with isolated cleft palate, there were no dental anomalies in both primary and secondary dentitions. In the group of infants with unilateral cleft of the lip without cleft palate, dental anomalies in the primary and secondary dentitions were dissimilar, the primary dentition group was unoperated and the secondary dentition group was the same group after surgery. Almost one third of infants with isolated unilateral cleft lip had supernumeraries of the primary maxillary lateral incisors associated with increased severity of the cleft lip. It suggested a cleft of the dental epithelium in a UCL subphenotype with forme fruste cleft dentoalveolus. Dental maturation delay was detected in both groups of infants with isolated unilateral cleft lip and isolated cleft palate. Increased cleft severity was associated with greater delayed maturation in the primary dentition but not in the secondary dentition.

6.3 STUDY IV & V
In operated Southeast Asian children with UCLP, about two thirds had dental anomalies of the secondary maxillary lateral incisors at the cleft site, commonly microdontia and missing teeth. Asymmetric dental maturation occurred in children with UCLP with delayed tooth development on the cleft-side of the maxilla. Dental maturational delay occurred in the 5 to 9-year-old children with UCLP, which normalised to match that of children without clefts by the time they were 9 to 13 years of age.
7 POINTS OF PERSPECTIVE

The multiethnic population-based findings revealed high prevalence of live births with OFC in specific ethnic groups. High infant mortality was a crucial point for concern with a rising trend of infants with cleft palate and medical comorbidities, a common phenotype in the population. The survival of these infants with associated malformations in their first year is precarious and requires the use of highly specialized manpower, facilities and equipment. It comes at much cost to the parents and families, emotionally and financially, with a significant burden of care in long-term treatment and habilitation.

Orofacial cleft phenotypes are variable in traits that are not immediately obvious as they are developmental in nature. As orofacial clefts and associated malformations occurred early in pregnancy, continued surveillance for data is important in decision-making for upstream management and prevention programmes of at-risk individuals. More information from research is needed to establish the variants and phenotypes with implications in reproductive health. Aggregated data and population-based information are not applicable to the individual and not all at-risk individuals are similar. As such, detailed evaluation of the clinical phenotype and molecular tests are necessary for personalised diagnosis and treatment indications.

Several processes are necessary to enable the practice of precision medicine in this field:

1. Early detection of a developing foetus with orofacial cleft and services in counselling and support for parents and their families.
2. Personalised genetic service with molecular testing and detailed characterisations of the non-syndromic orofacial cleft phenotype, family and medical histories for recurrence risks.
3. Facilitate informed decisions through education of those affected or are at-risk to provide support groups and research resources for information to help individuals, parents and families in understanding future implications: the downstream effects of the affected individual with orofacial clefts, general health and wellbeing, growth and development with and without treatment, schedule of coordinated multidisciplinary treatment, treatment procedures, possible complications, and estimated expenditure.
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10 PAPERS I-V